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

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
04 November 2021 (04.11.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/222065 A8

(51) International Patent Classification:
C12N 15/113 (2010.01) *C07H 21/00* (2006.01)
A61K 31/713 (2006.01) *A61P 25/28* (2006.01)

(21) International Application Number:
PCT/US2021/029081

(22) International Filing Date:
26 April 2021 (26.04.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/015,867 27 April 2020 (27.04.2020) US

(71) Applicant: **ALNYLAM PHARMACEUTICALS, INC.** [US/US]; 675 West Kendall Street, Henri A. Termeer Square, Cambridge, MA 02142 (US).

(72) Inventors; and
(71) Applicants: **BOSTWICK, Bret, Lee** [US/US]; 675 West Kendall Street, Henri A. Termeer Square, Cambridge, MA 02142 (US). **PENG, Haiyan** [CN/US]; 675 West Kendall Street, Henri A. Termeer Square, Cambridge, MA 02142 (US). **MCININCH, James, D.** [US/US]; 675 West Kendall Street, Henri A. Termeer Square, Cambridge, MA 02142 (US). **CASTORENO, Adam** [US/US]; 675 West Kendall Street, Henri A. Termeer Square, Cambridge, MA 02142 (US). **SCHLEGEL, Mark, K.** [US/US]; 675 West Kendall Street, Henri A. Termeer Square, Cambridge, MA 02142 (US).

(74) Agent: **ZACHARAKIS, Maria Laccotripe** et al.; McCarter & English, LLP, 265 Franklin Street, Boston, MA 02110 (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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(48) Date of publication of this corrected version:
09 December 2021 (09.12.2021)

(15) Information about Correction:
see Notice of 09 December 2021 (09.12.2021)

(54) Title: APOLIPOPROTEIN E (APOE) IRNA AGENT COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: The disclosure relates to double stranded ribonucleic acid (dsRNAi) agents and compositions targeting an APOE gene, as well as methods of inhibiting expression of an APOE gene and methods of treating subjects having an APOE-associated neurodegenerative disease or disorder, e.g., Alzheimer's disease and Parkinson's disease, using such dsRNAi agents and compositions.

WO 2021/222065 A8

APOLIPOPROTEIN E (APOE) iRNA AGENT COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 63/015,867, filed on April 27, 2020, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 20, 2021, is named 121301_11220_SL.txt and is 200,944 bytes in size.

BACKGROUND OF THE INVENTION

The *apolipoprotein E* gene encodes the Apolipoprotein E (APOE) protein, a glycoprotein that, following cleavage of an 18 amino acid signal peptide, is composed of 299 amino acids. There are three common isoforms of APOE, APOE2, APOE3, and APOE4, encoded by three corresponding alleles. The three APOE isofroms, ApoE ε2 (APOE2), ApoE ε3 (APOE3), ApoE ε4 (APOE4), differ from one another only at amino acid positions 112 and 158; APOE2 has a Cys112 and a Cys158, APOE3 has a Cys112 and an Arg 158, and APOE4 has an Arg112 and an Arg158. APOE is widely expressed, but is primarily expressed peripherally in liver hepatocytes and in glial cells in the central nervous system (CNS).

In the periphery, APOE functions in lipid homeostasis. These lipoprotein particles cannot cross the blood-brain barrier; studies have shown that apoE-containing particles released by astrocytes and microglia are the main sources of brain apoE (Bjorkhem I, *et al.* (1998) *J Lipid Research* 39(8):1594–1600; Pitas RE, *et al.* (1987) *Biochimica Biophysica Acta.* 13;917(1):148–161; Krasemann S, *et al.* (2017) *Immunity.* 47(3):566–581.e9. doi:10.1016/j.immuni.2017.08.008). In the brain, APOE modulates multiple pathways including lipid transport, synaptic integrity and plasticity, glucose metabolism, neuroinflammation, and cerebrovascular integrity. For example, once APOE is secreted from cells, several transporters (*e.g.,* ATB-binding cssestte transporters) transfer cholesterol and phospholipids to nascent APOE to form lipoprotein particles which APOE subsequently distributes to neurons through binding to APOE receptors, such as LDL receptor (LDLR) family members. Furthermore, it has been observed that the serum APOE phenotype but not the cerebrospinal fluid (CSF) ApoE phenotype of a recipient completely converted to that of donor following liver transplantation. In addition, astrocytes produce APOE in high-density lipoprotein (HDL)-like particles that have distinct properties from APOE derived from other sources (see, *e.g.*, Morikawa, *et al.*, *Neurobiol Dis.*. Jun-Jul 2005;19(1-2): 66-76). Therefore, the APOE in CSF cannot

be derived from the plasma pool and therefore must be synthesized locally (Linton MF, *et al.* (1991) *J Clin Invest.* 88(1):270–281. doi:10.1172/JCI115288).

Polymorphism in the APOE gene has been associated with multiple proteinopathies. The best established link between APOE polymorphism and disease is between APOE genotype and Alzheimer's disease (AD) which has been shown to be a major risk determinant of late-onset Alzheimer's disease, the symptoms of which develop after age 65. Additionally, recent work from Haltzman lab described that possession of the ε4 allele significantly accelerated disease progression (p=0.02), with one ε4 allele increasing progression rate by 14% and two ε4 alleles increasing the rate by 23% compared to non-carriers (Holtzman, *et al.* (2017) *Nature* 549:523). AD is the leading cause of dementia in elderly individuals and its pathological hallmarks include the deposition of extracellular amyloid-β (Aβ) aggregates as amylod plaques and intracellular hyperphosphorylated tau aggregates as neurofilbrillary tangles along with neuronal loss and glial activation. As individuals with late-onset AD account for 95% or more of the total AD population, various efforts to elucidate the role of APOE in AD are ongoing.

More specifically, it has been shown that subjects having one copy of APOE4 have a greater than three-fold risk of developing AD and subjects having two copies of APOE4 have a greater than 12-fold increased risk of developing AD, while two copies of APOE2 are protective in subjects from AD development (Reiman EM, *et al.* (2020) *Nature Communications* 11 (1); 667). In addition, there have been three APOE knockout human cases reported and none of these subjects experienced dementia at the time of hospital visit (age 40-60) (Ghiselli, *et al.* (1981) *Science* 214(4526):1239; Mak, *et al.* (2014) *JAMA Neurol* 71:1228; and Lohse, *et al.* (1992) *J Lipid Res.* (11):1583). In one of the three cases (40 years old male), MRI and Cerebral Spinal Fluid (CSF) biomarker tests demonstrated no signs of neurodegenerations with intact brain structure and normal range of Tau and p-Tau levels. Furthermore, a recent case study, has shown that the Christchurch mutation in ApoE3 may be protective again presenilin 1 driven dementia as evident by preserved cognitive function and limited Taupathy by PET. The presence of the Christchurch mutation led to loss of function of ApoE3 binding to HSPGs and LDL receptors and the patient has hyperlipoproteinemia type III but no cardiovascular disease (Arboleda-Velasquez, *et al.* (2019) *Nature Medicien* 25:1680).

It has also been demonstrated in ApoE inducible amyloid mouse models that increased expression of ApoE4 accelerates amyloid accumulation and neuritic dystrophy (Liu, *et al.* (2017) *Neuron* 96:1024) and Huynh, *et al.* (*Neuron* (2017) 96:1013) and that antisense inhibition of APOE4 is protective in transgenic amyloid precursor protein (APP)/presenillin 1 (PS1-21) mice. In addition, it has been shown that deletion of ApoE4 in a tauopathy mouse model was protective of neurodegeneration (Holtzman, *et al.* (2017) *Nature* 549:523) and that reintroduction of APOE4 expression in human neurons derived from induced pluripotent stem cells that expressed APOE4 but were made APOE null results in a gain of toxic effect from APOE4 (Wang, *et al.* (2018) *Nat Medicine* 24:647). Furthermore, it has been shown that restricting expression of APOE4 to the liver of mice can still have an effect on cognitive abilities and can compromise the blood brain barrier and

increase neuroinflammation (alzforum.org/news/research-news/apoe-has-hand-Alzheimer'ss-beyond-av-beyond-brain).

Currently, there are no cures or preventative treatments for subjects having an APOE-associated neurodegenerative disease, such as AD, and supportive and symptomatic management is the mainstay of treatment. Therefore, there is a need in the art for compositions and methods for the treatment of subjects that have or are at risk of developing a neurodegenerative disease.

BRIEF SUMMARY OF THE INVENTION

The present disclosure provides RNAi agent compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an apolipoprotein E (APOE) gene. The APOE gene may be within a cell, *e.g.,* a cell within a subject, such as a human. The present disclosure also provides methods of using the RNAi agent compositions of the disclosure for inhibiting the expression of an APOE gene or for treating a subject who would benefit from inhibiting or reducing the expression of an APOE gene, *e.g.,* a pathogenic APOE allele, *i.e.,* APOE4, *e.g.,* a subject suffering or prone to suffering from an APOE-associated neurodegenerative disease, such as an amyloid-β-mediated disease or a tau-mediated disease. In particular, the RNAi agent compositions herein are capable of affecting the unique APOE expression by astrocytes within the CNS for the treatment of APOE-associated neurodegenerative disease.

Accordingly, in one aspect, the instant disclosure provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of an apolipoprotein E (APOE) gene, where the RNAi agent includes a sense strand and an antisense strand, and where the antisense strand includes a region of complementarity which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the antisense sequences listed in any one of Tables 2-5 and 7-10. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 15 contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 2-5 and 7-10. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 15 contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 7 and 8. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 15 contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 9 and 10. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the antisense sequences listed in any one of Tables 2-5 and 7-10. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19 contiguous nucleotide (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of the antisense sequences listed in any one of Tables 7 and 8. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19 contiguous nucleotide (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of the antisense sequences listed in any one of Tables 9 and 10. In certain

embodiments, the antisense strand includes a region of complementarity which includes at least 19 contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 2-5 and 7-10. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19 contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 7 and 8. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19 contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 9 and 10. In certain embodiments, thymine-to-uracil or uracil-to-thymine differences between aligned (compared) sequences are not counted as nucleotides that differ between the aligned (compared) sequences.

In some embodiments, the agents include one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In yet other embodiments, the agents further comprise one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a apolipoprotein E (APOE) gene, where the dsRNA agent includes a sense strand and an antisense strand, where the sense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the sense strand sequences presented in Tables 2-5 and 7-10; and where the antisense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of antisense strand nucleotide sequences presented in Tables 2-5 and 7-10. In certain embodiments, the sense strand includes at least 15 contiguous nucleotides of any one of the sense strand sequences presented in Tables 2-5 and 7-10; and where the antisense strand includes at least 15 contiguous nucleotides of any one of antisense strand nucleotide sequences presented in Tables 2-5 and 7-10. In certain embodiments, the sense strand includes at least 15 contiguous nucleotides of any one of the sense strand sequences presented in Tables 7 and 8; and where the antisense strand includes at least 15 contiguous nucleotides of any one of antisense strand nucleotide sequences presented in Tables 7 and 8. In certain embodiments, the sense strand includes at least 15 contiguous nucleotides of any one of the sense strand sequences presented in Tables 9 and 10; and where the antisense strand includes at least 15 contiguous nucleotides of any one of antisense strand nucleotide sequences presented in

Tables 9 and 10. In certain embodiments, the sense strand includes at least 19 contiguous nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of the sense strand sequences presented in Tables 2-5 and 7-10; and where the antisense strand includes at least 19 contiguous nucleotides of any one of antisense strand nucleotide sequences presented in Tables 2-5 and 7-10 (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of antisense strand nucleotide sequences presented in Tables 2-5 and 7-10. In certain embodiments, the sense strand includes at least 19 contiguous nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of the sense strand sequences presented in Tables 7 and 8; and where the antisense strand includes at least 19 contiguous nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of antisense strand nucleotide sequences presented in Tables 7 and 8. In certain embodiments, the sense strand includes at least 19 contiguous nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of the sense strand sequences presented in Tables 9 and 10; and where the antisense strand includes at least 19 contiguous nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) of any one of antisense strand nucleotide sequences presented in Tables 9 and 10.

In some embodiments, the agents include one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In yet other embodiments, the agents further comprise one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

An additional aspect of the disclosure provides a double stranded RNAi agent for inhibiting expression of an apolipoprotein E (APOE) gene, where the dsRNA agent includes a sense strand and an antisense strand, where the sense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, or 9, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9, where a substitution of a uracil for any thymine of SEQ ID NOs: 1, 3, 5, 7, and 9 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, and 9 or the nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or

9; and where the antisense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, or 10 or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, where a substitution of a uracil for any thymine of SEQ ID NOs: 2, 4, 6, 8, and 10, (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, and 10, or the nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, where at least one of the sense strand and the antisense strand includes one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the nucleotide sequence of the sense strand nucleotide sequence of a duplex in Tables 2-5 and 7-10. In one embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the nucleotide sequence of the sense strand nucleotide sequence of a duplex in Tables 7 and 8. In one embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the nucleotide sequence of the sense strand nucleotide sequence of a duplex in Tables 9 and 10.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises an antisense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the antisense nucleotide sequence of any one of the duplexes in one of Tables 2-5 and 7-10. In one embodiment, the double stranded RNAi agent targeted to APOE comprises an antisense strandwhich includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the antisense nucleotide sequence of duplex in one of Tables 7 and 8. In one embodiment, the double stranded RNAi agent targeted to APOE comprises an antisense strandwhich includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the antisense nucleotide sequence of duplex in one of Tables 9 and 10.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (i.e., differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of nucleotides 50-113, 59-97, 59-90, 107-177, 107-153, 124-153, 198-240, 203-240, 209-240, 283-378, 283-312, 307-378, 322-369, 330-357, 394-419, 568-600, 568-594, 841-879, 900-926, 997-1055, 1002-1044, 1014-1044,

1019-1044, 1120-1166, 1130-1166, 1130-1155 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (i.e., differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of nucleotides 59-90, 330-357, 568-594, 1019-1044, 1130-1155 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of nucleotides 57-79, 62-84, 75-97, 86-108, 207-229, 213-235, 218-240, 898-920, 1128-1150, 637-659 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In another embodiment, the double stranded RNAi agent targeted to APOE comprises a sense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) fromfrom any one of the nucleotide sequences of nucleotides 57-79, 62-84, 207-229, 1128-1150 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises an antisense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1204704, AD-1204705, AD-1204705, AD-1204706 AD-1204707, AD-1204708, AD-1204709, AD-1204710, AD-1204711, AD-1204712, and AD-1204713.

In one embodiment, the double stranded RNAi agent targeted to APOE comprises an antisense strand which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1204704, AD-1204705, AD-1204708, and AD-1204712.

In some embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In certain embodiments of the invention, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

Optionally, the double stranded RNAi agent includes at least one modified nucleotide.

In certain embodiments, the lipophilicity of the lipophilic moiety, measured by $\log K_{ow}$, exceeds 0.

In some embodiments, the hydrophobicity of the double-stranded RNAi agent, measured by the unbound fraction in a plasma protein binding assay of the double-stranded RNAi agent, exceeds 0.2. In a related embodiment, the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

In certain embodiments, substantiallyll of the nucleotides of the sense strand are modified nucleotides. Optionally, all of the nucleotides of the sense strand are modified nucleotides.

In some embodiments, substantially all of the nucleotides of the antisense strand are modified nucleotides. Optionally, all of the nucleotides of the antisense strand are modified nucleotides.

Optionally, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

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

In a related embodiment, the modified nucleotide is a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxy-thymidine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide.

In one embodiment, the modified nucleotide includes a short sequence of 3'-terminal deoxy-thymidine nucleotides (dT).

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

In an additional embodiment, the double stranded RNAi agent includes at least one phosphorothioate internucleotide linkage. Optionally, the double stranded RNAi agent includes 6-8 (*e.g.,* 6, 7, or 8) phosphorothioate internucleotide linkages.

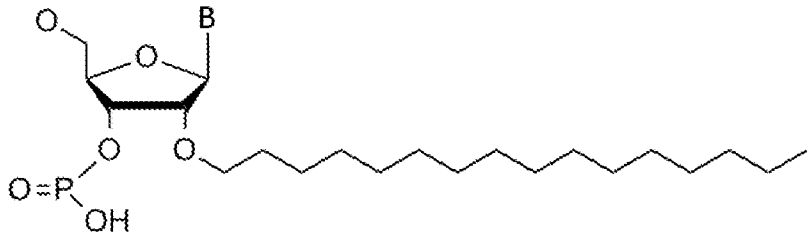
In certain embodiments, the region of complementarity is at least 17 nucleotides in length. Optionally, the region of complementarity is 19-23 nucleotides in length. Optionally, the region of complementarity is 19 nucleotides in length.

In one embodiment, each strand is no more than 30 nucleotides in length.

In another embodiment, at least one strand includes a 3' overhang of at least 1 nucleotide. Optionally, at least one strand includes a 3' overhang of at least 2 nucleotides.

In certain embodiments, the double stranded RNAi agent further includes a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker. In certain embodiments, the double stranded RNAi agent further includes a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to an internal nucleotide positon, *e.g.*, through a monovalent or branched bivalent or trivalent linker.

In one embodiment, the ligand is



,

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

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.*, one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In yet other embodiments, the agents further comprise a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to an internal nucleotide position, *e.g.*, through a monovalent or branched bivalent or trivalent linker, and a targeting ligand that targets a liver tissue, *e.g.*, one or more GalNAc derivatives conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker.

In another embodiment, the region of complementarity to APOE includes any one of the antisense sequences in any one of Tables 2-5 and 7-10. In certain embodiments, the region of complementarity to APOE includes any one of the antisense sequences in any one of Tables 7 and 8. In certain embodiments, the region of complementarity to APOE includes any one of the antisense sequences in any one of Tables 9 and 10.

In an additional embodiment, the region of complementarity to APOE is that of any one of the antisense sequences in any one of Tables 2-5 and 7-10. In certain embodiments, the region of complementarity to APOE is that of any one of the antisense sequences in any one of Tables 7 and 8. In some embodiments, the internal nucleotide positions include all positions except the terminal two positions from each end of the strand. In certain embodiments, the region of complementarity to

APOE is that of any one of the antisense sequences in any one of Tables 9 and 10. In some embodiments, the internal nucleotide positions include all positions except the terminal two positions from each end of the strand.

In a related embodiment, the internal positions include all positions except terminal three positions from each end of the strand. Optionally, the internal positions exclude the cleavage site region of the sense strand.

In some embodiments, the internal positions exclude positions 9-12, counting from the 5'-end of the sense strand. In certain emodiments, the sense strand is 21 nucleotides in length.

In other embodiments, the internal positions exclude positions 11-13, counting from the 3'-end of the sense strand. Optionally, the internal positions exclude the cleavage site region of the antisense strand. In certain emodiments, the sense strand is 21 nucleotides in length.

In some embodiments, the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand. In certain emodiments, the antisense strand is 23 nucleotides in length.

In another embodiment, the internal positions excluding positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end. In certain emodiments, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

In an additional embodiment, one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand. Optionally, one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand. In certain emodiments, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

In certain embodiments, the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound. Optionally, the lipophilic moiety is lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.

In some embodiments, the lipophilic moiety contains a saturated or unsaturated C_4-C_{30} hydrocarbon chain, and an optional functional group selected that is hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, or alkyne.

In certain embodiments, the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain. Optionally, the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain. In a related embodiment, the lipophilic moiety is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s). In certain embodiments, the carrier is a cyclic group that is pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl,

piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, or decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

In some embodiments, the lipophilic moiety is conjugated to the double-stranded RNAi agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

In one embodiment, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

In another embodiment, the double-stranded RNAi agent further includes a phosphate or phosphate mimic at the 5'-end of the antisense strand. Optionally, the phosphate mimic is a 5'-vinyl phosphonate (VP).

In certain embodiments, the double-stranded RNAi agent further includes a targeting ligand that targets a receptor which mediates delivery to a CNS tissue, *e.g.*, a hydrophilic ligand. In certain embodiments, the targeting ligand is a C16 ligand.

In some embodiments, the double-stranded RNAi agent further includes a targeting ligand that targets a brain tissue, *e.g.,* striatum.

In some embodiments, the double-stranded RNAi agent further includes a targeting ligand that targets a liver tissue, *e.g.,* hepatocytes.

In one embodiment, the lipophilic moeity or targeting ligand is conjugated via a bio-cleavable linker that is DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, or a combination thereof.

In a related embodiment, the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, the cyclic group being pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, or decalinyl.

In one embodiment, the RNAi agent includes at least one modified nucleotide that is a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a nucleotide that includes a glycol nucleic acid (GNA) or a nucleotide that includes a vinyl phosphonate. Optionally, the RNAi agent includes at least one of each of the following modifications: 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA) and a nucleotide comprising vinyl phosphonate.

In another embodiment, the RNAi agent includes a pattern of modified nucleotides as provided below in Tables 2-5 and 7-10 where locations of 2'-C16, 2'-O-methyl, GNA, phosphorothioate and 2'-fluoro modifications, irrespective of the individual nucleotide base sequences of the displayed RNAi agents. In one embodiment, the RNAi agent includes a pattern of modified nucleotides as provided below in Tables 7 and 8 where locations of 2'-C16, 2'-O-methyl, GNA, phosphorothioate and 2'-fluoro modifications, irrespective of the individual nucleotide base sequences

of the displayed RNAi agents. In one embodiment, the RNAi agent includes a pattern of modified nucleotides as provided below in Tables 9 and 10 where locations of 2'-C16, 2'-O-methyl, GNA, phosphorothioate and 2'-fluoro modifications, irrespective of the individual nucleotide base sequences of the displayed RNAi agents.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene, where the double stranded RNAi agent includes a sense strand complementary to an antisense strand, where the antisense strand includes a region complementary to part of an mRNA encoding APOE, where each strand is about 14 to about 30 nucleotides in length, where the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

where:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence including 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

where the sense strand is conjugated to at least one ligand.

In one embodiment, i is 0; j is 0; i is 1; j is 1; both i and j are 0; or both i and j are 1.

In another embodiment, k is 0; l is 0; k is 1; l is 1; both k and l are 0; or both k and l are 1.

In certain embodiments, XXX is complementary to X'X'X', YYY is complementary to Y'Y'Y', and ZZZ is complementary to Z'Z'Z'.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

In another embodiment, the YYY motif occurs at or near the cleavage site of the sense strand.

In an additional embodiment, the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end. Optionally, the Y' is 2'-O-methyl.

In some embodiments, formula (III) is represented by formula (IIIa):

sense: 5' n_p -N_a -Y Y Y -N_a - n_q 3'

antisense: 3' $n_{p'}$-$N_{a'}$- Y'Y'Y'- $N_{a'}$- $n_{q'}$ 5' (IIIa).

In another embodiment, formula (III) is represented by formula (IIIb):

sense: 5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a - n_q 3'

antisense: 3' $n_{p'}$-$N_{a'}$- Y'Y'Y'-$N_{b'}$-Z'Z'Z'- $N_{a'}$- $n_{q'}$ 5' (IIIb)

where each N_b and N_b' independently represents an oligonucleotide sequence including 1-5 modified nucleotides.

In an additional embodiment, formula (III) is represented by formula (IIIc):

sense: 5' n_p -N_a –X X X -N_b -Y Y Y -N_a - n_q 3'

antisense: 3' $n_{p'}$-$N_{a'}$- X'X'X'-$N_{b'}$- Y'Y'Y'- $N_{a'}$- $n_{q'}$ 5' (IIIc)

where each N_b and N_b' independently represents an oligonucleotide sequence including 1-5 modified nucleotides.

In certain embodiments, formula (III) is represented by formula (IIId):

sense: 5' n_p -N_a –X X X- N_b -Y Y Y -N_b -Z Z Z -N_a - n_q 3'

antisense: 3' $n_{p'}$-$N_{a'}$- X'X'X'- $N_{b'}$-Y'Y'Y'-$N_{b'}$-Z'Z'Z'- $N_{a'}$- $n_{q'}$ 5' (IIId)

where each N_b and N_b' independently represents an oligonucleotide sequence including 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence including 2-10 modified nucleotides.

In another embodiment, the double stranded region is 15-30 nucleotide pairs in length. Optionally, the double stranded region is 17-23 nucleotide pairs in length.

In certain embodiments, the double stranded region is 17-25 nucleotide pairs in length. Optionally, the double stranded region is 23-27 nucleotide pairs in length.

In some embodiments, the double stranded region is 19-21 nucleotide pairs in length. Optionally, the double stranded region is 21-23 nucleotide pairs in length.

In certain embodiments, each strand has 15-30 nucleotides. Optionally, each strand has 19-30 nucleotides. Optionally, each strand has 19-23 nucleotides.

In certain embodiments, the double stranded region is 19-21 nucleotide pairs in length and each strand has 19-23 nucleotides.

In another embodiment, the modifications on the nucleotides of the RNAi agent are LNA, glycol nucleic acid (GNA), HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy or 2'-hydroxyl, and combinations thereof. Optionally, the modifications on nucleotides include 2'-O-methyl, 2'-fluoro or GNA, and combinations thereof. In a related embodiment, the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

In one embodiment the RNAi agent includes a ligand that is or includes one or more lipophilic, *e.g.,* C16, moieties attached through a bivalent or trivalent branched linker.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives.

In yet other embodiments, the agents further comprise a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker.

In certain embodiments, the ligand is attached to the 3′ end of the sense strand.

In some embodiments, the RNAi agent further includes at least one phosphorothioate or methylphosphonate internucleotide linkage. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

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

In an additional embodiment, the base pair at the 1 position of the 5′-end of the antisense strand of the RNAi agent duplex is an A:U base pair.

In certain embodiments, the Y nucleotides contain a 2′-fluoro modification.

In some embodiments, the Y′ nucleotides contain a 2′-O-methyl modification.

In certain embodiments, p'>0. Optionally, p'=2.

In some embodiments, q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA.

In certain embodiments, q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

In one embodiment, the sense strand of the RNAi agent has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In another embodiment, at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage. Optionally, all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

In certain embodiments, the APOE RNAi agent of the instant disclosure is one of those listed in Tables 2-5 and 7-10. In certain embodiments, the APOE RNAi agent of the instant disclosure is one of those listed in Tables 7 and 8. In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand include a modification. In certain embodiments, the APOE RNAi agent of the instant disclosure is one of those listed in Tables 9 and 10. In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand include a modification.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene in a cell, where the double stranded RNAi agent includes a sense strand

complementary to an antisense strand, where the antisense strand includes a region complementary to part of an mRNA encoding an APOE gene, where each strand is about 14 to about 30 nucleotides in length, where the double stranded RNAi agent is represented by formula (III):

sense: 5' n_p -N_a -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- n_q' 5' (III)

where:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence including 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q, and n_q', each of which may or may not be present independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and where the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

where the sense strand is conjugated to at least one ligand, optionally where the ligand is one or more lipophilic, *e.g.*, C16, ligands, and/or one or more GalNAc derivatives.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

An additional aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene in a cell, where the double stranded RNAi agent includes a sense strand complementary to an antisense strand, where the antisense strand includes a region complementary to part of an mRNA encoding APOE, where each strand is about 14 to about 30 nucleotides in length, where the double stranded RNAi agent is represented by formula (III):

sense: 5' n_p -N_a -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- n_q' 5' (III)

where:

i, j, k, and l are each independently 0 or 1;

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence including 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and where the modifications are 2′-O-methyl, glycol nucleic acid (GNA) or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

where the sense strand is conjugated to at least one ligand, optionally where the ligand is one or more lipophilic, *e.g.*, C16, ligands, and/or one or more GalNAc derivatives.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene in a cell, where the double stranded RNAi agent includes a sense strand complementary to an antisense strand, where the antisense strand includes a region complementary to part of an mRNA encoding APOE (SEQ ID NO: 1, , or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of SEQ ID NO:1), where each strand is about 14 to about 30 nucleotides in length, where the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

where:

i, j, k, and l are each independently 0 or 1;

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q′ are each independently 0-6;

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence including 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and where the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

where the sense strand is conjugated to at least one ligand, optionally where the ligand is one or more lipophilic, *e.g.*, C16, ligands, and/or one or more GalNAc derivatives.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

An additional aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene in a cell, where the double stranded RNAi agent includes a sense strand complementary to an antisense strand, where the antisense strand includes a region complementary to part of an mRNA encoding APOE (SEQ ID NO: 1, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of SEQ ID NO: 1), where each strand is about 14 to about 30 nucleotides in length, where the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

where:

i, j, k, and l are each independently 0 or 1;

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence including 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and where the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y';

where the sense strand includes at least one phosphorothioate linkage; and

where the sense strand is conjugated to at least one ligand, optionally where the ligand is one or more lipophilic, *e.g.*, C16, ligands and/or one or more GalNAc derivatives.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene in a cell, where the double stranded RNAi agent includes a sense strand complementary to an antisense strand, where the antisense strand includes a region complementary to part of an mRNA encoding APOE (SEQ ID NO: 1, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of SEQ ID NO: 1), where each strand is about 14 to about 30 nucleotides in length, where the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}Y\ Y\ Y\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}Y'Y'Y'\text{-}N_a'\text{-}n_q'\ 5'$ (IIIa)

where:

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q′ are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and where the modifications are 2′-O-methyl or 2′-fluoro modifications;

where the sense strand includes at least one phosphorothioate linkage; and

where the sense strand is conjugated to at least one ligand, optionally where the ligand is one or more lipophilic, *e.g.*, C16 ligands, and/or one or more GalNAc derivatives.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

An additional aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene, where the double stranded RNAi agent targeted to APOE includes a sense strand and an antisense strand forming a double stranded region, where the sense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, and 9, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9, and the antisense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, and 10, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, and 10; where a substitution of a uracil for any thymine in the sequences provided in the SEQ ID NOs: 1-10 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences provided in SEQ ID NOs: 1-10, where substantially all of the nucleotides of the sense strand include a modification that is a 2'-O-methyl modification, a GNA or a 2'-fluoro modification, where the sense strand includes two phosphorothioate internucleotide linkages at the 5'-terminus, where substantially all of the nucleotides of the antisense strand include a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, where the antisense strand includes two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and where the sense strand is conjugated to one or more lipophilic, *e.g.*, C16, ligands, optionally, further comprising a liver targeting ligand, *e.g.,* a ligand comprising one or more GalNAc derivatives.

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of an APOE gene, where the double stranded RNAi agent targeted to APOE includes a sense strand and an antisense strand forming a double stranded region, where the sense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, and 9, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9, and the antisense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, and 10, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence

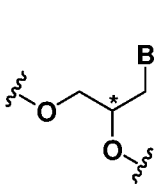
of any one of SEQ ID NOs: 2, 4, 6, 8, and 10, where a substitution of a uracil for any thymine in the sequences provided in the SEQ ID NOs: 1-10 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences provided in SEQ ID NOs:1-10; where the sense strand includes at least one 3'-terminal deoxy-thymidine nucleotide (dT), and where the antisense strand includes at least one 3'-terminal deoxy-thymidine nucleotide (dT).

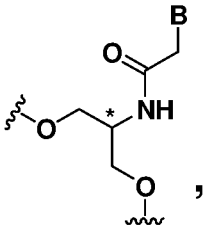
In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

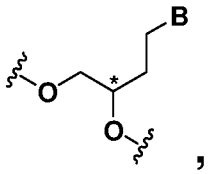
In one embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

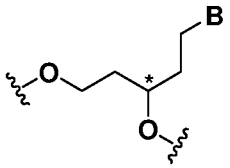
In another embodiment, each strand has 19-30 nucleotides.

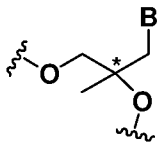
In certain embodiments, the antisense strand of the RNAi agent includes at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5′ region or a precursor thereof. Optionally, the thermally destabilizing modification of the duplex is one or more of

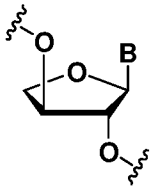


,


,


,


,


, and


where B is nucleobase.

Another aspect of the instant disclosure provides a cell containing a double stranded RNAi agent of the instant disclosure.

An additional aspect of the instant disclosure provides a pharmaceutical composition for inhibiting expression of an APOE gene that includes a double stranded RNAi agent of the instant disclosure.

In one embodiment, the double stranded RNAi agent is administered in an unbuffered solution. Optionally, the unbuffered solution is saline or water.

In another embodiment, the double stranded RNAi agent is administered with a buffer solution. Optionally, the buffer solution includes acetate, citrate, prolamine, carbonate, or phosphate

or any combination thereof. In another embodiment, the buffer solution is phosphate buffered saline (PBS).

Another aspect of the disclosure provides a pharmaceutical composition that includes a double stranded RNAi agent of the instant disclosure and a lipid formulation.

In one embodiment, the lipid formulation includes a lipid nanoparticle (LNP).

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

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

In certain embodiments, the subject is a rhesus monkey, a cynomolgous monkey, a mouse, or a rat.

In certain embodiments, the human subject suffers from an APOE-associated neurodegenerative disease, *e.g.,* an amyloid-β-mediated disease, such as Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy, or a tau-mediated disease, *e.g.*, a primary tauopathy, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), or a secondary tauopathy, *e.g.*,AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

In certain embodiments, the method further involves administering an additional therapeutic agent to the subject, such as a cholinesterase inhibitors and/or memantine.

In certain embodiments, the double stranded RNAi agent is administered at a dose of about 0.01 mg/kg to about 50 mg/kg.

In some embodiments, the double stranded RNAi agent is administered to the subject intrathecally.

In one embodiment, the method reduces the expression of an APOE gene in a brain (*e.g.,* striatum) or spine tissue. Optionally, the brain or spine tissue is striatum, cortex, cerebellum, cervical spine, lumbar spine, or thoracic spine.

In some embodiments, the double stranded RNAi agent is administered to the subject subcutaneously.

In one embodiment, the method reduces the expression of an APOE gene in the liver.

In other embodiments, the method reduces the expression of an APOE gene in the liver and the brain.

Another aspect of the instant disclosure provides a method of inhibiting the expression of APOE in a subject, the method involving: administering to the subject a therapeutically effective

amount of a double stranded RNAi agent of the disclosure or a pharmaceutical composition of the disclosure, thereby inhibiting the expression of APOE in the subject.

An additional aspect of the disclosure provides a method for treating or preventing an disorder or APOE-associated neurodegenerative disease or disorder in a subject, the method involving administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the disclosure or a pharmaceutical composition of the disclosure, thereby treating or preventing an APOE-associated neurodegenerative disease or disorder in the subject.

In certain embodiments, the APOE-associated neurodegenerative disease is an amyloid-β-mediated disease, such as an amyloid-β-mediated disease selected from the group consisting of Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy.

In certain embodiments, the APOE-associated neurodegenerative disease is a tau-mediated disease, such as a primary tauopathy or a seconday tauopathy.

In certain embodiments, the primary tauopathy is selected from the group consisting of Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART).

In certain embodiments, the secondary tauopathy is selected from the group consisting of AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

Another aspect of the instant disclosure provides a kit for performing a method of the instant disclosure, the kit including: a) a double stranded RNAi agent of the instant disclosure, and b) instructions for use, and c) optionally, a device for administering the double stranded RNAi agent to the subject.

An additional aspect of the instant disclosure provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of an APOE gene, where the RNAi agent possesses a sense strand and an antisense strand, and where the antisense strand includes a region of complementarity which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.*, differing by 3, 2, 1, or 0 nucleotides), *e.g.*, at least 15 nucleotides (*i.e.*, differing by 3, 2, 1, or 0 nucleotides), at least 19 nucleotides (*i.e.*, differing by 3, 2, 1, or 0 nucleotides), from any one of the antisense strand nucleobase sequences of Tables 2-5 and 7-10. In one embodiment, the RNAi agent includes one or more of the following modifications: a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-C-alkyl-modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA), a phosphorothioate (PS) and a vinyl phosphonate (VP). Optionally, the RNAi agent includes at least one of each of the following modifications: a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-C-alkyl-modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA), a phosphorothioate and a vinyl phosphonate (VP).

In certain embodiments, the double stranded RNAi agents inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the double stranded RNAi agents inhibit the expression of APOE4 but do not substantially inhibit the expression of APOE2 and APOE3, *e.g.,* the expression of APOE2 and APOE3 is inhibited by no more than about 10%.

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

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

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

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

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

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

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

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

In one aspect, the present invention provides a method of inhibiting expression of an APOE gene in an astrocyte. The method includes contacting the astrocyte with the dsRNA agent or pharmaceutical composition of the invention; and maintaining the astrocyte produced for a time sufficient to obtain degradation of the mRNA transcript of the APOE gene, thereby inhibiting expression of the APOE gene in the astrocyte.

In certain embodiments, the cell is within a subject, *e.g.,* a human subject.

In some embodiment, the contacting the astrocyte is by inthrathecal administration of the pharmaceutical composition.

In certain embodiments, the antisense strand of the dsRNA agent comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1204704, AD-1204705, AD-1204708, and AD-1204712.

BRIEF DESCRIPTIONS OF THE FIGURES

Figure 1A is a graph depicting the percent of APOE mRNA remaining in the right hemisphere of the brain (BRH) of homozygous humanized APOE knock-in mice administered a single 300 μg dose of the indicated duplexes or artificial CSF (aCSF) control by intracerebroventricular injection (ICV) at day 14 post-dose.

Figure 1B is a graph depicting the percent of APOE mRNA remaining in the liver of homozygous humanized APOE knock-in mice administered a single 300 μg dose of the indicated duplexes or artificial CSF (aCSF) control by intracerebroventricular injection (ICV) at day 14 post-dose.

Figure 2 is a graph depicting the correlation of the activity of the agents AD-1204704, AD-1204705, AD-1204705, AD-1204706 AD-1204707, AD-1204708, AD-1204709, AD-1204710, AD-1204711, AD-1204712, and AD-1204713 *in vitro* to the activity of the agents *in vivo*.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides RNAi compositions, which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an gene. The APOE gene may be within a cell, *e.g.,* a cell within a subject, such as a human. The present disclosure also provides methods of using the RNAi compositions of the disclosure for inhibiting the expression of an APOE gene or for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an APOE gene, *e.g.,* a pathogenic APOE allele, *i.e.,* APOE4, *e.g.,* an APOE-associated neurodegenerative disesase, for example, an amyloid-β-mediated disease or a tau-mediated disease..

The RNAi agents of the disclosure include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25,

15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an APOE gene. In certain embodiments, the RNAi agents of the disclosure include an RNA strand (the antisense strand) having a region which is about 21-23 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an APOE gene.

In certain embodiments, the RNAi agents of the disclosure include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of an APOE gene. These RNAi agents with the longer length antisense strands preferably include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of these RNAi agents enables the targeted degradation of mRNAs of an APOE gene in mammals. Thus, methods and compositions including these RNAi agents are useful for treating a subject who would benefit by a reduction in the levels or activity of an APOE protein, such as a subject having an APOE-associated neurodegenerative disease, *e.g.* an amyloid-β-mediated disease or a tau-mediated disease.

The following detailed description discloses how to make and use compositions containing RNAi agents to inhibit the expression of an APOE gene, as well as compositions and methods for treating subjects having diseases and disorders that would benefit from inhibition or reduction of the expression of the genes.

I. Definitions

In order that the present disclosure may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this disclosure.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to". The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. In certain embodiments, about means ±10%. In certain embodiments, about means ±5%. When about is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least", "no less than", or "or more"prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21 nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or intergers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range.

As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

In the event of a conflict between a chemical structure and a chemical name, the chemical structure takes precedence.

The terms "APOE" or "APOE", also known as "Apolipoprotein E," "Alzheimer's Disease 2," "LPG" and "LDLCQ5," refer to the well-known gene that encodes the protein, APOE. APOE is synthesized throughout the body, primarily in the liver and functions as a lipid transport protein and is a major ligand for low density lipoprotein (LDL) receptors. APOE has been shown to play a role in cholesterol metabolism and cardiovascular disease and, more recently, has emerged as a major risk factor for Alzheimer's disease and is associated with the pathology of other neurodegenerative diseases.

Nucleotide and amino acid sequences of APOE can be found, for example, at GenBank Accession No. NM_000041.4 (Homo sapiens APOE, SEQ ID NO: 1, reverse complement, SEQ ID NO: 2); GenBank Accession No. NM_001270681.1 (*Rattus norvegicus* APOE, SEQ ID NO: 3; reverse complement, SEQ ID NO: 4); GenBank Accession No. NM_001305843.1 (*Mus musculus* APOE, SEQ ID NO: 5, reverse complement, SEQ ID NO: 6); GenBank Accession No. XM_028839202.1 (*Macaca mulatta* APOE, SEQ ID NO: 7, reverse complement, SEQ ID NO: 8); and GenBank Accession No. XM_005589554.2 (*Macaca fascicularis* APOE, SEQ ID NO: 9; reverse complement, SEQ ID NO: 10).

Additional examples of APOE sequences can be found in publically available databases, for example, GenBank, OMIM, and UniProt. Additional information on APOE can be found, for example, at www.ncbi.nlm.nih.gov/gene/348.

The term APOE as used herein also refers to variations of the APOE gene including variants of human APOE provided in the SNP database, for example, at ncbi.nlm.nih.gov/clinvar/?term=APOE[gene].

The human APOE gene contains two single-nucleotide polymorphisms that result in the three most common variants, APOE2 (also referred to as APOE*ε2 or ε2; Cys112, Cys158), APOE3 (also referred to as APOE*ε3 or ε3; Cys112, Arg 158), and APOE4 (also referred to as APOE*ε4 or ε4 (Arg112, Arg158). GenBank Accession No. NM_000041.4 (*Homo sapiens* APOE, SEQ ID NO: 1, reverse complement, SEQ ID NO: 2) is the nucleotide sequence of the APOE*ε3 (APOE3) variant; the APOE*ε2 (APOE2) variant has a single nucleotide change at nucleotide 595C>T of SEQ ID NO:1, and the APOE*ε4 (APOE4) variant has a single nucleotide change at nucleotide 457T>C of SEQ ID NO:1.

It is to be understood that, unless specified herein, the term "APOE," "ApoE," or the like, refers to any one or more of the three APOE variants or alleles. For example, as used herein, the term "an APOE gene" refers to an APOE2 allele, an APOE3 allele, and/or an APOE4 allele" while the term "APOE4 allele," or the like, only refers to an APOE4 allele.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an APOE gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodment, the target portion of the sequence will be at least long enough to serve as a substrate for RNAi-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an APOE gene.

The target sequence is about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In certain embodiments, the target sequence is 19-23 nucleotides in length, optionally 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T", and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively in the context of a modified or unmodified nucleotide. However, it will be understood that the term "ribonucleotide" or "nucleotide"

can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.,* Table 1). The skilled person is well aware that guanine, cytosine, adenine, thymidine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the disclosure by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the disclosure.

The terms "iRNA", "RNAi agent," "iRNA agent," "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. RNA interference (RNAi) is a process that directs the sequence-specific degradation of mRNA. RNAi modulates, *e.g.,* inhibits, the expression of APOE in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the disclosure includes a single stranded RNAi that interacts with a target RNA sequence, *e.g.,* an APOE target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into double-stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). These siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the disclosure relates to a single stranded RNA (ssRNA) (the antisense strand of a siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* an APOE gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded RNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded RNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense

nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In another embodiment, a "RNAi agent" for use in the compositions and methods of the disclosure is a double stranded RNA and is referred to herein as a "double stranded RNAi agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA" refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* an APOE gene. In some embodiments of the disclosure, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.*, an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, a dsRNA molecule can include ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide, a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides. As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or a modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the disclosure include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide – which is acknowledged as a naturally occurring form of nucleotide – if present within a RNAi agent can be considered to constitute a modified nucleotide.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 15-36 base pairs in length, for example, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex region is 19-21 base pairs in length, *e.g.*, 21 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of

one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides or nucleotides not directed to the target site of the dsRNA. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. In certain embodiments where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker" (though it is noted that certain other structures defined elsewhere herein can also be referred to as a "linker"). The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In one embodiment, an RNAi agent of the disclosure is a dsRNA, each strand of which independently comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, an APOE target mRNA sequence, to direct the cleavage of the target RNA.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a RNAi agent, *e.g.*, a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively, the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the overhang on the sense strand or the antisense strand can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

The terms "blunt" or "blunt ended" as used herein in reference to a dsRNA mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a dsRNA, *i.e.*, no nucleotide overhang. One or both ends of a dsRNA can be blunt. Where both ends of a dsRNA are blunt, the dsRNA is said to be blunt ended. To be clear, a "blunt ended" dsRNA is a dsRNA that is blunt at both ends, *i.e.*, no nucleotide overhang at either end of the molecule. Most often such a molecule will be double stranded over its entire length.

The term "antisense strand" or "guide strand" refers to the strand of a RNAi agent, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* an APOE mRNA.

As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* an APOE nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- or 3'-terminus of the RNAi agent.

The term “sense strand” or "passenger strand" as used herein, refers to the strand of a RNAi agent that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, the term “cleavage region” refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person.

Complementary sequences within a RNAi agent, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as “fully complementary” with respect to each other herein. However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as “fully complementary” for the purposes described herein.

“Complementary” sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

The terms “complementary,” “fully complementary” and “substantially complementary” herein can be used with respect to the base matching between two oligonucleotides or polynucleotides, such as the sense strand and the antisense strand of a dsRNA, or between the

antisense strand of a RNAi agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding APOE). For example, a polynucleotide is complementary to at least a part of an APOE mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding APOE.

Accordingly, in some embodiments, the antisense strand polynucleotides disclosed herein are fully complementary to the target APOE sequence. In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target APOE sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 1, 3, 5, 7, or 9 for APOE, or a fragment of SEQ ID NOs: 1, 3, 5, 7, or 9 for APOE , such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target APOE sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 2-5 and 7-10 for APOE, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-5 and 7-10 for APOE, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target APOE sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, or 10, or a fragment of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, ssuch as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, at least partial suppression of the expression of an APOE gene, is assessed by a reduction of the amount of APOE mRNA which can be isolated from or detected in a first cell or group of cells in which an APOE gene is transcribed and which has or have been treated such that the expression of an APOE gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

The phrase "contacting a cell with an RNAi agent," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an RNAi agent includes contacting a cell *in vitro* with the RNAi agent or contacting a cell *in vivo* with the RNAi agent. The contacting may be done directly or indirectly. Thus, for example, the RNAi agent may be put into physical contact with the cell by the individual performing the method, or alternatively, the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, the central nervous system (CNS), optionally *via* intrathecal, intravitreal or other injection, or to the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain or be coupled to a ligand, *e.g.*, a lipophilic moiety or moieties as described below and further detailed, *e.g.*, in PCT/US2019/031170, which is incorporated herein by reference, that directs or otherwise stabilizes the RNAi agent at a site of interest, *e.g.*, the CNS. In some embodiments, the RNAi agent may contain or be coupled to a ligand, *e.g.*, one or more GalNAc derivatives as described below, that directs or otherwise stabilizes the RNAi agent at a site of interest, *e.g.*, the liver. In other embodiments, the RNAi agent may contain or be coupled to a lipophilic moiety or moieties and one or more GalNAc derivatives. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

In one embodiment, contacting a cell with an RNAi agent includes "introducing" or "delivering the RNAi agent into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of a RNAi agent can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing a RNAi agent into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, a RNAi agent can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

The term "lipophile" or "lipophilic moiety" broadly refers to any compound or chemical moiety having an affinity for lipids. One way to characterize the lipophilicity of the lipophilic moiety is by the octanol-water partition coefficient, $\log K_{ow}$, where K_{ow} is the ratio of a chemical's concentration in the octanol-phase to its concentration in the aqueous phase of a two-phase system at equilibrium. The octanol-water partition coefficient is a laboratory-measured property of a substance. However, it may also be predicted by using coefficients attributed to the structural components of a chemical which are calculated using first-principle or empirical methods (see, for example, Tetko *et al.*, *J. Chem. Inf. Comput. Sci.* 41:1407-21 (2001), which is incorporated herein by reference in its entirety). It provides a thermodynamic measure of the tendency of the substance to prefer a non-aqueous or oily milieu rather than water (*i.e.* its hydrophilic/lipophilic balance). In principle, a

chemical substance is lipophilic in character when its $logK_{ow}$ exceeds 0. Typically, the lipophilic moiety possesses a $logK_{ow}$ exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10. For instance, the $logK_{ow}$ of 6-amino hexanol, for instance, is predicted to be approximately 0.7. Using the same method, the $logK_{ow}$ of cholesteryl N-(hexan-6-ol) carbamate is predicted to be 10.7.

The lipophilicity of a molecule can change with respect to the functional group it carries. For instance, adding a hydroxyl group or amine group to the end of a lipophilic moiety can increase or decrease the partition coefficient (*e.g.*, $logK_{ow}$) value of the lipophilic moiety.

Alternatively, the hydrophobicity of the double-stranded RNAi agent, conjugated to one or more lipophilic moieties, can be measured by its protein binding characteristics. For instance, in certain embodiments, the unbound fraction in the plasma protein binding assay of the double-stranded RNAi agent could be determined to positively correlate to the relative hydrophobicity of the double-stranded RNAi agent, which could then positively correlate to the silencing activity of the double-stranded RNAi agent.

In one embodiment, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. An exemplary protocol of this binding assay is illustrated in detail in, *e.g.*, PCT/US2019/031170. The hydrophobicity of the double-stranded RNAi agent, measured by fraction of unbound dsRNA in the binding assay, exceeds 0.15, exceeds 0.2, exceeds 0.25, exceeds 0.3, exceeds 0.35, exceeds 0.4, exceeds 0.45, or exceeds 0.5 for an enhanced *in vivo* delivery of dsRNA.

Accordingly, conjugating the lipophilic moieties to the internal position(s) of the double-stranded RNAi agent provides optimal hydrophobicity for the enhanced *in vivo* delivery of siRNA.

The term "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.,* a rNAi agent or a plasmid from which a RNAi agent is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), or a non-primate (such as a a rat, or a mouse). In a preferred embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder, or condition that would benefit from reduction in APOE expression; a human at risk for a disease, disorder, or condition that would benefit from reduction in APOE expression; a human having a disease, disorder, or condition that would benefit from reduction in APOE expression; or human being treated for a disease, disorder, or condition that would benefit from reduction in APOE expression as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more signs or symptoms associated with APOE gene expression or APOE protein production, *e.g.,* APOE-associated neurodegenerative

disease, such as an amyloid-β-mediated disease, *e.g.* Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy, or a tau-mediated disease, *e.g.* a primary tauopathy, such as frontotemporal dementia, Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Chronic traumatic encelopathy (CTE), Frontotemporal dementia (FTD, FTDP-17), Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), Primary age-related tauopathy (PART), and Globular glial tauopathies (GGTs), or a secondary tauopathy, *e.g.*, AD, Creuzfeld Jakob's disease, Down's Syndrome, Familial British Dementia, and Dementia pugilistica. Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of APOE in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.*, protein or gene expression level. "Lower" in the context of the level of APOE in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, "lower" is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual, *e.g.*, the level of decrease in bodyweight between an obese individual and an individual having a weight accepted within the range of normal. As used herein, lowering can refer to lowering or predominantly lowering the level of mRNA of an APOE gene having a nucleotide repeat expansion.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder, or condition thereof, that would benefit from a reduction in expression of an APOE gene or production of an APOE protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.*, a symptom of an APOE-associated neurodegenerative disease. The failure to develop a disease, disorder, or condition, or the reduction in the development of a symptom associated with such a disease, disorder, or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months or years) is considered effective prevention.

As used herein, the term "APOE-associated neurodegenerative disease" or "APOE-associated neurodegenerative disorder" is understood as any disease or disorder that would benefit from reduction in the expression and/or activity of APOE. Exemplary APOE-associated neurodegenerative diseases include amyloid-β-mediated diseases, such as, Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy, and tau-mediated diseases, *e.g.* primary tauopathies, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica,

Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), and secondary tauopathies, e.g.,AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

As used herein, the term "amyloid-β-mediated disease" is a disorder resulting from extracellular accumulation of amyloid-β, which leads to formation of amyloid plaques in brain tissue. Exemplary amyloid-β-mediated diseases include Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy (CAA).

As used herein, the term "tau-mediated disease" is a disorder resulting from the aggregation of tau protein into neurofibrillary tangles. Tangles are formed by hyperphosphorylation of tau, causing the protein to dissociate from microtubules and from aggregates. Tauopathies can be divided into "primary tauopathies", in which the pathology is driven primarily by tau aggregation, and "secondary tauopathies", in which another factor drives the disease (for example, amyloid-β plaques in Alzheimer's disease) and the presence of tauopathies worsens disease progression. Examples of primary tauopathies include Frontotemporal dementia (FTD) Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), Frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART). Examples of secondary tauopathies include AD, Creuzfeld Jakob's disease, Down's Syndrome and Familial British Dementia.

APOE polymorphism has been associated with multiple tauopathies. The APOE4 allele was found to accelerate neurodegeneration and lower age at onset in frontotemporal dementia (FTD) in patients with MAPT mutations (Koriath, C. *et al.* (2019) *Alzheimers Dement* 11:277-280). In addition, the presence of APOE4 correlated with more advanced chronic traumatic encephalopathy (CTE) in autopsy brains of football players with low exposure of repetitive head impacts (Verscaj, C. *et al.* (2017) *Neurology* 88 (16) Supplement S9.001) and in brains of boxers (Jordan, B.D. *et al.* (1997) *JAMA* 278(2): 136-140). The presence f the APOE4 allele is also associated with increased risk of Creutzfeldt-Jakob disease (CJD) while the presence of theAPOE3 allele is associated with protection against susceptibility to Creutzfeldt-Jakob disease (CJD) (Wei, Y. *et al.* (2013) *J Clinical Neuroscience* 21(3): 390-394).

Furthermore, Shi *et al.* described that in the P301S mouse model of FTD, when APOE4 was present there was a marked increase in tau levels, brain atrophy and neuroinflammation as compared the when APOE2, APOE3 or an APOE knockout were present (Shi *et al.*, (2017) *Nature* 549: 523–527). In another studying using a mouse model that expresses human tau with the P301L mutation found in FTD with parkinsonism, hyperphosphorylated tau, tau aggregation, behavioral abnormalities were worsened on an APOE2 background (Zhao, N. *et al.*, (2018) *Nat Commun* 9:4388). Zhao *et al.* further identified an association between the APOE ε2/ε2 genotype with risk of tauopathies in confirmed cases of progressive supranuclear palsy (PSP) and corticobasal degeneration, suggesting

that APOE2 might be protective in when amyloid pathology is present, APOE2 is related to increased severity of tau pathology in the absence of amyloid pathology.

"Alzheimer's disease" ("AD") is a chronic neurodegenerative disease that usually starts slowly and gradually worsens over time. The most common early symptom is difficulty in remembering recent events. As the disease advances, symptoms can include problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self-care, and behavioral issues. As a person's condition declines, they often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death.

Neuropathologically, AD is characterised by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus. Degeneration is also present in brainstem nuclei like the locus coeruleus. Studies using MRI and PET have documented reductions in the size of specific brain regions in people with AD as they progressed from mild cognitive impairment to Alzheimer's disease, and in comparison with similar images from healthy older adults.

Both amyloid plaques and neurofibrillary tangles are clearly visible by microscopy in brains of those afflicted by AD. Plaques are dense, mostly insoluble deposits of beta-amyloid peptide and cellular material outside and around neurons. Tangles (neurofibrillary tangles) are aggregates of the microtubule-associated protein tau which has become hyperphosphorylated and accumulate inside the cells themselves. Although many older individuals develop some plaques and tangles as a consequence of ageing, the brains of people with AD have a greater number of them in specific brain regions such as the temporal lobe. Lewy bodies are not rare in the brains of people with AD.

The National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA, now known as the Alzheimer's Association) established the most commonly used NINCDS-ADRDA Alzheimer's Criteria for diagnosis in 1984, extensively updated in 2007. These criteria require that the presence of cognitive impairment, and a suspected dementia syndrome, be confirmed by neuropsychological testing for a clinical diagnosis of possible or probable AD. A histopathologic confirmation including a microscopic examination of brain tissue is required for a definitive diagnosis. Good statistical reliability and validity have been shown between the diagnostic criteria and definitive histopathological confirmation. Eight intellectual domains are most commonly impaired in AD—memory, language, perceptual skills, attention, motor skills, orientation, problem solving and executive functional abilities. These domains are equivalent to the NINCDS-ADRDA Alzheimer's Criteria as listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) published by the American Psychiatric Association.

At present, drugs available to treat AD patients include cholinesterase inhibitors and memantine. These drugs can improve quality of life of patients by treating symptoms related to, for

example, memory, thinking, and language, however, they do not change the progression of the disease or the rate of decline.

The cause of AD is poorly understood but, as discussed above, the presence of APOE4 has shown to be a major risk determinant of late-onset Alzheimer's disease (AD), the symptoms of which develop after age 65, and numerous studies in non-human animal models of amyloid-β-mediated disease (AD) and tau-mediated disease have demonstrated that inhibiting APOE, *e.g.,* APOE4, has a beneficial effect on the formation of amyloid plaques and cognitive abilities.

"Down's syndrome" ("DS"), also known as trisomy 21, is a genetic order caused by the presence of all or part of a third copy of chromosome 21. DS is a life-long condition associated with intellectual disability, a characteristic facial appearance, weak muscle tone in infancy, and people with DS often experience a gradual decline in cognitive ability. The third chromosome 21 carries an extra amyloid precursor protein (APP) gene, and excess amyloid production leading to buildup of amyloid-β plaques and consequently increased risk of early-onset Alzheimer's disease (AD) to more than 50%. Another gene that is triplicated in DS is DYRK1A, which affects alternative splicing of tau, priming tau for abnormal hyperphosphorylation and promote neurofibrillary degeneration (Hartley D. *et al.* (2016) *Alzheimers Dement* 11(6): 700-709). DS individuals with AD have neuropathological changes similar to general AD patients, including amyloid plaques, tau neurofibrillary tangles, oxidative damage, and neuron loss. Elevated levels of both amyloid and tau are found in cerebrospinal fluid of DS individuals (Lee, N.C. *et al.* (2017) *Neurology and Therapy* 6: 69-81).

"Cerebral amyloid angiopathy" ("CAA") is a form of angiopathy in which amyloid plaques are deposited in the walls of small to medium blood vessels and certain areas of the brain. The amyloid plaques damage brain cells and impair various parts of the brain. In addition, the amyloid deposits in blood vessels replace the muscle and elastic fibers that give the blood vessels flexibility, causing them to become prone to breakage. CAA may lead to dementia, intracranial hemorrhage and transient neurologic events. CAA has been recognized as one of the morphologic hallmarks of Alzheimer's disease. Mutations in the amyloid-β precursor protein (APP) gene are the most common cause of hereditary CAA (Desimone C.V. *et al.* (2017) *J Am Coll Cardiol* 70(9): 1173-1182).

"Frontotemporal dementia" ("FTD"), which encompasses diseases such as Pick's disease, Progressive supranuclear palsy (PSP), and Cordicobasal denegearion (CBD). FTD is a common type of dementia in patients younger than 65 years of age and encompasses a group of neurodegenerative diseases characterized by progressive decline in behavior, executive function, or language. In FTD, nerve cells in the frontal and temporal lobes of the brain are lost, and therefore FTD is also called Frontotemporal lobar degeneration (FTLD). Mutations in the microtubule-associated protein tau (MAPT) gene and accumulation of tau are found in several subtypes of FTD, including Pick's disease, Progressive supranuclear palsy (PSP), and Cordicobasal denegearion (CBD).

"Pick's disease" is characterized by striking knife-edge atrophy of frontal, temporal, and cingulate gyri where the parietal lobe is better preserved.

"Corticobasal degeneration" ("CBD") is characterized by predominant loss of cells in the dorsal prefrontal cortex, supplemental motor area, peri-Rolandic cortex, and subcortical nuclei.

"Progressive supranuclear palsy" ("PSP") is associated with atrophy of the frontal convexity; subcortical atrophy is severe at the level of globus pallidus, subthalamic nucleus, and brainstem nuclei (Olney, N.T. *et al.* (2017) *Neurol Clin* 35(2): 339-374).

"Globular glial tauopathies" ("GGTs") are a type of rare frontotemporal lobar degeneration (FLD) that have widespread, globular inclusions in astrocytes and oligodendrocytes containing the 4-repeat tau isoform. These cases are associated with a range of clinical presentations that correlate with the severity and distribution of underlying tau pathology and neurodegeneration (Ahmed, Z. *et al.* (2013) *Acta Neuropathol* 126(4): 537-544).

"Frontotemporal dementia with parkinsonism" ("FTDP") is a less common type of FTD that also affects movement. Chromosome 17 was found be linked to FTDP (FTDP-17) and mutations on the microtubule-associated protein tau (MAPT) on chromosome 17 were found in many kindreds with familial FTDP-17. FTDP-17 due to mutations in MAPT starts between 25-65 years of age, and penetrance is close to 100%. Symptoms involve executive dysfunction and altered personality and behavior with aphasia and parkinsonism evolving in many individuals (Boeve, B.F. *et al.* (2008) Arch Neurol 65(4): 460-464).

"Chronic traumatic encephalopathy" ("CTE") is a debilitating neurodegenerative disease resulting from repetitive mild traumatic brain injuries found in many athletes, especially football players. The neuropathological signature of CTE includes accumulation of phosphorylated tau in sulci and peri-vascular regions, microgliosis, and astrocytosis; from some tau deposits at early stage, the disease can progress to global brain atrophy at late stage. CTE can progress through many years from mild symptoms such as short-term memory deficits and mild aggression to advanced language deficits and psychotic symptoms including paranoia and parkinsonism (Fesharaki-Zadeh, A.(2019) *Front Neurol* 10:713).

"Dementia pugilistica" is a form of CTE that involves gross impairment of cognitive and motor functions due to repetitive blows to the head from boxing (Castellani. R.J *et al.* (2017) *J Alzheimers Dis* 60(4): 1209-1221).

"Argyrophilic grain disease" ("AGD") is a highly frequent sporadic tauopathy and the second-most-common neurodegenerative disease after Alzheimer's disease in several studies. AGD is a late-onset neurodegenerative disease characterized by small spindle- or comma-shaped, silver stain positive lesions in neuronal processes referred to as argyrophilic grains (AG). Phosphorylated-tau is a major component of AG. The most common AGD manifestation is slowly progressive, amnestic and mild cognitive impairment, accompanied by a high prevalence of neuropsychiatric symptoms. Due to the lack of prominent clinical features, AGD is often only diagnosed postmortem based on three pathologic features: AG, oligodendrocytic coiled bodies and neuronal tangles (Rodriguez, R.D. *et al.*(2015) *Dement Neuropsychol* 9(1): 2-8).

"Primary age-related tauopathy" ("PART") is a pathology commonly observed postmortem in the brains of aged individuals whose cognitive functions are normal or only mildly impaired. PART brains have tau neurofibrillary tangles indistinguishable from that of Alzheimer's disease but do not have amyloid-β plaques (Crary, J.F. *et al.* (2014) *Acta Neuropathol* 128(6): 755-66).

"Creuzfeld Jakob's disease" ("CJD") belongs to a family of human and animal diseases known as the transmissible spongiform encephalopathies (TSEs) or prion diseases. A prion—derived from "protein" and "infectious"—causes CJD in people and TSEs in animals. Spongiform refers to the characteristic appearance of infected brains, which become filled with holes until they resemble sponges when examined under a microscope. CJD is a rare, degenerative and fatal brain disorder, usually appears in later life and runs a rapid course. Typical onset of symptoms occurs at about age 60, and about 70 percent of individuals die within one year. In the early stages of the disease, people may have failing memory, behavioral changes, lack of coordination, and visual disturbances. As the illness progresses, mental deterioration becomes pronounced and involuntary movements, blindness, weakness of extremities, and coma may occur. In addition to prion plaques, tau pathology is also observed in several brain regions of CJD patients, and the cerebrospinal fluid of patients with widespread taupathology also has elevated total tau protein (Kovacs, G.G et al. (2017) Brain Pathol 3: 332-344).

"Familial British dementia" ("FBD") is a type of cerebral amyloid angiopathy that was first documented in affected members of a large British pedigree with clinical presentations including dementia, spastic tetreparesis and cerebellar ataxia. FBD is caused by a mutation in the BRI2 gene. Amyloid plaques in FBD are made up of amyloid-Bri, and tau positive neurofibrillary tangles are found in areas affected by amyloid-Bri lesions. Immunoblotting of tau in FBD is similar to the patterns of tau in Alzheimer's disease (Holton J.L. *et al.* (2001) *Am J Patho* 2: 515-526).

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having an APOE-associated neurodegenerative disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of a RNAi agent that, when administered to a subject having an APOE-associated neurodegenerative disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of a RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. A RNAi agent employed in the methods of the present disclosure may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the brain (*e.g.*, whole brain or certain segments of brain, *e.g.,* striatum, or certain types of cells in the brain, such as, *e.g.*, neurons and glial cells (astrocytes, oligodendrocytes, microglial cells)). In other embodiments, a "sample derived from a subject" refers to liver tissue (or subcomponents thereof)

derived from the subject. In some embodiments, a "sample derived from a subject" refers to blood drawn from the subject or plasma or serum derived therefrom. In further embodiments, a "sample derived from a subject" refers to brain tissue (or subcomponents thereof) or retinal tissue (or subcomponents thereof) derived from the subject.

II. RNAi Agents of the Disclosure

Described herein are RNAi agents which inhibit the expression of an APOE gene. In some embodiments, the RNAi agents provided herein inhibit the expression of an APOE2 allele, an APOE3 allele, and an APOE4 allele. In other embodiments, the RNAi agent provided herein inhibit the expression of an APOE4 allele, *e.g.,* the RNAi agents do not substantially inhibit the expression of an APOE2 allele or an APOE3 allele, *e.g.,* the inhibition of APOE2 and/or APOE3 expression is no more than about 10%. In one embodiment, the RNAi agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an APOE gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human having an APOE-associated neurodegenerative disease *e.g.*, an amyloid-β-mediated disease, such as, Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy, and tau-mediated diseases, *e.g.* a primary tauopathy, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), or a secondary tauopathy, *e.g.,*AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia. The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an APOE gene, The region of complementarity is about 15-30 nucleotides or less in length. Upon contact with a cell expressing the APOE gene, the RNAi agent inhibits the expression of the APOE gene (*e.g.,* a human gene, a primate gene, a non-primate gene) by at least 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flowcytometric techniques. In one embodiment, the level of knockdown is assayed at a 10 nM concentration of siRNA in human neuroblastoma BE(2)-C cells using a Dual-Luciferase assay method provided in Example 1 below.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of an APOE gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-

complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain preferred embodiments, the duplex structure is 18 to 25 base pairs in length, *e.g.*, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-25, 20-24,20-23, 20-22, 20-21, 21-25, 21-24, 21-23, 21-22, 22-25, 22-24, 22-23, 23-25, 23-24 or 24-25 base pairs in length, for example, 19-21 basepairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

Similarly, the region of complementarity to the target sequence is 15 to 30 nucleotides in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, for example 19-23 nucleotides in length or 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

In some embodiments, the dsRNA is 15 to 23 nucleotides in length, or 25 to 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well known in the art that dsRNAs longer than about 21-23 nucleotides can serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 15 to 36 base pairs, *e.g.*, 15-36, 15-35, 15-34, 15-33, 15-32, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs, for example, 19-21 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another

embodiment, a RNAi agent useful to target APOE expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.,* 1, 2, 3, or 4 nucleotides. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art.

In one aspect, a dsRNA of the disclosure includes at least two nucleotide sequences, a sense sequence and an antisense sequence. The sense strand sequence for APOE may be selected from the group of sequences provided in any one of Tables 2-5 and 7-10, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected from the group of sequences of any one of Tables 2-5 and 7-10. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of an APOE gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in any one of Tables 2-5 and 7-10, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 2-5 and 7-10 for APOE.

In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although the sequences in Tables 3, 5, 8, and 10 are described as modified or conjugated sequences and the sequences in Tables 2, 4, 7, and 9 are described as unmodified, the RNA of the RNAi agent of the disclosure *e.g.*, a dsRNA of the disclosure, may comprise any one of the sequences set forth in any one of Tables 2-5 and 7-10 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. One or more lipophilic ligands and/or one or more GalNAc ligands can be included in any of the positions of the RNAi agents provided in the instant application.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs

described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of an APOE gene by not more than 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence using the *in vitro* assay with Cos7 and a 10 nM concentration of the RNA agent and the PCR assay as provided in the examples herein, are contemplated to be within the scope of the present disclosure.

In addition, the RNAs described herein identify a site(s) in an APOE transcript that is susceptible to RISC-mediated cleavage. As such, the present disclosure further features RNAi agents that target within this site(s). As used herein, a RNAi agent is said to target within a particular site of an RNA transcript if the RNAi agent promotes cleavage of the transcript anywhere within that particular site. Such a RNAi agent will generally include at least about 15 contiguous nucleotides, preferably at least 19 nucleotides, from one of the sequences provided herein coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an APOE gene.

An RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of an APOE gene generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of an APOE gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of an APOE gene is important, especially if the particular region of complementarity in an APOE gene is known to have polymorphic sequence variation within the population.

III. Modified RNAi Agents of the Disclosure

In one embodiment, the RNA of the RNAi agent of the disclosure *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications or conjugations known in the art and described herein. In preferred embodiments, the RNA of an RNAi agent of the disclosure, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the disclosure, substantially all of the nucleotides of an RNAi agent of the disclosure are modified. In other embodiments of the disclosure, all of the nucleotides of an RNAi agent of the disclosure are modified. RNAi agents of the disclosure in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1

unmodified nucleotides. In still other embodiments of the disclosure, RNAi agents of the disclosure can include not more than 5, 4, 3, 2 or 1 modified nucleotides.

The nucleic acids featured in the disclosure can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of RNAi agents useful in the embodiments described herein include, but are not limited to, RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified RNAi agent will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, *e.g.,* sodium salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent 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 RE39,464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms 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; 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.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent 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.

In other embodiments, suitable RNA mimetics are contemplated for use in RNAi agents, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the RNAi agents of the disclosure are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the disclosure include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2-, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2-- the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced US5,034,506. The native phosphodiester backbone can be represented as O-P(O)(OH)-OCH_2-.

Modified RNAs can also contain one or more substituted sugar moieties. The RNAi agents, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: 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 can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include O[$(CH_2)_nO$] $_mCH_3$, O$(CH_2)_nOCH_3$, O$(CH_2)_nNH_2$, O$(CH_2)_nCH_3$, O$(CH_2)_nONH_2$, and O$(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl,

substituted lower alkyl, 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, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of a RNAi agent, or a group for improving the pharmacodynamic properties of a RNAi agent, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$), 2'-*O*-hexadecyl, and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of a RNAi agent, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. RNAi agents can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

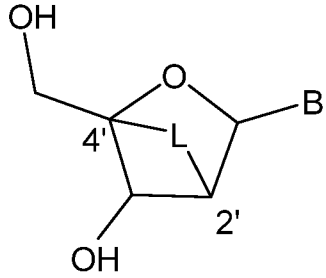
An RNAi agent of the disclosure can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases 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 uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal 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, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry,

Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, (1991) *Angewandte Chemie, International Edition*, 30:613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the disclosure. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, an RNAi agent of the disclosure can also be modified to include one or more bicyclic sugar moieties. A "bicyclic sugar" is a furanosyl ring modified by a ring formed by the bridging of two carbons, whether adjacent or non-adjacent. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a ring formed by bridging two carbons, whether adjacent or non-adjacent, of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring, optionally, via the 2'-acyclic oxygen atom. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH_2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4′ to 2′ bridge.

A locked nucleoside can be represented by the structure (omitting stereochemistry),



wherein B is a nucleobase or modified nucleobase and L is the linking group that joins the 2'-carbon to the 4'-carbon of the ribose ring. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2′; 4′-(CH_2)$_2$—O-2′ (ENA); 4′-$CH(CH_3)$—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-$CH(CH_2OCH_3)$—O-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4′-$C(CH_3)(CH_3)$—O-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4′-CH_2—$N(OCH_3)$-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4′-CH_2—O—$N(CH_3)$-2′ (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4′-CH_2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4′-CH_2—$C(H)(CH_3)$-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH_2—$C(=CH_2)$-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative US Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: US Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133;7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

An RNAi agent of the disclosure can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH3)-0-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An RNAi agent of the disclosure may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2'and C4' carbons of ribose or the C3 and -C5′ carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US 2013/0190383; and WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, a RNAi agent of the disclosure comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series,* 52, 133-134 (2008) and Fluiter et al., *Mol. Biosyst.,* 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"- phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in WO 2011/005861.

Other modifications of a RNAi agent of the disclosure include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of a RNAi agent. Suitable phosphate mimics are disclosed in, for example US 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified RNAi agents Comprising Motifs of the Disclosure

In certain aspects of the disclosure, the double-stranded RNAi agents of the disclosure include agents with chemical modifications as disclosed, for example, in WO 2013/075035, the entire contents of which are incorporated herein by reference. As shown herein and in WO 2013/075035, a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The RNAi agent may be optionally conjugated with a lipophilic ligand, *e.g.*, a C16 ligand, for instance on the sense strand. The RNAi agent may be optionally modified with a (*S*)-glycol nucleic acid (GNA) modification, for instance on one or more residues of the antisense strand. The resulting RNAi agents present superior gene silencing activity.

Accordingly, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, an APOE gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be 15-30 nucleotides in length. For example, each strand may be 16-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length. In certain embodiments, each strand is 19-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent may be 15-30 nucleotide pairs in length. For example, the duplex region can be 16-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17 - 23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length. In preferred embodiments, the duplex region is 19-21 nucleotide pairs in length.

In one embodiment, the RNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In preferred embodiments, the nucleotide overhang region is 2 nucleotides in length. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In one embodiment, the nucleotides in the overhang region of the RNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2-F, 2'-O-methyl, thymidine (T), and any combinations thereof.

For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand or both strands of the RNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3'-end of the sense strand, antisense

strand, or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

The RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'-terminal end of the sense strand or, alternatively, at the 3'-terminal end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the RNAi has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In one embodiment, the RNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In another embodiment, the RNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In yet another embodiment, the RNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand. When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the

motifs are modified nucleotides. In one embodiment each residue is independently modified with a 2'-O-methyl or 2'-fluoro, *e.g.,* in an alternating motif. Optionally, the RNAi agent further comprises a ligand (*e.g.*, a lipophilic ligand, optionally a C16 ligand).

In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

In one embodiment, the sense strand of the RNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In one embodiment, the antisense strand of the RNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

For an RNAi agent having a duplex region of 17-23 nucleotide in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the antisense strand, the count starting from the 1st nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the RNAi from the 5'-end.

The sense strand of the RNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In one embodiment, the sense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adajacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistry of the motifs are distinct from each other and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In one embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end or both ends of the strand.

In another embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end or both ends of the strand.

When the sense strand and the antisense strand of the RNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two or three nucleotides.

When the sense strand and the antisense strand of the RNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two, or three nucleotides in the duplex region.

In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In one embodiment, the RNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'- end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair.

In another embodiment, the nucleotide at the 3'-end of the sense strand is deoxy-thymidine (dT). In another embodiment, the nucleotide at the 3'-end of the antisense strand is deoxy-thymidine (dT). In one embodiment, there is a short sequence of deoxy-thymidine nucleotides, for example, two dT nucleotides on the 3'-end of the sense or antisense strand.

In one embodiment, the sense strand sequence may be represented by formula (I):

5' n_p-N_a-(X X X)$_i$-N_b-Y Y Y -N_b-(Z Z Z)$_j$-N_a-n_q 3' (I)

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In one embodiment, the N_a or N_b comprise modifications of alternating pattern.

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' n_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib);

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic); or

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id).

When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides.

Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

5' n_p-N_a-YYY- N_a-n_q 3' (Ia).

When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

5' $n_{q'}$-N_a'-(Z'Z'Z')$_k$-N_b'-Y'Y'Y'-N_b'-(X'X'X')$_l$-N'_a-n_p' 3' (II)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification;

and

X'X'X', Y'Y'Y' and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, the N_a' or N_b' comprise modifications of alternating pattern.

The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23nucleotidein length, the Y'Y'Y' motif can occur at positions 9, 10, 11;10, 11, 12; 11, 12, 13; 12, 13, 14 ; or 13, 14, 15 of the antisense strand, with the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

In one embodiment, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:

5' $n_{q'}$-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'-$n_{p'}$ 3' (IIb);

5' $n_{q'}$-N_a'-Y'Y'Y'-N_b'-X'X'X'-$n_{p'}$ 3' (IIc); or

5' $n_{q'}$-N_a'- Z'Z'Z'-N_b'-Y'Y'Y'-N_b'- X'X'X'-N_a'-$n_{p'}$ 3' (IId).

When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a'

independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

5' $n_{p'}$-$N_{a'}$-Y'Y'Y'- $N_{a'}$-$n_{q'}$ 3' (Ia).

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X′, Y′ and Z′ may be the same or different from each other.

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X′, Y′ and Z′, in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In one embodiment, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In one embodiment the antisense strand may contain Y′Y′Y′ motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y′ represents 2'-O-methyl modification. The antisense strand may additionally contain X′X′X′ motif or Z′Z′Z′ motifs as wing modifications at the opposite end of the duplex region; and X′X′X′ and Z′Z′Z′ each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the RNAi agents for use in the methods of the disclosure may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):

sense: 5' n_p -N_a-(X X X)$_i$ -N_b- Y Y Y -N_b -(Z Z Z)$_j$-N_a-n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'-n_q' 5'

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein

each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

5' n_p - N_a -Y Y Y -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y' -N_a'n_q' 5'

(IIIa)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y'-N_b'-Z'Z'Z'-N_a'n_q' 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_b'-Z'Z'Z'-N_a-n_q' 5'

(IIId)

When the RNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b and N_b' independently comprises modifications of alternating pattern.

In one embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more C16 (or related) moieties attached through a bivalent or trivalent branched linker (described below). In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more lipophilic, *e.g.*, C16 (or related) moieties, optionally attached through a bivalent or trivalent branched linker.

In one embodiment, when the RNAi agent is represented by formula (IIIa), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more lipophilic, *e.g.*, C16 (or related) moieties attached through a bivalent or trivalent branched linker.

In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

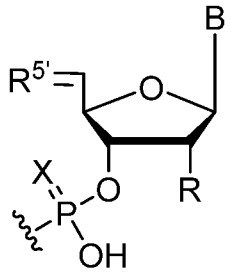
In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are

connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, two RNAi agents represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

Various publications describe multimeric RNAi agents that can be used in the methods of the disclosure. Such publications include WO2007/091269, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520; and US 7858769, the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a 5' vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or C_{1-20}alkoxy (*e.g.*, methoxy or n-hexadecyloxy);

$R^{5'}$ is $=C(H)-P(O)(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the *E* or *Z* orientation (*e.g.*, *E* orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate structure includes the preceding structure, where $R^{5'}$ is $=C(H)-OP(O)(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the E or Z orientation (*e.g.*, E orientation).

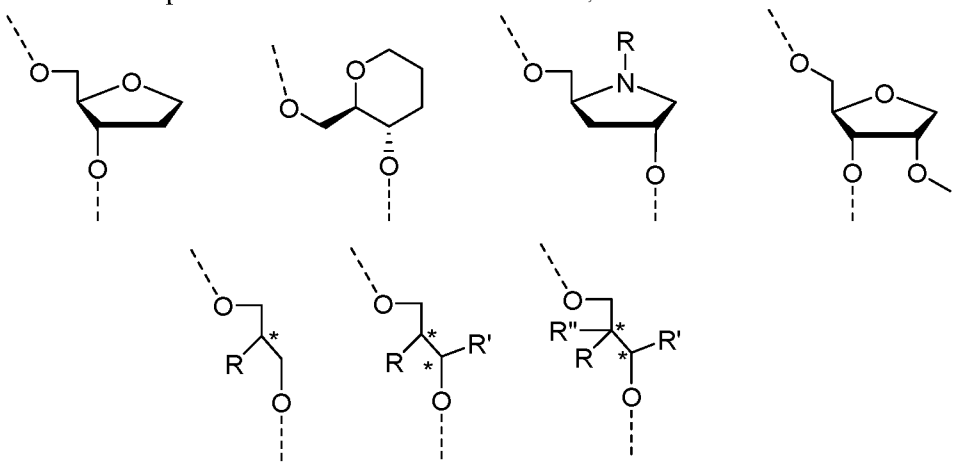
E. *Thermally Destabilizing Modifications*

In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand (*i.e.*, at positions 2-9 of the 5'-end of the antisense strand) to reduce or inhibit off-target gene silencing. It has

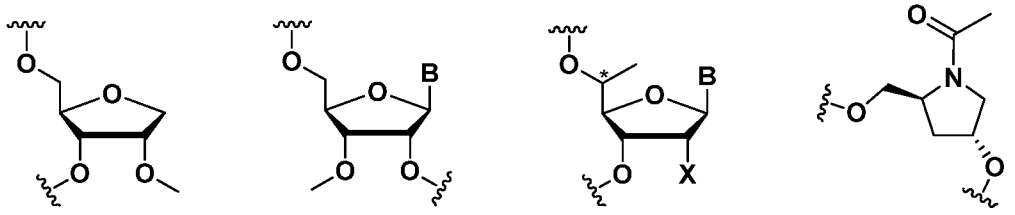
been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (*e.g.*, one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, or preferably positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. The term "thermally destabilizing modification(s)" includes modification(s) that would result with a dsRNA with a lower overall melting temperature (Tm) (preferably a Tm with one, two, three or four degrees lower than the Tm of the dsRNA without having such modification(s). In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

The thermally destabilizing modifications can include, but are not limited to, abasic modification; mismatch with the opposing nucleotide in the opposing strand; and sugar modification such as 2'-deoxy modification or acyclic nucleotide, *e.g.*, unlocked nucleic acids (UNA) or glycol nucleic acid (GNA).

Exemplified abasic modifications include, but are not limited to the following:



Wherein R = H, Me, Et or OMe; R' = H, Me, Et or OMe; R" = H, Me, Et or OMe



Mod2 (2'-OMe Abasic Spacer)

Mod3 (3'-OMe)

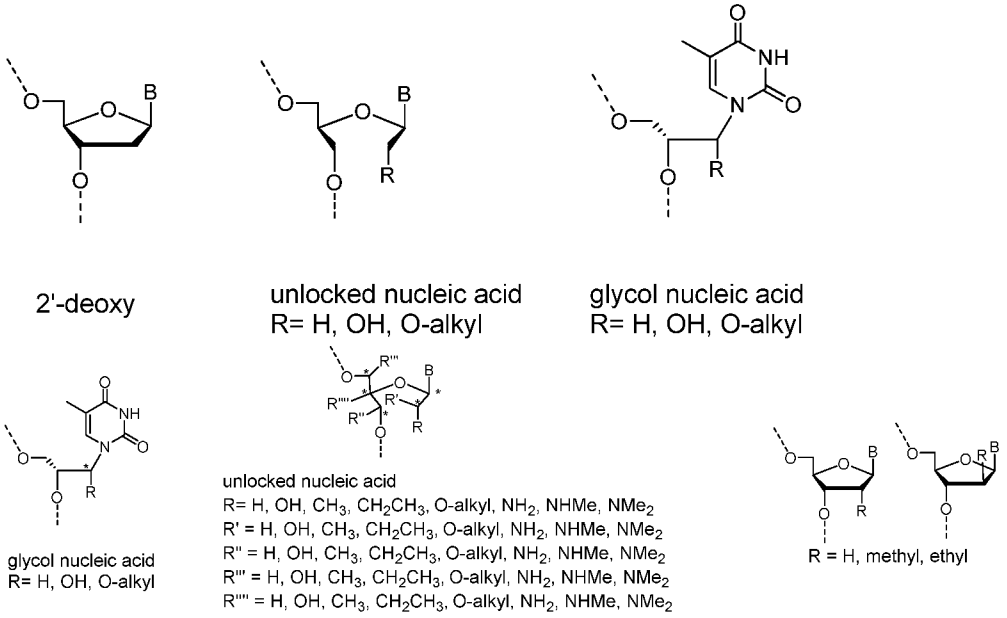
Mod4 (5'-Me)

X = OMe, F

Mod5 (Hyp-spacer)

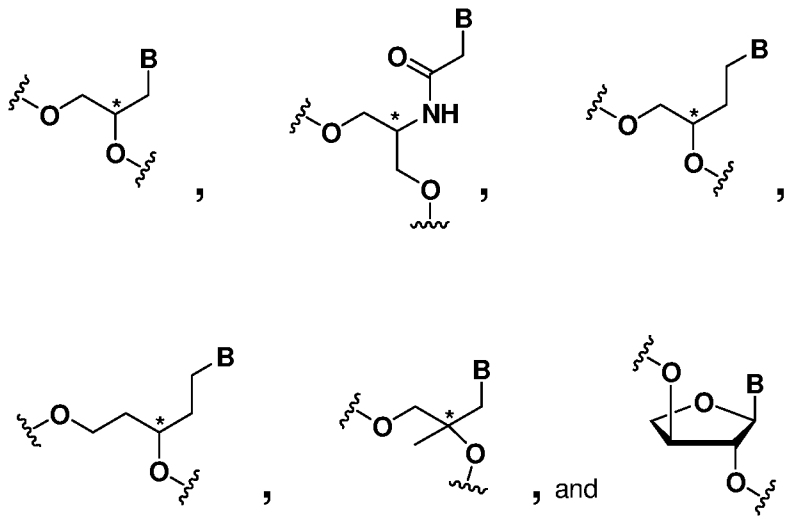
wherein B is a modified or unmodified nucleobase.

Exemplified sugar modifications include, but are not limited to the following:



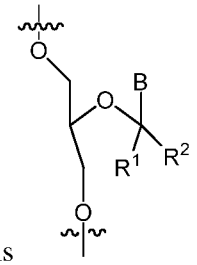
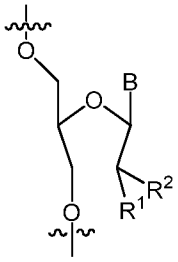
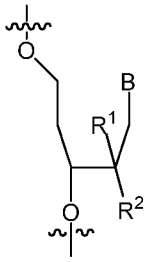
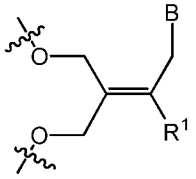
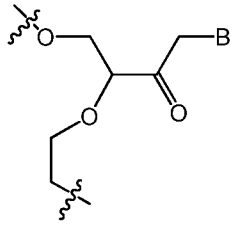
wherein B is a modified or unmodified nucleobase.

In some embodiments the thermally destabilizing modification of the duplex is selected from the group consisting of:

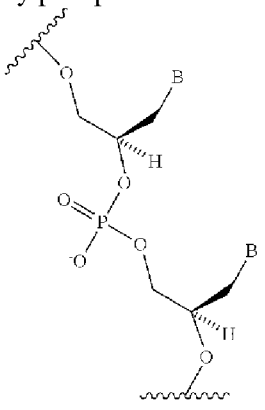


wherein B is a modified or unmodified nucleobase and the asterisk on each structure represents either *R*, *S* or *racemic*.

The term "acyclic nucleotide" refers to any nucleotide having an acyclic ribose sugar, for example, where any of bonds between the ribose carbons (*e.g.*, C1'-C2', C2'-C3', C3'-C4', C4'-O4', or C1'-O4') is absent or at least one of ribose carbons or oxygen (*e.g.*, C1', C2', C3', C4' or O4') are independently or in combination absent from the nucleotide. In some embodiments, acyclic nucleotide

is  ,  ,  ,  or  , wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H, halogen, OR_3, or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar). The term "UNA" refers to unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomers with bonds between C1'-C4' being removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar is removed (see Mikhailov et. al., Tetrahedron Letters, 26 (17): 2059 (1985); and Fluiter et al., Mol. Biosyst., 10: 1039 (2009), which are hereby incorporated by reference in their entirety). The acyclic derivative provides greater backbone flexibility without affecting the Watson-Crick pairings. The acyclic nucleotide can be linked via 2'-5' or 3'-5' linkage.

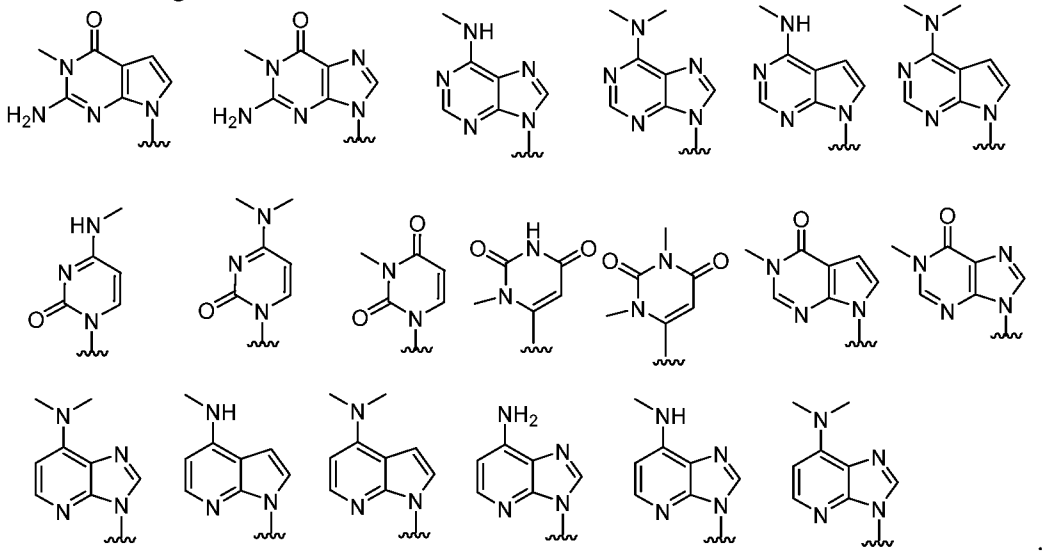
The term 'GNA' refers to glycol nucleic acid which is a polymer similar to DNA or RNA but differing in the composition of its "backbone" in that is composed of repeating glycerol units linked by phosphodiester bonds:



(R)-GNA .

The thermally destabilizing modification of the duplex can be mismatches (*i.e.*, noncomplementary base pairs) between the thermally destabilizing nucleotide and the opposing nucleotide in the opposite strand within the dsRNA duplex. Exemplary mismatch base pairs include G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, U:T, or a combination thereof. Other mismatch base pairings known in the art are also amenable to the present invention. A mismatch can occur between nucleotides that are either naturally occurring nucleotides or modified nucleotides, *i.e.*, the mismatch base pairing can occur between the nucleobases from respective nucleotides independent of the modifications on the ribose sugars of the nucleotides. In certain embodiments, the dsRNA molecule contains at least one nucleobase in the mismatch pairing that is a 2'-deoxy nucleobase; *e.g.*, the 2'-deoxy nucleobase is in the sense strand.

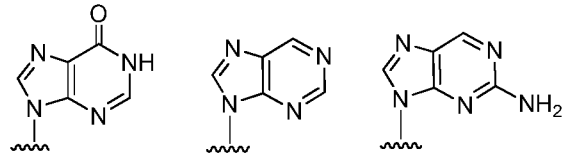
In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes nucleotides with impaired W-C H-bonding to complementary base on the target mRNA, such as:



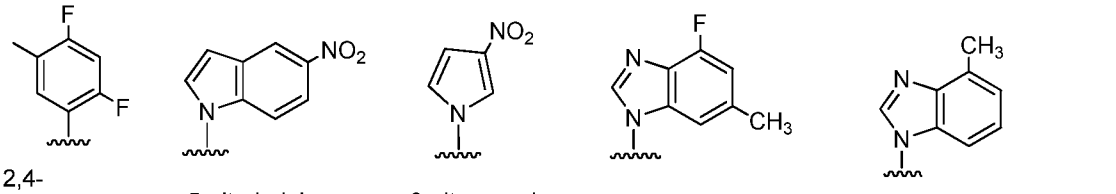
More examples of abasic nucleotide, acyclic nucleotide modifications (including UNA and GNA), and mismatch modifications have been described in detail in WO 2011/133876, which is herein incorporated by reference in its entirety.

The thermally destabilizing modifications may also include universal base with reduced or abolished capability to form hydrogen bonds with the opposing bases, and phosphate modifications.

In some embodiments, the thermally destabilizing modification of the duplex includes nucleotides with non-canonical bases such as, but not limited to, nucleobase modifications with impaired or completely abolished capability to form hydrogen bonds with bases in the opposite strand. These nucleobase modifications have been evaluated for destabilization of the central region of the dsRNA duplex as described in WO 2010/0011895, which is herein incorporated by reference in its entirety. Exemplary nucleobase modifications are:

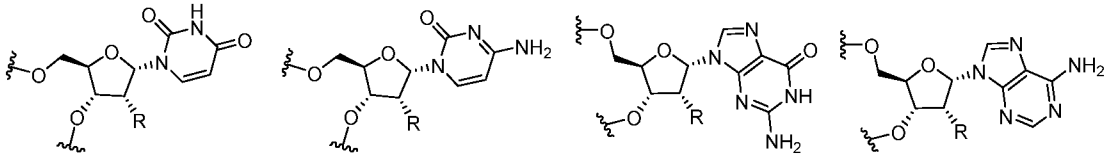


inosine nebularine 2-aminopurine



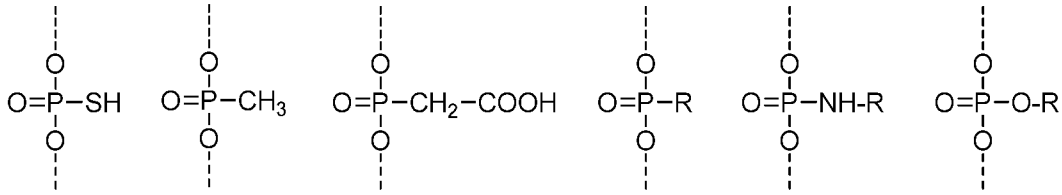
2,4-difluorotoluene 5-nitroindole 3-nitropyrrole 4-Fluoro-6-methylbenzimidazole 4-Methylbenzimidazole

In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes one or more α-nucleotide complementary to the base on the target mRNA, such as:



wherein R is H, OH, OCH_3, F, NH_2, NHMe, NMe_2 or O-alkyl.

Exemplary phosphate modifications known to decrease the thermal stability of dsRNA duplexes compared to natural phosphodiester linkages are:



R = alkyl

The alkyl for the R group can be a C_1-C_6alkyl. Specific alkyls for the R group include, but are not limited to methyl, ethyl, propyl, isopropyl, butyl, pentyl and hexyl.

As the skilled artisan will recognize, in view of the functional role of nucleobases is defining specificity of a RNAi agent of the disclosure, while nucleobase modifications can be performed in the various manners as described herein, *e.g.*, to introduce destabilizing modifications into a RNAi agent of the disclosure, *e.g.*, for purpose of enhancing on-target effect relative to off-target effect, the range of modifications available and, in general, present upon RNAi agents of the disclosure tends to be much greater for non-nucleobase modifications, *e.g.*, modifications to sugar groups or phosphate backbones of polyribonucleotides. Such modifications are described in greater detail in other sections of the instant disclosure and are expressly contemplated for RNAi agents of the disclosure, either possessing native nucleobases or modified nucleobases as described above or elsewhere herein.

In addition to the antisense strand comprising a thermally destabilizing modification, the dsRNA can also comprise one or more stabilizing modifications. For example, the dsRNA can comprise at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, the stabilizing modifications all can be present in one strand. In some embodiments, both the sense and the antisense strands comprise at least two stabilizing modifications. The stabilizing modification can occur on any nucleotide of the sense strand or antisense strand. For instance, the stabilizing modification can occur on every nucleotide on the sense strand or antisense strand; each stabilizing modification can occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both stabilizing modification in an alternating pattern. The alternating pattern of the stabilizing modifications on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the stabilizing modifications on the sense strand can have a shift relative to the alternating pattern of the stabilizing modifications on the antisense strand.

In some embodiments, the antisense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, a stabilizing modification in the antisense strand can be present at any positions. In some embodiments, the antisense comprises stabilizing modifications at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense comprises stabilizing modifications at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense comprises stabilizing modifications at positions 2, 14, and 16 from the 5'-end.

In some embodiments, the antisense strand comprises at least one stabilizing modification adjacent to the destabilizing modification. For example, the stabilizing modification can be the nucleotide at the 5'-end or the 3'-end of the destabilizing modification, *i.e.*, at position -1 or +1 from the position of the destabilizing modification. In some embodiments, the antisense strand comprises a stabilizing modification at each of the 5'-end and the 3'-end of the destabilizing modification, *i.e.*, positions -1 and +1 from the position of the destabilizing modification.

In some embodiments, the antisense strand comprises at least two stabilizing modifications at the 3'-end of the destabilizing modification, *i.e.*, at positions +1 and +2 from the position of the destabilizing modification.

In some embodiments, the sense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, a stabilizing modification in the sense strand can be present at any positions. In some embodiments, the sense strand comprises stabilizing modifications at positions 7, 10, and 11 from the 5'-end. In some other embodiments, the sense strand comprises stabilizing modifications at positions 7, 9, 10, and 11 from the 5'-end. In some embodiments, the sense strand comprises stabilizing modifications at positions opposite or complimentary to positions 11, 12, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some other embodiments, the sense strand comprises stabilizing modifications at positions opposite or complimentary to positions 11, 12, 13, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some embodiments, the sense strand comprises a block of two, three, or four stabilizing modifications.

In some embodiments, the sense strand does not comprise a stabilizing modification in position opposite or complimentary to the thermally destabilizing modification of the duplex in the antisense strand.

Exemplary thermally stabilizing modifications include, but are not limited to, 2'-fluoro modifications. Other thermally stabilizing modifications include, but are not limited to, LNA.

In some embodiments, the dsRNA of the disclosure comprises at least four (*e.g.*, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, the 2'-fluoro nucleotides all can be present in one strand. In some embodiments, both the sense and the antisense strands comprise at least two 2'-fluoro nucleotides. The 2'-fluoro modification can occur on any nucleotide of the sense strand or antisense strand. For instance, the 2'-fluoro modification can occur on every nucleotide on the sense strand or antisense strand; each 2'-fluoro modification can occur in

an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both 2'-fluoro modifications in an alternating pattern. The alternating pattern of the 2'-fluoro modifications on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the 2'-fluoro modifications on the sense strand can have a shift relative to the alternating pattern of the 2'-fluoro modifications on the antisense strand.

In some embodiments, the antisense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, a 2'-fluoro modification in the antisense strand can be present at any positions. In some embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 14, and 16 from the 5'-end.

In some embodiments, the antisense strand comprises at least one 2'-fluoro nucleotide adjacent to the destabilizing modification. For example, the 2'-fluoro nucleotide can be the nucleotide at the 5'-end or the 3'-end of the destabilizing modification, *i.e.*, at position -1 or +1 from the position of the destabilizing modification. In some embodiments, the antisense strand comprises a 2'-fluoro nucleotide at each of the 5'-end and the 3'-end of the destabilizing modification, *i.e.*, positions -1 and +1 from the position of the destabilizing modification.

In some embodiments, the antisense strand comprises at least two 2'-fluoro nucleotides at the 3'-end of the destabilizing modification, *i.e.*, at positions +1 and +2 from the position of the destabilizing modification.

In some embodiments, the sense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) 2'-fluoro nucleotides. Without limitations, a 2'-fluoro modification in the sense strand can be present at any positions. In some embodiments, the antisense comprises 2'-fluoro nucleotides at positions 7, 10, and 11 from the 5'-end. In some other embodiments, the sense strand comprises 2'-fluoro nucleotides at positions 7, 9, 10, and 11 from the 5'-end. In some embodiments, the sense strand comprises 2'-fluoro nucleotides at positions opposite or complimentary to positions 11, 12, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some other embodiments, the sense strand comprises 2'-fluoro nucleotides at positions opposite or complimentary to positions 11, 12, 13, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some embodiments, the sense strand comprises a block of two, three or four 2'-fluoro nucleotides.

In some embodiments, the sense strand does not comprise a 2'-fluoro nucleotide in position opposite or complimentary to the thermally destabilizing modification of the duplex in the antisense strand.

In some embodiments, the dsRNA molecule of the disclosure comprises a 21 nucleotides (nt) sense strand and a 23 nucleotides (nt) antisense, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide occurs in

the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein one end of the dsRNA is blunt, while the other end is comprises a 2 nt overhang, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4 or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4 or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a blunt end at 5'-end of the antisense strand. Preferably, the 2 nt overhang is at the 3'-end of the antisense.

In some embodiments, the dsRNA molecule of the disclosure comprising a sense and antisense strands, wherein: the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1), positions 1 to 23 of said sense strand comprise at least 8 ribonucleotides; antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when said double stranded nucleic acid is introduced into a mammalian cell; and wherein the antisense strand contains at least one thermally destabilizing nucleotide, where at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.* at position 2-9 of the 5'-end of the antisense strand). For example, the thermally destabilizing nucleotide occurs between positions opposite or complimentary to positions 14-17 of the 5'-end of the sense strand, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a duplex region of 12-30 nucleotide pairs in length.

In some embodiments, the dsRNA molecule of the disclosure comprises a sense and antisense strands, wherein said dsRNA molecule comprises a sense strand having a length which is at least 25 and at most 29 nucleotides and an antisense strand having a length which is at most 30 nucleotides

with the sense strand comprises a modified nucleotide that is susceptible to enzymatic degradation at position 11 from the 5'end, wherein the 3' end of said sense strand and the 5' end of said antisense strand form a blunt end and said antisense strand is 1-4 nucleotides longer at its 3' end than the sense strand, wherein the duplex region which is at least 25 nucleotides in length, and said antisense strand is sufficiently complementary to a target mRNA along at least 19 nt of said antisense strand length to reduce target gene expression when said dsRNA molecule is introduced into a mammalian cell, and wherein dicer cleavage of said dsRNA preferentially results in an siRNA comprising said 3' end of said antisense strand, thereby reducing expression of the target gene in the mammal, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.* at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA has a duplex region of 12-29 nucleotide pairs in length.

In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNA molecule may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2′ hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases, the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or 5' terminal position, may only occur in a terminal region, *e.g.,* at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of an RNA or may only occur in a single strand region of an RNA. *E.g.*, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5' end or ends can be phosphorylated.

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5' or 3'

overhang, or in both. *E.g.*, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, or 2'-fluoro. The strands can contain more than one modification. In some embodiments, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. It is to be understood that these modifications are in addition to the at least one thermally destabilizing modification of the duplex present in the antisense strand.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-deoxy, 2'- O-methyl or 2'-fluoro modifications, acyclic nucleotides or others. In some embodiments, the sense strand and antisense strand each comprises two differently modified nucleotides selected from 2'-O-methyl or 2'-deoxy. In some embodiments, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl nucleotide, 2'-deoxy nucleotide, 2´-deoxy-2'-fluoro nucleotide, 2'-O-N-methylacetamido (2'-O-NMA) nucleotide, a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) nucleotide, 2'-O-aminopropyl (2'-O-AP) nucleotide, or 2'-ara-F nucleotide. Again, it is to be understood that these modifications are in addition to the at least one thermally destabilizing modification of the duplex present in the antisense strand.

In some embodiments, the dsRNA molecule of the disclosure comprises modifications of an alternating pattern, particular in the B1, B2, B3, B1', B2', B3', B4' regions. The term "alternating motif" or "alternative pattern" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB…," "AABBAABBAABB…," "AABAABAABAAB…," "AAABAAABAAAB…," "AAABBBAAABBB…," or "ABCABCABCABC…," etc.
The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB…", "ACACAC…" "BDBDBD…" or "CDCDCD…," etc.

In some embodiments, the dsRNA molecule of the disclosure comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the

alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and vice versa. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 3'-5'of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 3'-5'of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

The dsRNA molecule of the disclosure may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand.

In some embodiments, the dsRNA molecule comprises the phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region comprises two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide. Preferably, these terminal three nucleotides may be at the 3'-end of the antisense strand.

In some embodiments, the sense strand of the dsRNA molecule comprises 1-10 blocks of two to ten phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said sense strand is paired with an antisense strand comprising any

combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of two phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of three phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of four phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of five phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of six phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of seven phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, or 8 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of eight phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, or 6 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of nine phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, or 4 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the termini position(s) of the sense or antisense strand. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage at one end or both ends of the sense or antisense strand.

In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the internal region of the duplex of each of the sense or antisense strand. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides may be linked through phosphorothioate methylphosphonate internucleotide linkage at position 8-16 of the duplex region counting from the 5'-end of the sense strand; the dsRNA molecule can optionally further comprise one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the termini position(s).

In some embodiments, the dsRNA molecule of the disclosure further comprises one to five phosphorothioate or methylphosphonate internucleotide linkage modification(s) within position 1-5 and one to five phosphorothioate or methylphosphonate internucleotide linkage modification(s) within position 18-23 of the sense strand (counting from the 5'-end), and one to five phosphorothioate or

methylphosphonate internucleotide linkage modification at positions 1 and 2 and one to five within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one phosphorothioate or methylphosphonate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate or methylphosphonate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and two phosphorothioate internucleotide linkage modifications within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and two phosphorothioate internucleotide linkage modifications within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modification at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 (counting from the 5'-end) of the sense strand, and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 (counting from the 5'-end) of the sense strand, and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 20 and 21 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one at position 21 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 20 and 21 the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 21 and 22 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one phosphorothioate internucleotide linkage modification at position 21 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 21 and 22 the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 22 and 23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one phosphorothioate internucleotide linkage modification at position 21 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 23 and 23 the antisense strand (counting from the 5'-end).

In some embodiments, compound of the disclosure comprises a pattern of backbone chiral centers. In some embodiments, a common pattern of backbone chiral centers comprises at least 5 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 6 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 7 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 8 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 9 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the Sp configuration. In

some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 16 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 17 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 18 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 19 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages which are not chiral (as a non-limiting example, a phosphodiester). In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the Sp configuration, and no more than 8 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the Sp configuration, and no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least

12 internucleotidic linkages in the Sp configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the Sp configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the Sp configuration, and no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the Sp configuration, and no more than 4 internucleotidic linkages which are not chiral. In some embodiments, the internucleotidic linkages in the Sp configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages in the Rp configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages which are not chiral are optionally contiguous or not contiguous.

In some embodiments, compound of the disclosure comprises a block is a stereochemistry block. In some embodiments, a block is an Rp block in that each internucleotidic linkage of the block is Rp. In some embodiments, a 5'-block is an Rp block. In some embodiments, a 3'-block is an Rp block. In some embodiments, a block is an Sp block in that each internucleotidic linkage of the block is Sp. In some embodiments, a 5'-block is an Sp block. In some embodiments, a 3'-block is an Sp block. In some embodiments, provided oligonucleotides comprise both Rp and Sp blocks. In some embodiments, provided oligonucleotides comprise one or more Rp but no Sp blocks. In some embodiments, provided oligonucleotides comprise one or more Sp but no Rp blocks. In some embodiments, provided oligonucleotides comprise one or more PO blocks wherein each internucleotidic linkage in a natural phosphate linkage.

In some embodiments, compound of the disclosure comprises a 5'-block is an Sp block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an Sp block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an Sp block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block comprises 4 or more nucleoside units. In some embodiments, a 5'-block comprises 5 or more nucleoside units. In some embodiments, a 5'-block comprises 6 or more nucleoside units. In some embodiments, a 5'-block comprises 7 or more nucleoside units. In some embodiments, a 3'-block is an Sp block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an Sp block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an Sp block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block comprises 4 or more nucleoside units. In some embodiments, a 3'-block comprises 5 or more nucleoside units. In some embodiments, a 3'-block comprises 6 or more nucleoside units. In some embodiments, a 3'-block comprises 7 or more nucleoside units.

In some embodiments, compound of the disclosure comprises a type of nucleoside in a region or an oligonucleotide is followed by a specific type of internucleotidic linkage, *e.g.*, natural phosphate linkage, modified internucleotidic linkage, Rp chiral internucleotidic linkage, Sp chiral internucleotidic linkage, etc. In some embodiments, A is followed by Sp. In some embodiments, A is followed by Rp. In some embodiments, A is followed by natural phosphate linkage (PO). In some embodiments, U is followed by Sp. In some embodiments, U is followed by Rp. In some embodiments, U is followed by natural phosphate linkage (PO). In some embodiments, C is followed by Sp. In some embodiments, C is followed by Rp. In some embodiments, C is followed by natural phosphate linkage (PO). In some embodiments, G is followed by Sp. In some embodiments, G is followed by Rp. In some embodiments, G is followed by natural phosphate linkage (PO). In some embodiments, C and U are followed by Sp. In some embodiments, C and U are followed by Rp. In some embodiments, C and U are followed by natural phosphate linkage (PO). In some embodiments, A and G are followed by Sp. In some embodiments, A and G are followed by Rp.

In some embodiments, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4 or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 1, 2, 3, 4 or 5 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4 or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (vii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the dsRNA molecule of the disclosure comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch can occur in the overhang region or the duplex region. The base pair can be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In some embodiments, the dsRNA molecule of the disclosure comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'- end of the antisense strand can be chosen independently from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In some embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair.

It was found that introducing 4'-modified or 5'-modified nucleotide to the 3'-end of a phosphodiester (PO), phosphorothioate (PS), or phosphorodithioate (PS2) linkage of a dinucleotide at any position of single stranded or double stranded oligonucleotide can exert steric effect to the internucleotide linkage and, hence, protecting or stabilizing it against nucleases.

In some embodiments, 5'-modified nucleoside is introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. For instance, a 5'-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The alkyl group at the 5' position of the ribose sugar can be racemic or chirally pure *R* or *S* isomer. An exemplary 5'-alkylated nucleoside is 5'-methyl nucleoside. The 5'-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 4'-modified nucleoside is introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. For instance, a 4'-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The alkyl group at the 4' position of the ribose sugar can be racemic or chirally pure *R* or *S* isomer. An exemplary 4'-alkylated nucleoside is 4'-methyl nucleoside. The 4'-methyl can be either racemic or chirally pure *R* or *S* isomer. Alternatively, a 4'-*O*-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The 4'-*O*-alkyl of the ribose sugar can be racemic or chirally pure *R* or *S* isomer. An exemplary 4'-*O*-alkylated nucleoside is 4'-*O*-methyl nucleoside. The 4'-*O*-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 5'-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 5'-alkyl can be either racemic or chirally pure *R* or *S* isomer. An exemplary 5'-alkylated nucleoside is 5'-methyl nucleoside. The 5'-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 4'-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 4'-alkyl can be either racemic or chirally pure *R* or *S* isomer. An exemplary 4'-alkylated nucleoside is 4'-methyl nucleoside. The 4'-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 4'-*O*-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 5'-alkyl can be either racemic or chirally pure *R* or *S* isomer. An exemplary 4'-*O*-

alkylated nucleoside is 4'-*O*-methyl nucleoside. The 4'-*O*-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, the dsRNA molecule of the disclosure can comprise 2'-5' linkages (with 2'-H, 2'-OH and 2'-OMe and with P=O or P=S). For example, the 2'-5' linkages modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

In another embodiment, the dsRNA molecule of the disclosure can comprise L sugars (*e.g.*, L ribose, L-arabinose with 2'-H, 2'-OH and 2'-OMe). For example, these L sugars modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

Various publications describe multimeric siRNA which can all be used with the dsRNA of the disclosure. Such publications include WO2007/091269, US 7858769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 which are hereby incorporated by their entirely.

As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to an RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose sugar of one or more ribonucleotide subunits of a dsRNA agent can be replaced with another moiety, *e.g.*, a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

The RNAi agents may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

In certain specific embodiments, the RNAi agent for use in the methods of the disclosure is an agent selected from the group of agents listed in any one of Tables 2-5 and 7-10. These agents may further comprise a ligand, such as one or more lipophilic moieties, one or more GalNAc derivatives, or both of one of more lipophilic moieties and one or more GalNAc derivatives.

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA, *e.g.*, into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In certain embodiments, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In some embodiments, a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Typical ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly

L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an α helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a glial cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, the ligand is a multivalent galactose, *e.g.*, an N-acetyl-galactosamine.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a brain cell or a glial cell. Ligands may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxon,

vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc*. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems® (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule can typically bind a serum protein, such as human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid-based ligand can be used to modulate, *e.g.*, control (*e.g.*, inhibit) the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In certain embodiments, the lipid-based ligand binds HSA. For example, the ligand can bind HSA with a sufficient affinity such that distribution of the conjugate to a non-kidney tissue is enhanced. However, the affinity is typically not so strong that the HSA-ligand binding cannot be reversed.

In certain embodiments, the lipid-based ligand binds HSA weakly or not at all, such that distribution of the conjugate to the kidney is enhanced. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid-based ligand.

In certain embodiments, the lipid-based ligand binds HSA weakly or not at all, such that distribution of the conjugate to the kidney is enhanced. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid-based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by cancer cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, such as a helical cell-permeation agent. In certain embodiments, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is typically an α-helical agent and can have a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO:11). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:12)) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:13)) and the *Drosophila Antennapedia* protein (RQIKIWFQNRRMKWKK (SEQ ID NO:14)) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, *Nature*, 354:82-84, 1991). Typically, the peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit is a cell targeting peptide such as an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

An RGD peptide moiety can be used to target a particular cell type, *e.g.*, a tumor cell, such as an endothelial tumor cell or a breast cancer tumor cell (Zitzmann *et al.*, *Cancer Res.*, 62:5139-43, 2002). An RGD peptide can facilitate targeting of an dsRNA agent to tumors of a variety of other tissues, including the lung, kidney, spleen, or liver (Aoki *et al.*, *Cancer Gene Therapy* 8:783-787, 2001). Typically, the RGD peptide will facilitate targeting of an iRNA agent to the kidney. The RGD peptide can be linear or cyclic, and can be modified, *e.g.*, glycosylated or methylated to facilitate targeting to specific tissues. For example, a glycosylated RGD peptide can deliver an iRNA agent to a tumor cell expressing $\alpha_V\beta_3$ (Haubner *et al.*, *Jour. Nucl. Med.*, 42:326-336, 2001).

A "cell permeation peptide" is capable of permeating a cell, *e.g.,* a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, *Nucl. Acids Res.* 31:2717-2724, 2003).

C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.,* C5, C6, C7, or C8) sugars; di- and tri-saccharides include sugars having two or three monosaccharide units (*e.g.,* C5, C6, C7, or C8).

In certain embodiments, a carbohydrate conjugate comprises a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, *e.g.*, by serving as a ligand for the asialoglycoprotein receptor of liver cells (*e.g.*, hepatocytes).

In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached via a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 3' end of the sense strand) via a linker, *e.g.*, a linker as described herein. In some embodiments the GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) via a linker, *e.g.*, a linker as described herein.

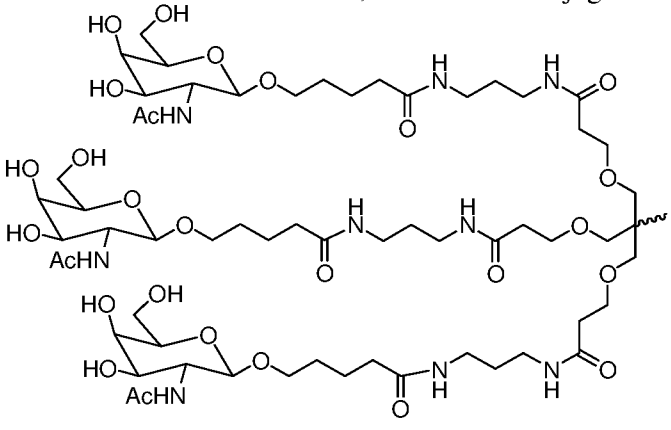
In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a tetravalent linker.

In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

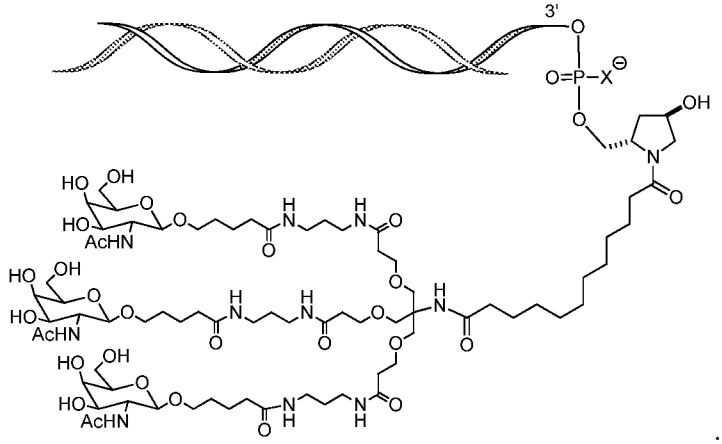
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, the GalNAc conjugate is

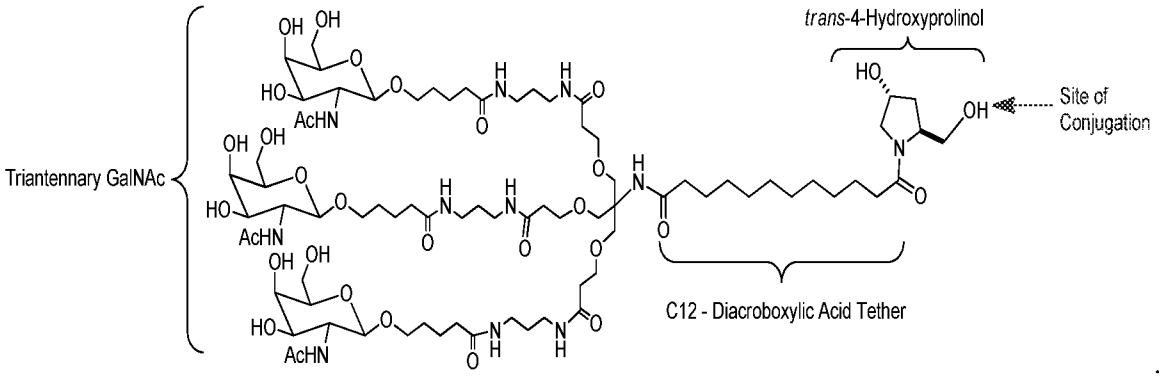


Formula II.

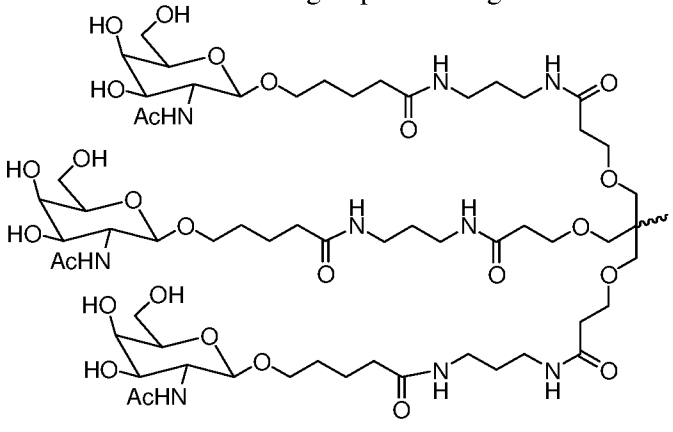
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate via a linker as shown in the following schematic, wherein X is O or S



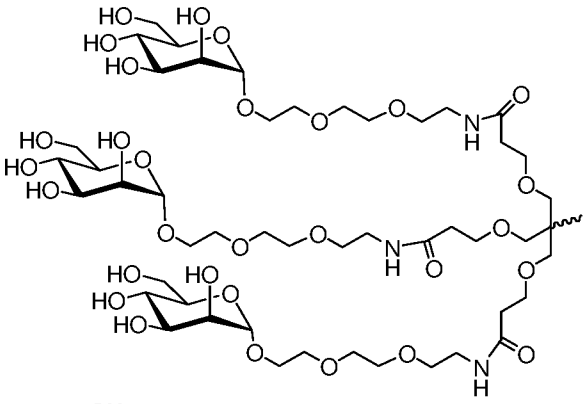
In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:



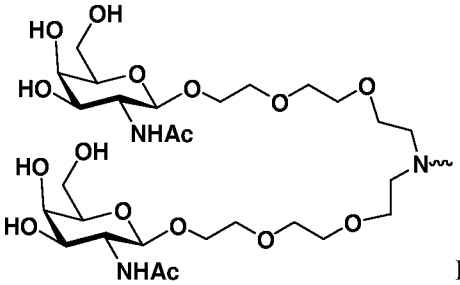
In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



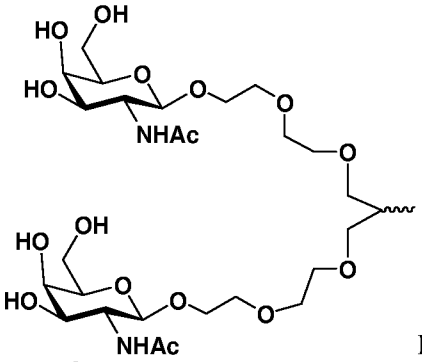
Formula II,



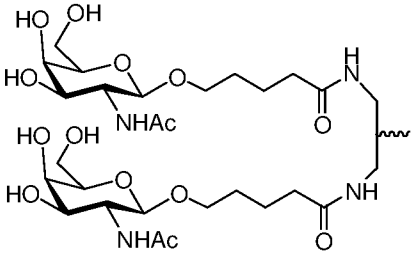
Formula III,



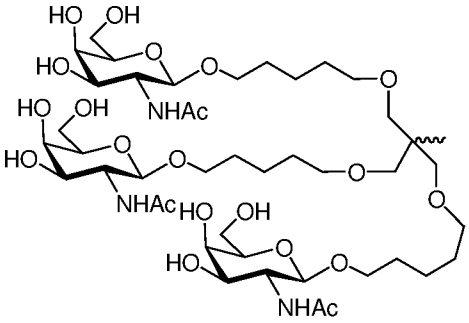
Formula IV,



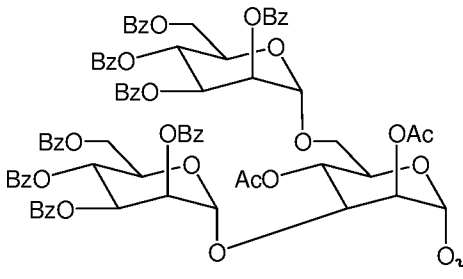
Formula V,



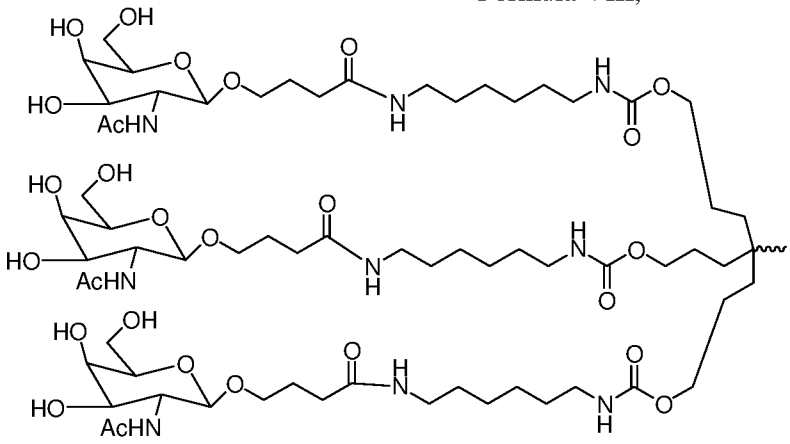
Formula VI,



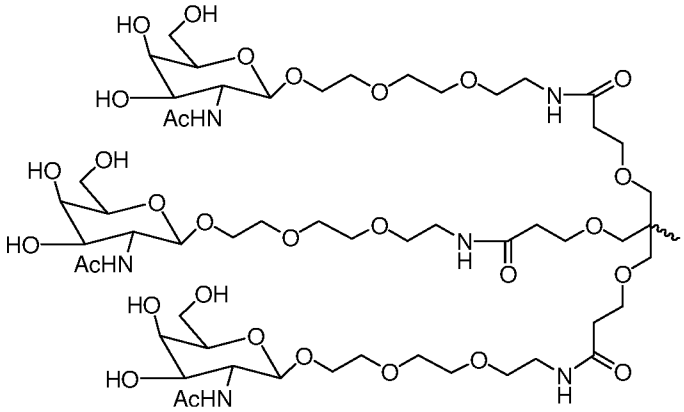
Formula VII,



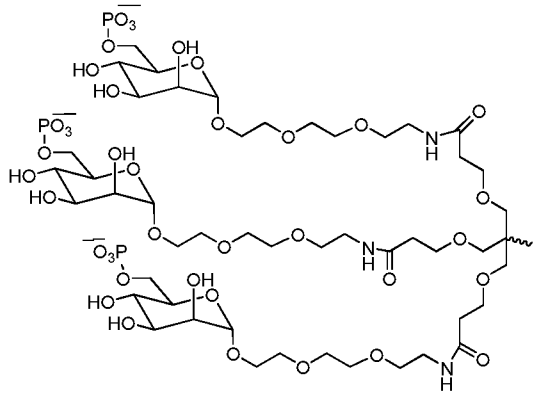
Formula VIII,



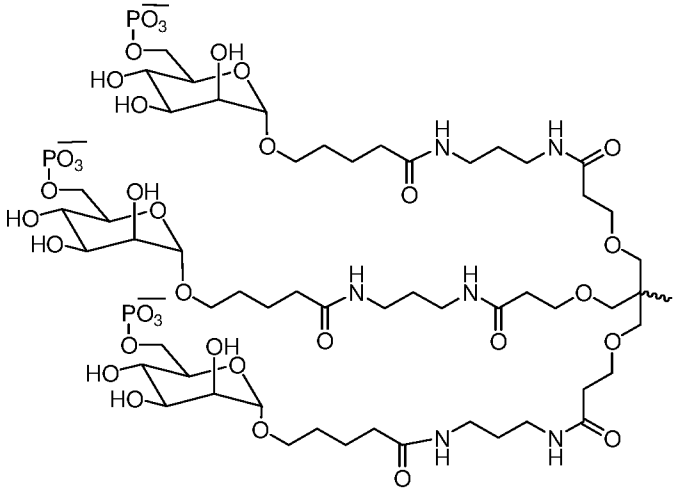
Formula IX,



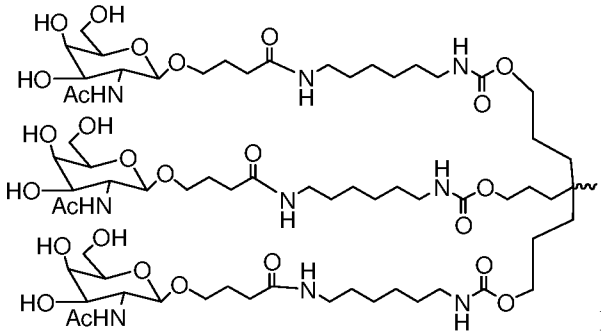
Formula X,



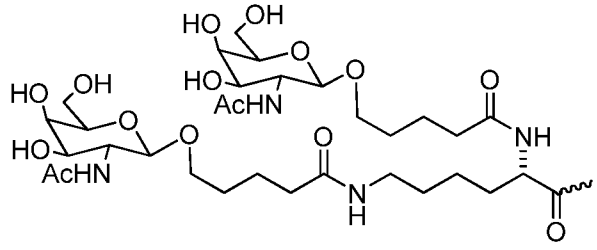
Formula XI,



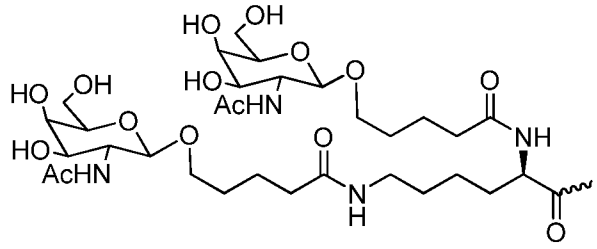
Formula XII,



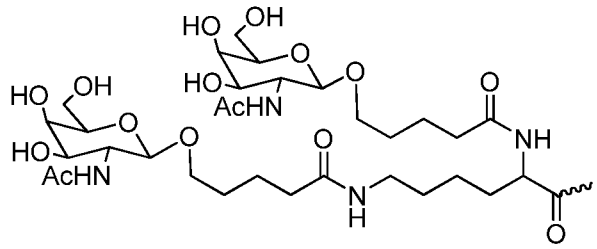
Formula XIII,



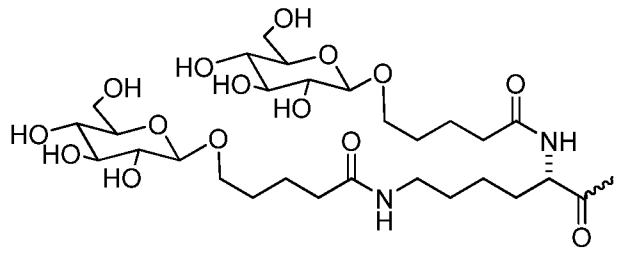
Formula XIV,



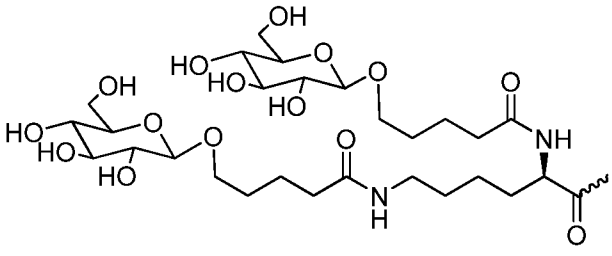
Formula XV,



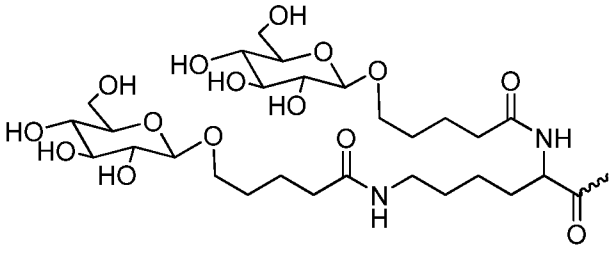
Formula XVI,



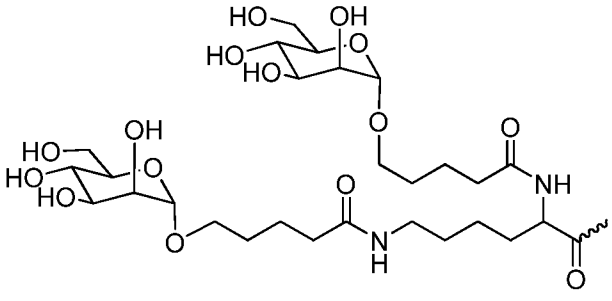
Formula XVII,



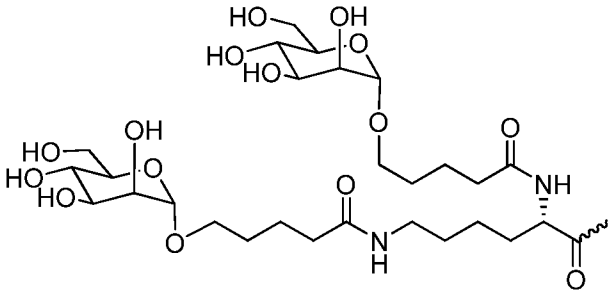
Formula XVIII,



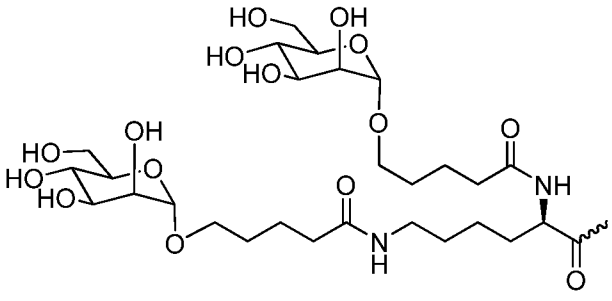
Formula XIX,



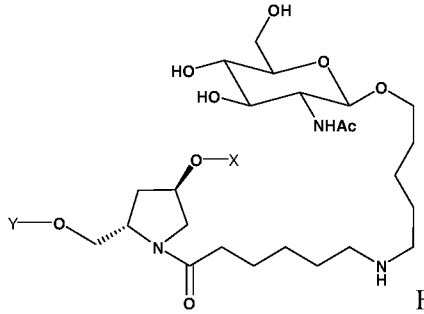
Formula XX,



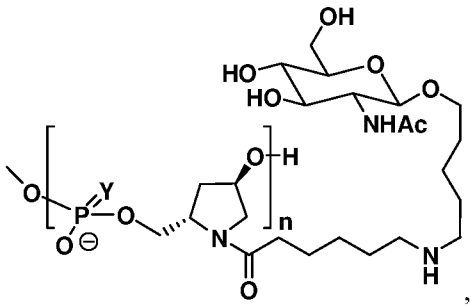
Formula XXI,



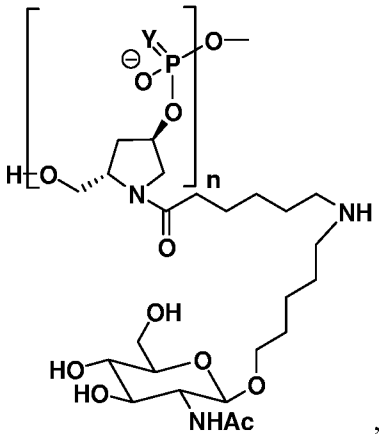
Formula XXII,



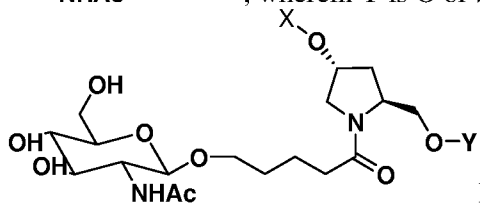
Formula XXIII;



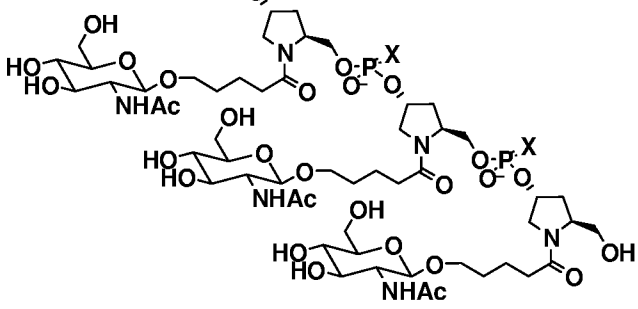
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



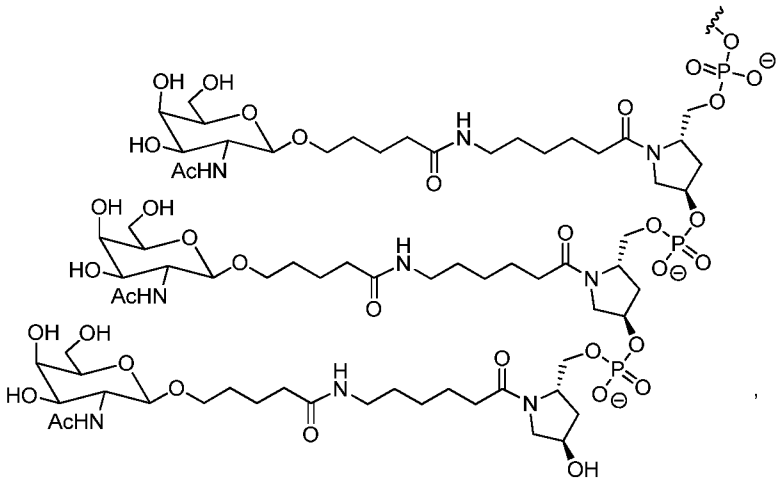
, wherein Y is O or S and n is 3-6 (Formula XXV);

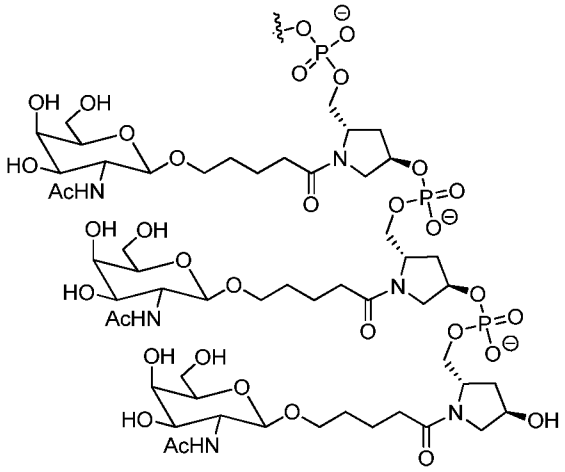


Formula XXVI;

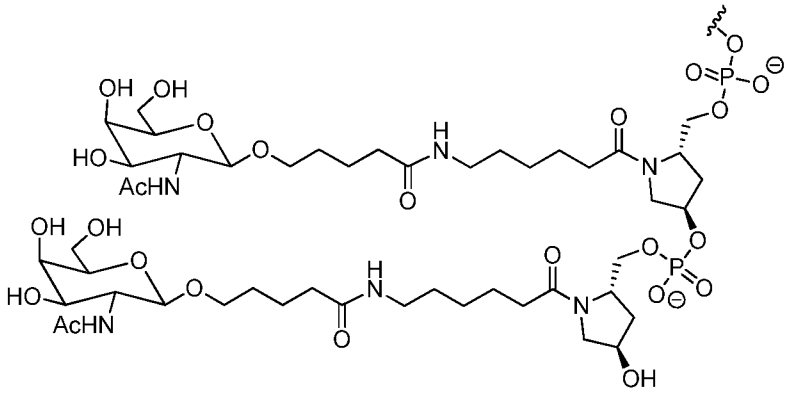


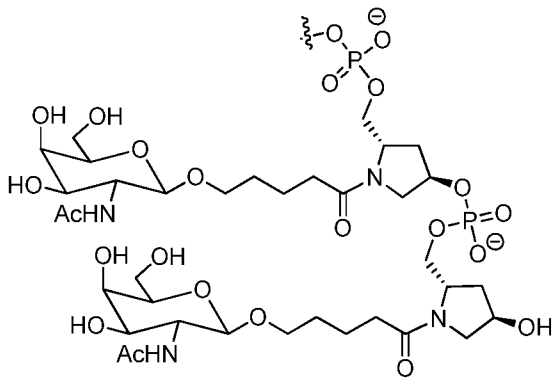
, wherein X is O or S (Formula XXVII);





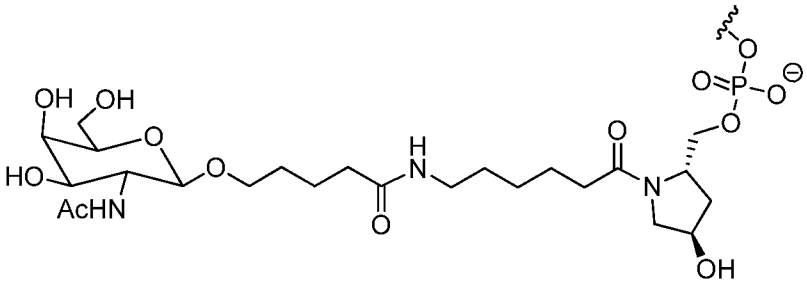
Formula XXVII; Formula XXIX;



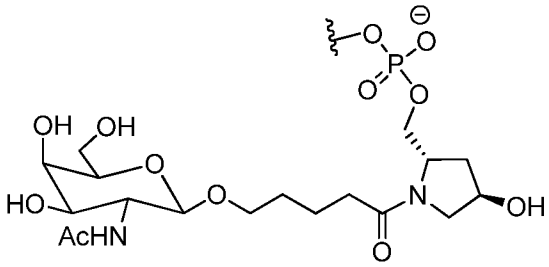


Formula XXX;

Formula XXXI;

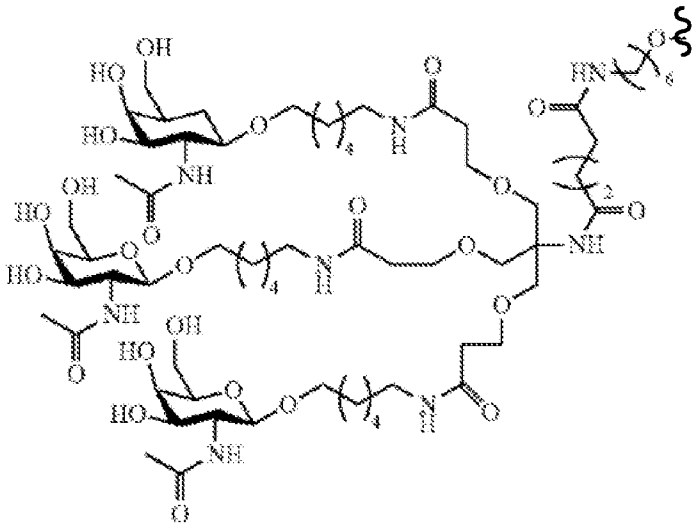


, and



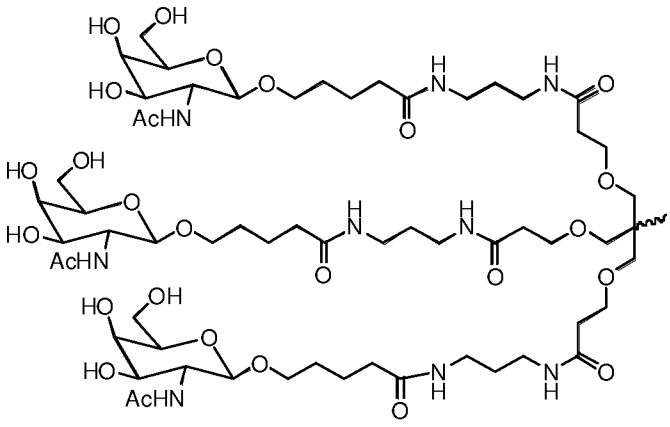
Formula XXXII;

Formula XXXIII.



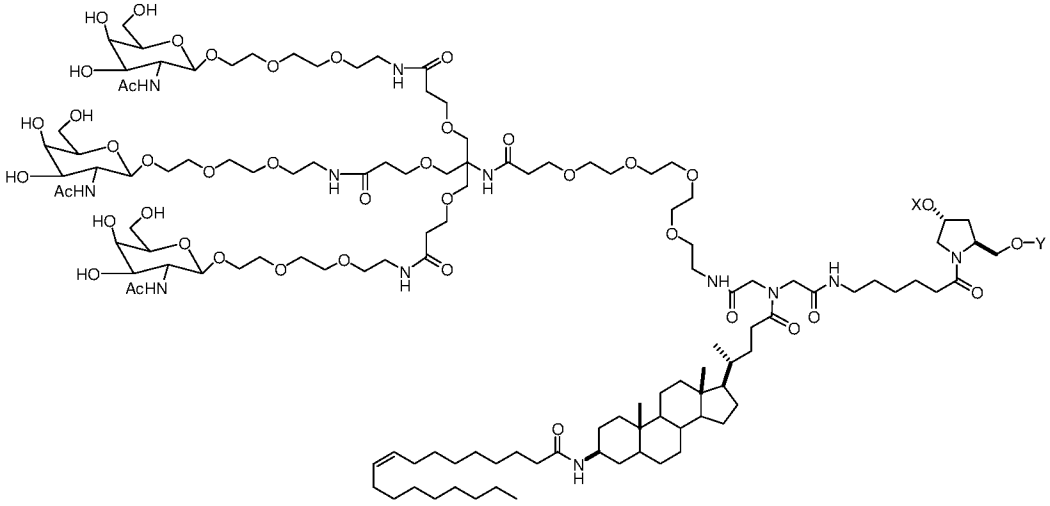
Formula XXXIV.

In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In certain embodiments, the monosaccharide is an N-acetylgalactosamine, such as



Formula II.

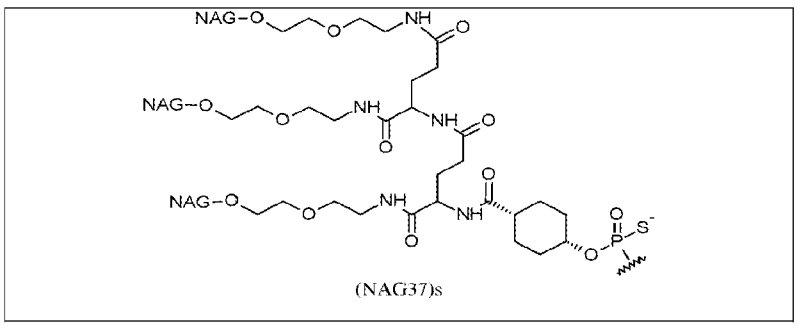
Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXXVI),

when one of X or Y is an oligonucleotide, the other is a hydrogen.

In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises the structure below:



In certain embodiments, the RNAi agents of the disclosure may include GalNAc ligands, even if such GalNAc ligands are currently projected to be of limited value for the preferred intrathecal/CNS delivery route(s) of the instant disclosure.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a tetravalent linker.

In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent, *e.g.,* the 5'end of the sense strand of a dsRNA agent, or the 5' end of one or both sense strands of a dual targeting RNAi agent as described herein. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.,* 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

Additional carbohydrate conjugates and linkers suitable for use in the present invention include those described in WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl,

alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In certain embodiments, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

In certain embodiments, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

In certain embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S. Exemplary embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-

O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-, wherein Rk at each occurrence can be, independently, C1-C20 alkyl, C1-C20 haloalkyl, C6-C10 aryl, or C7-C12 aralkyl. In certain preferred embodiments a phosphate-based linking group is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In certain embodiments, a cleavable linker comprises an acid cleavable linking group. An *a*cid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

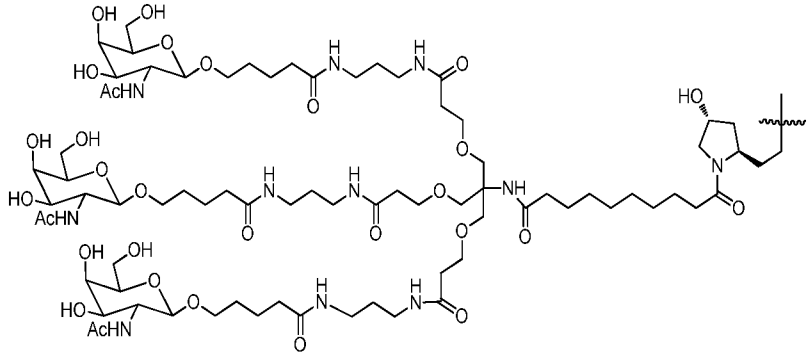
iv. Ester-based cleavable linking groups

In certain embodiments, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated using methods analogous to those described above.

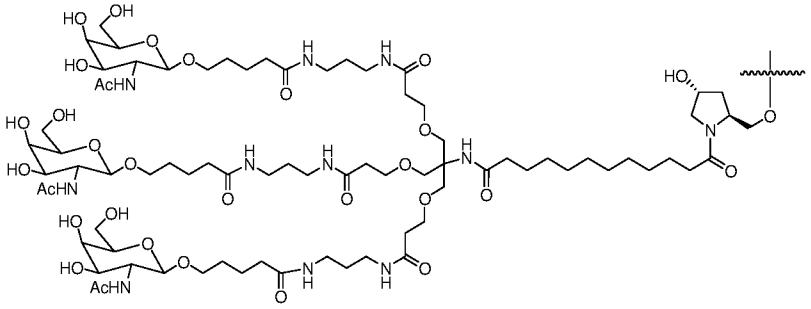
v. Peptide-based cleavable linking groups

In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

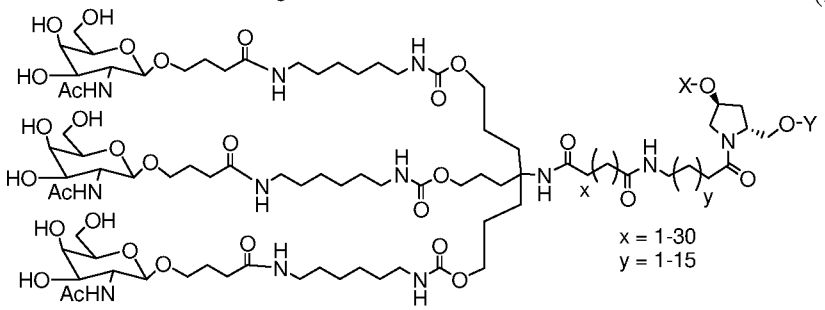
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



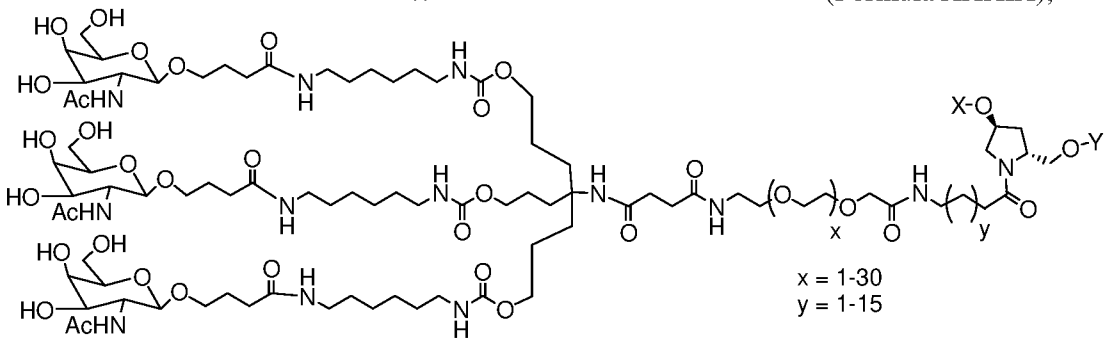
(Formula XXXVII),



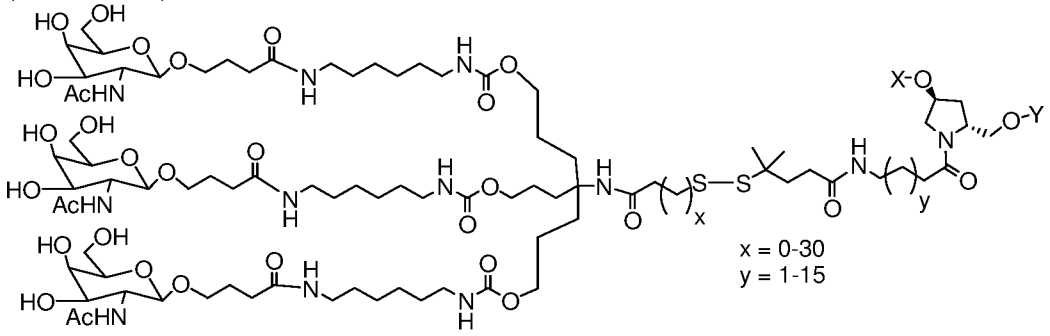
(Formula XXXVIII),



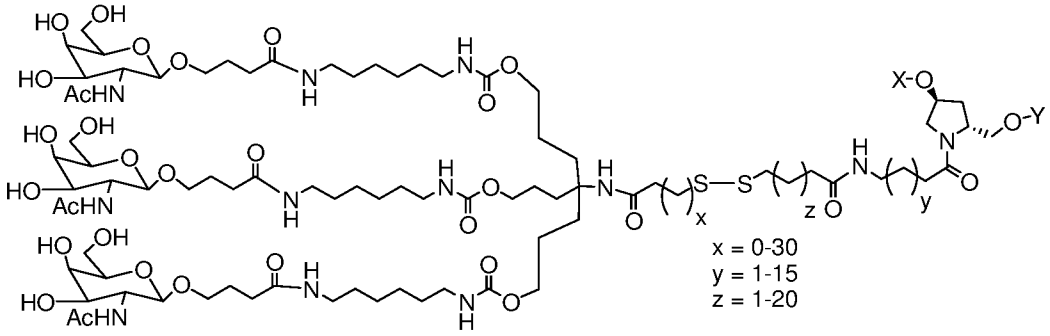
(Formula XXXIX),



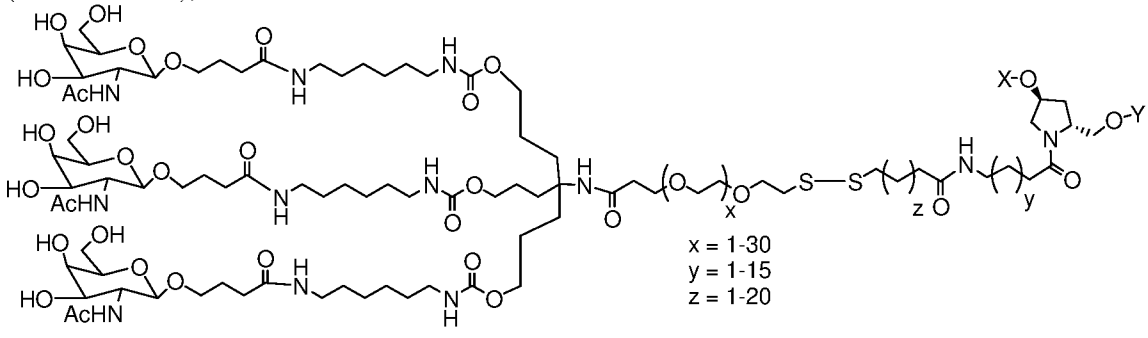
(Formula XL),



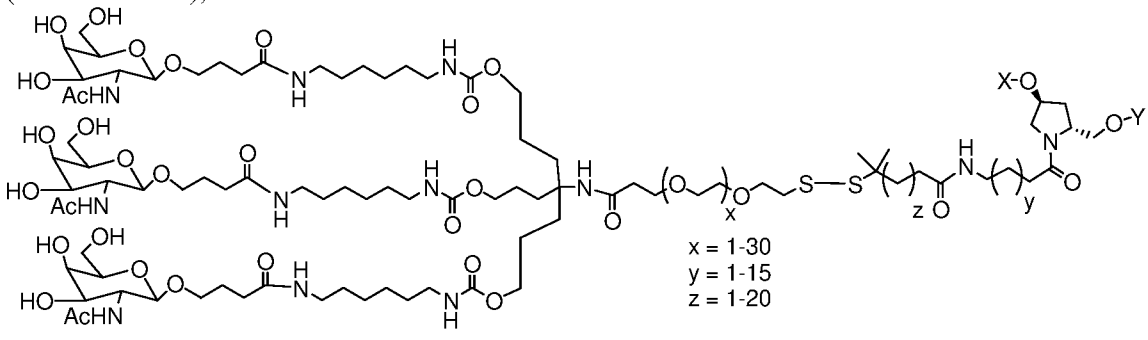
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

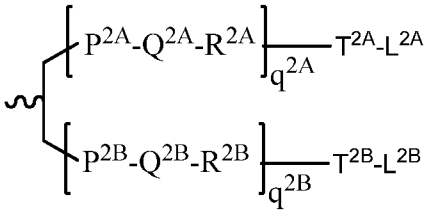


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In certain embodiments, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

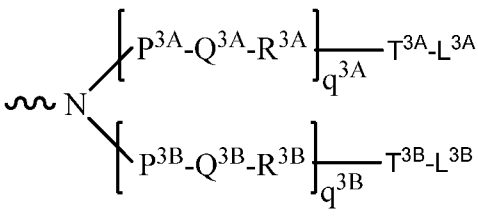
Formula XXXXV



(IV)

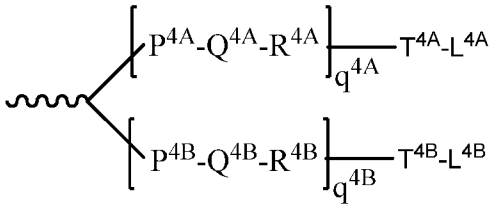
,

Formula XLVI

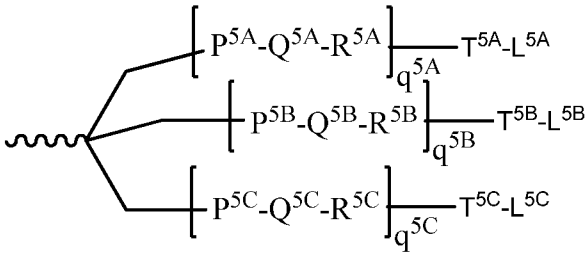


V)

,



,



;

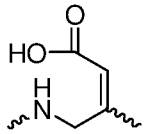
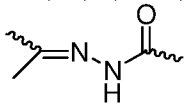
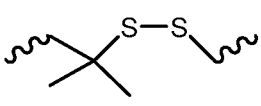
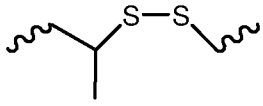
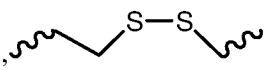
Formula XLVII Formula XLVIII

wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

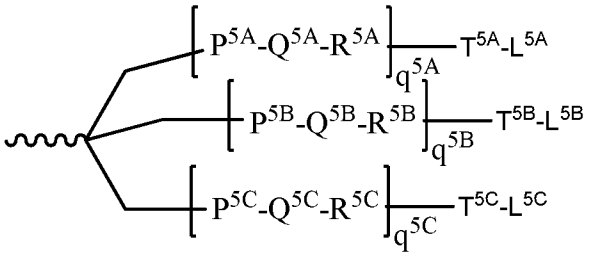
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, -C(O)-$CH(R^a)$-NH-, CO, CH=N-O, , , , ,  or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; andR^a is H or amino acid side chain.Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XLIX):

Formula XLIX



wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Patent Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928;5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds or "chimeras," in the context of this invention, are iRNA compounds, preferably dsRNA agents, that contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to

the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al., Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. *In Vivo* Testing of APOE Knockdown

Human APOE knock-in mouse models, including transgenic mice expressing one or more human APOE isoforms (APOE2, APOE3, and APOE4) have been generated (see, *e.g.,* Trommer, *et al.* (2005) *Neuroreport* 15:2655-2658) and can be used to demonstrate the *in vivo* efficacy of the RNAi agents provided herein.

Mouse models of APOE-associated neurodegenerative disease (*e.g.,* Alzheimer's disease) have also been generated and can further be used to demonstrate the *in vivo* efficacy of the RNAi agents provided herein. Such models may combine transgenic expression of one or more isoforms of human APOE with constituitive or inducible expression, *e.g.,* overexpression, of, for example, human amyloid precursor protein (APP), in some instances comprising a pathogenic mutation (*e.g.,* a Swedish mutation (KM670/671NL)), constituitive or inducible expression, *e.g.,* overexpression, of, human presenilin 1 (PS1), in some instances comprising a pathogenic mutation (*e.g.,* L166P) mutation

(see, *e.g.,* Huynh, *et al.* (2017) *Neuron* 96: 1013-1023), and/or constituitive or inducible expression, *e.g.,* overexpression, of 1N4R human tau protein, in some instances comprising a pathogenic mutation (*e.g.,* a P301S mutation) (Shi, *et al.* (2017) *Nature* 549: 523–527).

VI. Delivery of an RNAi Agent of the Disclosure

The delivery of a RNAi agent of the disclosure to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject having an APOE-associated neurodegenerative disorder, *e.g.,* an amyloid-β-mediated disease, such as, Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy, or a tau-mediated disease, *e.g.* a primary tauopathy, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), or a secondary tauopathy, *e.g.,* AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an RNAi agent of the disclosure either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an RNAi agent, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the RNAi agent. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with a RNAi agent of the disclosure (see *e.g.*, Akhtar S. and Julian RL., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an RNAi agent include, for example, biological stability of the delivered agent, prevention of non-specific effects, and accumulation of the delivered agent in the target tissue. The non-specific effects of an RNAi agent can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the RNAi agent to be administered. Several studies have shown successful knockdown of gene products when an RNAi agent is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ. *et al.*, (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ. *et al.* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. *et al.* (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ. *et al.*, (2006) *Mol. Ther.* 14:343-350; Li, S. *et al.*, (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success

with local delivery to the CNS by direct injection (Dorn, G. *et al.,* (2004) *Nucleic Acids* 32:e49; Tan, PH. *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H. *et a.l* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al.* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA. *et al.*, (2006) *Mol. Ther.* 14:476-484; Zhang, X. *et al.*, (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V. *et al.*, (2005) *Nat. Med.* 11:50-55). For administering a RNAi agent systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the RNAi agent to the target tissue and avoid undesirable off-target effects (*e.g.*, without wishing to be bound by theory, use of GNAs as described herein has been identified to destabilize the seed region of a dsRNA, resulting in enhanced preference of such dsRNAs for on-target effectiveness, relative to off-target effects, as such off-target effects are significantly weakened by such seed region destabilization). RNAi agents can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, a RNAi agent directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. *et al.*, (2004) *Nature* 432:173-178). Conjugation of an RNAi agent to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO. *et al.*, (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the RNAi agent can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of molecule RNAi agent (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an RNAi agent by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an RNAi agent, or induced to form a vesicle or micelle (see *e.g.*, Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases an RNAi agent. The formation of vesicles or micelles further prevents degradation of the RNAi agent when administered systemically. Methods for making and administering cationic- RNAi agent complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of RNAi agents include DOTAP (Sorensen, DR., et al (2003), supra; Verma, UN. *et al.*, (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.,* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc.*

Trans. 35:61-67; Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, a RNAi agent forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAi agents and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

Certain aspects of the instant disclosure relate to a method of reducing the expression of an APOE target gene in a cell, comprising contacting said cell with the double-stranded RNAi agent of the disclosure. In one embodiment, the cell is a hepatic cell, optionally a hepatocyte. In one embodiment, the cell is an extrahepatic cell, optionally a CNS cell.

Another aspect of the disclosure relates to a method of reducing the expression of an APOE target gene in a subject, comprising administering to the subject the double-stranded RNAi agent of the disclosure.

Another aspect of the disclosure relates to a method of treating a subject having an APOE-associated neurodegenerative disorder, comprising administering to the subject a therapeutically effective amount of the double-stranded RNAi agent of the disclosure, thereby treating the subject. Exemplary CNS disorders that can be treated by the method of the disclosure include amyloid-β-mediated diseases, such as, Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy, and tau-mediated diseases, *e.g.* primary tauopathies, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), and secondary tauopathies, *e.g.,* AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia. In one embodiment, the double-stranded RNAi agent is administered subcutaneously.

In one embodiment, the double-stranded RNAi agent is administered intrathecally. By intrathecal administration of the double-stranded RNAi agent, the method can reduce the expression of an APOE target gene in a brain (*e.g.,* striatum) or spine tissue, for instance, cortex, cerebellum, cervical spine, lumbar spine, and thoracic spine.

For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to modified siRNA compounds. It may be understood, however, that these formulations, compositions and methods can be practiced with other siRNA compounds, *e.g.*, unmodified siRNA compounds, and such practice is within the disclosure. A composition that includes a RNAi agent can be delivered to a subject by a variety of routes. Exemplary routes include: intrathecal, intravenous, topical, rectal, anal, vaginal, nasal, pulmonary, and ocular.

The RNAi agents of the disclosure can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of RNAi agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with

pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The pharmaceutical compositions of the present disclosure may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral, or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, or intrathecal or intraventricular administration.

The route and site of administration may be chosen to enhance targeting. For example, to target muscle cells, intramuscular injection into the muscles of interest would be a logical choice. Lung cells might be targeted by administering the RNAi agent in aerosol form. The vascular endothelial cells could be targeted by coating a balloon catheter with the RNAi agent and mechanically introducing the RNA.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening or flavoring agents can be added.

Compositions for intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

In one embodiment, the administration of the siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, composition is parenteral, *e.g.,* intravenous (*e.g.*, as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary, intranasal, urethral, or ocular. Administration can be provided by the subject or by another person, *e.g.*, a health care provider. The medication can be provided in measured doses or in a

dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

Intrathecal Administration.

In one embodiment, the double-stranded RNAi agent is delivered by intrathecal injection (*i.e.*, injection into the spinal fluid which bathes the brain and spinal cord tissue). Intrathecal injection of RNAi agents into the spinal fluid can be performed as a bolus injection or via minipumps which can be implanted beneath the skin, providing a regular and constant delivery of siRNA into the spinal fluid. The circulation of the spinal fluid from the choroid plexus, where it is produced, down around the spinal chord and dorsal root ganglia and subsequently up past the cerebellum and over the cortex to the arachnoid granulations, where the fluid can exit the CNS, that, depending upon size, stability, and solubility of the compounds injected, molecules delivered intrathecally could hit targets throughout the entire CNS.

In some embodiments, the intrathecal administration is via a pump. The pump may be a surgically implanted osmotic pump. In one embodiment, the osmotic pump is implanted into the subarachnoid space of the spinal canal to facilitate intrathecal administration.

In some embodiments, the intrathecal administration is via an intrathecal delivery system for a pharmaceutical including a reservoir containing a volume of the pharmaceutical agent, and a pump configured to deliver a portion of the pharmaceutical agent contained in the reservoir. More details about this intrathecal delivery system may be found in WO 2015/116658, which is incorporated by reference in its entirety.

The amount of intrathecally injected RNAi agents may vary from one target gene to another target gene and the appropriate amount that has to be applied may have to be determined individually for each target gene. Typically, this amount ranges from 10 μg to 2 mg, preferably 50 μg to 1500 μg, more preferably 100 μg to 1000 μg.

Vector encoded RNAi agents of the Disclosure

RNAi agents targeting the APOE gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; WO 00/22113, WO 00/22114, and US 6,054,299). Expression is preferablysustained (months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

The individual strand or strands of a RNAi agent can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively, each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted

repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

RNAi agent expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of a RNAi agent as described herein. Delivery of RNAi agent expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of a RNAi agent will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the RNAi agent in target cells. Other aspects to consider for vectors and constructs are known in the art.

VII. Pharmaceutical Compositions of the Invention

The present disclosure also includes pharmaceutical compositions and formulations which include the RNAi agents of the disclosure. In one embodiment, provided herein are pharmaceutical compositions containing an RNAi agent, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the RNAi agent are useful for treating a disease or disorder associated with the expression or activity of APOE, *e.g.*, an APOE-associated neurodegenerative disease, such as an amyloid-β-mediated disease, *e.g.* Alzheimer's disease, Down's syndrome, and cerebral amyloid angiopathy, a tau-mediated disease, *e.g.* a primary tauopathy, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), or a secondary tauopathy, *e.g.,* AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV), intramuscular (IM), or for subcutaneous (subQ) delivery. Another example is

compositions that are formulated for direct delivery into the CNS, *e.g.*, by intrathecal or intravitreal routes of injection, optionally by infusion into the brain (*e.g.,* striatum), such as by continuous pump infusion.

In some embodiments, the pharmaceutical compositions of the invention are pyrogen free or non-pyrogenic.

The pharmaceutical compositions of the disclosure may be administered in dosages sufficient to inhibit expression of an APOE gene. In general, a suitable dose of an RNAi agent of the disclosure will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day.

A repeat-dose regimen may include administration of a therapeutic amount of a RNAi agent on a regular basis, such as monthly to once every six months. In certain embodiments, the RNAi agent is administered about once per quarter (*i.e.*, about once every three months) to about twice per year.

After an initial treatment regimen (*e.g.*, loading dose), the treatments can be administered on a less frequent basis.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that subsequent doses are administered at not more than 1, 2, 3, or 4 or more month intervals. In some embodiments of the disclosure, a single dose of the pharmaceutical compositions of the disclosure is administered once per month. In other embodiments of the disclosure, a single dose of the pharmaceutical compositions of the disclosure is administered once per quarter to twice per year.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments.

Advances in mouse genetics have generated a number of mouse models for the study of various APOE-associated neurodegenerative diseases that would benefit from reduction in the expression of APOE. Such models can be used for *in vivo* testing of RNAi agents, as well as for determining a therapeutically effective dose. Suitable mouse models are known in the art and include, for example, the mouse models described elsewhere herein.

The pharmaceutical compositions of the present disclosure can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (*e.g.*, by a transdermal patch), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The RNAi agents can be delivered in a manner to target a particular tissue, such as the liver, the CNS (*e.g.*, neuronal, glial or vascular tissue of the brain), or both the liver and CNS.

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the RNAi agents featured in the disclosure are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). RNAi agents featured in the disclosure can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi agents can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in US 6,747,014, which is incorporated herein by reference.

A. RNAi Agent Formulations Comprising Membranous Molecular Assemblies

A RNAi agent for use in the compositions and methods of the disclosure can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the RNAi agent composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the RNAi agent composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the RNAi agent are delivered into the cell where the RNAi agent can specifically bind to a target RNA and can mediate RNAi. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the RNAi agent to particular cell types.

A liposome containing an RNAi agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or

lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The RNAi agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the RNAi agent and condense around the RNAi agent to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of RNAi agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). pH can also adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, *e.g.*, WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 8:7413-7417; United States Patent No. 4,897,355; United States Patent No. 5,171,678; Bangham *et al.*, (1965) *M. Mol. Biol.* 23:238; Olson *et al.*, (1979) *Biochim. Biophys. Acta* 557:9; Szoka *et al.*, (1978) *Proc. Natl. Acad. Sci.* 75: 4194; Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169; Kim *et al.*, (1983) *Biochim. Biophys. Acta* 728:339; and Fukunaga *et al.*, (1984) *Endocrinol.* 115:757. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.*, Mayer *et al.*, (1986) *Biochim. Biophys. Acta* 858:161. Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169. These methods are readily adapted to packaging RNAi agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.* (1987) *Biochem. Biophys. Res. Commun.*, 147:980-985).

Liposomes, which are pH-sensitive or negatively charged, entrap nucleic acids rather than complex with them. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.* (1992) *Journal of Controlled Release*, 19:269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl

phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid or phosphatidylcholine or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include United States Patent No. 5,283,185; United States Patent No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, (1994) *J. Biol. Chem.* 269:2550; Nabel, (1993) *Proc. Natl. Acad. Sci.* 90:11307; Nabel, (1992) *Human Gene Ther.* 3:649; Gershon, (1993) *Biochem.* 32:7143; and Strauss, (1992) *EMBO J.* 11:417.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu *et al.*, (1994) *S.T.P.Pharma. Sci.*, 4(6):466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, (1987) *FEBS Letters,* 223:42; Wu *et al.*, (1993) *Cancer Research*, 53:3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, (1987), 507:64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, (1988), 85,:6949). United States Patent No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. United States Patent No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin.

Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al*).

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages in vivo and can be used to deliver RNAi agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated RNAi agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of RNAi agent (see, *e.g.*, Felgner, P. L. *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 8:7413-7417, and United States Patent No.4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, *e.g.*, United States Patent No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., (1991) *Biochim. Biophys. Res. Commun.* 179:280). Lipopolylysine, made by conjugating

polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. *et al.*, (1991) *Biochim. Biophys. Acta* 1065:8). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer RNAi agent into the skin. In some implementations, liposomes are used for delivering RNAi agent to epidermal cells and also to enhance the penetration of RNAi agent into dermal tissues, *e.g.*, into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner *et al.*, (1992) *Journal of Drug Targeting*, vol. 2,405-410 and du Plessis *et al.*, (1992) *Antiviral Research*, 18:259-265; Mannino, R. J. and Fould-Fogerite, S., (1998) *Biotechniques* 6:682-690; Itani, T. *et al.*, (1987) *Gene* 56:267-276; Nicolau, C. *et al.* (1987) *Meth. Enzymol.* 149:157-176; Straubinger, R. M. and Papahadjopoulos, D. (1983) *Meth. Enzymol.* 101:512-527; Wang, C. Y. and Huang, L., (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with RNAi agent are useful for treating a dermatological disorder.

Liposomes that include RNAi agents can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transferosomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include RNAi agent can be delivered, for example, subcutaneously by infection in order to deliver RNAi agent to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transferosomes can be self-optimizing (adaptive to the shape of pores, *e.g.*, in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present disclosure are described in United States provisional application serial Nos. 61/018,616, filed January 2, 2008; 61/018,611, filed January 2, 2008; 61/039,748, filed March 26, 2008; 61/047,087, filed April 22, 2008 and 61/051,528, filed May

8, 2008. PCT application number PCT/US2007/080331, filed October 3, 2007, also describes formulations that are amenable to the present disclosure.

Transfersomes, yet another type of liposomes, are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, *e.g.,* they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as those described herein, particularlay in emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general, their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The RNAi agent for use in the methods of the disclosure can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C_8 to C_{22} alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the siRNA composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the siRNA composition, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, *i.e.,* there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.,* through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, *e.g.,* at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

Lipid particles

RNAi agents, *e.g.,* dsRNAs of in the disclosure may be fully encapsulated in a lipid formulation, *e.g.,* a LNP, or other nucleic acid-lipid particle.

As used herein, the term "LNP" refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in WO 00/03683. The particles of the present disclosure typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present disclosure are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; United States Patent publication No. 2010/0324120 and WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the disclosure.

Certain specific LNP formulations for delivery of RNAi agents have been described in the art, including, *e.g.*, "LNP01" formulations as described in, *e.g.*, WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are identified in the table below.

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
SNALP-1	l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1

LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1

LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in WO 2009/127060, which is hereby incorporated by reference.

XTC comprising formulations are described in WO 2010/088537, the entire contents of which are hereby incorporated herein by reference.

MC3 comprising formulations are described, *e.g.*, in United States Patent Publication No. 2010/0324120, the entire contents of which are hereby incorporated by reference.

ALNY-100 comprising formulations are described in WO 2010/054406, the entire contents of which are hereby incorporated herein by reference.

C12-200 comprising formulations are described in WO 2010/129709, the entire contents of which are hereby incorporated herein by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the disclosure are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids or esters or salts thereof, bile acids or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the disclosure can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, U.S. 2003/0027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present disclosure include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated

from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the brain when treating APP-associated diseases or disorders.

The pharmaceutical formulations of the present disclosure, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present disclosure can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present disclosure can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol or dextran. The suspension can also contain stabilizers.

Additional Formulations

i. Emulsions

The compositions of the present disclosure can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w)

emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise, a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

ii. Microemulsions

In one embodiment of the present disclosure, the compositions of RNAi agents and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically, microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used, and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can

typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385; Ho *et al.*, J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or RNAi agents. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present disclosure will facilitate the increased systemic absorption of RNAi agents and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of RNAi agents and nucleic acids.

Microemulsions of the present disclosure can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the RNAi agents and nucleic acids of the present disclosure. Penetration enhancers used in the microemulsions of the present disclosure can be classified as belonging to one of five broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

iii. Microparticles

An RNAi agent of the disclosure may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present disclosure employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly RNAi agents, to the skin of animals. Most drugs are

present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of RNAi agents through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi *et al.*, J. Pharm. Pharmacol., 1988, 40, 252).

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-20} alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (see *e.g.*, Touitou, E., *et al.* Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri *et al.*, J. Pharm. Pharmacol., 1992, 44, 651-654).

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium

taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita *et al.*, J. Pharm. Sci., 1990, 79, 579-583).

Chelating agents, as used in connection with the present disclosure, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of RNAi agents through the mucosa is enhanced. With regards to their use as penetration enhancers in the present disclosure, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(see *e.g.*, Katdare, A. *et al.*, Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, J. Control Rel., 1990, 14, 43-51).

As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of RNAi agents through the alimentary mucosa (see *e.g.*, Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, J. Pharm. Pharmacol., 1987, 39, 621-626).

Agents that enhance uptake of RNAi agents at the cellular level can also be added to the pharmaceutical and other compositions of the present disclosure. For example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.

Other agents can be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

vi. Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, *etc.*, when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc*).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present disclosure. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

vii. Other Components

The compositions of the present disclosure can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present disclosure, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present disclosure. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers,

colorings, flavorings or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the disclosure include (a) one or more RNAi agents and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating an APOE-associated neurodegenerative disorder. Examples of such agents include, but are not lmited to SSRIs, venlafaxine, bupropion, and atypical antipsychotics.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the disclosure lies generally within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the RNAi agents featured in the disclosure can be administered in combination with other known agents effective in treatment of pathological processes mediated by nucleotide repeat expression. In any event, the administering physician can adjust the amount and timing of RNAi agent administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VIII. Kits

In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a siRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, or precursor thereof). In certain embodiments the

individual components of the pharmaceutical formulation may be provided in one container. Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*, one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

IX. Methods for Inhibiting APOE Expression

The present disclosure also provides methods of inhibiting expression of an APOE gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNAi agent, in an amount effective to inhibit expression of APOE in the cell, thereby inhibiting expression of APOE in the cell. In certain embodiments of the disclosure, APOE is inhibited preferentially in CNS (*e.g.*, brain) cells. In other embodiments of the disclosure, APOE is inhibited preferentially in the liver (*e.g.*, hepatocytes). In certain embodiments of the disclosure, APOE is inhibited in CNS (*e.g.*, brain) cells and in liver (*e.g.*, hepatocytes) cells.

In some embodiments, the expression of APOE2 is inhibited. In some embodiments, the expression of APOE3 is inhibited. In some embodiments, the expression of APOE4 is inhibited. In some embodiments, the expression of APOE2 and APOE3 is inhibited. In some embodiments, the expression of APOE2, APOE3, and APOE4 is inhibited. In some embodiments, the expression of APOE4 is inhibited and the expression of APOE2 and APOE3 is substantially not inhibited, *e.g.*, expression of APOE2 and APOE3 is inhibited by no more than 10%.

Contacting of a cell with a RNAi agent, *e.g.*, a double stranded RNAi agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the RNAi agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the RNAi agent. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible.

Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc ligand, or any other ligand that directs the RNAi agent to a site of interest.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating," "suppressing" and other similar terms, and includes any level of inhibition. In certain embodiments, a level of inhibition, *e.g.*, for an RNAi agent of the instant disclosure, can be assessed in cell culture conditions, *e.g.*, wherein cells in cell culture are transfected *via* Lipofectamine™-mediated transfection at a concentration in the vicinity of a cell of 10 nM or less, 1 nM or less, *etc*. Knockdown of a given RNAi agent can be determined *via* comparison of pre-treated levels in cell culture versus post-treated levels in cell culture, optionally also comparing against cells

treated in parallel with a scrambled or other form of control RNAi agent. Knockdown in cell culture of, *e.g.*, preferably 50% or more, can thereby be identified as indicative of "inhibiting" or "reducing", "downregulating" or "suppressing", *etc.* having occurred. It is expressly contemplated that assessment of targeted mRNA or encoded protein levels (and therefore an extent of "inhibiting", *etc.* caused by a RNAi agent of the disclosure) can also be assessed in *in vivo* systems for the RNAi agents of the instant disclosure, under properly controlled conditions as described in the art.

The phrase "inhibiting expression of an APOE gene" or "inhibiting expression of APOE," as used herein, includes inhibition of expression of any APOE gene (such as, *e.g.*, a mouse APOE gene, a rat APOE gene, a monkey APOE gene, or a human APOE gene) as well as variants or mutants of an APOE gene that encode an APOE protein. Thus, the APOE gene may be a wild-type APOE gene, a mutant APOE gene, or a transgenic APOE gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of an APOE gene" includes any level of inhibition of an APOE gene, *e.g.*, at least partial suppression of the expression of an APOE gene, such as an inhibition by at least 20%. In certain embodiments, inhibition is by at least 30%, at least 40%, preferably at least 50%, at least about 60%, at least 70%, at least about 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%; or to below the level of detection of the assay method.

The expression of an APOE gene may be assessed based on the level of any variable associated with APOE gene expression, *e.g.*, APOE mRNA level or APOE protein level, or, for example, the level of amyloid or tau deposition.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the disclosure, expression of an APOE gene is inhibited by at least 20%, 30%, 40%, preferably at least 50%, 60%, 70%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In certain embodiments, the methods include a clinically relevant inhibition of expression of APOE, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of APOE.

Inhibition of the expression of an APOE gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which an APOE gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with a RNAi agent of the disclosure, or by administering a RNAi agent of the disclosure to a subject in which the cells are or were present) such that the expression of an APOE gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)

not treated with a RNAi agent or not treated with a RNAi agent targeted to the gene of interest). The degree of inhibition may be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

In other embodiments, inhibition of the expression of an APOE gene may be assessed in terms of a reduction of a parameter that is functionally linked to an APOE gene expression, *e.g.*, APOE protein expression. APOE gene silencing may be determined in any cell expressing APOE, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of an APOE protein may be manifested by a reduction in the level of the APOE protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of an APOE gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the disclosure. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of APOE mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of APOE in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the APOE gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, and microarray analysis. Circulating APOE mRNA may be detected using methods the described in WO2012/177906, the entire contents of which are hereby incorporated herein by reference.

In some embodiments, the level of expression of APOE is determined using a nucleic acid probe. The term “probe”, as used herein, refers to any molecule that is capable of selectively binding to a specific APOE nucleic acid or protein, or fragment thereof. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to APOE mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated

mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of APOE mRNA.

An alternative method for determining the level of expression of APOE in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, US Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, US Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the disclosure, the level of expression of APOE is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System), by a Dual-Glo® Luciferase assay, or by other art-recognized method for measurement of APOE expression or mRNA level.

The expression level of APOE mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See US Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of APOE expression level may also comprise using nucleic acid probes in solution.

In some embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of this PCR method is described and exemplified in the Examples presented herein. Such methods can also be used for the detection of APOE nucleic acids.

The level of APOE protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like. Such assays can also be used for the detection of proteins indicative of the presence or replication of APOE proteins.

In some embodiments, the efficacy of the methods of the disclosure in the treatment of an APOE-related disease is assessed by a decrease in APOE mRNA level (*e.g*, by assessment of a CSF sample for APOE level, by brain biopsy, or otherwise).

In some embodiments, the efficacy of the methods of the disclosure in the treatment of an APOE-related disease is assessed by a decrease in APOE mRNA level (*e.g*, by assessment of a liver sample for APOE level, by biopsy, or otherwise).

In some embodiments of the methods of the disclosure, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of APOE may be assessed using measurements of the level or change in the level of APOE mRNA or APOE protein in a sample derived from a specific site within the subject, *e.g.*, CNS cells. In certain embodiments, the methods include a clinically relevant inhibition of expression of APOE, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of APOE.

As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

X. Methods of Treating or Preventing APOE-Associated Neurodegenerative Diseases

The present disclosure also provides methods of using a RNAi agent of the disclosure or a composition containing a RNAi agent of the disclosure to reduce or inhibit APOE expression in a cell. The methods include contacting the cell with a dsRNA of the disclosure and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an APOE gene, thereby inhibiting expression of the APOE gene in the cell. Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of APOE may be determined by determining the mRNA expression level of APOE using methods routine to one of ordinary skill in the art, *e.g.,* northern blotting, qRT-PCR; by determining the protein level of APOE using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques.

In the methods of the disclosure the cell may be contacted *in vitro* or *in vivo, i.e.,* the cell may be within a subject.

A cell suitable for treatment using the methods of the disclosure may be any cell that expresses an APOE gene. A cell suitable for use in the methods of the disclosure may be a mammalian cell, *e.g.,* a primate cell (such as a human cell or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a a rat cell, or a mouse cell. In one embodiment, the cell is a human cell, *e.g.,* a human CNS cell. In one embodiment, the cell is a human cell, *e.g.,* a human liver cell. In one embodiment, the cell is a human cell, *e.g.,* a human CNS cell and a human liver cell.

APOE expression is inhibited in the cell by at least about 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or about 100%, *i.e.*, to below the level of detection. In preferred embodiments, APOE expression is inhibited by at least 50 %.

The *in vivo* methods of the disclosure may include administering to a subject a composition containing a RNAi agent, where the RNAi agent includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the APOE gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, intravitreal, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered by intrathecal injection.

In some embodiments, the administration is *via* a depot injection. A depot injection may release the RNAi agent in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of APOE, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is *via* a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intracranial, intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the RNAi agent to the CNS.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present disclosure also provides methods for inhibiting the expression of an APOE gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets an APOE gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the APOE gene, thereby inhibiting expression of the APOE gene in the cell. Reduction in gene expression can be assessed by any methods known it the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, *e.g.* ELISA, described herein. In one embodiment, a CNS biopsy sample or a cerebrospinal fluid (CSF) sample serves as the

tissue material for monitoring the reduction in APOE gene or protein expression (or of a proxy therefore).

The present disclosure further provides methods of treatment of a subject in need thereof. The treatment methods of the disclosure include administering an RNAi agent of the disclosure to a subject, *e.g.*, a subject that would benefit from inhibition of APOE expression, in a therapeutically effective amount of a RNAi agent targeting an APOE gene or a pharmaceutical composition comprising a RNAi agent targeting aAPOE gene.

In addition, the present disclosure provides methods of preventing, treating or inhibiting the progression of an APOE-associated neurodegenerative disease or disorder, such as an amyloid-β–mediated disease, *e.g.,* Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy, or a tau-mediated disease, *e.g.* a primary tauopathy, such as Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART), or a secondary tauopathy, *e.g.,* AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

. The methods include administering to the subject a therapeutically effective amount of any of the RNAi agent, *e.g.,* dsRNA agents, or the pharmaceutical composition provided herein, thereby preventing, treating or inhibiting the progression of the APOE-associated neurodegenerative disease or disorder in the subject.

An RNAi agent of the disclosure may be administered as a "free RNAi agent." A free RNAi agent is administered in the absence of a pharmaceutical composition. The naked RNAi agent may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the RNAi agent can be adjusted such that it is suitable for administering to a subject.

Alternatively, an RNAi agent of the disclosure may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from a reduction or inhibition of APOE gene expression are those having an APOE-associated neurodegenerative disease.

The disclosure further provides methods for the use of a RNAi agent or a pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction or inhibition of APOE expression, *e.g.,* a subject having an APOE-associated neurodegenerative disorder, in combination with other pharmaceuticals or other therapeutic methods, *e.g.*, with known pharmaceuticals or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an RNAi agent targeting APOE is administered in combination with, *e.g.*, an agent useful in treating an APOE-associated

neurodegenerative disorder as described elsewhere herein or as otherwise known in the art. For example, additional agents suitable for treating a subject that would benefit from reducton in APOE expression, *e.g.,* a subject having an APOE-associated neurodegenerative disorder, may include agents currently used to treat symptoms of APOE. The RNAi agent and additional therapeutic agents may be administered at the same time or in the same combination, *e.g.*, intrathecally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein.

In one embodiment, the method includes administering a composition featured herein such that expression of the target APOE gene is decreased, for at least one month. In preferred embodiments, expression is decreased for at least 2 months, 3 months, or 6 months.

Preferably, the RNAi agents useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target APOE gene. Compositions and methods for inhibiting the expression of these genes using RNAi agents can be prepared and performed as described herein.

Administration of the dsRNA according to the methods of the disclosure may result in a reduction of the severity, signs, symptoms, or markers of such diseases or disorders in a patient with an APOE-associated neurodegenerative disorder. By "reduction" in this context is meant a statistically significant or clinically significant decrease in such level. The reduction can be, for example, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of an APOE-associated neurodegenerative disorder may be assessed, for example, by periodic monitoring of a subject's cognition, learning, and/or memory. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of a RNAi agent targeting APOE or pharmaceutical composition thereof, "effective against" an APOE-associated neurodegenerative disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating APOE-associated neurodegenerative disorders and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given RNAi agent drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using a RNAi agent or RNAi agent formulation as described herein.

Subjects can be administered a therapeutic amount of dsRNA, such as about 0.01 mg/kg to about 200 mg/kg.

The RNAi agent can be administered intrathecally, via intravitreal injection, or by intravenous infusion over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the RNAi agent can reduceAPOE levels, *e.g.*, in a cell, tissue, blood, CSF sample or other compartment of the patient by at least 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70,% 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99% or more. In a preferred embodiment, administration of the RNAi agent can reduce APOE levels, *e.g.*, in a cell, tissue, blood, CSF sample or other compartment of the patient by at least 50%.

Before administration of a full dose of the RNAi agent, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

Alternatively, the RNAi agent can be administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired, *e.g.*, monthly dose of RNAi agent to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimine may include administration of a therapeutic amount of RNAi agent on a regular basis, such as monthly or extending to once a quarter, twice per year, once per year. In certain embodiments, the RNAi agent is administered about once per month to about once per quarter (*i.e.*, about once every three months).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the RNAi agents and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

An inforrmal Sequence Listing is filed herewith and forms part of the specification as filed.

EXAMPLES

Example 1. RNAi Agent Design, Synthesis, Selection, and *In Vitro* Evaluation

This Example describes methods for the design, synthesis, selection, and *in vitro* evaluation of APOE RNAi agents.

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Bioinformatics

A set of siRNAs targeting the human apolipoprotein E (APOE; human NCBI refseqID NM_000041.4; NCBI GeneID: 348) was designed using custom R and Python scripts. The human NM_000041 REFSEQ mRNA, version 4, has a length of 1166 nucleotides.

APOE single strands and duplexes were made using routine methods known in the art. A detailed list of the unmodified APOE sense and antisense strand sequences is shown in Tables 2 and 4 and a detailed list of the modified APOE sense and antisense strand sequences is shown in Tables 3 and 5.

Table 7 provides a detailed list of the unmodified APOE sense and antisense strand sequences of those agents in Table 2 that target the pathogenic APOE4 allele and Table 8 provides a detailed list of the modified APOE sense and antisense strand sequences of those agents in Table 3 that target the pathogenic APOE4 allele.

siRNA Synthesis

siRNAs were synthesized and annealed using routine methods known in the art. Briefly, siRNA sequences were synthesized on a 1 µmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington,

MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 µL of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Cell culture and transfections

Cells were transfected by adding 4.9 µL of Opti-MEM plus 0.1 µL of RNAiMAX per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µL of siRNA duplexes per well, with 4 replicates of each siRNA duplex, into a 96-well plate, and incubated at room temperature for 15 minutes. Forty µL of MEDIA containing ~1.5 x10^4 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Experiments were performed at 10nM. Transfection experiments are performed in human hepatoma Hep3B cells (ATCC HB-8064) with EMEM (ATCC catalog no. 30-2003).

Total RNA isolation using DYNABEADS mRNA Isolation Kit

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADs (Invitrogen, cat#61012). Briefly, 70 µL of Lysis/Binding Buffer and 10 µL of lysis buffer containing 3 µL of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured

and the supernatant was removed. Bead-bound RNA were then washed 2 times with 150 µL Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 µL Elution Buffer, re-captured and supernatant removed.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

Ten µL of a master mix containing 1 µL 10X Buffer, 0.4 µL 25X dNTPs, 1 µL 10x Random primers, 0.5 µL Reverse Transcriptase, 0.5 µL RNase inhibitor and 6.6 µL of H_2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hour incubation at 37°C.

Real time PCR

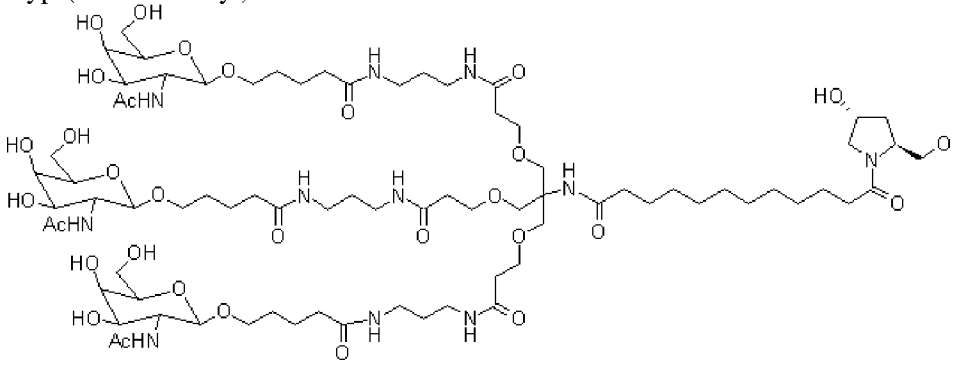
Two µL of cDNA were added to a master mix containing 0.5 µL of human or mouse GAPDH TaqMan Probe (ThermoFisher cat 4352934E or 4351309) and 0.5 µL of appropriate APOE probe (commercially available, *e.g.*, from Thermo Fisher) and 5 µL Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche). Each duplex was tested with N=4 and data were normalized to cells transfected with a non-targeting control siRNA. To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with a non-targeting control siRNA.

The results of a single dose screen in Hep3B cells with the agents in Table 5 are provided in Table 6.

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds; and it is understood that when the nucleotide contains a 2'-fluoro modification, then the fluoro replaces the hydroxy at that position in the parent nucleotide (*i.e.*, it is a 2'-deoxy-2'-fluoronucleotide).

Abbreviation	**Nucleotide(s)**
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Abs	beta-L-adenosine-3`-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3`-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate

Abbreviation	Nucleotide(s)
Cf	2’-fluorocytidine-3’-phosphate
Cfs	2’-fluorocytidine-3’-phosphorothioate
Cs	cytidine-3’-phosphorothioate
G	guanosine-3’-phosphate
Gb	beta-L-guanosine-3`-phosphate
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2’-fluoroguanosine-3’-phosphate
Gfs	2’-fluoroguanosine-3’-phosphorothioate
Gs	guanosine-3’-phosphorothioate
T	5’-methyluridine-3’-phosphate
Tf	2’-fluoro-5-methyluridine-3’-phosphate
Tfs	2’-fluoro-5-methyluridine-3’-phosphorothioate
Ts	5-methyluridine-3’-phosphorothioate
U	Uridine-3’-phosphate
Uf	2’-fluorouridine-3’-phosphate
Ufs	2’-fluorouridine -3’-phosphorothioate
Us	uridine -3’-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3’-phosphate
as	2'-O-methyladenosine-3’- phosphorothioate
c	2'-O-methylcytidine-3’-phosphate
cs	2'-O-methylcytidine-3’- phosphorothioate
g	2'-O-methylguanosine-3’-phosphate
gs	2'-O-methylguanosine-3’- phosphorothioate
t	2’-O-methyl-5-methyluridine-3’-phosphate
ts	2’-O-methyl-5-methyluridine-3’-phosphorothioate
u	2'-O-methyluridine-3’-phosphate
us	2'-O-methyluridine-3’-phosphorothioate
s	phosphorothioate linkage

Abbreviation	Nucleotide(s)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3 
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Cgn)	Cytidine-glycol nucleic acid (GNA)
(Ggn)	Guanosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
(Aam)	2`-O-(N-methylacetamide)adenosine-3`-phosphate
(Aams)	2`-O-(N-methylacetamide)adenosine-3`-phosphorothioate
(Gam)	2`-O-(N-methylacetamide)guanosine-3`-phosphate
(Gams)	2`-O-(N-methylacetamide)guanosine-3`-phosphorothioate
(Tam)	2`-O-(N-methylacetamide)thymidine-3`-phosphate
(Tams)	2`-O-(N-methylacetamide)thymidine-3`-phosphorothioate
dA	2`-deoxyadenosine-3`-phosphate
dAs	2`-deoxyadenosine-3`-phosphorothioate
dC	2`-deoxycytidine-3`-phosphate
dCs	2`-deoxycytidine-3`-phosphorothioate
dG	2`-deoxyguanosine-3`-phosphate
dGs	2`-deoxyguanosine-3`-phosphorothioate
dT	2`-deoxythymidine-3`-phosphate
dTs	2`-deoxythymidine-3`-phosphorothioate
dU	2`-deoxyuridine
dUs	2`-deoxyuridine-3`-phosphorothioate
(Aeo)	2`-O-methoxyethyladenosine-3`-phosphate
(Aeos)	2`-O-methoxyethyladenosine-3`-phosphorothioate

Abbreviation	Nucleotide(s)
(Geo)	2`-O-methoxyethylguanosine-3`-phosphate
(Geos)	2`-O-methoxyethylguanosine-3`-phosphorothioate
(Teo)	2`-O-methoxyethyl-5-methyluridine-3`-phosphate
(Teos)	2`-O-methoxyethyl-5-methyluridine-3`-phosphorothioate
(m5Ceo)	2`-O-methoxyethyl-5-methylcytidine-3`-phosphate
(m5Ceos)	2`-O-methoxyethyl-5-methylcytidine-3`-phosphorothioate
(A3m)	3`-O-methyladenosine-2`-phosphate
(A3mx)	3`-O-methyl-xylofuranosyladenosine-2`-phosphate
(G3m)	3`-O-methylguanosine-2`-phosphate
(G3mx)	3`-O-methyl-xylofuranosylguanosine-2`-phosphate
(C3m)	3`-O-methylcytidine-2`-phosphate
(C3mx)	3`-O-methyl-xylofuranosylcytidine-2`-phosphate
(U3m)	3`-O-methyluridine-2`-phosphate
U3mx)	3`-O-methyl-xylofuranosyluridine-2`-phosphate
(m5Cam)	2`-O-(N-methylacetamide)-5-methylcytidine-3`-phosphate
(m5Cams)	2`-O-(N-methylacetamide)-5-methylcytidine-3`-phosphorothioate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Chds)	2'-O-hexadecyl-cytidine-3'-phosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Uhds)	2'-O-hexadecyl-uridine-3'-phosphorothioate
(pshe)	Hydroxyethylphosphorothioate

Table 2. APOE Unmodified Sense and Antisense Strand Sequences

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
GGCCAAUCACAGGCAGGAAGU	15	ACUUCCUGCCUGUGAUUGGCCAG	150	NM_000041.4_50-70_A21U_s	NM_000041.4_48-70_U1A_as
CAGGCAGGAAGAUGAAGGUUU	16	AAACCUUCAUCUUCCUGCCUGUG	151	NM_000041.4_59-79_C21U_s	NM_000041.4_57-79_G1A_as
AGGAAGAUGAAGGUUCUGUGU	17	ACACAGAACCUUCAUCUUCCUGC	152	NM_000041.4_64-84_G21U_s	NM_000041.4_62-84_C1A_as
AUGAAGGUUCUGUGGGCUGCU	18	AGCAGCCCACAGAACCUUCAUCU	153	NM_000041.4_70-90_G21U_s	NM_000041.4_68-90_C1A_as
UUCUGUGGGCUGCGUUGCUGU	19	ACAGCAACGCAGCCCACAGAACC	154	NM_000041.4_77-97_G21U_s	NM_000041.4_75-97_C1A_as
UGGGCUGCGUUGCUGGUCACU	20	AGUGACCAGCAACGCAGCCCACA	155	NM_000041.4_82-102_A21U_s	NM_000041.4_80-102_U1A_as
GCGUUGCUGGUCACAUUCCUU	21	AAGGAAUGUGACCAGCAACGCAG	156	NM_000041.4_88-108_G21U_s	NM_000041.4_86-108_C1A_as
GCUGGUCACAUUCCUGGCAGU	22	ACUGCCAGGAAUGUGACCAGCAA	157	NM_000041.4_93-113_G21U_s	NM_000041.4_91-113_C1A_as
UCACAUUCCUGGCAGGAUGCU	23	AGCAUCCUGCCAGGAAUGUGACC	158	NM_000041.4_98-118_C21U_s	NM_000041.4_96-118_G1A_as
UGGCAGGAUGCCAGGCCAAGU	24	ACUUGGCCUGGCAUCCUGCCAGG	159	NM_000041.4_107-127_G21U_s	NM_000041.4_105-127_C1A_as
CAGGCCAAGGUGGAGCAAGCU	25	AGCUUGCUCCACCUUGGCCUGGC	160	NM_000041.4_118-138_G21U_s	NM_000041.4_116-138_C1A_as
AAGGUGGAGCAAGCGGUGGAU	26	AUCCACCGCUUGCUCCACCUUGG	161	NM_000041.4_124-144_G21U_s	NM_000041.4_122-144_C1A_as
CAAGCGGUGGAGACAGAGCCU	27	AGGCUCUGUCUCCACCGCUUGCU	162	NM_000041.4_133-153_G21U_s	NM_000041.4_131-153_C1A_as
GGUGGAGACAGAGCCGGAGCU	28	AGCUCCGGCUCUGUCUCCACCGC	163	NM_000041.4_138-158_C21U_s	NM_000041.4_136-158_G1A_as
CCCGAGCUGCGCCAGCAGACU	29	AGUCUGCUGGCGCAGCUCGGGCU	164	NM_000041.4_157-177_C21U_s	NM_000041.4_155-177_G1A_as

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
GCUGCGCCAGCAGACCGAGUU	30	AACUCGGUCUGCUGGCGCAGCUC	165	NM_000041.4_162-182_G21U_s	NM_000041.4_160-182_C1A_as
CCAGCAGACCGAGUGGCAGAU	31	AUCUGCCACUCGGUCUGCUGGCG	166	NM_000041.4_168-188_G21U_s	NM_000041.4_166-188_C1A_as
CAGCGCUGGGAACUGGCACUU	32	AAGUGCCAGUUCCCAGCGCUGGC	167	NM_000041.4_193-213_G21U_s	NM_000041.4_191-213_C1A_as
CUGGGAACUGGCACUGGGUCU	33	AGACCCAGUGCCAGUUCCCAGCG	168	NM_000041.4_198-218_G21U_s	NM_000041.4_196-218_C1A_as
AACUGGCACUGGGUCGCUUUU	34	AAAAGCGACCCAGUGCCAGUUCC	169	NM_000041.4_203-223_s	NM_000041.4_201-223_as
CACUGGGUCGCUUUUGGGAUU	35	AAUCCCAAAAGCGACCCAGUGCC	170	NM_000041.4_209-229_s	NM_000041.4_207-229_as
GUCGCUUUUGGGAUUACCUGU	36	ACAGGUAAUCCCAAAAGCGACCC	171	NM_000041.4_215-235_C21U_s	NM_000041.4_213-235_G1A_as
UUUUGGGAUUACCUGCGCUGU	37	ACAGCGCAGGUAAUCCCAAAAGC	172	NM_000041.4_220-240_G21U_s	NM_000041.4_218-240_C1A_as
CUGCGCUGGGUGCAGACACUU	38	AAGUGUCUGCACCCAGCGCAGGU	173	NM_000041.4_232-252_G21U_s	NM_000041.4_230-252_C1A_as
UGGGUGCAGACACUGUCUGAU	39	AUCAGACAGUGUCUGCACCCAGC	174	NM_000041.4_238-258_G21U_s	NM_000041.4_236-258_C1A_as
CAGACACUGUCUGAGCAGGUU	40	AACCUGCUCAGACAGUGUCUGCA	175	NM_000041.4_244-264_G21U_s	NM_000041.4_242-264_C1A_as
CAGGAGGAGCUGCUCAGCUCU	41	AGAGCUGAGCAGCUCCUCCUGCA	176	NM_000041.4_265-285_C21U_s	NM_000041.4_263-285_G1A_as
CUGCUCAGCUCCCAGGUCACU	42	AGUGACCUGGGAGCUGAGCAGCU	177	NM_000041.4_274-294_C21U_s	NM_000041.4_272-294_G1A_as
UCCCAGGUCACCCAGGAACUU	43	AAGUUCCUGGGUGACCUGGGAGC	178	NM_000041.4_283-303_G21U_s	NM_000041.4_281-303_C1A_as
ACCCAGGAACUGAGGGCGCUU	44	AAGCGCCCUCAGUUCCUGGGUGA	179	NM_000041.4_292-312_G21U_s	NM_000041.4_290-312_C1A_as
UGAGGGCGCUGAUGGACGAGU	45	ACUCGUCCAUCAGCGCCCUCAGU	180	NM_000041.4_302-	NM_000041.4_300-

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
				322_A21U_s	322_U1A_as
GCGCUGAUGGACGAGACCAUU	46	AAUGGUCUCGUCCAUCAGCGCCC	181	NM_000041.4_307-327_G21U_s	NM_000041.4_305-327_C1A_as
GACGAGACCAUGAAGGAGUUU	47	AAACUCCUUCAUGGUCUCGUCCA	182	NM_000041.4_316-336_G21U_s	NM_000041.4_314-336_C1A_as
ACCAUGAAGGAGUUGAAGGCU	48	AGCCUUCAACUCCUUCAUGGUCU	183	NM_000041.4_322-342_C21U_s	NM_000041.4_320-342_G1A_as
GGAGUUGAAGGCCUACAAAUU	49	AAUUUGUAGGCCUUCAACUCCUU	184	NM_000041.4_330-350_C21U_s	NM_000041.4_328-350_G1A_as
AAGGCCUACAAAUCGGAACUU	50	AAGUUCCGAUUUGUAGGCCUUCA	185	NM_000041.4_337-357_G21U_s	NM_000041.4_335-357_C1A_as
ACAAAUCGGAACUGGAGGAAU	51	AUUCCUCCAGUUCCGAUUUGUAG	186	NM_000041.4_344-364_C21U_s	NM_000041.4_342-364_G1A_as
UCGGAACUGGAGGAACAACUU	52	AAGUUGUUCCUCCAGUUCCGAUU	187	NM_000041.4_349-369_G21U_s	NM_000041.4_347-369_C1A_as
GAGGAACAACUGACCCCGGUU	53	AACCGGGGUCAGUUGUUCCUCCA	188	NM_000041.4_358-378_G21U_s	NM_000041.4_356-378_C1A_as
CGCGGGCACGGCUGUCCAAGU	54	ACUUGGACAGCCGUGCCCGCGUC	189	NM_000041.4_389-409_G21U_s	NM_000041.4_387-409_C1A_as
GCACGGCUGUCCAAGGAGCUU	55	AAGCUCCUUGGACAGCCGUGCCC	190	NM_000041.4_394-414_G21U_s	NM_000041.4_392-414_C1A_as
GCUGUCCAAGGAGCUGCAGGU	56	ACCUGCAGCUCCUUGGACAGCCG	191	NM_000041.4_399-419_C21U_s	NM_000041.4_397-419_G1A_as
GCCCGGCUGGGCGCGGACAUU	57	AAUGUCCGCGCCCAGCCGGGCCU	192	NM_000041.4_427-447_G21U_s	NM_000041.4_425-447_C1A_as
CUGGGCGCGGACAUGGAGGAU	58	AUCCUCCAUGUCCGCGCCCAGCC	193	NM_000041.4_433-453_C21U_s	NM_000041.4_431-453_G1A_as
CGCGGACAUGGAGGACGUGCU	59	AGCACGUCCUCCAUGUCCGCGCC	194	NM_000041.4_438-458_U20C_G21U_s	NM_000041.4_436-458_C1A_A2G_as
GCGGACAUGGAGGACGUGUGU	60	ACACACGUCCUCCAUGUCCGCGC	195	NM_000041.4_439-459_C21U_s	NM_000041.4_437-459_G1A_as

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
GCGGACAUGGAGGACGUGCGU	61	ACGCACGUCCUCCAUGUCCGCGC	196	NM_000041.4_439-459_U19C_C21U_s	NM_000041.4_437-459_G1A_A3G_as
CGGACAUGGAGGACGUGCGCU	62	AGCGCACGUCCUCCAUGUCCGCG	197	NM_000041.4_440-460_U18C_G21U_s	NM_000041.4_438-460_C1A_A4G_as
GGACAUGGAGGACGUGCGCGU	63	ACGCGCACGUCCUCCAUGUCCGC	198	NM_000041.4_441-461_U17C_G21U_s	NM_000041.4_439-461_C1A_A5G_as
GACAUGGAGGACGUGCGCGGU	64	ACCGCGCACGUCCUCCAUGUCCG	199	NM_000041.4_442-462_U16C_C21U_s	NM_000041.4_440-462_G1A_A6G_as
ACAUGGAGGACGUGCGCGGCU	65	AGCCGCGCACGUCCUCCAUGUCC	200	NM_000041.4_443-463_U15C_C21U_s	NM_000041.4_441-463_G1A_A7G_as
CAUGGAGGACGUGCGCGGCCU	66	AGGCCGCGCACGUCCUCCAUGUC	201	NM_000041.4_444-464_U14C_G21U_s	NM_000041.4_442-464_C1A_A8G_as
AUGGAGGACGUGCGCGGCCGU	67	ACGGCCGCGCACGUCCUCCAUGU	202	NM_000041.4_445-465_U13C_C21U_s	NM_000041.4_443-465_G1A_A9G_as
UGGAGGACGUGCGCGGCCGCU	68	AGCGGCCGCGCACGUCCUCCAUG	203	NM_000041.4_446-466_U12C_C21U_s	NM_000041.4_444-466_G1A_A10G_as
GGAGGACGUGCGCGGCCGCCU	69	AGGCGGCCGCGCACGUCCUCCAU	204	NM_000041.4_447-467_U11C_s	NM_000041.4_445-467_A11G_as
GAGGACGUGCGCGGCCGCCUU	70	AAGGCGGCCGCGCACGUCCUCCA	205	NM_000041.4_448-468_U10C_G21U_s	NM_000041.4_446-468_C1A_A12G_as
AGGACGUGCGCGGCCGCCUGU	71	ACAGGCGGCCGCGCACGUCCUCC	206	NM_000041.4_449-469_U9C_G21U_s	NM_000041.4_447-469_C1A_A13G_as
GGACGUGCGCGGCCGCCUGGU	72	ACCAGGCGGCCGCGCACGUCCUC	207	NM_000041.4_450-470_U8C_s	NM_000041.4_448-470_A14G_as
GACGUGCGCGGCCGCCUGGUU	73	AACCAGGCGGCCGCGCACGUCCU	208	NM_000041.4_451-471_U7C_G21U_s	NM_000041.4_449-471_C1A_A15G_as
ACGUGCGCGGCCGCCUGGUGU	74	ACACCAGGCGGCCGCGCACGUCC	209	NM_000041.4_452-472_U6C_C21U_s	NM_000041.4_450-472_G1A_A16G_as
CGUGCGCGGCCGCCUGGUGCU	75	AGCACCAGGCGGCCGCGCACGUC	210	NM_000041.4_453-473_U5C_A21U_s	NM_000041.4_451-473_U1A_A17G_as
GUGCGCGGCCGCCUGGUGCAU	76	AUGCACCAGGCGGCCGCGCACGU	211	NM_000041.4_454-	NM_000041.4_452-

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
				474_U4C_G21U_s	474_C1A_A18G_as
GCGGCCGCCUGGUGCAGUACU	77	AGUACUGCACCAGGCCGGCCGCAC	212	NM_000041.4_458-478_C21U_s	NM_000041.4_456-478_G1A_as
CGCCUGGUGCAGUACCGCGGU	78	ACCGCGGUACUGCACCAGGCGGC	213	NM_000041.4_463-483_C21U_s	NM_000041.4_461-483_G1A_as
GAGGUGCAGGCCAUGCUCGGU	79	ACCGAGCAUGGCCUGCACCUCGC	214	NM_000041.4_484-504_C21U_s	NM_000041.4_482-504_G1A_as
GCCAUGCUCGGCCAGAGCACU	80	AGUGCUCUGGCCGAGCAUGGCCU	215	NM_000041.4_493-513_C21U_s	NM_000041.4_491-513_G1A_as
GGCCAGAGCACCGAGGAGCUU	81	AAGCUCCUCGGUGCUCUGGCCGA	216	NM_000041.4_502-522_G21U_s	NM_000041.4_500-522_C1A_as
GCUGCGGGUGCGCCUCGCCUU	82	AAGGCGAGGCGCACCCGCAGCUC	217	NM_000041.4_519-539_C21U_s	NM_000041.4_517-539_G1A_as
GUGCGCCUCGCCUCCCACCUU	83	AAGGUGGGAGGCGAGGCGCACCC	218	NM_000041.4_526-546_G21U_s	NM_000041.4_524-546_C1A_as
CGCCUCCCACCUGCGCAAGCU	84	AGCUUGCGCAGGUGGGAGGCGAG	219	NM_000041.4_534-554_s	NM_000041.4_532-554_as
CCCACCUGCGCAAGCUGCGUU	85	AACGCAGCUUGCGCAGGUGGGAG	220	NM_000041.4_539-559_A21U_s	NM_000041.4_537-559_U1A_as
CUGCGCAAGCUGCGUAAGCGU	86	ACGCUUACGCAGCUUGCGCAGGU	221	NM_000041.4_544-564_G21U_s	NM_000041.4_542-564_C1A_as
AAGCUGCGUAAGCGGCUCCUU	87	AAGGAGCCGCUUACGCAGCUUGC	222	NM_000041.4_550-570_C21U_s	NM_000041.4_548-570_G1A_as
GUAAGCGGCUCCUCCGCGAUU	88	AAUCGCGGAGGAGCCGCUUACGC	223	NM_000041.4_557-577_G21U_s	NM_000041.4_555-577_C1A_as
GGCUCCUCCGCGAUGCCGAUU	89	AAUCGGCAUCGCGGAGGAGCCGC	224	NM_000041.4_563-583_G21U_s	NM_000041.4_561-583_C1A_as
CUCCGCGAUGCCGAUGACCUU	90	AAGGUCAUCGGCAUCGCGGAGGA	225	NM_000041.4_568-588_G21U_s	NM_000041.4_566-588_C1A_as
GAUGCCGAUGACCUGCAGAAU	91	AUUCUGCAGGUCAUCGGCAUCGC	226	NM_000041.4_574-594_G21U_s	NM_000041.4_572-594_C1A_as

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
GAUGACCUGCAGAAGCGCCUU	92	AAGGCGCUUCUGCAGGUCAUCGG	227	NM_000041.4_580-600_G21U_s	NM_000041.4_578-600_C1A_as
CUGCAGAAGCGCCUGGCAGUU	93	AACUGCCAGGCGCUUCUGCAGGU	228	NM_000041.4_586-606_G21U_s	NM_000041.4_584-606_C1A_as
AAGCGCCUGGCAGUGUACCAU	94	AUGGUACACUGCCAGGCGCUUCU	229	NM_000041.4_592-612_G21U_s	NM_000041.4_590-612_C1A_as
CUGGCAGUGUACCAGGCCGGU	95	ACCGGCCUGGUACACUGCCAGGC	230	NM_000041.4_598-618_G21U_s	NM_000041.4_596-618_C1A_as
GAGCGCGGCCUCAGCGCCAUU	96	AAUGGCGCUGAGGCCGCGCUCGG	231	NM_000041.4_634-654_C21U_s	NM_000041.4_632-654_G1A_as
CGGCCUCAGCGCCAUCCGCGU	97	ACGCGGAUGGCGCUGAGGCCGCG	232	NM_000041.4_639-659_A21U_s	NM_000041.4_637-659_U1A_as
AGCGCCAUCCGCGAGCGCCUU	98	AAGGCGCUCGCGGAUGGCGCUGA	233	NM_000041.4_646-666_G21U_s	NM_000041.4_644-666_C1A_as
UGGGGCCCCUGGUGGAACAGU	99	ACUGUUCCACCAGGGGCCCCAGG	234	NM_000041.4_665-685_G21U_s	NM_000041.4_663-685_C1A_as
CCCCUGGUGGAACAGGGCCGU	100	ACGGCCCUGUUCCACCAGGGGCC	235	NM_000041.4_670-690_C21U_s	NM_000041.4_668-690_G1A_as
CGCGUGCGGGCCGCCACUGUU	101	AACAGUGGCGGCCCGCACGCGGC	236	NM_000041.4_688-708_G21U_s	NM_000041.4_686-708_C1A_as
GCCGCCACUGUGGGCUCCCUU	102	AAGGGAGCCCACAGUGGCGGCCC	237	NM_000041.4_697-717_G21U_s	NM_000041.4_695-717_C1A_as
CCUGGCCGGCCAGCCGCUACU	103	AGUAGCGGCUGGCCGGCCAGGGA	238	NM_000041.4_714-734_A21U_s	NM_000041.4_712-734_U1A_as
GGCCAGCCGCUACAGGAGCGU	104	ACGCUCCUGUAGCGGCUGGCCGG	239	NM_000041.4_721-741_G21U_s	NM_000041.4_719-741_C1A_as
GCCGCUACAGGAGCGGGCCCU	105	AGGGCCCGCUCCUGUAGCGGCUG	240	NM_000041.4_726-746_A21U_s	NM_000041.4_724-746_U1A_as
GCGCGGAUGGAGGAGAUGGGU	106	ACCCAUCUCCUCCAUCCGCGCGC	241	NM_000041.4_769-789_C21U_s	NM_000041.4_767-789_G1A_as
CGCGACCGCCUGGACGAGGUU	107	AACCUCGUCCAGGCGGUCGCGGG	242	NM_000041.4_799-	NM_000041.4_797-

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
				819_G21U_s	819_C1A_as
GCCUGGACGAGGUGAAGGAGU	108	ACUCCUUCACCUCGUCCAGGCGG	243	NM_000041.4_806-826_C21U_s	NM_000041.4_804-826_G1A_as
GACGAGGUGAAGGAGCAGGUU	109	AACCUGCUCCUUCACCUCGUCCA	244	NM_000041.4_811-831_G21U_s	NM_000041.4_809-831_C1A_as
GGAGGUGCGCGCCAAGCUGGU	110	ACCAGCUUGGCGCGCACCUCCGC	245	NM_000041.4_834-854_A21U_s	NM_000041.4_832-854_U1A_as
CGCGCCAAGCUGGAGGAGCAU	111	AUGCUCCUCCAGCUUGGCGCGCA	246	NM_000041.4_841-861_G21U_s	NM_000041.4_839-861_C1A_as
CAGGCCCAGCAGAUACGCCUU	112	AAGGCGUAUCUGCUGGGCCUGCU	247	NM_000041.4_859-879_G21U_s	NM_000041.4_857-879_C1A_as
CCAGCAGAUACGCCUGCAGGU	113	ACCUGCAGGCGUAUCUGCUGGGC	248	NM_000041.4_864-884_C21U_s	NM_000041.4_862-884_G1A_as
CGCCUGCAGGCCGAGGCCUUU	114	AAAGGCCUCGGCCUGCAGGCGUA	249	NM_000041.4_874-894_C21U_s	NM_000041.4_872-894_G1A_as
GCAGGCCGAGGCCUUCCAGGU	115	ACCUGGAAGGCCUCGGCCUGCAG	250	NM_000041.4_879-899_C21U_s	NM_000041.4_877-899_G1A_as
CCGAGGCCUUCCAGGCCCGCU	116	AGCGGGCCUGGAAGGCCUCGGCC	251	NM_000041.4_884-904_C21U_s	NM_000041.4_882-904_G1A_as
GCCUUCCAGGCCCGCCUCAAU	117	AUUGAGGCGGGCCUGGAAGGCCU	252	NM_000041.4_889-909_G21U_s	NM_000041.4_887-909_C1A_as
CCAGGCCCGCCUCAAGAGCUU	118	AAGCUCUUGAGGCGGGCCUGGAA	253	NM_000041.4_894-914_G21U_s	NM_000041.4_892-914_C1A_as
CCGCCUCAAGAGCUGGUUCGU	119	ACGAACCAGCUCUUGAGGCGGGC	254	NM_000041.4_900-920_A21U_s	NM_000041.4_898-920_U1A_as
CAAGAGCUGGUUCGAGCCCCU	120	AGGGGCUCGAACCAGCUCUUGAG	255	NM_000041.4_906-926_s	NM_000041.4_904-926_as
AGCCCCUGGUGGAAGACAUGU	121	ACAUGUCUUCCACCAGGGGCUCG	256	NM_000041.4_920-940_C21U_s	NM_000041.4_918-940_G1A_as
CUGGUGGAAGACAUGCAGCGU	122	ACGCUGCAUGUCUUCCACCAGGG	257	NM_000041.4_925-945_C21U_s	NM_000041.4_923-945_G1A_as

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
GGAAGACAUGCAGCGCCAGUU	123	AACUGGCGCUGCAUGUCUUCCAC	258	NM_000041.4_930-950_G21U_s	NM_000041.4_928-950_C1A_as
GCCGGGCUGGUGGAGAAGGUU	124	AACCUUCUCCACCAGCCCGGCCC	259	NM_000041.4_952-972_G21U_s	NM_000041.4_950-972_C1A_as
GCUGGUGGAGAAGGUGCAGGU	125	ACCUGCACCUUCUCCACCAGCCC	260	NM_000041.4_957-977_C21U_s	NM_000041.4_955-977_G1A_as
ACCAGCGCCGCCCCUGUGCCU	126	AGGCACAGGGGCGGCGCUGGUGC	261	NM_000041.4_988-1008_C21U_s	NM_000041.4_986-1008_G1A_as
GCCCCUGUGCCCAGCGACAAU	127	AUUGUCGCUGGGCACAGGGGCGG	262	NM_000041.4_997-1017_s	NM_000041.4_995-1017_as
UGUGCCCAGCGACAAUCACUU	128	AAGUGAUUGUCGCUGGGGCACAGG	263	NM_000041.4_1002-1022_G21U_s	NM_000041.4_1000-1022_C1A_as
CAGCGACAAUCACUGAACGCU	129	AGCGUUCAGUGAUUGUCGCUGGG	264	NM_000041.4_1008-1028_C21U_s	NM_000041.4_1006-1028_G1A_as
CAAUCACUGAACGCCGAAGCU	130	AGCUUCGGCGUUCAGUGAUUGUC	265	NM_000041.4_1014-1034_C21U_s	NM_000041.4_1012-1034_G1A_as
ACUGAACGCCGAAGCCUGCAU	131	AUGCAGGCUUCGGCGUUCAGUGA	266	NM_000041.4_1019-1039_G21U_s	NM_000041.4_1017-1039_C1A_as
ACGCCGAAGCCUGCAGCCAUU	132	AAUGGCUGCAGGCUUCGGCGUUC	267	NM_000041.4_1024-1044_G21U_s	NM_000041.4_1022-1044_C1A_as
GAAGCCUGCAGCCAUGCGACU	133	AGUCGCAUGGCUGCAGGCUUCGG	268	NM_000041.4_1029-1049_C21U_s	NM_000041.4_1027-1049_G1A_as
UGCAGCCAUGCGACCCCACGU	134	ACGUGGGGUCGCAUGGCUGCAGG	269	NM_000041.4_1035-1055_C21U_s	NM_000041.4_1033-1055_G1A_as
GCGACCCCACGCCACCCCGUU	135	AACGGGGUGGCGUGGGGUCGCAU	270	NM_000041.4_1044-1064_G21U_s	NM_000041.4_1042-1064_C1A_as
CCCACGCCACCCCGUGCCUCU	136	AGAGGCACGGGGUGGCGUGGGGU	271	NM_000041.4_1049-1069_C21U_s	NM_000041.4_1047-1069_G1A_as
CCACCCCGUGCCUCCUGCCUU	137	AAGGCAGGAGGCACGGGGUGGCG	272	NM_000041.4_1055-1075_C21U_s	NM_000041.4_1053-1075_G1A_as
CGUGCCUCCUGCCUCCGCGCU	138	AGCGCGGAGGCAGGAGGCACGGG	273	NM_000041.4_1061-	NM_000041.4_1059-

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
				1081_A21U_s	1081_U1A_as
CUCCUGCCUCCGCGCAGCCUU	139	AAGGCUGCGCGGAGGCAGGAGGC	274	NM_000041.4_1066-1086_G21U_s	NM_000041.4_1064-1086_C1A_as
GCCUCCGCGCAGCCUGCAGCU	140	AGCUGCAGGCUGCGCGGAGGCAG	275	NM_000041.4_1071-1091_G21U_s	NM_000041.4_1069-1091_C1A_as
CCUGUCCCCGCCCCAGCCGUU	141	AACGGCUGGGGCGGGGACAGGGU	276	NM_000041.4_1098-1118_C21U_s	NM_000041.4_1096-1118_G1A_as
CCCGCCCCAGCCGUCCUCCUU	142	AAGGAGGACGGCUGGGGCGGGGA	277	NM_000041.4_1104-1124_G21U_s	NM_000041.4_1102-1124_C1A_as
CCCAGCCGUCCUCCUGGGGUU	143	AACCCCAGGAGGACGGCUGGGGC	278	NM_000041.4_1109-1129_G21U_s	NM_000041.4_1107-1129_C1A_as
UCCUGGGGUGGACCCUAGUUU	144	AAACUAGGGUCCACCCCAGGAGG	279	NM_000041.4_1120-1140_s	NM_000041.4_1118-1140_as
GGGUGGACCCUAGUUUAAUAU	145	AUAUUAAACUAGGGUCCACCCCA	280	NM_000041.4_1125-1145_A21U_s	NM_000041.4_1123-1145_U1A_as
GACCCUAGUUUAAUAAAGAUU	146	AAUCUUUAUUAAACUAGGGUCCA	281	NM_000041.4_1130-1150_s	NM_000041.4_1128-1150_as
UAGUUUAAUAAAGAUUCACCU	147	AGGUGAAUCUUUAUUAAACUAGG	282	NM_000041.4_1135-1155_A21U_s	NM_000041.4_1133-1155_U1A_as
UAAUAAAGAUUCACCAAGUUU	148	AAACUUGGUGAAUCUUUAUUAAA	283	NM_000041.4_1140-1160_s	NM_000041.4_1138-1160_as
AGAUUCACCAAGUUUCACGCU	149	AGCGUGAAACUUGGUGAAUCUUU	284	NM_000041.4_1146-1166_A21U_s	NM_000041.4_1144-1166_U1A_as

Table 3. APOE Modified Sense and Antisense Strand Sequences

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
gsgsccaaUfcAfCfAfggcaggaaguL96	285	asCfsuucCfuGfCfcuguGfaUfuggccsasg	420	CTGGCCAATCACAGGCAGGAAGA	555
csasggcaGfgAfAfGfaugaagguuuL96	286	asAfsaccUfuCfAfucuuCfcUfgccugsusg	421	CACAGGCAGGAAGATGAAGGTTC	556

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
asgsgaagAfuGfAfAfgguucugugguL96	287	asCfsacaGfaAfCfcuucAfuCfuuccusgsc	422	GCAGGAAGATGAAGGTTCTGTGG	557
asusgaagGfuUfCfUfgugggcugcuL96	288	asGfscagCfcCfAfcagaAfcCfuucauscsu	423	AGATGAAGGTTCTGTGGGCTGCG	558
ususcuguGfgGfCfUfgcguugcuguL96	289	asCfsagcAfaCfGfcagcCfcAfcagaascsc	424	GGTTCTGTGGGCTGCGTTGCTGG	559
usgsggcuGfcGfUfUfgcuggucacuL96	290	asGfsugaCfcAfGfcaacGfcAfgccccascsa	425	TGTGGGCTGCGTTGCTGGTCACA	560
gscsguugCfuGfGfUfcacauuccuuL96	291	asAfsggaAfuGfUfgaccAfgCfaacgcsasg	426	CTGCGTTGCTGGTCACATTCCTG	561
gscsuggguCfaCfAfUfuccuggcaguL96	292	asCfsugcCfaGfGfaaugUfgAfccagcsasa	427	TTGCTGGTCACATTCCTGGCAGG	562
uscsacauUfcCfUfGfgcaggaugcuL96	293	asGfscauCfcUfGfccagGfaAfugugascsc	428	GGTCACATTCCTGGCAGGATGCC	563
usgsgcagGfaUfGfGfCfcaggccaagL96	294	asCfsuugGfcCfUfggcaUfcCfugccasgsg	429	CCTGGCAGGATGCCAGGCCAAGG	564
csasggccAfaGfGfUfggagcaagcuL96	295	asGfscuuGfcUfCfcaccUfuGfgccugsgsc	430	GCCAGGCCAAGGTGGAGCAAGCG	565
asasggugGfaGfCfAfagcgguggauL96	296	asUfsccaCfcGfCfuugcUfcCfaccuusgsg	431	CCAAGGTGGAGCAAGCGGTGGAG	566
csasagcgGfuGfGfAfgacagagccuL96	297	asGfsgcuCfuGfUfcuccAfcCfgcuugscsu	432	AGCAAGCGGTGGAGACAGAGCCG	567
gsgsuggaGfaCfAfGfagccggagcuL96	298	asGfscucCfgGfCfucugUfcUfccaccsgsc	433	GCGGTGGAGACAGAGCCGGAGCC	568
cscscgagCfuGfCfGfccagcagacuL96	299	asGfsucuGfcUfGfgcgcAfgCfucgggscsu	434	AGCCCGAGCTGCGCCAGCAGACC	569
gscsugcgCfcAfGfCfagaccgaguuL96	300	asAfscucGfgUfCfugcuGfgCfgcagcsusc	435	GAGCTGCGCCAGCAGACCGAGTG	570
cscsagcaGfaCfCfGfaguggcagauL96	301	asUfscugCfcAfCfucggUfcUfgcuggscsg	436	CGCCAGCAGACCGAGTGGCAGAG	571
csasgcgcUfgGfGfAfacuggcacuuL96	302	asAfsgugCfcAfGfuuccCfaGfcgcugsgsc	437	GCCAGCGCTGGGAACTGGCACTG	572
csusgggaAfcUfGfGfcacugggucuL96	303	asGfsaccCfaGfUfgccaGfuUfcccagscsg	438	CGCTGGGAACTGGCACTGGGTCG	573
asascuggCfaCfUfGfggucgcuuuuL96	304	asAfsaagCfgAfCfccagUfgCfcaguuscsc	439	GGAACTGGCACTGGGTCGCTTTT	574
csascuggGfuCfGfCfuuuuggggauuL96	305	asAfsuccCfaAfAfagcgAfcCfcagugscsc	440	GGCACTGGGTCGCTTTTGGGATT	575
gsuscgcuUfuUfGfGfgauuaccuguL96	306	asCfsaggUfaAfUfcccaAfaAfgcgacscsc	441	GGGTCGCTTTTGGGATTACCTGC	576
ususuuggGfaUfUfUfAfccugcgcuguL96	307	asCfsagcGfcAfGfguaaUfcCfcaaaasgsc	442	GCTTTTGGGATTACCTGCGCTGG	577
csusgcgcUfgGfGfUfgcagacacuuL96	308	asAfsgugUfcUfGfcaccCfaGfcgcagsgsu	443	ACCTGCGCTGGGTGCAGACACTG	578
usgsggugCfaGfAfCfacugucugauL96	309	asUfscagAfcAfGfugucUfgCfacccasgsc	444	GCTGGGTGCAGACACTGTCTGAG	579
csasgacaCfuGfUfCfugagcagguuL96	310	asAfsccuGfcUfCfagacAfgUfgucugscsa	445	TGCAGACACTGTCTGAGGCAGGTG	580
csasggagGfaGfCfUfgcucagcucuL96	311	asGfsagcUfgAfGfcagcUfcCfuccugscsa	446	TGCAGGAGGAGCTGCTCAGCTCC	581
csusgcucAfgCfUfCfccaggucacuL96	312	asGfsugaCfcUfGfggagCfuGfagcagscsu	447	AGCTGCTCAGCTCCCAGGTCACC	582

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
uscsccagGfuCfAfCfccaggaacuuL96	313	asAfsguuCfcUfGfggugAfcCfugggasgsc	448	GCTCCCAGGTCACCCAGGAACTG	583
ascscccagGfaAfCfUfgagggcgcuuL96	314	asAfsgcgCfcCfUfcaguUfcCfugggusgsa	449	TCACCCAGGAACTGAGGGCGCTG	584
usgsagggCfgCfUfGfauggacgagguL96	315	asCfsucgUfcCfAfucagCfgCfccucasgsu	450	ACTGAGGGCGCTGATGGACGAGA	585
gscsgcugAfuGfGfAfcgagaccauuL96	316	asAfsuggUfcUfCfguccAfuCfagcgcscsc	451	GGGCGCTGATGGACGAGACCATG	586
gsascgagAfcCfAfUfgaaggaguuuL96	317	asAfsacuCfcUfUfcaugGfuCfucgucscsa	452	TGGACGAGACCATGAAGGAGTTG	587
ascscaugAfaGfGfAfguugaaggcuL96	318	asGfsccuUfcAfAfcuccUfuCfauggucscsu	453	AGACCATGAAGGAGTTGAAGGCC	588
gsgsaguuGfaAfGfGfccuacaaauuL96	319	asAfsuuuGfuAfGfgccuUfcAfacuccsusu	454	AAGGAGTTGAAGGCCTACAAATC	589
asasggccUfaCfAfAfaucggaacuuL96	320	asAfsguuCfcGfAfuuugUfaGfgccuuscsa	455	TGAAGGCCTACAAATCGGAACTG	590
ascsaaauCfgGfAfAfcuggaggaauL96	321	asUfsuccUfcCfAfguucCfgAfuuugusasg	456	CTACAAATCGGAACTGGAGGAAC	591
uscsggaaCfuGfGfAfggaacaacuuL96	322	asAfsguuGfuUfCfcuccAfgUfuccgasusu	457	AATCGGAACTGGAGGAACAACTG	592
gsasggaaCfaAfCfUfgaccccggguuL96	323	asAfsccgGfgGfUfcaguUfgUfuccucscsa	458	TGGAGGAACAACTGACCCCGGTG	593
csgscgggCfaCfGfGfcuguccaaguL96	324	asCfsuugGfaCfAfgccgUfgCfccgcgsusc	459	GACGCGGGCACGGCTGTCCAAGG	594
gscsacggCfuGfUfCfcaaggagcuuL96	325	asAfsgcuCfcUfUfggacAfgCfcgugcscsc	460	GGGCACGGCTGTCCAAGGAGCTG	595
gscsugucCfaAfGfGfagcugcaggguL96	326	asCfscugCfaGfCfuccuUfgGfacagcscsg	461	CGGCTGTCCAAGGAGCTGCAGGC	596
gscscggCfuGfGfGfcgcggacauuL96	327	asAfsuguCfcGfCfgcccAfgCfcgggcscsu	462	AGGCCCGGCTGGGCGCGGACATG	597
csusgggcGfcGfGfAfcauggaggauL96	328	asUfsccuCfcAfUfguccGfcGfcccagscsc	463	GGCTGGGCGCGGACATGGAGGAC	598
csgscggaCfaUfGfGfaggacgugcuL96	329	asGfscacGfuCfCfuccaUfgUfccgcgscsc	464	GGCGCCGGACATGGAGGACGTGCG	599
gscsggacAfuGfGfAfggacgugugguL96	330	asCfsacaCfgUfCfcuccAfuGfuccgcsgsc	465	GCGCGGACATGGAGGACGTGTGC	600
gscsggacAfuGfGfAfggacgugcguL96	331	asCfsgcaCfgUfCfcuccAfuGfuccgcsgsc	466	GCGCGGACATGGAGGACGTGCGC	601
csgsgacaUfgGfAfGfgacgugcgcuL96	332	asGfscgcAfcGfUfccucCfaUfguccgscsg	467	CGCGGACATGGAGGACGTGCGCG	602
gsgsacauGfgAfGfGfacgugcgcguL96	333	asCfsgcgCfaCfGfuccuCfcAfuguccsgsc	468	GCGGACATGGAGGACGTGCGCGG	603
gsascaugGfaGfGfAfcgugcgcggguL96	334	asCfscgcGfcAfCfguccUfcCfauguscscsg	469	CGGACATGGAGGACGTGCGCGGC	604
ascsauggAfgGfAfCfgugcgcggcuL96	335	asGfsccgCfgCfAfcgucCfuCfcauguscsc	470	GGACATGGAGGACGTGCGCGGCC	605
csasuggaGfgAfCfGfugcgcggccuL96	336	asGfsgccGfcGfCfacguCfcUfccaugsusc	471	GACATGGAGGACGTGCGCGGCCG	606
asusggagGfaCfGfUfgcgcggccguL96	337	asCfsggcCfgCfGfcacgUfcCfuccausgsu	472	ACATGGAGGACGTGCGCGGCCGC	607
usgsgaggAfcGfUfGfcgcggccgcuL96	338	asGfscggCfcGfCfgcacGfuCfcuccasusg	473	CATGGAGGACGTGCGCGGCCGCC	608

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
gsgsaggaCfgUfGfCfgcggccgccuL96	339	asGfsgcgGfcCfGfcgcaCfgUfccuccsasu	474	ATGGAGGACGTGCGCCGGCCGCCT	609
gsasggacGfuGfCfGfcggccgccuuL96	340	asAfsggcGfgCfCfgcgcAfcGfuccucscsa	475	TGGAGGACGTGCGCCGGCCGCCTG	610
asgsgacgUfgCfGfCfggccgccugL96	341	asCfsaggCfgGfCfcgcgCfaCfguccuscsc	476	GGAGGACGTGCGCCGGCCGCCTGG	611
gsgsacguGfcGfCfGfgccgccuggL96	342	asCfscagGfcGfGfccgcGfcAfcguccsusc	477	GAGGACGTGCGCCGGCCGCCTGGT	612
gsascgugCfgCfGfGfccgccuggL96	343	asAfsccaGfgCfGfgccgCfgCfacgucscsu	478	AGGACGTGCGCCGGCCGCCTGGTG	613
ascsgugcGfcGfGfGfCfcgccuggL96	344	asCfsaccAfgGfGfCfggccGfcGfcacgusccsc	479	GGACGTGCGCCGGCCGCCTGGTGC	614
csgsugcgCfgGfCfCfGfccuggugcL96	345	asGfscacCfaGfGfcggcCfgCfgcacgsusc	480	GACGTGCGCCGGCCGCCTGGTGCA	615
gsusgcgcGfgCfCfGfccuggugcauL96	346	asUfsgcaCfcAfGfgcggCfcGfGfcgcacsgsu	481	ACGTGCGCCGGCCGCCTGGTGCAG	616
gscsggccGfcCfUfGfgugcaguacuL96	347	asGfsuacUfgCfAfccagGfcGfgccgcsasc	482	GTGCGGCCGGCCGCCTGGTGCAGTACC	617
csgsccugGfuGfCfAfguaccgcgguL96	348	asCfscgcGfgUfAfcugcAfcCfaggcgsgsc	483	GCCGCCTGGTGCAGTACCGCGGC	618
gsasgguGfaGfGfCfcaugcucgguL96	349	asCfscgaGfcAfUfggccUfgCfaccucsgsc	484	GCGAGGTGCAGGCCATGCTCGGC	619
gscscaugCfuCfGfGfccagagcacuL96	350	asGfsugcUfcUfGfgccgAfgCfauggcscsu	485	AGGCCATGCTCGGCCAGAGCACC	620
gsgsccagAfgCfAfCfcgaggagcuuL96	351	asAfsgcuCfcUfCfggugCfuCfuggccsgsa	486	TCGGCCAGAGCACCGAGGAGCTG	621
gscsugcgGfgUfGfCfgccucgccuuL96	352	asAfsggcGfaGfGfcgcaCfcCfgcagcsusc	487	GAGCTGCGGGTGCGCCTCGCCTC	622
gsusgcgcCfuCfGfCfcucccaccuuL96	353	asAfsgguGfgGfAfggcgAfgGfcgcacsccsc	488	GGGTGCGCCTCGCCTCCCACCTG	623
csgsccucCfcAfCfCfcugcgcaagcuL96	354	asGfscuuGfcGfCfagguGfgGfaggcgsasg	489	CTCGCCTCCCACCTGCGCAAGCT	624
cscscaccUfgCfGfCfaagcugcguuL96	355	asAfscgcAfgCfUfugcgCfaGfguggsasg	490	CTCCCACCTGCGCAAGCTGCGTA	625
csusgcgcAfaGfCfUfgcguaagcguL96	356	asCfsgcuUfaCfGfcagcUfuGfcgcagsgsu	491	ACCTGCGCAAGCTGCGTAAGCGG	626
asasgcugCfgUfAfAfgcggcuccuuL96	357	asAfsggaGfcCfGfcuuaCfgCfagcuusgsc	492	GCAAGCTGCGTAAGCGGCTCCTC	627
gsusaagcGfgCfUfCfcuccgcgauuL96	358	asAfsucgCfgGfAfggagCfcGfcuuacsgsc	493	GCGTAAGCGGCTCCTCCGCGATG	628
gsgscuccUfcCfGfCfgaugccgauuL96	359	asAfsucgGfcAfUfcgcgGfaGfgagccsgsc	494	GCGGCTCCTCCGCGATGCCGATG	629
csusccgcGfaUfGfCfcgaugaccuuL96	360	asAfsgguCfaUfCfggcaUfcGfcggagsgsa	495	TCCTCCGCGATGCCGATGACCTG	630
gsasugccGfaUfGfAfccugcagaauL96	361	asUfsucuGfcAfGfgucaUfcGfgcaucsgsc	496	GCGATGCCGATGACCTGCAGAAG	631
gsasugacCfuGfCfAfgaagcgccuuL96	362	asAfsggcGfcUfUfcugcAfgGfucaucsgsg	497	CCGATGACCTGCAGAAGCGCCTG	632
csusgcagAfaGfCfGfccuggcaguuL96	363	asAfscugCfcAfGfgcgcUfuCfugcagsgsu	498	ACCTGCAGAAGCGCCTGGCAGTG	633
asasgcgcCfuGfGfCfaguguaccauL96	364	asUfsgguAfcAfCfugccAfgGfcgcuuscsu	499	AGAAGCGCCTGGCAGTGTACCAG	634

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
csusggcaGfuGfUfAfccaggccgguL96	365	asCfscggCfcUfGfguacAfcUfgccagsgsc	500	GCCTGGCAGTGTACCAGGCCGGG	635
gsasgcgcGfgCfCfUfcagcgccauuL96	366	asAfsuggCfgCfUfgaggCfcGfcgcucsgsg	501	CCGAGCGCGGCCTCAGCGCCATC	636
csgsgccuCfaGfCfGfccauccgcguL96	367	asCfsgcgGfaUfGfgcgcUfgAfggccgscsg	502	CGCGGCCTCAGCGCCATCCGCGA	637
asgscgccAfuCfCfGfcgagcgccuuL96	368	asAfsggcGfcUfCfgcggAfuGfgcgcusgsa	503	TCAGCGCCATCCGCGAGCGCCTG	638
usgsggggCfcCfUfGfguggaacagL96	369	asCfsuguUfcCfAfccagGfgGfccccasgsg	504	CCTGGGGGCCCCTGGTGGAACAGG	639
cscsccugGfuGfGfAfacagggccguL96	370	asCfsggcCfcUfGfuuccAfcCfaggggscsc	505	GGCCCCTGGTGGAACAGGGCCGC	640
csgscgugCfgGfGfCfcgccacuguuL96	371	asAfscagUfgGfCfggccCfgCfacgcgsgsc	506	GCCGCGTGCGGGCCGCCACTGTG	641
gscscgccAfcUfGfUfgggcuccccuuL96	372	asAfsgggAfgCfCfcacaGfuGfgcggcscsc	507	GGGCCGCCACTGTGGGCTCCCTG	642
cscsuggcCfgGfCfCfagccgcuacuL96	373	asGfsuagCfgGfCfuggcCfgGfccaggsgsa	508	TCCCTGGCCGGCCAGCCGCTACA	643
gsgsccagCfcGfCfUfacaggagcgcguL96	374	asCfsgcuCfcUfGfuagcGfgCfuggccsggg	509	CCGGCCAGCCGCTACAGGAGCGG	644
gscscgcuAfcAfGfGfagcgggcccuL96	375	asGfsggcCfcGfCfuccuGfuAfgcggcsusg	510	CAGCCGCTACAGGAGCGGGCCCA	645
gscsgcggAfuGfGfAfggagaugggguL96	376	asCfsccaUfcUfCfcuccAfuCfcgcgcsgsc	511	GCGCGCGGATGGAGGAGATGGGC	646
csgscgacCfgCfCfUfggacgaggauuL96	377	asAfsccuCfgUfCfcaggCfgGfucgcgsgsg	512	CCCGCGACCGCCTGGACGAGGTG	647
gscscuggAfcGfAfGfgugaaggagguL96	378	asCfsuccUfuCfAfccucGfuCfcaggcsgsg	513	CCGCCTGGACGAGGTGAAGGAGC	648
gsascgagGfuGfAfAfggagcagguuL96	379	asAfsccuGfcUfCfcuucAfcCfucgucscsa	514	TGGACGAGGTGAAGGAGCAGGTG	649
gsgsagguGfcGfCfGfccaagcuggguL96	380	asCfscagCfuUfGfgcgcGfcAfccuccsgsc	515	GCGGAGGTGCGCGCCAAGCTGGA	650
csgscgccAfaGfCfCfUfggaggagcauL96	381	asUfsgcuCfcUfCfcagcUfuGfgcgcgscsa	516	TGCGCGCCAAGCTGGAGGAGCAG	651
csasggccCfaGfCfCfAfgauacgccuuL96	382	asAfsggcGfuAfUfcugcUfgGfgccugscsu	517	AGCAGGCCCAGCAGATACGCCTG	652
cscsagcaGfaUfAfCfgccugcaggauL96	383	asCfscugCfaGfGfcguaUfcUfgcuggsgsc	518	GCCCAGCAGATACGGCCTGCAGGC	653
csgsccugCfaGfGfCfcgaggccuuuL96	384	asAfsaggCfcUfCfggccUfgCfaggcgsusa	519	TACGCCTGCAGGCCGAGGCCTTC	654
gscsaggcCfgAfGfGfccuuccaggauL96	385	asCfscugGfaAfGfgccuCfgGfccugcsasg	520	CTGCAGGCCGAGGCCTTCCAGGC	655
cscsgaggCfcUfUfCfcaggcccgcuL96	386	asGfscggGfcCfUfggaaGfgCfcucggscsc	521	GGCCGAGGCCTTCCAGGCCCGCC	656
gscscuucCfaGfGfCfccgccucaauL96	387	asUfsugaGfgCfGfggccUfgGfaaggcscsu	522	AGGCCTTCCAGGCCCGCCTCAAG	657
cscsaggcCfcGfCfCfcuucaagagcuuL96	388	asAfsgcuCfuUfGfaggcGfgGfccuggsasa	523	TTCCAGGCCCGCCTCAAGAGGCTG	658
cscsgccuCfaAfGfAfgcuggguucguL96	389	asCfsgaaCfcAfGfcucuUfgAfggcggsgsc	524	GCCCGCCTCAAGAGCTGGTTCGA	659
csasagagCfuGfGfUfucgagcccccuL96	390	asGfsgggCfuCfGfaaccAfgCfucuugsasg	525	CTCAAGAGCTGGTTCGAGCCCCT	660

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
asgsccccUfgGfUfGfgaagacauguL96	391	asCfsaugUfcUfUfccacCfaGfgggcuscsg	526	CGAGCCCCTGGTGGAAGACATGC	661
csusggugGfaAfGfAfcaugcagcguL96	392	asCfsgcuGfcAfUfgucuUfcCfaccagsgsg	527	CCCTGGTGGAAGACATGCAGCGC	662
gsgsaagaCfaUfGfCfagccaguuL96	393	asAfscugGfcGfCfugcaUfgUfcuuccsasc	528	GTGGAAGACATGCAGCGCCAGTG	663
gscscgggCfuGfGfUfggagaagguuL96	394	asAfsccuUfcUfCfcaccAfgCfccggcscsc	529	GGGCCGGGCTGGTGGAGAAGGTG	664
gscsugguGfgAfGfAfaggugcagguL96	395	asCfscugCfaCfCfuucuCfcAfccagcscsc	530	GGGCTGGTGGAGAAGGTGCAGGC	665
ascscagcGfcCfGfCfcccugugccuL96	396	asGfsgcaCfaGfGfggcgGfcGfcuggusgsc	531	GCACCAGCGCCGCCCCTGTGCCC	666
gscscccuGfuGfCfCfcagcgacaauL96	397	asUfsuguCfgCfUfgggcAfcAfgggcsgsg	532	CCGCCCCTGTGCCCAGCGACAAT	667
usgsugccCfaGfCfCfGfacaaucacuuL96	398	asAfsgugAfuUfGfucgcUfgGfgcacasgsg	533	CCTGTGCCCAGCGACAATCACTG	668
csasgcgaCfaAfUfCfacugaacgcuL96	399	asGfscguUfcAfGfugauUfgUfcgcugsgsg	534	CCCAGCGACAATCACTGAACGCC	669
csasaucaCfuGfAfAfcgccgaagcuL96	400	asGfscuuCfgGfCfguucAfgUfgauugsusc	535	GACAATCACTGAACGCCGAAGCC	670
ascsugaaCfgCfCfCfGfaagccugcauL96	401	asUfsgcaGfgCfUfucggCfgUfucagusgsa	536	TCACTGAACGCCGAAGCCTGCAG	671
ascsgccgAfaGfCfCfUfgcagccauuL96	402	asAfsuggCfuGfCfaggcUfuCfggcgususc	537	GAACGCCGAAGCCTGCAGCCATG	672
gsasagccUfgCfAfGfccaugcgacuL96	403	asGfsucgCfaUfGfgcugCfaGfgcuucsgsg	538	CCGAAGCCTGCAGCCATGCGACC	673
usgscagcCfaUfGfCfgaccccacguL96	404	asCfsgugGfgGfUfcgcaUfgGfcugcasgsg	539	CCTGCAGCCATGCGACCCCACGC	674
gscsgaccCfcAfCfGfccacccguuL96	405	asAfscggGfgUfGfgcguGfgGfgucgcsasu	540	ATGCGACCCCACGCCACCCCGTG	675
cscscacgCfcAfCfCfcgugccucuL96	406	asGfsaggCfaCfGfggguGfgCfguggggsgsu	541	ACCCCACGCCACCCCGTGCCTCC	676
cscsacccCfgUfGfGfCfcuccugccuuL96	407	asAfsggcAfgGfGfAfggcaCfgGfgguggscsg	542	CGCCACCCCGTGCCTCCTGCCTC	677
csgsugccUfcCfUfGfGfccuccgcgcuL96	408	asGfscgcGfgAfGfGfcagGfaGfgcacgsgsg	543	CCCGTGCCTCCTGCCTCCGCGCA	678
csuscccugCfcUfCfCfCfgcgcagccuuL96	409	asAfsggcUfgCfCfGfcggaGfgCfaggagsgsc	544	GCCTCCTGCCTCCGCGGCAGCCTG	679
gscscuccGfcGfCfCfAfgccugcagcuL96	410	asGfscugCfaGfGfGfcugcGfcGfgaggcsasg	545	CTGCCTCCGCGGCAGCCTGCAGCG	680
cscsugucCfcCfGfCfCfcccagccguuL96	411	asAfscggCfuGfGfGfggcgGfgGfacaggsgsu	546	ACCCTGTCCCCGCCCCCAGCCGTC	681
cscscgccCfcAfGfCfCfcguccuccuuL96	412	asAfsggaGfgAfCfggcuGfgGfgcgggsgsa	547	TCCCCGCCCCCAGCCGTCCTCCTG	682
cscscagcCfgUfCfCfCfuccuggggguuL96	413	asAfscccCfaGfGfGfaggaCfgGfcuggggsgsc	548	GCCCCAGCCGTCCTCCTGGGGGTG	683
uscscuggGfgUfGfGfGfacccuaguuuL96	414	asAfsacuAfgGfGfGfuccaCfcCfcaggasgsg	549	CCTCCTGGGGGTGGACCCTAGTTT	684
gsgsguggAfcCfCfUfUfaguuuaauauL96	415	asUfsauuAfaAfCfUfuaggGfuCfcacccscsa	550	TGGGGTGGACCCTAGTTTAATAA	685
gsascccuAfgUfUfUfUfaauaaagauuL96	416	asAfsucuUfuAfUfUfuaaaCfuAfgggucscsa	551	TGGACCCTAGTTTAATAAAGATT	686

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
usasguuuAfaUfAfAfagauucaccuL96	417	asGfsgugAfaUfCfuuuaUfuAfaacuasgsg	552	CCTAGTTTAATAAAGATTCACCA	687
usasauaaAfgAfUfUfcaccaaguuuL96	418	asAfsacuUfgGfUfgaauCfuUfuauuasasa	553	TTTAATAAAGATTCACCAAGTTT	688
asgsauucAfcCfAfAfguuucacgcuL96	419	asGfscguGfaAfAfcuugGfuGfaaucususu	554	AAAGATTCACCAAGTTTCACGCA	689

Table 4. APOE Unmodified Sense and Antisense Strand Sequences

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072375.1	GGAGUUGAAGGCCUACAAAUU	49	AAUUUGUAGGCCUUCAACUCCUU	184
AD-1072352.1	GCGCUGAUGGACGAGACCAUU	46	AAUGGUCUCGUCCAUCAGCGCCC	181
AD-1072394.1	UCGGAACUGGAGGAACAACUU	52	AAGUUGUUCCUCCAGUUCCGAUU	187
AD-1072721.1	ACGCCGAAGCCUGCAGCCAUU	132	AAUGGCUGCAGGCUUCGGCGUUC	267
AD-1072254.1	CACUGGGUCGCUUUUGGGAUU	35	AAUCCCAAAAGCGACCCAGUGCC	170
AD-1072189.1	AAGGUGGAGCAAGCGGUGGAU	26	AUCCACCGCUUGCUCCACCUUGG	161
AD-1072741.1	GACCCUAGUUUAAUAAAGAUU	146	AAUCUUUAUUAAACUAGGGUCCA	281
AD-1072382.1	AAGGCCUACAAAUCGGAACUU	50	AAGUUCCGAUUUGUAGGCCUUCA	185
AD-1072129.1	AGGAAGAUGAAGGUUCUGUGU	17	ACACAGAACCUUCAUCUUCCUGC	152
AD-1072514.1	CUCCGCGAUGCCGAUGACCUU	90	AAGGUCAUCGGCAUCGCGGAGGA	225
AD-1072124.1	CAGGCAGGAAGAUGAAGGUUU	16	AAACCUUCAUCUUCCUGCCUGUG	151
AD-1072337.1	ACCCAGGAACUGAGGGCGCUU	44	AAGCGCCCUCAGUUCCUGGGUGA	179
AD-1072135.1	AUGAAGGUUCUGUGGGCUGCU	18	AGCAGCCCACAGAACCUUCAUCU	153
AD-1072745.1	UAGUUUAAUAAAGAUUCACCU	147	AGGUGAAUCUUUAUUAAACUAGG	282
AD-1072153.1	GCGUUGCUGGUCACAUUCCUU	21	AAGGAAUGUGACCAGCAACGCAG	156
AD-1072520.1	GAUGCCGAUGACCUGCAGAAU	91	AUUCUGCAGGUCAUCGGCAUCGC	226
AD-1072389.1	ACAAAUCGGAACUGGAGGAAU	51	AUUCCUCCAGUUCCGAUUUGUAG	186
AD-1072716.1	ACUGAACGCCGAAGCCUGCAU	131	AUGCAGGCUUCGGCGUUCAGUGA	266

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072222.1	CCAGCAGACCGAGUGGGCAGAU	31	AUCUGCCACUCGGUCUGCUGGCG	166
AD-1072367.1	ACCAUGAAGGAGUUGAAGGCU	48	AGCCUUCAACUCCUUCAUGGUCU	183
AD-1103894.1	AGAUUCACCAAGUUUCACGCU	149	AGCGUGAAACUUGGUGAAUCUUU	284
AD-1072651.1	GCCUUCCAGGCCCGCCUCAAU	117	AUUGAGGCGGGCCUGGAAGGCCU	252
AD-1072260.1	GUCGCUUUUGGGAUUACCUGU	36	ACAGGUAAUCCCAAAAGCGACCC	171
AD-1072265.1	UUUUGGGAUUACCUGCGCUGU	37	ACAGCGCAGGUAAUCCCAAAAGC	172
AD-1072750.1	UAAUAAAGAUUCACCAAGUUU	148	AAACUUGGUGAAUCUUUAUUAAA	283
AD-1072142.1	UUCUGUGGGCUGCGUUGCUGU	19	ACAGCAACGCAGCCCACAGAACC	154
AD-1072328.1	UCCCAGGUCACCCAGGAACUU	43	AAGUUCCUGGGUGACCUGGGAGC	178
AD-1072198.1	CAAGCGGUGGAGACAGAGCCU	27	AGGCUCUGUCUCCACCGCUUGCU	162
AD-1072361.1	GACGAGACCAUGAAGGAGUUU	47	AAACUCCUUCAUGGUCUCGUCCA	182
AD-1072526.1	GAUGACCUGCAGAAGCGCCUU	92	AAGGCGCUUCUGCAGGUCAUCGG	227
AD-1072699.1	UGUGCCCAGCGACAAUCACUU	128	AAGUGAUUGUCGCUGGGCACAGG	263
AD-1072711.1	CAAUCACUGAACGCCGAAGCU	130	AGCUUCGGCGUUCAGUGAUUGUC	265
AD-1103879.1	CAAGAGCUGGUUCGAGCCCCU	120	AGGGGCUCGAACCAGCUCUUGAG	255
AD-1103849.1	GAGGAACAACUGACCCCGGUU	53	AACCGGGGUCAGUUGUUCCUCCA	188
AD-1072662.1	CCGCCUCAAGAGCUGGUUCGU	119	ACGAACCAGCUCUUGAGGCGGGC	254
AD-1103883.1	UGCAGCCAUGCGACCCCACGU	134	ACGUGGGGUCGCAUGGCUGCAGG	269
AD-1072172.1	UGGCAGGAUGCCAGGCCAAGU	24	ACUUGGCCUGGCAUCCUGCCAGG	159
AD-1072158.1	GCUGGUCACAUUCCUGGCAGU	22	ACUGCCAGGAAUGUGACCAGCAA	157
AD-1072705.1	CAGCGACAAUCACUGAACGCU	129	AGCGUUCAGUGAUUGUCGCUGGG	264
AD-1072147.1	UGGGCUGCGUUGCUGGUCACU	20	AGUGACCAGCAACGCAGCCCACA	155
AD-1072593.1	GACGAGGUGAAGGAGCAGGUU	109	AACCUGCUCCUUCACCUCGUCCA	244
AD-1072115.1	GGCCAAUCACAGGCAGGAAGU	15	ACUUCCUGCCUGUGAUUGGCCAG	150
AD-1072289.1	CAGACACUGUCUGAGCAGGUU	40	AACCUGCUCAGACAGUGUCUGCA	175
AD-1072283.1	UGGGUGCAGACACUGUCUGAU	39	AUCAGACAGUGUCUGCACCCAGC	174
AD-1103871.1	CGGCCUCAGCGCCAUCCGCGU	97	ACGCGGAUGGCGCUGAGGCCGCG	232

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072183.1	CAGGCCAAGGUGGAGCAAGCU	25	AGCUUGCUCCACCUUGGCCUGGC	160
AD-1072667.1	CUGGUGGAAGACAUGCAGCGU	122	ACGCUGCAUGUCUUCCACCAGGG	257
AD-1072614.1	CGCGCCAAGCUGGAGGAGCAU	111	AUGCUCCUCCAGCUUGGCGCGCA	246
AD-1072632.1	CAGGCCCAGCAGAUACGCCUU	112	AAGGCGUAUCUGCUGGGCCUGCU	247
AD-1072211.1	CCCGAGCUGCGCCAGCAGACU	29	AGUCUGCUGGCGCAGCUCGGGCU	164
AD-1072406.1	GCACGGCUGUCCAAGGAGCUU	55	AAGCUCCUUGGACAGCCGUGCCC	190
AD-1103882.1	GCCCCUGUGCCCAGCGACAAU	127	AUUGUCGCUGGGCACAGGGGCGG	262
AD-1103855.1	GACAUGGAGGACGUGCGCGGU	64	ACCGCGCACGUCCUCCAUGUCCG	199
AD-1072726.1	GAAGCCUGCAGCCAUGCGACU	133	AGUCGCAUGGCUGCAGGCUUCGG	268
AD-1103893.1	UCCUGGGGUGGGACCCUAGUUU	144	AAACUAGGGUCCACCCCAGGAGG	279
AD-1072736.1	GGGUGGGACCCUAGUUUAAUAU	145	AUAUUAAACUAGGGUCCACCCCA	280
AD-1072426.1	GCGGACAUGGAGGACGUGUGU	60	ACACACGUCCUCCCAUGUCCGCGC	195
AD-1072506.1	GUAAGCGGCUCCUCCGCGAUU	88	AAUCGCGGAGGAGCCGCUUACGC	223
AD-1072248.1	AACUGGCACUGGGUCCGCUUUU	34	AAAAGCGACCCAGUGCCAGUUCC	169
AD-1072203.1	GGUGGAGACAGAGCCGGAGCU	28	AGCUCCGGCUCUGUCUCCACCGC	163
AD-1103888.1	CUCCUGCCUCCGCGCAGCCUU	139	AAGGCUGCGCGGAGGCAGGAGGC	274
AD-1072243.1	CUGGGAACUGGCACUGGGUCU	33	AGACCCAGUGCCAGUUCCCAGCG	168
AD-1103886.1	CCACCCCGUGCCUCCUGCCUU	137	AAGGCAGGAGGCACGGGGUGGCG	272
AD-1072347.1	UGAGGGGCGCUGAUGGACGAGU	45	ACUCGUCCAUCAGCGCCCUCAGU	180
AD-1072411.1	GCUGUCCAAGGAGCUGCAGGU	56	ACCUGCAGCUCCUUGGACAGCCG	191
AD-1072319.1	CUGCUCAGCUCCCAGGUCACU	42	AGUGACCUGGGAGCUGAGCAGCU	177
AD-1103885.1	CCCACGCCACCCCGUGCCUCU	136	AGAGGCACGGGGUGGCGUGGGGU	271
AD-1072488.1	CCCACCUGCGCAAGCUGCGUU	85	AACGCAGCUUGCGCAGGUGGGAG	220
AD-1072532.1	CUGCAGAAGCGCCUGGCAGUU	93	AACUGCCAGGCGCUUCUGCAGGU	228
AD-1072493.1	CUGCGCAAGCUGCGUAAGCGU	86	ACGCUUACGCAGCUUGCGCAGGU	221
AD-1072686.1	GCUGGUGGAGAAGGUGCAGGU	125	ACCUGCACCUUCUCCACCAGCCC	260
AD-1072656.1	CCAGGCCCGCCUCAAGAGCUU	118	AAGCUCUUGAGGCGGGCCUGGAA	253

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1103851.1	CGCGGACAUGGAGGACGUGCU	59	AGCACGUCCUCCAUGUCCGCGCC	194
AD-1072216.1	GCUGCGCCAGCAGACCGAGUU	30	AACUCGGUCUGCUGGCGCAGCUC	165
AD-1072455.1	GAGGUGCAGGCCAUGCUCGGU	79	ACCGAGCAUGGCCUGCACCUCGC	214
AD-1072581.1	CGCGACCGCCUGGACGAGGUU	107	AACCUCGUCCAGGCGGUCGCGGG	242
AD-1072499.1	AAGCUGCGUAAGCGGCUCCUU	87	AAGGAGCCGCUUACGCAGCUUGC	222
AD-1103854.1	GGACAUGGAGGACGUGCGCGU	63	ACGCGCACGUCCUCCAUGUCCGC	198
AD-1072672.1	GGAAGACAUGCAGCGCCAGUU	123	AACUGGCGCUGCAUGUCUUCCAC	258
AD-1072644.1	CGCCUGCAGGCCGAGGCCUUU	114	AAAGGCCUCGGCCUGCAGGCGUA	249
AD-1103850.1	GCCCGGCUGGGCGCGGACAUU	57	AAUGUCCGCGCCCAGCCGGGCCU	192
AD-1103875.1	CGCGUGCGGGCCGCCACUGUU	101	AACAGUGGCGGCCCGCACGCGGC	236
AD-1072401.1	CGCGGGCACGGCUGUCCAAGU	54	ACUUGGACAGCCGUGCCCGCGUC	189
AD-1072438.1	CGCCUGGUGCAGUACCGCGGU	78	ACCGCGGUACUGCACCAGGCGGC	213
AD-1072310.1	CAGGAGGAGCUGCUCAGCUCU	41	AGAGCUGAGGCAGCUCCUCCUGCA	176
AD-1072277.1	CUGCGCUGGGUGCAGACACUU	38	AAGUGUCUGCACCCAGCGCAGGU	173
AD-1103852.1	GCGGACAUGGAGGACGUGCGU	61	ACGCACGUCCUCCAUGUCCGCGC	196
AD-1072483.1	CGCCUCCCACCUGCGCAAGCU	84	AGCUUGCGCAGGUGGGAGGCGAG	219
AD-1072569.1	GCGCGGAUGGAGGAGAUGGGU	106	ACCCAUCUCCUCCAUCCGCGCGC	241
AD-1072473.1	GGCCAGAGCACCGAGGAGCUU	81	AAGCUCCUCGGUGCUCUGGCCGA	216
AD-1103868.1	GCUGCGGGUGCGCCUCGCCUU	82	AAGGCGAGGCGCACCCGCAGCUC	217
AD-1103858.1	AUGGAGGACGUGCGCGGCCGU	67	ACGGCCGCGCACGUCCUCCAUGU	202
AD-1103857.1	CAUGGAGGACGUGCGCGCCU	66	AGGCCGCGCACGUCCUCCAUGUC	201
AD-1103861.1	GAGGACGUGCGCGGCCGCCUU	70	AAGGCGGCCGCGCACGUCCUCCA	205
AD-1103891.1	CCCGCCCCAGCCGUCCUCCUU	142	AAGGAGGACGGCUGGGGCGGGGA	277
AD-1103870.1	GAGCGCGGCCUCAGCGCCAUU	96	AAUGGCGCUGAGGCCGCGCUCGG	231
AD-1103867.1	GUGCGCGGCCGCCUGGUGCAU	76	AUGCACCAGGCGGCCGCGCACGU	211
AD-1072433.1	GCGGCCGCCUGGUGCAGUACU	77	AGUACUGCACCAGGCGGCCGCAC	212
AD-1103872.1	AGCGCCAUCCGCGAGCGCCUU	98	AAGGCGCUCGCGGAUGGCGCUGA	233

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1103889.1	GCCUCCGCGCAGCCUGCAGCU	140	AGCUGCAGGCUGCGCGGAGGCAG	275
AD-1103864.1	GACGUGCGCGGCCGCCUGGUU	73	AACCAGGCGGCCGCGCACGUCCU	208
AD-1072464.1	GCCAUGCUCGGCCAGAGCACU	80	AGUGCUCUGGCCGAGCAUGGCCU	215
AD-1103862.1	AGGACGUGCGCGGCCGCCUGU	71	ACAGGCGGCCGCGCACGUCCUCC	206
AD-1072637.1	CCAGCAGAUACGCCUGCAGGU	113	ACCUGCAGGCGUAUCUGCUGGGC	248
AD-1072420.1	CUGGGCGCGGACAUGGAGGAU	58	AUCCUCCAUGUCCGCGCCCAGCC	193
AD-1072238.1	CAGCGCUGGGAACUGGCACUU	32	AAGUGCCAGUUCCCAGCGCUGGC	167
AD-1103866.1	CGUGCGCGGCCGCCUGGUGCU	75	AGCACCAGGCGGCCGCGCACGUC	210
AD-1103873.1	UGGGGCCCCCUGGUGGAACAGU	99	ACUGUUCCACCAGGGGCCCCAGG	234
AD-1103869.1	GUGCGCCUCCGCCUCCCACCUU	83	AAGGUGGGAGGCGAGGCGCACCC	218
AD-1072588.1	GCCUGGACGAGGUGAAGGAGU	108	ACUCCUUCACCUCGUCCAGGCGG	243
AD-1072544.1	CUGGCAGUGUACCAGGCCGGU	95	ACCGGCCUGGUACACUGCCAGGC	230
AD-1103859.1	UGGAGGACGUGCGCGGCCGCU	68	AGCGGCCGCGCACGUCCUCCAUG	203
AD-1103853.1	CGGACAUGGAGGACGUGCGCU	62	AGCGCACGUCCUCCAUGUCCGCG	197
AD-1103856.1	ACAUGGAGGACGUGCGCGGCU	65	AGCCGCGCACGUCCUCCAUGUCC	200
AD-1072607.1	GGAGGUGCGCGCCAAGCUGGU	110	ACCAGCUUGGCGCGCACCUCCGC	245
AD-1103860.1	GGAGGACGUGCGCGGCCGCCU	69	AGGCGGCCGCGCACGUCCUCCAU	204
AD-1103884.1	GCGACCCCACGCCACCCCGUU	135	AACGGGGUGGGCGUGGGGUCGCAU	270
AD-1072551.1	GCCGCCACUGUGGGGCUCCCUU	102	AAGGGAGCCCCACAGUGGCGGCCC	237
AD-1103874.1	CCCCUGGUGGGAACAGGGCCGU	100	ACGGCCCCUGUUCCACCAGGGGCC	235
AD-1103890.1	CCUGUCCCCCGCCCCAGCCGUU	141	AACGGCUGGGGGCGGGGACAGGGU	276
AD-1103881.1	ACCAGCGCCCGCCCCUGUGCCU	126	AGGCACAGGGGCGGCGCUGGUGC	261
AD-1103887.1	CGUGCCUCCUGCCUCCCGCGCU	138	AGCGCGGAGGCAGGAGGCACGGG	273
AD-1103863.1	GGACGUGCGCGGCCGCCUGGU	72	ACCAGGCGGCCGCGCACGUCCUC	207
AD-1072509.1	GGCUCCUCCGCGAUGCCGAUU	89	AAUCGGCAUCGCGGAGGAGCCGC	224
AD-1103892.1	CCCAGCCGUCCUCCUGGGGUU	143	AACCCCAGGAGGACGGCUGGGGC	278
AD-1103877.1	GCCGCUACAGGAGCGGGCCCU	105	AGGGCCCGCUCCUGUAGCGGCUG	240

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1103865.1	ACGUGCGCGGCCGCCUGGUGU	74	ACACCAGGCGGCCGCGCACGUCC	209
AD-1072649.1	GCAGGCCGAGGCCUUCCAGGU	115	ACCUGGAAGGCCUCGGCCUGCAG	250
AD-1103880.1	AGCCCCUGGUGGAAGACAUGU	121	ACAUGUCUUCCACCAGGGGCUCG	256
AD-1103878.1	CCGAGGCCUUCCAGGCCCGCU	116	AGCGGGCCUGGAAGGCCUCGGCC	251
AD-1072163.1	UCACAUUCCUGGCAGGAUGCU	23	AGCAUCCUGCCAGGAAUGUGACC	158
AD-1072681.1	GCCGGGCUGGUGGAGAAGGUU	124	AACCUUCUCCACCAGCCCGGCCC	259
AD-1072559.1	GGCCAGCCGCUACAGGAGCGU	104	ACGCUCCUGUAGCGGCUGGCCGG	239
AD-1072538.1	AAGCGCCUGGCAGUGUACCAU	94	AUGGUACACUGCCAGGCGCUUCU	229
AD-1103876.1	CCUGGCCGGCCAGCCGCUACU	103	AGUAGCGGCUGGCCGGCCAGGGA	238

Table 5. APOE Modified Duplex Sequences

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072375.1	gsgsaguuGfaAfGfGfccuacaaauu(L96)	319	asAfsuuuGfuAfGfgccuUfcAfacuccsusu	454
AD-1072352.1	gscsgcugAfuGfGfAfcgagaccauu(L96)	316	asAfsuggUfcUfCfguccAfuCfagcgcscsc	451
AD-1072394.1	uscsggaaCfuGfGfAfggaacaacuu(L96)	322	asAfsguuGfuUfCfcuccAfgUfuccgasusu	457
AD-1072721.1	ascsgccgAfaGfCfCfugcagccauu(L96)	402	asAfsuggCfuGfCfaggcUfuCfggcgususc	537
AD-1072254.1	csascuggGfuCfGfCfuuuugggauu(L96)	305	asAfsuccCfaAfAfagcgAfcCfcagugscsc	440
AD-1072189.1	asasggugGfaGfCfAfagcgguggau(L96)	296	asUfsccaCfcGfCfuugcUfcCfaccuusgsg	431
AD-1072741.1	gsascccuAfgUfUfUfaauaaagauu(L96)	416	asAfsucuUfuAfUfuaaaCfuAfgggucscsa	551
AD-1072382.1	asasggccUfaCfAfAfaucggaacuu(L96)	320	asAfsguuCfcGfAfuuugUfaGfgccuuscsa	455
AD-1072129.1	asgsgaagAfuGfAfAfgguucugugu(L96)	287	asCfsacaGfaAfCfcuucAfuCfuuccusgsc	422
AD-1072514.1	csusccgcGfaUfGfCfcgaugaccuu(L96)	360	asAfsgguCfaUfCfggcaUfcGfcggagsgsa	495
AD-1072124.1	csasggcaGfgAfAfGfaugaagguuu(L96)	286	asAfsaccUfuCfAfucuuCfcUfgccugsusg	421
AD-1072337.1	ascsccagGfaAfCfUfgagggcgcuu(L96)	314	asAfsgcgCfcCfUfcaguUfcCfugggusgsa	449
AD-1072135.1	asusgaagGfuUfCfUfgugggcugcu(L96)	288	asGfscagCfcCfAfcagaAfcCfuucauscsu	423
AD-1072745.1	usasguuuAfaUfAfAfagauucaccu(L96)	417	asGfsgugAfaUfCfuuuaUfuAfaacuasgsg	552

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072153.1	gscsgugCfuGfGfUfcacauuccuu(L96)	291	asAfsggaAfuGfUfgaccAfgCfaacgcsasg	426
AD-1072520.1	gsasugccGfaUfGfAfcugcagaau(L96)	361	asUfsucuGfcAfGfgucaUfcGfgcaucsgsc	496
AD-1072389.1	ascsaaauCfgGfAfAfcuggaggaau(L96)	321	asUfsuccUfcCfAfguucCfgAfuuugusasg	456
AD-1072716.1	ascsugaaCfgCfCfGfaagccugcau(L96)	401	asUfsgcaGfgCfUfucggCfgUfucagusgsa	536
AD-1072222.1	cscsagcaGfaCfCfGfaguggcagau(L96)	301	asUfscugCfcAfCfucggUfcUfgcuggcscsg	436
AD-1072367.1	ascscaugAfaGfGfAfguugaaggcu(L96)	318	asGfsccuUfcAfAfcuccUfuCfaugguscsu	453
AD-1103894.1	asgsauucAfcCfAfAfguuucacgcu(L96)	419	asGfscguGfaAfAfcuugGfuGfaaucususu	554
AD-1072651.1	gscscuucCfaGfGfCfcgccucaau(L96)	387	asUfsugaGfgCfGfggccUfgGfaaggcscsu	522
AD-1072260.1	gsuscgcuUfuUfGfGfgauuaccugu(L96)	306	asCfsaggUfaAfUfcccaAfaAfgcgacscsc	441
AD-1072265.1	ususuuggGfaUfUfAfccugcgcugu(L96)	307	asCfsagcGfcAfGfguaaUfcCfcaaaasgsc	442
AD-1072750.1	usasauaaAfgAfUfUfcaccaaguuu(L96)	418	asAfsacuUfgGfUfgaauCfuUfuauuasasa	553
AD-1072142.1	ususcuguGfgGfCfUfgcguugcugu(L96)	289	asCfsagcAfaCfGfcagcCfcAfcagaascsc	424
AD-1072328.1	uscscccagGfuCfAfCfccaggaacuu(L96)	313	asAfsguuCfcUfGfggugAfcCfugggasgsc	448
AD-1072198.1	csasagcgGfuGfGfAfgacagagccu(L96)	297	asGfsgcuCfuGfUfcuccAfcCfgcuugscsu	432
AD-1072361.1	gsascgagAfcCfAfUfgaaggaguuu(L96)	317	asAfsacuCfcUfUfcaugGfuCfucgucscsa	452
AD-1072526.1	gsasugacCfuGfCfAfgaagcgccuu(L96)	362	asAfsggcGfcUfUfcugcAfgGfucaucsgsg	497
AD-1072699.1	usgsugccCfaGfCfGfacaaucacuu(L96)	398	asAfsgugAfuUfGfucgcUfgGfgcacasgsg	533
AD-1072711.1	csasaucaCfuGfAfAfcgccgaagcu(L96)	400	asGfscuuCfgGfCfguucAfgUfgauugsusc	535
AD-1103879.1	csasagagCfuGfGfUfucgagcccccu(L96)	390	asGfsgggCfuCfCfGfaaccAfgCfucuugsasg	525
AD-1103849.1	gsasggaaCfaAfCfUfgaccccgguu(L96)	323	asAfsccgGfgGfUfcaguUfgUfuccucscsa	458
AD-1072662.1	cscsgccuCfaAfGfAfgcugguucgu(L96)	389	asCfsgaaCfcAfGfcucuUfgAfggcgggsgsc	524
AD-1103883.1	usgscagcCfaUfGfGfCfgaccccacgu(L96)	404	asCfsgugGfgGfUfcgcaUfgGfcugcasgsg	539
AD-1072172.1	usgsgcagGfaUfGfGfCfcaggccaagu(L96)	294	asCfsuugGfcCfUfggcaUfcCfugccasgsg	429
AD-1072158.1	gscsugguCfaCfAfUfuccuggcagu(L96)	292	asCfsugcCfaGfGfaaugUfgAfccagcsasa	427
AD-1072705.1	csasgcgaCfaAfUfCfacugaacgcu(L96)	399	asGfscguUfcAfGfugauUfgUfcgcugsgsg	534
AD-1072147.1	usgsggcuGfcGfUfUfgcuggucacu(L96)	290	asGfsugaCfcAfGfcaacGfcAfgcccascsa	425
AD-1072593.1	gsascgagGfuGfAfAfggagcagguu(L96)	379	asAfsccuGfcUfCfcuucAfcCfucgucscsa	514

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072115.1	gsgsccaaUfcAfCfAfggcaggaagu(L96)	285	asCfsuucCfuGfCfcuguGfaUfuggccsasg	420
AD-1072289.1	csasgacaCfuGfUfCfugagcagguu(L96)	310	asAfsccuGfcUfCfagacAfgUfgucugscsa	445
AD-1072283.1	usgsggugCfaGfAfCfacugucugau(L96)	309	asUfscagAfcAfGfugucUfgCfacccasgsc	444
AD-1103871.1	csgsgccuCfaGfCfGfccauccgcgu(L96)	367	asCfsgcgGfaUfGfgcgcUfgAfggccgscsg	502
AD-1072183.1	csasggccAfaGfGfUfggagcaagcu(L96)	295	asGfscuuGfcUfCfcaccUfuGfgccugsgsc	430
AD-1072667.1	csusggugGfaAfGfAfcaugcagcgu(L96)	392	asCfsgcuGfcAfUfgucuUfcCfaccagsgsg	527
AD-1072614.1	csgscgccAfaGfCfUfggaggagcau(L96)	381	asUfsgcuCfcUfCfcagcUfuGfgcgcgscsa	516
AD-1072632.1	csasggccCfaGfCfAfgauacgccuu(L96)	382	asAfsggcGfuAfUfcugcUfgGfgccugscsu	517
AD-1072211.1	cscscgagCfuGfCfGfccagcagacu(L96)	299	asGfsucuGfcUfGfgcgcAfgCfucgggscsu	434
AD-1072406.1	gscsacggCfuGfUfCfcaaggagccuu(L96)	325	asAfsgcuCfcUfUfggacAfgCfcgugcscsc	460
AD-1103882.1	gscscccuGfuGfCfCfcagcgacaau(L96)	397	asUfsuguCfgCfUfgggcAfcAfgggcgsgsg	532
AD-1103855.1	gsascaugGfaGfGfAfcgugcgcggu(L96)	334	asCfscgcGfcAfCfguccUfcCfaugucscsg	469
AD-1072726.1	gsasagccUfgCfAfGfccaugcgacu(L96)	403	asGfsucgCfaUfGfgcugCfaGfgcuucsgsg	538
AD-1103893.1	uscscuggGfgUfGfGfcaccuaguuu(L96)	414	asAfsacuAfgGfGfuccaCfcCfcaggasgsg	549
AD-1072736.1	gsgsguggAfcCfCfUfaguuuaauau(L96)	415	asUfsauuAfaAfCfuaggGfuCfcacccscsa	550
AD-1072426.1	gscsggacAfuGfGfAfggacgugugu(L96)	330	asCfsacaCfgUfCfcuccAfuGfuccgcsgsc	465
AD-1072506.1	gsusaagcGfgCfUfCfcuccgcgauu(L96)	358	asAfsucgCfgGfAfggagCfcGfcuuacsgsc	493
AD-1072248.1	asascuggCfaCfUfGfggucgcuuuu(L96)	304	asAfsaagCfgAfCfccagUfgCfcaguuscsc	439
AD-1072203.1	gsgsuggaGfaCfAfGfagccggagcu(L96)	298	asGfscucCfgGfCfucugUfcUfccaccsgsc	433
AD-1103888.1	csusccugCfcUfCfCfgcgcagccuu(L96)	409	asAfsggcUfgCfGfcggaGfgCfaggagsgsc	544
AD-1072243.1	csusgggaAfcUfGfGfcacugggucu(L96)	303	asGfsaccCfaGfUfgccaGfuUfcccagscsg	438
AD-1103886.1	cscsacccCfgUfGfCfcuccugccuu(L96)	407	asAfsggcAfgGfAfggcaCfgGfgguggscsg	542
AD-1072347.1	usgsagggCfgCfUfGfauggacgagu(L96)	315	asCfsucgUfcCfAfucagCfgCfccucasgsu	450
AD-1072411.1	gscsugucCfaAfGfGfagcugcaggu(L96)	326	asCfscugCfaGfCfuccuUfgGfacagcscsg	461
AD-1072319.1	csusgcucAfgCfUfCfccaggucacu(L96)	312	asGfsugaCfcUfGfggagCfuGfagcagscsu	447
AD-1103885.1	cscscacgCfcAfCfCfccgugccucu(L96)	406	asGfsaggCfaCfGfggguGfgCfguggggsgsu	541
AD-1072488.1	cscscaccUfgCfGfCfaagcugcguu(L96)	355	asAfscgcAfgCfUfugcgCfaGfgugggsasg	490

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1072532.1	csusgcagAfaGfCfGfccuggcaguu(L96)	363	asAfscugCfcAfGfgcgcUfuCfugcagsgsu	498
AD-1072493.1	csusgcgcAfaGfCfUfgcguaagcgu(L96)	356	asCfsgcuUfaCfGfcagcUfuGfcgcagsgsu	491
AD-1072686.1	gscsugguGfgAfGfAfaggugcaggu(L96)	395	asCfscugCfaCfCfuucuCfcAfccagcscsc	530
AD-1072656.1	cscsaggcCfcGfCfCfucaagagcuu(L96)	388	asAfsgcuCfuUfGfaggcGfgGfccuggsasa	523
AD-1103851.1	csgscggaCfaUfGfGfaggacgugcu(L96)	329	asGfscacGfuCfCfuccaUfgUfccgcgcscsc	464
AD-1072216.1	gscsugcgCfcAfGfCfagaccgaguu(L96)	300	asAfscucGfgUfCfugcuGfgCfgcagcsusc	435
AD-1072455.1	gsasggugCfaGfGfCfcaugcucggu(L96)	349	asCfscgaGfcAfUfggccUfgCfaccucsgsc	484
AD-1072581.1	csgscgacCfgCfCfUfggacgagguu(L96)	377	asAfsccuCfgUfCfcaggCfgGfucgcgsgsg	512
AD-1072499.1	asasgcugCfgUfAfAfgcggcuccuu(L96)	357	asAfsggaGfcCfGfcuuaCfgCfagcuusgsc	492
AD-1103854.1	gsgsacauGfgAfGfGfacgugcgcgu(L96)	333	asCfsgcgCfaCfGfuccuCfcAfuguccsgsc	468
AD-1072672.1	gsgsaagaCfaUfGfCfagcgccaguu(L96)	393	asAfscugGfcGfCfugcaUfgUfcuuccsasc	528
AD-1072644.1	csgsccugCfaGfGfCfcgaggccuuu(L96)	384	asAfsaggCfcUfCfggccUfgCfaggcgsusa	519
AD-1103850.1	gscsccggCfuGfGfGfcgcggacauu(L96)	327	asAfsuguCfcGfCfgcccAfgCfcgggcscsu	462
AD-1103875.1	csgscgugCfgGfGfCfcgccacuguu(L96)	371	asAfscagUfgGfCfggccCfgCfacgcgsgsc	506
AD-1072401.1	csgscgggCfaCfGfGfcuguccaagu(L96)	324	asCfsuugGfaCfAfgccgUfgCfccgcgsusc	459
AD-1072438.1	csgsccugGfuGfCfCfAfguaccgcggu(L96)	348	asCfscgcGfgUfAfcugcAfcCfaggcgsgsc	483
AD-1072310.1	csasggagGfaGfCfCfUfgcucagcucu(L96)	311	asGfsagcUfgAfGfcagcUfcCfuccugscsa	446
AD-1072277.1	csusgcgcUfgGfGfGfUfgcagacacuu(L96)	308	asAfsgugUfcUfGfcaccCfaGfcgcagsgsu	443
AD-1103852.1	gscsggacAfuGfGfGfAfggacgugcgu(L96)	331	asCfsgcaCfgUfUfCfcuccAfuGfuccgcsgsc	466
AD-1072483.1	csgsccucCfcAfCfCfUfugcgcaagcu(L96)	354	asGfscuuGfcGfCfagguGfgGfaggcgsasg	489
AD-1072569.1	gscsgcggAfuGfGfGfAfggagaugggu(L96)	376	asCfsccaUfcUfCfcuccAfuCfcgcgcsgsc	511
AD-1072473.1	gsgsccagAfgCfAfCfCfgaggagcuu(L96)	351	asAfsgcuCfcUfUfCfggugCfuCfuggccsgsa	486
AD-1103868.1	gscsugcgGfgUfGfCfCfgccucgccuu(L96)	352	asAfsggcGfaGfGfCfgcaCfcCfgcagcsusc	487
AD-1103858.1	asusggagGfaCfGfUfUfgcgcggccgu(L96)	337	asCfsggcCfgCfGfcacgUfcCfuccausgsu	472
AD-1103857.1	csasuggaGfgAfCfGfUfugcgcggccu(L96)	336	asGfsgccGfcGfCfacguCfcUfccaugsusc	471
AD-1103861.1	gsasggacGfuGfCfCfGfcggccgccuu(L96)	340	asAfsggcGfgCfCfgcgcAfcGfuccucscsa	475
AD-1103891.1	cscscgccCfcAfGfCfcguccuccuu(L96)	412	asAfsggaGfgAfCfggcuGfgGfgcgggsgsa	547

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1103870.1	gsasgcgcGfgCfCfUfcagcgccauu(L96)	366	asAfsuggCfgCfUfgaggCfcGfcgcucsgsg	501
AD-1103867.1	gsusgcgcGfgCfCfGfccuggugcau(L96)	346	asUfsgcaCfcAfGfgcggCfcGfcgcacsgsu	481
AD-1072433.1	gscsggccGfcCfUfGfgugcaguacu(L96)	347	asGfsuacUfgCfAfccagGfcGfgccgcsasc	482
AD-1103872.1	asgscgccAfuCfCfGfcgagcgccuu(L96)	368	asAfsggcGfcUfCfgcggAfuGfgcgcusgsa	503
AD-1103889.1	gscscuccGfcGfCfAfgccugcagcu(L96)	410	asGfscugCfaGfGfcugcGfcGfgaggcsasg	545
AD-1103864.1	gsascgugCfgCfGfGfcgccuggguu(L96)	343	asAfsccaGfgCfGfgccgCfgCfacgucscsu	478
AD-1072464.1	gscscaugCfuCfGfGfccagagcacu(L96)	350	asGfsugcUfcUfGfgccgAfgCfauggcscsu	485
AD-1103862.1	asgsgacgUfgCfGfCfgccgccugu(L96)	341	asCfsaggCfgGfCfcgcgCfaCfguccuscsc	476
AD-1072637.1	cscsagcaGfaUfAfCfgccugcaggu(L96)	383	asCfscugCfaGfGfcguaUfcUfgcuggsgsc	518
AD-1072420.1	csusgggcGfcGfGfAfcauggaggau(L96)	328	asUfsccuCfcAfUfguccGfcGfcccagscsc	463
AD-1072238.1	csasgcgcUfgGfGfAfacuggcacuu(L96)	302	asAfsgugCfcAfGfuuccCfaGfcgcugsgsc	437
AD-1103866.1	csgsugcgCfgGfCfCfgccuggugcu(L96)	345	asGfscacCfaGfGfcggcCfgCfgcacgsusc	480
AD-1103873.1	usgsgggcCfcCfUfGfguggaacagu(L96)	369	asCfsuguUfcCfAfccagGfgGfccccasgsg	504
AD-1103869.1	gsusgcgcCfuCfGfCfCfcucccaccuu(L96)	353	asAfsgguGfgGfAfggcgAfgGfcgcacscsc	488
AD-1072588.1	gscscuggAfcGfAfGfgugaaggagu(L96)	378	asCfsuccUfuCfAfccucGfuCfcaggcsgsg	513
AD-1072544.1	csusggcaGfuGfUfAfccaggccggu(L96)	365	asCfscggCfcUfGfguacAfcUfgccagsgsc	500
AD-1103859.1	usgsgaggAfcGfUfGfcgcggccgcu(L96)	338	asGfscggCfcGfCfgcacGfuCfcuccasusg	473
AD-1103853.1	csgsgacaUfgGfAfGfgacgugcgcu(L96)	332	asGfscgcAfcGfUfccucCfaUfguccgscsg	467
AD-1103856.1	ascsauggAfgGfAfCfgugcgcggcu(L96)	335	asGfsccgCfgCfAfcgucCfuCfcauguscsc	470
AD-1072607.1	gsgsagguGfcGfCfCfGfccaagcuggu(L96)	380	asCfscagCfuUfGfgcgcGfcAfccuccsgsc	515
AD-1103860.1	gsgsaggaCfgUfGfCfgcggccgccu(L96)	339	asGfsgcgGfcCfGfcgcaCfgUfccuccsasu	474
AD-1103884.1	gscsgaccCfcAfCfGfccaccccguu(L96)	405	asAfscggGfgUfGfgcguGfgGfgucgcsasu	540
AD-1072551.1	gscscgccAfcUfGfUfgggcucccuu(L96)	372	asAfsgggAfgCfCfcacaGfuGfgcggcscsc	507
AD-1103874.1	cscsccugGfuGfGfAfacagggccgu(L96)	370	asCfsggcCfcUfGfuuccAfcCfaggggcscsc	505
AD-1103890.1	cscsugucCfcCfGfCfcccagccguu(L96)	411	asAfscggCfuGfGfgcgGfgGfacaggsgsu	546
AD-1103881.1	ascscagcGfcCfGfCfcccugugccu(L96)	396	asGfsgcaCfaGfGfggcgGfcCfgcuggusgsc	531
AD-1103887.1	csgsugccUfcCfUfGfccuccgcgcu(L96)	408	asGfscgcGfgAfGfgcagGfaGfgcacgsgsg	543

Duplex ID	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:
AD-1103863.1	gsgsacguGfcGfCfGfgccgccuggu(L96)	342	asCfscagGfcGfGfccgcGfcAfcguccsusc	477
AD-1072509.1	gsgscuccUfcCfGfCfgaugccgauu(L96)	359	asAfsucgGfcAfUfcgcgGfaGfgagccsgsc	494
AD-1103892.1	cscscagcCfgUfCfCfuccugggguu(L96)	413	asAfscccCfaGfGfaggaCfgGfcugggsgsc	548
AD-1103877.1	gscscgcuAfcAfGfGfagcgggcccu(L96)	375	asGfsggcCfcGfCfuccuGfuAfgcggcsusg	510
AD-1103865.1	ascsgugcGfcGfGfCfcgccuggugu(L96)	344	asCfsaccAfgGfCfggccGfcGfcacguscsc	479
AD-1072649.1	gscsaggcCfgAfGfGfccuuccaggu(L96)	385	asCfscugGfaAfGfgccuCfgGfccugcsasg	520
AD-1103880.1	asgsccccUfgGfUfGfgaagacaugu(L96)	391	asCfsaugUfcUfUfccacCfaGfgggcuscsg	526
AD-1103878.1	cscsgaggCfcUfUfCfcaggcccgcu(L96)	386	asGfscggGfcCfUfggaaGfgCfcucggscsc	521
AD-1072163.1	uscsacauUfcCfUfGfgcaggaugcu(L96)	293	asGfscauCfcUfGfccagGfaAfugugascsc	428
AD-1072681.1	gscscgggCfuGfGfUfggagaagguu(L96)	394	asAfsccuUfcUfCfcaccAfgCfccggcscsc	529
AD-1072559.1	gsgsccagCfcGfCfUfacaggagcgu(L96)	374	asCfsgcuCfcUfGfuagcGfgCfuggccsgsg	509
AD-1072538.1	asasgcgcCfuGfGfCfaguguaccau(L96)	364	asUfsgguAfcAfCfugccAfgGfcgcuuscsu	499
AD-1103876.1	cscsuggcCfgGfCfCfagccgcuacu(L96)	373	asGfsuagCfgGfCfuggcCfgGfccaggsgsa	508

Table 7. Selected APOE Unmodified Sense and Antisense Strand Sequences From Table 2 Targeting the Pathogenic APOE4 Allele

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
CGCGGACAUGGAGGACGUGCU	59	AGCACGUCCUCCAUGUCCGCGCC	194	NM_000041.4_438-458_U20C_G21U_s	NM_000041.4_436-458_C1A_A2G_as
GCGGACAUGGAGGACGUGCGU	61	ACGCACGUCCUCCAUGUCCGCGC	196	NM_000041.4_439-459_U19C_C21U_s	NM_000041.4_437-459_G1A_A3G_as
CGGACAUGGAGGACGUGCGCU	62	AGCGCACGUCCUCCAUGUCCGCG	197	NM_000041.4_440-460_U18C_G21U_s	NM_000041.4_438-460_C1A_A4G_as
GGACAUGGAGGACGUGCGCGU	63	ACGCGCACGUCCUCCAUGUCCGC	198	NM_000041.4_441-461_U17C_G21U_s	NM_000041.4_439-461_C1A_A5G_as
GACAUGGAGGACGUGCGCGGU	64	ACCGCGCACGUCCUCCAUGUCCG	199	NM_000041.4_442-	NM_000041.4_440-

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Sense Strand Target Site in NM_000041.4	Antisense Strand Target Site in NM_000041.4
				462_U16C_C21U_s	462_G1A_A6G_as
ACAUGGAGGACGUGCGCGGCU	65	AGCCGCGCACGUCCUCCAUGUCC	200	NM_000041.4_443-463_U15C_C21U_s	NM_000041.4_441-463_G1A_A7G_as
CAUGGAGGACGUGCGCGGCCU	66	AGGCCGCGCACGUCCUCCAUGUC	201	NM_000041.4_444-464_U14C_G21U_s	NM_000041.4_442-464_C1A_A8G_as
AUGGAGGACGUGCGCGGCCGU	67	ACGGCCGCGCACGUCCUCCAUGU	202	NM_000041.4_445-465_U13C_C21U_s	NM_000041.4_443-465_G1A_A9G_as
UGGAGGACGUGCGCGGCCGCU	68	AGCGGCCGCGCACGUCCUCCAUG	203	NM_000041.4_446-466_U12C_C21U_s	NM_000041.4_444-466_G1A_A10G_as
GGAGGACGUGCGCGGCCGCU	69	AGGCGGCCGCGCACGUCCUCCAU	204	NM_000041.4_447-467_U11C_s	NM_000041.4_445-467_A11G_as
GAGGACGUGCGCGGCCGCCUU	70	AAGGCGGCCGCGCACGUCCUCCA	205	NM_000041.4_448-468_U10C_G21U_s	NM_000041.4_446-468_C1A_A12G_as
AGGACGUGCGCGGCCGCCUGU	71	ACAGGCGGCCGCGCACGUCCUCC	206	NM_000041.4_449-469_U9C_G21U_s	NM_000041.4_447-469_C1A_A13G_as
GGACGUGCGCGGCCGCCUGGU	72	ACCAGGCGGCCGCGCACGUCCUC	207	NM_000041.4_450-470_U8C_s	NM_000041.4_448-470_A14G_as
GACGUGCGCGGCCGCCUGGUU	73	AACCAGGCGGCCGCGCACGUCCU	208	NM_000041.4_451-471_U7C_G21U_s	NM_000041.4_449-471_C1A_A15G_as
ACGUGCGCGGCCGCCUGGUGU	74	ACACCAGGCGGCCGCGCACGUCC	209	NM_000041.4_452-472_U6C_C21U_s	NM_000041.4_450-472_G1A_A16G_as
CGUGCGCGGCCGCCUGGUGCU	75	AGCACCAGGCGGCCGCGCACGUC	210	NM_000041.4_453-473_U5C_A21U_s	NM_000041.4_451-473_U1A_A17G_as
GUGCGCGGCCGCCUGGUGCAU	76	AUGCACCAGGCGGCCGCGCACGU	211	NM_000041.4_454-474_U4C_G21U_s	NM_000041.4_452-474_C1A_A18G_as

Table 8. Selected APOE Modified Sense and Antisense Strand Sequences From Table 2 Targeting the Pathogenic APOE4 Allele

Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
csgscggaCfaUfGfGfaggacgugcguL96	329	asGfscacGfuCfCfuccaUfgUfccgcgscsc	464	GGCGCCGGACATGGAGGACGTGCG	599
gscsggacAfuGfGfAfggacgugcguL96	331	asCfsgcaCfgUfCfcuccAfuGfuccgcsgsc	466	GCGCCGGACATGGAGGACGTGCGC	601
csgsgacaUfgGfAfGfgacgugcgcuL96	332	asGfscgcAfcGfUfccucCfaUfguccgscsg	467	CGCGGACATGGAGGACGTGCGCG	602
gsgsacauGfgAfGfGfacgugcgcguL96	333	asCfsgcgCfaCfGfuccuCfcAfuguccsgsc	468	GCGGACATGGAGGACGTGCGCGG	603
gsascaugGfaGfGfAfcgugcgcgguL96	334	asCfscgcGfcAfCfguccUfcCfaugucscsg	469	CGGACATGGAGGACGTGCGCGGC	604
ascsauggAfgGfAfCfgugcgcggcuL96	335	asGfsccgCfgCfAfcgucCfuCfcaguscsc	470	GGACATGGAGGACGTGCGCGGCC	605
csasuggaGfgAfCfGfugcgcggccuL96	336	asGfsgccGfcGfGfCfacguCfcUfccaugsusc	471	GACATGGAGGACGTGCGCGGCCG	606
asusggagGfaCfGfUfgcgcggccguL96	337	asCfsggcCfgCfGfcacgUfcCfuccausgsu	472	ACATGGAGGACGTGCGCGGCCGC	607
usgsgaggAfcGfUfGfcgcggccguL96	338	asGfscggCfcGfCfgcacGfuCfcuccasusg	473	CATGGAGGACGTGCGCGGCCGCC	608
gsgsaggaCfgUfGfCfgcggccguL96	339	asGfsgcgGfcCfGfcgcaCfgUfccuccsasu	474	ATGGAGGACGTGCGCGGCCGCCT	609
gsasggacGfuGfCfGfcggccguL96	340	asAfsggcGfgCfCfgcgcAfcGfuccucscsa	475	TGGAGGACGTGCGCGGCCGCCTG	610
asgsgacgUfgCfGfCfggccguL96	341	asCfsaggCfgGfCfcgcgCfaCfguccuscsc	476	GGAGGACGTGCGCGGCCGCCTGG	611
gsgsacguGfcGfCfGfgccguL96	342	asCfscagGfcGfGfccgcGfcAfcguccsusc	477	GAGGACGTGCGCGGCCGCCTGGT	612
gsascgugCfgCfGfGfccguL96	343	asAfsccaGfgCfGfgccgCfgCfacgucscsu	478	AGGACGTGCGCGGCCGCCTGGTG	613
ascsgugcGfcGfGfCfcguL96	344	asCfsaccAfgGfCfggccGfcGfcacguscsc	479	GGACGTGCGCGGCCGCCTGGTGC	614
csgsugcgCfgGfCfCfgccuL96	345	asGfscacCfaGfGfcggcCfgCfgcacgsusc	480	GACGTGCGCGGCCGCCTGGTGCA	615
gsusgcgcGfgCfCfGfccuL96	346	asUfsgcaCfcAfGfgcggCfcGfcgcacsgsu	481	ACGTGCGCGGCCGCCTGGTGCAG	616

Table 6. 10 nM *In Vitro* Screening Data

Duplex ID	Mean	SD
AD-1072375.1	1.4	0.6
AD-1072352.1	2.4	0.3
AD-1072394.1	2.9	0.3
AD-1072721.1	3.2	0.5
AD-1072254.1	3.2	0.0
AD-1072189.1	3.5	0.3
AD-1072741.1	3.5	0.8
AD-1072382.1	3.7	1.0
AD-1072129.1	4.0	0.5
AD-1072514.1	4.2	0.3
AD-1072124.1	4.2	0.8
AD-1072337.1	4.3	0.6
AD-1072135.1	4.6	0.9
AD-1072745.1	4.7	1.1
AD-1072153.1	5.0	0.3
AD-1072520.1	5.7	1.2
AD-1072389.1	6.5	1.6
AD-1072716.1	6.5	1.3
AD-1072222.1	6.8	0.4
AD-1072367.1	7.1	1.0
AD-1103894.1	9.2	1.3
AD-1072651.1	9.2	0.8
AD-1072260.1	9.4	2.0
AD-1072265.1	9.4	0.9
AD-1072750.1	9.9	1.8
AD-1072142.1	10.1	1.3
AD-1072328.1	10.2	2.3
AD-1072198.1	10.3	1.7
AD-1072361.1	11.2	0.6
AD-1072526.1	11.4	1.5
AD-1072699.1	11.8	1.4
AD-1072711.1	11.8	4.0
AD-1103879.1	11.9	1.6
AD-1103849.1	12.1	0.4
AD-1072662.1	12.6	0.8
AD-1103883.1	12.7	3.2
AD-1072172.1	13.2	1.4
AD-1072158.1	15.4	5.8
AD-1072705.1	15.7	2.5
AD-1072147.1	16.6	0.7
AD-1072593.1	16.8	2.3
AD-1072115.1	17.0	0.4

Duplex ID	Mean	SD
AD-1072289.1	18.6	6.5
AD-1072283.1	18.9	1.6
AD-1103871.1	19.2	4.2
AD-1072183.1	20.2	0.5
AD-1072667.1	20.3	4.5
AD-1072614.1	20.4	1.7
AD-1072632.1	21.0	1.3
AD-1072211.1	21.2	7.2
AD-1072406.1	21.3	0.4
AD-1103882.1	21.9	0.9
AD-1103855.1	22.0	5.9
AD-1072726.1	22.8	1.5
AD-1103893.1	26.0	2.3
AD-1072736.1	31.5	5.0
AD-1072426.1	32.4	5.5
AD-1072506.1	33.7	6.4
AD-1072248.1	34.2	2.1
AD-1072203.1	35.4	3.7
AD-1103888.1	35.5	11.3
AD-1072243.1	35.8	0.6
AD-1103886.1	38.9	1.2
AD-1072347.1	39.4	3.1
AD-1072411.1	45.5	2.6
AD-1072319.1	53.0	17.3
AD-1103885.1	57.0	1.6
AD-1072488.1	57.3	4.4
AD-1072532.1	60.1	1.8
AD-1072493.1	60.5	5.4
AD-1072686.1	64.8	2.5
AD-1072656.1	66.4	7.3
AD-1103851.1	66.6	5.4
AD-1072216.1	66.6	5.1
AD-1072455.1	67.3	4.3
AD-1072581.1	68.5	9.7
AD-1072499.1	69.5	10.1
AD-1103854.1	70.4	4.7
AD-1072672.1	72.6	4.0
AD-1072644.1	73.6	5.8
AD-1103850.1	74.2	5.0
AD-1103875.1	74.5	2.4
AD-1072401.1	79.5	24.3
AD-1072438.1	79.5	5.7
AD-1072310.1	79.6	3.4

Duplex ID	Mean	SD
AD-1072277.1	81.9	5.0
AD-1103852.1	82.0	15.0
AD-1072483.1	86.5	3.1
AD-1072569.1	86.6	12.8
AD-1072473.1	87.1	5.6
AD-1103868.1	88.8	4.6
AD-1103858.1	88.9	4.4
AD-1103857.1	89.8	3.4
AD-1103861.1	90.3	6.5
AD-1103891.1	90.5	2.9
AD-1103870.1	92.2	5.7
AD-1103867.1	92.5	6.5
AD-1072433.1	92.5	6.5
AD-1103872.1	93.0	6.5
AD-1103889.1	93.6	3.6
AD-1103864.1	93.7	2.3
AD-1072464.1	94.1	6.3
AD-1103862.1	94.1	4.8
AD-1072637.1	94.7	12.4
AD-1072420.1	95.0	8.6
AD-1072238.1	95.4	5.0
AD-1103866.1	95.9	2.6
AD-1103873.1	96.5	5.7
AD-1103869.1	97.2	3.1
AD-1072588.1	97.8	5.4
AD-1072544.1	98.0	8.7
AD-1103859.1	98.0	6.2
AD-1103853.1	98.3	5.6
AD-1103856.1	99.3	5.1
AD-1072607.1	99.5	6.1
AD-1103860.1	99.6	16.5
AD-1103884.1	100.0	3.0
AD-1072551.1	101.2	3.1
AD-1103874.1	101.3	3.6
AD-1103890.1	103.4	3.4
AD-1103881.1	104.3	5.2
AD-1103887.1	104.9	2.6
AD-1103863.1	105.6	8.2
AD-1072509.1	106.5	19.7
AD-1103892.1	106.6	8.2
AD-1103877.1	106.9	12.2
AD-1103865.1	107.0	1.9
AD-1072649.1	108.5	3.9

Duplex ID	Mean	SD
AD-1103880.1	109.2	3.3
AD-1103878.1	110.2	5.8
AD-1072163.1	111.3	16.3
AD-1072681.1	114.6	6.8
AD-1072559.1	114.9	7.2
AD-1072538.1	119.3	8.0
AD-1103876.1	120.4	16.4

Example 2. *In Vivo* Evaluation in Transgenic Mice

This Example describes methods for the *in vivo* evaluation of APOE RNAi agents in the APPPS1-21 transgenic mouse model of Alzheimer's disease. These mice overexpress human amyloid precursor protein (APP) cDNA with a Swedish mutation (KM670/671NL) and mutant PS1 with the L166P mutation, The endogenous ApoE gene in these mice was replaced with either the human APOE3 allele or the human APOE4 allele (Huynh, et al. (2017) *Neuron* 96: 1013-1023).

The ability of selected dsRNA agents designed and assayed in Example 1 are assessed for their ability to reduce the level of APOE expression, *e.g.,* APOE2, APOE3, and APOE4 expression, in the brain and the liver of these animals.

Briefly, littermates are intrathecally or subcutaneously administered a single dose of the dsRNA agents of interest, or a placebo. Two weeks after administration, animals are sacrificed, blood and tissue samples, including cerebral cortex, spinal cord, liver, spleen, and cervical lymph nodes, are collected.

To determine the effect of administration of the dsRNA agents targeting APOE on the level APOE mRNA, mRNA levels are determined in cortex and liver samples by qRT-PCR.

The effect of administration of the agents targeting APOE on the pathology of Alzheimer's disease in this mouse model is also assessed as described in Huynh, *et al.* (*supra*).

Littermates are intrathecally or subcutaneously administered two doses of the dsRNA agents of interest, or a placebo, at birth and 8 weeks of age. At 16 weeks of age, animals are sacrificed, and blood and tissue samples, including cerebral cortex, spinal cord, liver, spleen, and cervical lymph nodes, are collected and appropriately processed.

The effect of the dsRNA agents on the deposition of Aβ plaques, the accumulation of Aβ, total neuritic dystrophy, the plaque size and the plaquedistribution are assessed. Briefly, for immunofluorescence analysis of tissue samples, after fixation and following immersion in sucrose for at least 24 hours, serial coronal sections are collected from frontal cortex to caudal hippocampus (right hemisphere) using a microtome. Three hippocampal-containing sections from the right hemisphere of each brain are stained with X34 dye to visualize fibrillary plaques or with commercially available antibodies against amyloid-β (such as 82E1) and corresponding fluorescently-labeled secondary antibodies. For analysis, stained sections are scanned at 20x maginification with a confocal microscope. Random windows containing clusters of plaques are

captured, spanning the same thickness in the z-plane for all sections. Using suitable software, the volume of the markers are quantified under the same threshold. Each data point represents the average value from three separate tissue sections from one single animal.

Example 3. *In Vivo* Evaluation in Humanized APOE Mice

Humanized ApoE4 knock-in mice (purchased from Jax; stock # 027894) for this study were generated by replacing exons 2, 3 and most of exon 4 of the mouse Apoe gene with the human APOE4 gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence) using standard techniques.

To assess the *in vivo* efficacy of duplexes of interest, at Day 0, 9-12 week old, male and female, Ketamine/ Xylazine anesthetized homozygous humanized APOE knock-in mice were administered a single 300 μg dose of AD-1204704, AD-1204705, AD-1204705, AD-1204706 AD-1204707, AD-1204708, AD-1204709, AD-1204710, AD-1204711, AD-1204712, or AD-1204713, or artificial CSF (aCSF) control by intracerebroventricular injection (ICV) into the right ventricle using a 25 μl Hamilton syringe and a custom angled 3.5mm needle.

Table 9 provides a detailed list of the unmodified APOE sense and antisense strand sequences of the agents used in this example and Table 10 provides a detailed list of the modified APOE sense and antisense strand sequences of the agents used in this example.

At day 14 post-dose, animals were sacrificed, both hemispheres of the brain and the liver were collected and snap-frozen in liquid nitrogen. mRNA was extracted from the tissue and analyzed by the RT-QPCR method.

The results of this analysis are provided in Figures 1A and 1B and demonstrate that a single 300 μg dose of AD-1204704, AD-1204705, AD-1204708, or AD-1204712 potently knocks down APOE expression in the brain (Figure 1A) with a lesser effect on peripheral APOE expression (Figure 1B).

Figure 2 depicts the correlation of the activity of the agents *in vitro* to the activity of the agents *in vivo*. Specifically, of the 45 duplexes that exhibited greater than 80% knockdown in Hep3B cells (at 10 nM), the top 4 duplexes identified in that *in vitro* screen showed the best activity *in vivo* (agents circled, *i.e.,* AD-1204704, AD-1204705, AD-1204708, or AD-1204712).

Table 9. Unodified Sense and Antisense Strand Sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000041.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range n NM_000041.4
AD-1204704	CAGGCAGGAAGAUGAAGGUUA	690	59-79	UAACCUUCAUCUUCCUGCCUGUG	700	57-79
AD-1204705	AGGAAGAUGAAGGUUCUGUGA	691	64-84	UCACAGAACCUUCAUCUUCCUGC	701	62-84
AD-1204706	UUCUGUGGGCUGCGUUGCUGA	692	77-97	UCAGCAACGCAGCCCACAGAACC	702	75-97
AD-1204707	GCGUUGCUGGUCACAUUCCUA	693	88-108	UAGGAAUGUGACCAGCAACGCAG	703	86-108
AD-1204708	CACUGGGUCGCUUUUGGGAUA	694	209-229	UAUCCCAAAAGCGACCCAGUGCC	704	207-229
AD-1204709	GUCGCUUUUGGGAUUACCUGA	695	215-235	UCAGGUAAUCCCAAAAGCGACCC	705	213-235
AD-1204710	UUUUGGGAUUACCUGCGCUGA	696	220-240	UCAGCGCAGGUAAUCCCAAAAGC	706	218-240
AD-1204711	CCGCCUCAAGAGCUGGUUCGA	697	900-920	UCGAACCAGCUCUUGAGGCGGGC	707	898-920
AD-1204712	GACCCUAGUUUAAUAAAGAUA	698	1130-1150	UAUCUUUAUUAAACUAGGGUCCA	708	1128-1150
AD-1204713	CGGCCUCAGCGCCAUCCGCGA	699	639-659	UCGCGGAUGGCGCUGAGGCCGCG	709	637-659

Table 10. Modified Sense and Antisense Strand Sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1204704	csasggc(Ahd)GfgAfAfGfaugaaggususa	710	VPusAfsaccUfuCfAfucuuCfcUfgccugsusg	720	CACAGGCAGGAAGAUGAAGGUUC	730
AD-1204705	asgsgaa(Ghd)AfuGfAfAfgguucugusgsa	711	VPusCfsacaGfaAfCfcuucAfuCfuuccusgsc	721	GCAGGAAGAUGAAGGUUCUGUGG	731
AD-1204706	ususcug(Uhd)GfgGfCfUfgcguugcusgsa	712	VPusCfsagcAfaCfGfcagcCfcAfcagaascsc	722	GGUUCUGUGGGCUGCGUUGCUGG	732
AD-1204707	gscsguu(Ghd)CfuGfGfUfcacauuccsusa	713	VPusAfsggaAfuGfUfgaccAfgCfaacgcsasg	723	CUGCGUUGCUGGUCACAUUCCUG	733
AD-	csascug(Ghd)GfuCfGfCfuuuuggga	714	VPusAfsuccCfaAfAfagcgAfcCfcagugs	724	GGCACUGGGUCGCUUUUGG	734

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1204708	susa		csc		GAUU	
AD-1204709	gsuscgc(Uhd)UfuUfGfGfgauuaccusgsa	715	VPusCfsaggUfaAfUfcccaAfaAfgcgacscsc	725	GGGUCGCUUUUGGGAUUACCUGC	735
AD-1204710	ususuug(Ghd)GfaUfUfAfccugcgcusgsa	716	VPusCfsagcGfcAfGfguaaUfcCfcaaaasgsc	726	GCUUUUGGGAUUACCUGCGCUGG	736
AD-1204711	cscsgcc(Uhd)CfaAfGfAfgcugguucsgsa	717	VPusCfsgaaCfcAfGfcucuUfgAfggcggsgsc	727	GCCCGCCUCAAGAGCUGGUUCGA	737
AD-1204712	gsasccc(Uhd)AfgUfUfUfaauaaagasusa	718	VPusAfsucuUfuAfUfuaaaCfuAfgggucscsa	728	UGGACCCUAGUUUAAUAAAGAUU	738
AD-1204713	csgsgcc(Uhd)CfaGfCfGfccauccgcsgsa	719	VPusCfsgcgGfaUfGfgcgUfgAfggccgscsg	729	CGCGGCCUCAGCGCCAUCCGCGA	739

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of APOE, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0 or 1 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:1, or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0 or 1 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:2, such that the sense strand is complementary to the at least 15 contiguous nucleotides in the antisense strand.

2. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0 or 1 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:1or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0 or 1 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide identity to the entire nucleotide sequence of SEQ ID NO:2, such that the sense strand is complementary to the at least 17 contiguous nucleotides in the antisense strand.

3. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0 or 1 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:1 or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0 or 1 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:2, such that the sense strand is complementary to the at least 19 contiguous nucleotides in the antisense strand.

4. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0 or 1 mismatches, of a portion of the nucleotide sequence of SEQ ID

NO:1or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0 or 1 mismatches, of the corresponding portion of nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide sequence identity to the entire nucleotide sequence of SEQ ID NO:2, such that the sense strand is complementary to the at least 21 contiguous nucleotides in the antisense strand.

5. The dsRNA agent of any one of claims 1-4, wherein the sense strand and/or the antisense strand is a sense strand and/or an antisense strand selected from the group consisting of any of the sense strands and antisense strands in any one of Tables 2-5 and 7-10.

6. The dsRNA agent of any one of claims 1-5, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of nucleotides 57-79, 62-84, 75-97, 86-108, 207-229, 213-235, 218-240, 898-920, 1128-1150, 637-659 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

7. The dsRNA agent of any one of claims 1-5, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of nucleotides 57-79, 62-84, 207-229, 1128-1150 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

8. The dsRNA agent of an one of claims 1-7, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1204704, AD-1204705, AD-1204705, AD-1204706 AD-1204707, AD-1204708, AD-1204709, AD-1204710, AD-1204711, AD-1204712, and AD-1204713.

9. The dsRNA agent of an one of claims 1-8, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1204704, AD-1204705, AD-1204708, and AD-1204712.

10. The dsRNA agent of any one of claims 1-9, which inhibits the expression of APOE4 but does not substantially inhibit the expression of APOE2 and the expression of APOE3.

11. The dsRNA agent of claim 10, wherein the sense strand and/or the antisense strand is a sense strand and/or an antisense strand selected from the group consisting of any of the sense strands and antisense strands in any one of Tables 7 and 8.

12. The dsRNA agent of any one of claims 1-11, wherein the sense strand, the antisense strand, or both the sense strand and the antisense strand is conjugated to one or more lipophilic moieties.

13. The dsRNA agent of claim 12, wherein the lipophilic moiety is conjugated to one or more positions in the double stranded region of the dsRNA agent.

14. The dsRNA agent of claim 12 or 13, wherein the lipophilic moiety is conjugated via a linker or a carrier.

15. The dsRNA agent of any one of claims 12-14, wherein lipophilicity of the lipophilic moiety, measured by logKow, exceeds 0.

16. The dsRNA agent of any one of claims 1-15, wherein the hydrophobicity of the double-stranded RNAi agent, measured by the unbound fraction in a plasma protein binding assay of the double-stranded RNAi agent, exceeds 0.2.

17. The dsRNA agent of claim 16, wherein the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

18. The dsRNA agent of any one of claims 1-17, wherein the dsRNA agent comprises at least one modified nucleotide.

19. The dsRNA agent of claim 18, wherein no more than five of the sense strand nucleotides and no more than five of the nucleotides of the antisense strand are unmodified nucleotides

20. The dsRNA agent of claim 18, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

21. The dsRNA agent of any one of claims 18-20, wherein at least one of the modified nucleotides is selected from the group a deoxy-nucleotide, a 3’-terminal deoxy-thymidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2’-amino-modified nucleotide, a 2’-O-allyl-

modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphonate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA) S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofurane-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, and a terminal nucleotide linked to a cholesteryl derivative and a dodecanoic acid bisdecylamide group; and combinations thereof.

22. The dsRNA agent of claim 21, wherein the modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxy-thymidine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

23. The dsRNA agent of claim 21, wherein the modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymidine nucleotides (dT).

24. The dsRNA agent of claim 21, wherein the modifications on the nucleotides are 2'-O-methyl, GNA and 2'fluoro modifications.

25. The dsRNA agent of claim 21, further comprising at least one phosphorothioate internucleotide linkage.

26. The dsRNA agent of claim 25, wherein the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages.

27. The dsRNA agent of any one of claims 1-26, wherein each strand is no more than 30 nucleotides in length.

28. The dsRNA agent of any one of claims 1-27, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

29. The dsRNA agent of any one of claims 1-27, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

30. The dsRNA agent of any one of claims 1-29, wherein the double stranded region is 15-30 nucleotide pairs in length.

31. The dsRNA agent of claim 30, wherein the double stranded region is 17-23 nucleotide pairs in length.

32. The dsRNA agent of claim 30, wherein the double stranded region is 17-25 nucleotide pairs in length.

33. The dsRNA agent of claim 30, wherein the double stranded region is 23-27 nucleotide pairs in length.

34. The dsRNA agent of claim 30, wherein the double stranded region is 19-21 nucleotide pairs in length.

35. The dsRNA agent of claim 30, wherein the double stranded region is 21-23 nucleotide pairs in length.

36. The dsRNA agent of any one of claims 1-35, wherein each strand has 19-30 nucleotides.

37. The dsRNA agent of any one of claims 1-35, wherein each strand has 19-23 nucleotides.

38. The dsRNA agent of any one of claims 1-35, wherein each strand has 21-23 nucleotides.

39. The dsRNA agent of any one of claims 12-38, wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand.

40. The dsRNA agent of claim 39, wherein the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand via a linker or carrier.

41. The dsRNA agent of claim 40, wherein the internal positions include all positions except the terminal two positions from each end of the at least one strand.

42. The dsRNA agent of claim 40, wherein the internal positions include all positions except the terminal three positions from each end of the at least one strand.

43. The dsRNA agent of claim 40-42, wherein the internal positions exclude a cleavage site region of the sense strand.

44. The dsRNA agent of claim 43, wherein the internal positions include all positions except positions 9-12, counting from the 5'-end of the sense strand.

45. The dsRNA agent of claim 43, wherein the internal positions include all positions except positions 11-13, counting from the 3'-end of the sense strand.

46. The dsRNA agent of claim 40-42, wherein the internal positions exclude a cleavage site region of the antisense strand.

47. The dsRNA agent of claim 46, wherein the internal positions include all positions except positions 12-14, counting from the 5'-end of the antisense strand.

48. The dsRNA agent of claim 40-42, wherein the internal positions include all positions except positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end.

49. The dsRNA agent of any one of claims 12-48, wherein the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

50. The dsRNA agent of claim 49, wherein the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

51. The dsRNA agent of claim 13, wherein the positions in the double stranded region exclude a cleavage site region of the sense strand.

52. The dsRNA agent of any one of claims 12-51, wherein the sense strand is 21 nucleotides in length, the antisense strand is 23 nucleotides in length, and the lipophilic moiety is conjugated to

position 21, position 20, position 15, position 1, position 7, position 6, or position 2 of the sense strand or position 16 of the antisense strand.

53. The dsRNA agent of claim 52, wherein the lipophilic moiety is conjugated to position 21, position 20, position 15, position 1, or position 7 of the sense strand.

54. The dsRNA agent of claim 52, wherein the lipophilic moiety is conjugated to position 21, position 20, or position 15 of the sense strand.

55. The dsRNA agent of claim 52, wherein the lipophilic moiety is conjugated to position 20 or position 15 of the sense strand.

56. The dsRNA agent of claim 52, wherein the lipophilic moiety is conjugated to position 16 of the antisense strand.

57. The dsRNA agent of any one of claims 12-56, wherein the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

58. The dsRNA agent of claim 57, wherein the lipophilic moiety is selected from the group consisting of lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.

59. The dsRNA agent of claim 58, wherein the lipophilic moiety contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

60. The dsRNA agent of claim 59, wherein the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

61. The dsRNA agent of claim 59, wherein the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

62. The dsRNA agent of claim 61, wherein the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6, counting from the 5'-end of the strand.

63. The dsRNA agent of any one of claims 12-60, wherein the lipophilic moiety is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s) or the double stranded region.

64. The dsRNA agent of claim 63, wherein the carrier is a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

65. The dsRNA agent of any one of claims 12-60, wherein the lipophilic moiety is conjugated to the double-stranded iRNA agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

66. The double-stranded iRNA agent of any one of claims 12-65, wherein the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

67. The dsRNA agent of any one of claims 12-66, wherein the lipophilic moeity or targeting ligand is conjugated via a bio-clevable linker selected from the group consisting of DNA, RNA, disulfide, amide, funtionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

68. The dsRNA agent of any one of claims 12-67, wherein the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

69. The dsRNA agent of any one of claims 12-68, further comprising a targeting ligand that targets a liver tissue.

70. The dsRNA agent of claim 69, wherein the targeting ligand is a GalNAc conjugate.

71. The dsRNA agent of any one of claims 1-70 further comprising
a terminal, chiral modification occurring at the first internucleotide linkage at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration,

a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp configuration or Sp configuration.

72. The dsRNA agent of any one of claims 1-70 further comprising
a terminal, chiral modification occurring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration,
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

73. The dsRNA agent of any one of claims 1-70 further comprising
a terminal, chiral modification occurring at the first, second and third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration,
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

74. The dsRNA agent of any one of claims 1-70 further comprising
a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration,
a terminal, chiral modification occurring at the third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Rp configuration,
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and
a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

75. The dsRNA agent of any one of claims 1-70 further comprising
a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration,
a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and

a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

76. The dsRNA agent of any one of claims 1-75, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

77. The dsRNA agent of claim 76, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

78. The dsRNA agent of any one of claims 1-77, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

79. The dsRNA agent of any one of claims 1-78, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

80. A cell containing the dsRNA agent of any one of claims 1-79.

81. A pharmaceutical composition for inhibiting expression of a gene encoding APOE, comprising the dsRNA agent of any one of claims 1-79.

82. A pharmaceutical composition comprising the dsRNA agent of any one of claims 1-79 and a lipid formulation.

83. A method of inhibiting expression of an APOE gene in a cell, the method comprising:
(a) contacting the cell with the dsRNA agent of any one of claims 1-79, or the pharmaceutical composition of claim 81 or 82; and
(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the APOE gene, thereby inhibiting expression of the APOE gene in the cell.

84. The method of claim 83, wherein the cell is within a subject.

85. The method of claim 84, wherein the subject is a human.

86. The method of any one of claims 83-85, wherein the expression of APOE is inhibited by at least 50%.

87. The method of claim 85, wherein the subject meets at least one diagnostic criterion for an APOE-associated neurodegenerative disease.

88. The method of claim 85, wherein the subject has been diagnosed with an APOE-associated neurodegenerative disease.

89. The method of claim 88, wherein the APOE-associated neurodegenerative disease is an amyloid-β-mediated disease.

90. The method of claim 89, wherein the amyloid-β-mediated disease is selected from the group consisting of Alzheimer's disease (AD), Down's syndrome, and cerebral amyloid angiopathy.

91. The method of claim 89, wherein the APOE-associated neurodegenerative disease is a tau-mediated disease.

92. The method of claim 91, wherein the tau-mediated disease is a primary tauopathy or a secondary tauopathy.

93. The method of claim 92, wherein the primary tauopathy is selected from the group consisting of Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART).

94. The method of claim 92, wherein the secondary tauopathy is selected from the group consisting of AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

95. A method of treating a subject diagnosed with an APOE-associated neurodegenerative disease, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-79 or the pharmaceutical composition of claim 81 or 82, thereby treating the subject.

96. The method of claim 95, wherein the subject is human.

97. The method of claim 95, wherein treating comprises amelioration of at least on sign or symptom of the disease.

98. The method of claim 96, where treating comprises prevention of progression of the disease.

99. The method of claim 95, wherein the subject has been diagnosed with an APOE-associated neurodegenerative disease.

100. The method of claim 98, wherein the APOE-associated neurodegenerative disease is an amyloid-β-mediated disease.

101. The method of claim 100, wherein the amyloid-β-mediated disease is selected from the group consisting of Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy.

102. The method of claim 98, wherein the APOE-associated neurodegenerative disease is a tau-mediated disease.

103. The method of claim 102, wherein the tau-mediated disease is a primary tauopathy or a secondary tauopathy.

104. The method of claim 103, wherein the primary tauopathy is selected from the group consisting of Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART).

105. The method of claim 103, wherein the secondary tauopathy is selected from the group consisting of AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

106. A method of preventing development of an APOE-associated neurodegenerative disease in a subject meeting at least one diagnostic criterion for an APOE-associated neurodegenerative disease, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-79 or the pharmaceutical composition of claim 81 or 82, thereby preventing the development of an APOE-associated neurodegenerative disease in the subject meeting at least one diagnostic criterion for an APOE-associated neurodegenerative disease.

107. The method of claim 106, wherein the subject is human.

108. The method of claim 106, wherein the subject has been diagnosed with an APOE-associated neurodegenerative disease.

109. The method of claim 106, wherein the APOE-associated neurodegenerative disease is an amyloid-β-mediated disease.

110. The method of claim 105, wherein the amyloid-β-mediated disease is selected from the group consisting of Alzheimer's's disease, Down's syndrome, and cerebral amyloid angiopathy.

111. The method of claim 106, wherein the APOE-associated neurodegenerative disease is a tau-mediated disease.

112. The method of claim 111, wherein the tau-mediated disease is a primary tauopathy or a secondary tauopathy.

113. The method of claim 112, wherein the primary tauopathy is selected from the group consisting of Frontotemporal dementia (FTD), Progressive supranuclear palsy (PSP), Cordicobasal degeneration (CBD), Pick's disease (PiD), Globular glial tauopathies (GGTs), frontotemporal dementia with parkinsonism (FTDP, FTDP-17), Chronic traumatic encelopathy (CTE), Dementia pugilistica, Frontotemporal lobar degeneration (FTLD), Argyrophilic grain disease (AGD), and Primary age-related tauopathy (PART).

114. The method of claim 112, wherein the secondary tauopathy is selected from the group consisting of AD, Creuzfeld Jakob's disease, Down's Syndrome, and Familial British Dementia.

115. The method of any one of claims 95-114, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

116. The method of any one of claims 95-115, wherein the dsRNA agent is administered to the subject intrathecally.

117. The method of any one of claims 95-116, further comprising measuring a level of one or more of APOE2, APOE3, and APOE4 protein.

118. The method of any one of claims 95-117, further comprising administering to the subject an additional agent suitable for treatment or prevention of an APOE-associated neurodegenerative disorder.

119. A method of inhibiting expression of an APOE gene in an astrocyte, the method comprising:

(a) contacting the astrocyte with the dsRNA agent of any one of claims 1-79, or the pharmaceutical composition of claim 81 or 82; and

(b) maintaining the astrocyte produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the APOE gene, thereby inhibiting expression of the APOE gene in the astrocyte.

120. The method of claim 119, wherein the astrocyte is within a subject.

121. The method of claim 120, wherein the subject is a human.

122. The method of any one of claims 119-121, wherein the contacting the astrocyte is by inthrathecal administration of the pharmaceutical composition.

123. The method of any one of claims 119-122, wherein the antisense strand of the dsRNA agent comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1204704, AD-1204705, AD-1204708, and AD-1204712.

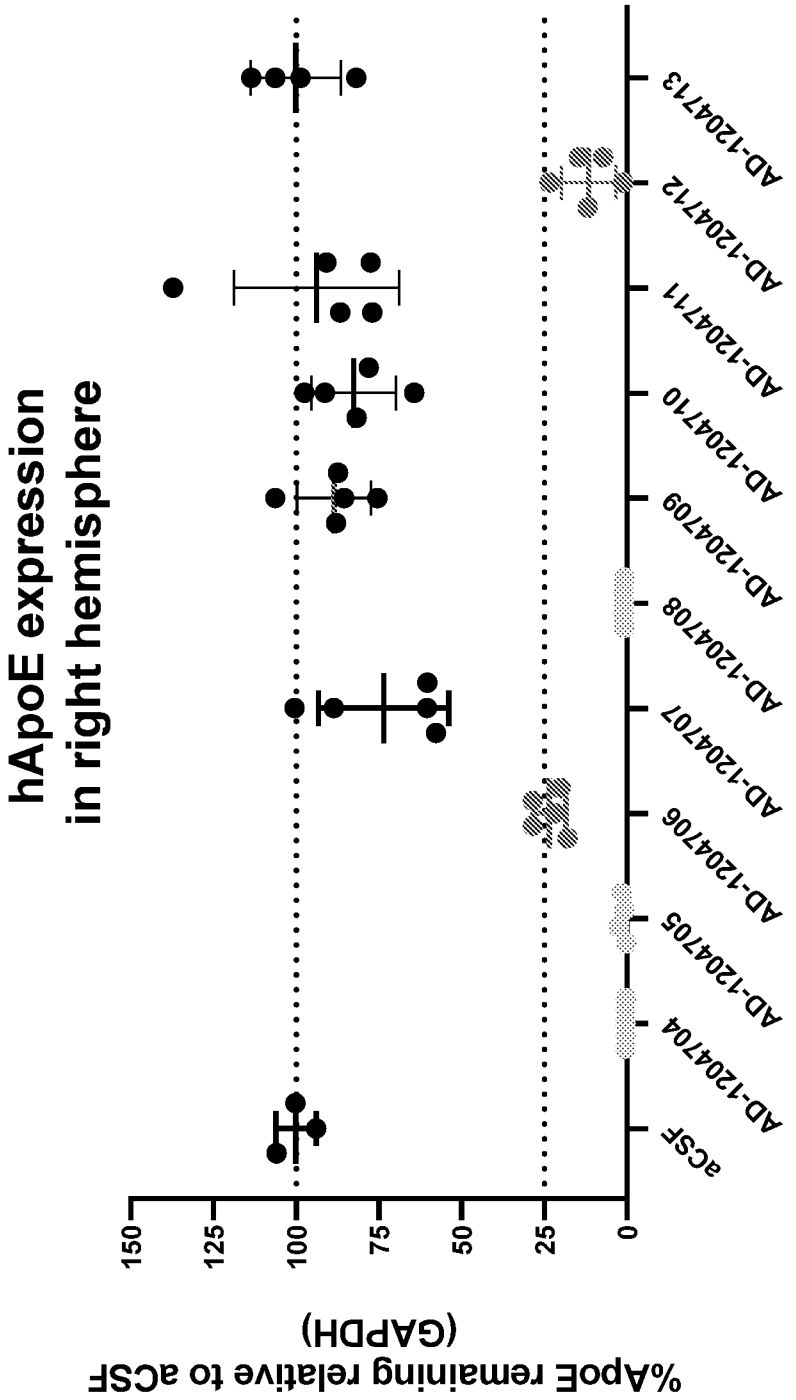

hApoE expression
in right hemisphere
%ApoE remaining relative to aCSF
(GAPDH)
150
125
100
75
50
25
0
aCSF
AD-1204704
AD-1204705
AD-1204706
AD-1204707
AD-1204708
AD-1204709
AD-1204710
AD-1204711
AD-1204712
AD-1204713

Figure 1A

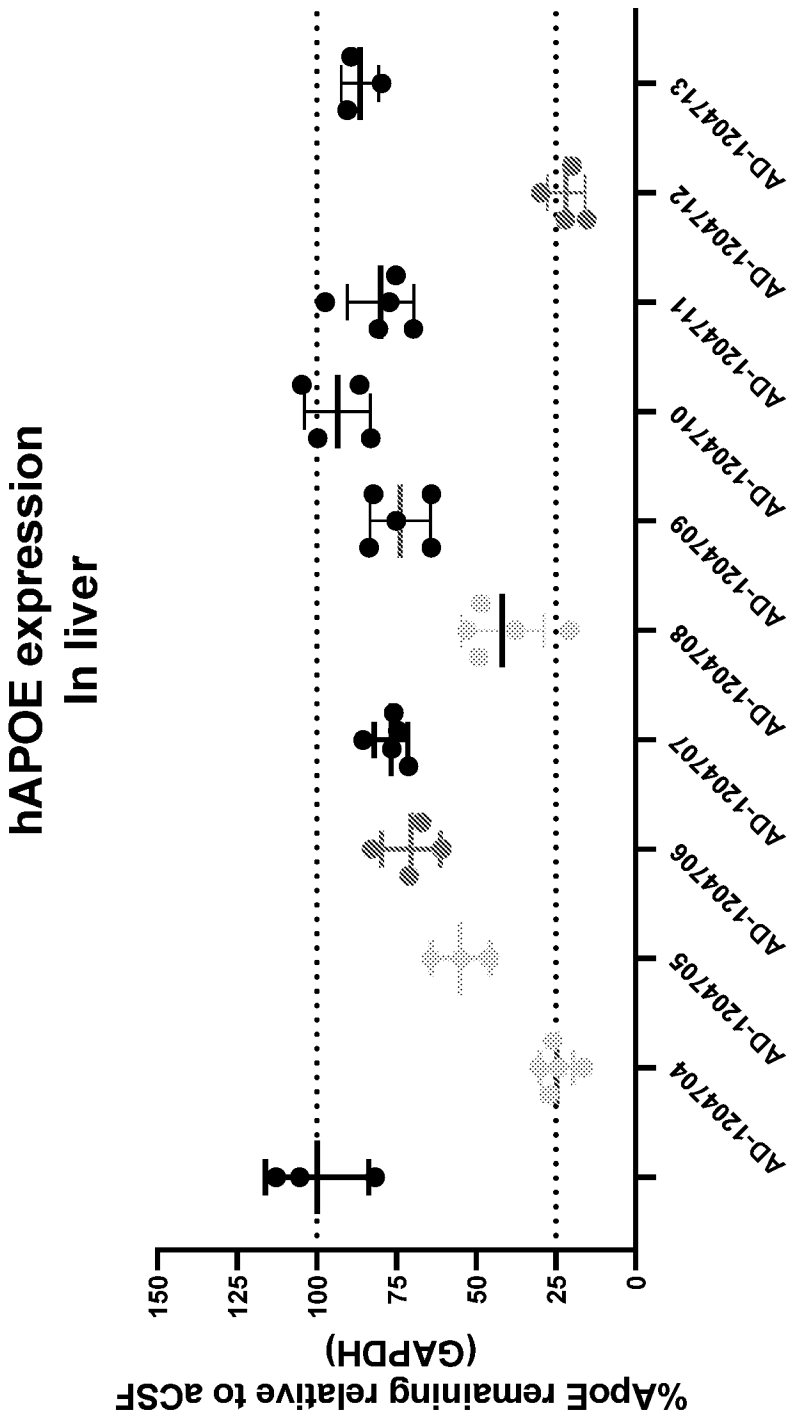

hAPOE expression
In liver
%ApoE remaining relative to aCSF
(GAPDH)
150
125
100
75
50
25
0
AD-1204704
AD-1204705
AD-1204706
AD-1204707
AD-1204708
AD-1204709
AD-1204710
AD-1204711
AD-1204712
AD-1204713

Figure 1B

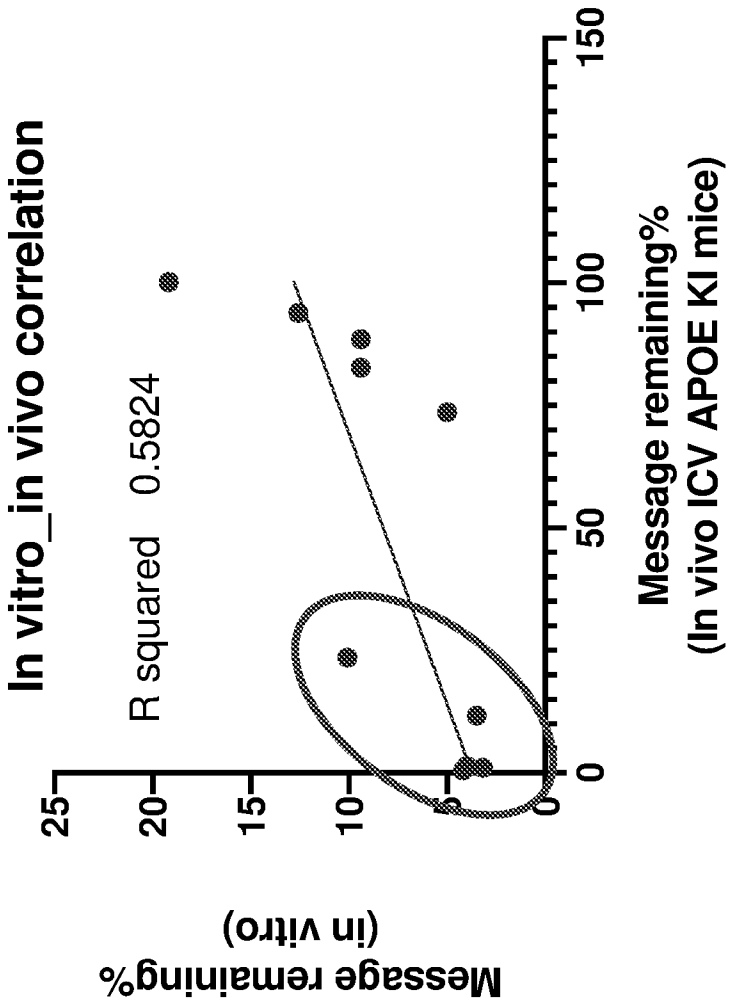

ApoE
In vitro_ in vivo correlation
R squared 0.5824
Message remaining%
(In vitro)
0
5
10
15
20
25
0
50
100
150
Message remaining%
(In vivo ICV APOE KI mice)

Figure 2

SEQUENCE LISTING

<110> ALNYLAM PHARMACEUTICALS, INC.
BOSTWICK, Bret Lee
PENG, Haiyan
MCININCH, James D.
CASTORENO, Adam
SCHLEGEL, Mark K.

<120> APOLIPOPROTEIN E (APOE) IRNA AGENT COMPOSITIONS AND METHODS OF USE THEREOF

<130> 121301-11220

<140>
<141>

<150> 63/015,867
<151> 2020-04-27

<160> 739

<170> PatentIn version 3.5

<210> 1
<211> 1166
<212> DNA
<213> Homo sapiens

<400> 1
ctactcagcc ccagcggagg tgaaggacgt ccttccccag gagccgactg gccaatcaca 60

ggcaggaaga tgaaggttct gtgggctgcg ttgctggtca cattcctggc aggatgccag 120

gccaaggtgg agcaagcggt ggagacagag ccggagcccg agctgcgcca gcagaccgag 180

tggcagagcg gccagcgctg ggaactggca ctgggtcgct tttgggatta cctgcgctgg 240

gtgcagacac tgtctgagca ggtgcaggag gagctgctca gctcccaggt cacccaggaa 300

ctgagggcgc tgatggacga gaccatgaag gagttgaagg cctacaaatc ggaactggag 360

gaacaactga ccccggtggc ggaggagacg cgggcacggc tgtccaagga gctgcaggcg 420

gcgcaggccc ggctgggcgc ggacatggag gacgtgtgcg gccgcctggt gcagtaccgc 480

ggcgaggtgc aggccatgct cggccagagc accgaggagc tgcgggtgcg cctcgcctcc 540

cacctgcgca agctgcgtaa gcggctcctc cgcgatgccg atgacctgca gaagcgcctg 600

gcagtgtacc aggccggggc ccgcgagggc gccgagcgcg gcctcagcgc catccgcgag 660

cgcctggggc ccctggtgga acagggccgc gtgcgggccg ccactgtggg ctccctggcc 720

ggccagccgc tacaggagcg ggcccaggcc tggggcgagc ggctgcgcgc gcggatggag 780

gagatgggca gccggacccg cgaccgcctg gacgaggtga aggagcaggt ggcggaggtg 840

cgcgccaagc tggaggagca ggcccagcag atacgcctgc aggccgaggc cttccaggcc 900

cgcctcaaga gctggttcga gcccctggtg gaagacatgc agcgccagtg ggccgggctg 960

gtggagaagg tgcaggctgc cgtgggcacc agcgccgccc ctgtgcccag cgacaatcac 1020

tgaacgccga agcctgcagc catgcgaccc cacgccaccc cgtgcctcct gcctccgcgc 1080

agcctgcagc gggagaccct gtccccgccc cagccgtcct cctggggtgg accctagttt 1140

aataaagatt caccaagttt cacgca 1166

<210> 2
<211> 1166
<212> DNA
<213> Homo sapiens

<400> 2
tgcgtgaaac ttggtgaatc tttattaaac tagggtccac cccaggagga cggctggggc 60

ggggacaggg tctcccgctg caggctgcgc ggaggcagga ggcacggggt ggcgtggggt 120

cgcatggctg caggcttcgg cgttcagtga ttgtcgctgg gcacaggggc ggcgctggtg 180

cccacggcag cctgcacctt ctccaccagc ccggcccact ggcgctgcat gtcttccacc 240

aggggctcga accagctctt gaggcgggcc tggaaggcct cggcctgcag gcgtatctgc 300

tgggcctgct cctccagctt ggcgcgcacc tccgccacct gctccttcac ctcgtccagg 360

cggtcgcggg tccggctgcc catctcctcc atccgcgcgc gcagccgctc gccccaggcc 420

tgggcccgct cctgtagcgg ctggccggcc agggagccca cagtggcggc ccgcacgcgg 480

ccctgttcca ccaggggccc caggcgctcg cggatggcgc tgaggccgcg ctcggcgccc 540

tcgcgggccc cggcctggta cactgccagg cgcttctgca ggtcatcggc atcgcggagg 600

agccgcttac gcagcttgcg caggtgggag gcgaggcgca cccgcagctc ctcggtgctc 660

tggccgagca tggcctgcac ctcgccgcgg tactgcacca ggcggccgca cacgtcctcc 720

atgtccgcgc ccagccgggc ctgcgccgcc tgcagctcct tggacagccg tgcccgcgtc 780

tcctccgcca ccggggtcag ttgttcctcc agttccgatt tgtaggcctt caactccttc 840

atggtctcgt ccatcagcgc cctcagttcc tgggtgacct gggagctgag cagctcctcc 900

tgcacctgct cagacagtgt ctgcacccag cgcaggtaat cccaaaagcg acccagtgcc 960

agttcccagc gctggccgct ctgccactcg gtctgctggc gcagctcggg ctccggctct 1020

gtctccaccg cttgctccac cttggcctgg catcctgcca ggaatgtgac cagcaacgca 1080

gcccacagaa ccttcatctt cctgcctgtg attggccagt cggctcctgg ggaaggacgt 1140

ccttcacctc cgctggggct gagtag 1166

<210> 3
<211> 1212
<212> DNA
<213> Rattus norvegicus

<400> 3
ataattggac aggtctggga tccggtcccc tgctcagacc ccggaggcta aggagttgtt 60

tcggaaggag ctggtaagac aagcttgggc tggcgattca cccaggggggc ttgactggcc 120

aatcacaact gggaagatga aggctctgtg ggccctgctg ttggtcccat tgctgacagg 180

atgcctggcc gagggagagc tggaggtgac agatcagctc ccagggcaaa gcgaccaacc 240

ctgggagcag gccctgaacc gcttctggga ttacctgcgc tgggtgcaga cgctttctga 300

ccaggtccag gaagagctgc agagctccca agtcacacag gaactgacgg tactgatgga 360

ggacactatg acggaagtaa aggcatacaa aaaggagctg gaggaacagc tgggcccagt 420

ggcggaggag acacgggcca ggctggctaa agaggtgcag gcggcacagg cccgtctggg 480

agctgacatg gaggatctac gcaaccgact cgggcagtac cgcaacgagg taaacaccat 540

gctgggccag agcacagagg agctgcggtc gcgcctctcc acacacctgc gcaagatgcg 600

caagcgcctg atgcgggatg cggatgatct gcagaagcgc ctggcggtgt acaaggccgg 660

ggcacaggag ggcgccgagc gcggtgtgag tgctatccgt gagcgcctgg ggccactggt 720

ggagcagggt cgtcagcgca cagccaacct aggcgctggc gccgcccagc ccctgcgcga 780

tcgcgcccag gctttgagtg accgcatccg agggcggctg gaggaagtgg gcaaccaggc 840

ccgagaccgc ctagaggagg tgcgtgagca gatggaggag gtgcgctcca agatggagga 900

gcagacccag cagatacgcc tgcaggccga gatcttccag gcccgcatca agggctggtt 960

cgagccgcta gtggaagaca tgcagcgcca gtgggcaaac ctaatggaga agatacaggc 1020

ctctgtggct accaactcca ttgcctccac cacagtgccc ctggagaatc aatgatcatc 1080

cctcacctac gccctgccgc aacatccatg accagccagg tggccctgtc ccaagcacca 1140

ctctggccct ctggtggccc ttgcttaata aagattctcc aagcaaaaaa aaaaaaaaaa 1200

aaaaaaaaaa aa 1212

<210> 4
<211> 1212
<212> DNA
<213> Rattus norvegicus

<400> 4
ttttttttttt ttttttttttt tttttttttgc ttggagaatc tttattaagc aagggccacc 60

agagggccag agtggtgctt gggacagggc cacctggctg gtcatggatg ttgcggcagg 120

gcgtaggtga gggatgatca ttgattctcc aggggcactg tggtggaggc aatggagttg 180

gtagccacag aggcctgtat cttctccatt aggtttgccc actggcgctg catgtcttcc 240

actagcggct cgaaccagcc cttgatgcgg gcctggaaga tctcggcctg caggcgtatc 300

tgctgggtct gctcctccat cttggagcgc acctcctcca tctgctcacg cacctcctct 360

aggcggtctc gggcctggtt gcccacttcc tccagccgcc ctcggatgcg gtcactcaaa 420

gcctgggcgc gatcgcgcag gggctgggcg gcgccagcgc ctaggttggc tgtgcgctga 480

cgaccctgct ccaccagtgg ccccaggcgc tcacggatag cactcacacc gcgctcggcg 540

ccctcctgtg ccccggcctt gtacaccgcc aggcgcttct gcagatcatc cgcatcccgc 600

atcaggcgct tgcgcatctt gcgcaggtgt gtggagaggc gcgaccgcag ctcctctgtg 660

ctctggccca gcatggtgtt tacctcgttg cggtactgcc cgagtcggtt gcgtagatcc 720

tccatgtcag ctcccagacg ggcctgtgcc gcctgcacct ctttagccag cctggcccgt 780

gtctcctccg ccactgggcc cagctgttcc tccagctcct ttttgtatgc ctttacttcc 840

gtcatagtgt cctccatcag taccgtcagt tcctgtgtga cttgggagct ctgcagctct 900

tcctggacct ggtcagaaag cgtctgcacc cagcgcaggt aatcccagaa gcggttcagg 960

gcctgctccc agggttggtc gctttgccct gggagctgat ctgtcacctc cagctctccc 1020

tcggccaggc atcctgtcag caatgggacc aacagcaggg cccacagagc cttcatcttc 1080

ccagttgtga ttggccagtc aagccccctg ggtgaatcgc cagcccaagc ttgtcttacc 1140

agctccttcc gaaacaactc cttagcctcc ggggtctgag caggggaccg gatcccagac 1200

ctgtccaatt at 1212

<210> 5
<211> 1358
<212> DNA
<213> Mus musculus

<400> 5
tttcctctgc cctgctgtga agggggagag aacaacccgc ctcgtgacag ggggctggca 60

cagcccgccc tagccctgag gagggggcgg gacaggggga gtcctataat tggaccggtc 120

tgggatccga tcccctgctc agaccctgga ggctaaggac ttgtttcgga aggagctgga 180

gagggagctg gaatttttgg cagcggatcc accccggggt gccgagatag cgaactcggc 240

aaggggagac tggccaatca caattgcgaa gatgaaggct ctgtgggccg tgctgttggt 300

cacattgctg acaggatgcc tagccgaggg agagccggag gtgacagatc agctcgagtg 360

gcaaagcaac caaccctggg agcaggccct gaaccgcttc tgggattacc tgcgctgggt 420

gcagacgctg tctgaccagg tccaggaaga gctgcagagc tcccaagtca cacaagaact 480

gacggcactg atggaggaca ctatgacgga agtaaaggct tacaaaaagg agctggagga 540

acagctgggt ccagtggcgg aggagacacg ggccaggctg ggcaaagagg tgcaggcggc 600

acaggcccga ctcggagccg acatggagga tctacgcaac cgactcgggc agtaccgcaa 660

cgaggtgcac accatgctgg gccagagcac agaggagata cgggcgcggc tctccacaca 720

cctgcgcaag atgcgcaagc gcttgatgcg ggatgccgag gatctgcaga agcgcctagc 780

tgtgtacaag gcaggggcac gcgagggcgc cgagcgcggt gtgagtgcca tccgtgagcg 840

cctggggcct ctggtggagc aaggtcgcca gcgcactgcc aacctaggcg ctggggccgc 900

ccagcctctg cgcgatcgcg cccaggcttt tggtgaccgc atccgagggc ggctggagga 960

agtgggcaac caggcccgtg accgcctaga ggaggtgcgt gagcacatgg aggaggtgcg 1020

ctccaagatg gaggaacaga cccagcaaat acgcctgcag gcggagatct tccaggcccg 1080

cctcaagggc tggttcgagc caatagtgga agacatgcat cgccagtggg caaacctgat 1140

ggagaagata caggcctctg tggctaccaa ccccatcatc accccagtgg cccaggagaa 1200

tcaatgagta tccttctcct gtcctgcaac aacatccata tccagccagg tggccctgtc 1260

tcaagcacct ctctggccct ctggtggccc ttgcttaata aagattctcc gagcacattc 1320

tgagtctctg tgagtgattc caaaaaaaaa aaaaaaaa 1358

<210> 6
<211> 1358
<212> DNA
<213> Mus musculus

<400> 6

tttttttttt tttttttgga atcactcaca gagactcaga atgtgctcgg agaatcttta 60

ttaagcaagg gccaccagag ggccagagag gtgcttgaga cagggccacc tggctggata 120

tggatgttgt tgcaggacag gagaaggata ctcattgatt ctcctgggcc actggggtga 180

tgatggggtt ggtagccaca gaggcctgta tcttctccat caggtttgcc cactggcgat 240

gcatgtcttc cactattggc tcgaaccagc ccttgaggcg ggcctggaag atctccgcct 300

gcaggcgtat ttgctgggtc tgttcctcca tcttggagcg cacctcctcc atgtgctcac 360

gcacctcctc taggcggtca cgggcctggt tgcccacttc ctccagccgc cctcggatgc 420

ggtcaccaaa agcctgggcg cgatcgcgca gaggctgggc ggccccagcg cctaggttgg 480

cagtgcgctg gcgaccttgc tccaccagag gccccaggcg ctcacggatg gcactcacac 540

cgcgctcggc gccctcgcgt gcccctgcct tgtacacagc taggcgcttc tgcagatcct 600

cggcatcccg catcaagcgc ttgcgcatct tgcgcaggtg tgtggagagc cgcgcccgta 660

tctcctctgt gctctggccc agcatggtgt gcacctcgtt gcggtactgc ccgagtcggt 720

tgcgtagatc ctccatgtcg gctccgagtc gggcctgtgc cgcctgcacc tctttgccca 780

gcctggcccg tgtctcctcc gccactggac ccagctgttc ctccagctcc tttttgtaag 840

cctttacttc cgtcatagtg tcctccatca gtgccgtcag ttcttgtgtg acttgggagc 900

tctgcagctc ttcctggacc tggtcagaca gcgtctgcac ccagcgcagg taatcccaga 960

agcggttcag ggcctgctcc cagggttggt tgctttgcca ctcgagctga tctgtcacct 1020

ccggctctcc ctcggctagg catcctgtca gcaatgtgac caacagcacg gcccacagag 1080

ccttcatctt cgcaattgtg attggccagt ctccccttgc cgagttcgct atctcggcac 1140

cccggggtgg atccgctgcc aaaaattcca gctccctctc cagctccttc cgaaacaagt 1200

ccttagcctc cagggtctga gcaggggatc ggatcccaga ccggtccaat tataggactc 1260

cccctgtccc gccccctcct cagggctagg gcgggctgtg ccagcccct gtcacgaggc 1320

gggttgttct ctccccccttc acagcagggc agaggaaa 1358

<210> 7
<211> 1179
<212> DNA
<213> Macaca mulatta

<400> 7
atgagctcag gggcctctag aaagtgtagc tgggacctcg ggaagccctg gcctccagac 60

tggccaatca caggcaggaa gatgaaggtt ctgtgggctg cgttgctggt cacattcctg 120

gcaggatgcc aggccaaggt ggagcaaccg gtggagccag agacggaacc cgagcttcgc 180

cagcaggctg aggggcagag cggccagccc tgggagctgg cactgggtcg cttttgggat 240

tacctgcgct gggtgcagac actgtctgag caggtgcagg aggagctgct cagcccccag 300

gtcacccagg aactgacgac gctgatggat gagaccatga aggagttgaa ggcctacaaa 360

tcggaactgg aggaacagct gagcccggtg gcggaggaga cgcgggcacg gctgtccaag 420

gagctacagg cggcgcaggc ccggctgggt gccgacatgg aggacgtgcg cagccgcctg 480

gtgcagtacc gcagcgaggt gcaggccatg ctgggccaga gtaccgagga gctgcgggcg 540

cgcctcgcct cccacctgcg caagctgcgc aagcggctcc tccgcgatgc tgatgacctg 600

cagaagcgcc tggcagtgta tcaggccggg gcccgcgagg gcgccgagcg cggggtcagc 660

gccatccgcg agcgcctggg acccctggtg gagcagggcc gcgtgcgggc cgccactgtg 720

ggctccctgg ccagccagcc gcttcaggag cgggcccagg ccttgggtga gcggcttcgc 780

gcacggatgg aggagatggg cagccggacc cgcgaccgcc tggacgaggt gaaggagcag 840

gtggcggagg tgcgcgccaa gctggaggaa caggcccagc agataagcct gcaggccgag 900

gccttccagg cccgcctcaa gagctggttc gagcccctgg tggaagatat gcagcgccag 960

tgggctgggc tggtggagaa ggtgcaggct gccgtgggcg ccagcaccgc ccctgtgccc 1020

agcgacaatc actgaacgcc caggcctaca gccatgcgac ccgactccac cccatgcctc 1080

ctctctccgc tcagcctgca gcgggagacc ctgtccccac cccagccgtc ctccaggggt 1140

gggccctagt ttaataaaga ttcgccaagt ttcaccgca 1179

<210> 8
<211> 1179
<212> DNA
<213> Macaca mulatta

<400> 8
tgcggtgaaa cttggcgaat ctttattaaa ctagggccca cccctggagg acggctgggg 60

tggggacagg gtctcccgct gcaggctgag cggagagagg aggcatgggg tggagtcggg 120

tcgcatggct gtaggcctgg gcgttcagtg attgtcgctg ggcacagggg cggtgctggc 180

gcccacggca gcctgcacct tctccaccag cccagcccac tggcgctgca tatcttccac 240

caggggctcg aaccagctct tgaggcgggc ctggaaggcc tcggcctgca ggcttatctg 300

ctgggcctgt tcctccagct tggcgcgcac ctccgccacc tgctccttca cctcgtccag 360

gcggtcgcgg gtccggctgc ccatctcctc catccgtgcg cgaagccgct cacccaaggc 420

ctgggcccgc tcctgaagcg gctggctggc cagggagccc acagtggcgg cccgcacgcg 480

gccctgctcc accaggggtc ccaggcgctc gcggatggcg ctgaccccgc gctcggcgcc 540

ctcgcgggcc ccggcctgat acactgccag gcgcttctgc aggtcatcag catcgcggag 600

gagccgcttg cgcagcttgc gcaggtggga ggcgaggcgc gcccgcagct cctcggtact 660

ctggcccagc atggcctgca cctcgctgcg gtactgcacc aggcggctgc gcacgtcctc 720

catgtcggca cccagccggg cctgcgccgc ctgtagctcc ttggacagcc gtgcccgcgt 780

ctcctccgcc accgggctca gctgttcctc cagttccgat ttgtaggcct tcaactcctt 840

catggtctca tccatcagcg tcgtcagttc ctgggtgacc tgggggctga gcagctcctc 900

ctgcacctgc tcagacagtg tctgcaccca gcgcaggtaa tcccaaaagc gacccagtgc 960

cagctcccag ggctggccgc tctgcccctc agcctgctgg cgaagctcgg gttccgtctc 1020

tggctccacc ggttgctcca ccttggcctg gcatcctgcc aggaatgtga ccagcaacgc 1080

agcccacaga accttcatct tcctgcctgt gattggccag tctggaggcc agggcttccc 1140

gaggtcccag ctacactttc tagaggcccc tgagctcat 1179

<210> 9
<211> 1179
<212> DNA
<213> Macaca fascicularis

<400> 9
atgagctcag gcgcctctag aaagtgtagc tgggacctcg ggaagccctg gcctccagac 60

tggccaatca caggcaggaa gatgaaggtt ctgtgggctg cgttgctggt cacattcctg 120

gcaggatgcc aggccaaggt ggagcaaccg gtggagccag agacggaacc cgagcttcgc 180

cagcaggctg aggggcagag cggccagccc tgggagctgg cactgggtcg cttttgggat 240

tacctgcgct gggtgcagac actgtctgag caggtgcagg aggagctgct cagcccccag 300

gtcacccagg aactgacgac gctgatggat gagaccatga aggagttgaa ggcctacaaa 360

tcggaactgg aggaacagct gagcccggtg gcggaggaga cgcgggcacg gctgtccaag 420

gagctgcagg cggcgcaggc ccggctgggt gccgacatgg aggacgtgcg cagccgcctg 480

gtgcagtacc gcagcgaggt gcaggccatg ctgggccaga gtaccgagga gctgcgggcg 540

cgcctcgcct cccacctgcg caagctgcgc aagcggctcc tccgcgatgc tgatgacctg 600

cagaagcgcc tggcagtgta tcaggccggg gcccgcgagg gcgccgagcg cggggtcagc 660

gccatccgcg agcgcctggg acccctggtg gagcagggcc gcgtgcgggc cgccactgtg 720

ggctccctgg ccagccagcc gcttcaggag cgggcccagg ccttgggtga gcggcttcgc 780

gcacggatgg aggagatggg cagccggacc cgcgaccgcc tggacgaggt gaaggagcag 840

gtggcggagg tgcgcgccaa gctggaggaa caggcccagc agataagcct gcaggccgag 900

gccttccagg cccgcctcaa gagctggttc gagcccctcg tggaagatat gcagcgccag 960

tgggctgggc tggtggagaa ggtgcaggct gccgtgggcg ccagcaccgc ccctgtgccc 1020

agcgacaatc actgaacgcc caggcctaca gccatgcgac ccgactccac cccatgcctc 1080

ctctctccgc tcagcctgca gcgggagacc ctgtccccgc cccagccgtc ctccaggggt 1140

gggccctagt ttaataaaga ttcgccaagt ttcaccgca 1179

<210> 10
<211> 1179
<212> DNA
<213> Macaca fascicularis

<400> 10
tgcggtgaaa cttggcgaat ctttattaaa ctagggccca cccctggagg acggctgggg 60

cggggacagg gtctcccgct gcaggctgag cggagagagg aggcatgggg tggagtcggg 120

tcgcatggct gtaggcctgg gcgttcagtg attgtcgctg ggcacagggg cggtgctggc 180

gcccacggca gcctgcacct tctccaccag cccagcccac tggcgctgca tatcttccac 240

gaggggctcg aaccagctct tgaggcgggc ctggaaggcc tcggcctgca ggcttatctg 300

ctgggcctgt tcctccagct tggcgcgcac ctccgccacc tgctccttca cctcgtccag 360

gcggtcgcgg gtccggctgc ccatctcctc catccgtgcg cgaagccgct cacccaaggc 420

ctgggcccgc tcctgaagcg gctggctggc cagggagccc acagtggcgg cccgcacgcg 480

gccctgctcc accaggggtc ccaggcgctc gcggatggcg ctgaccccgc gctcggcgcc 540

ctcgcgggcc ccggcctgat acactgccag gcgcttctgc aggtcatcag catcgcggag 600

gagccgcttg cgcagcttgc gcaggtggga ggcgaggcgc gcccgcagct cctcggtact 660

ctggcccagc atggcctgca cctcgctgcg gtactgcacc aggcggctgc gcacgtcctc 720

catgtcggca cccagccggg cctgcgccgc ctgcagctcc ttggacagcc gtgcccgcgt 780

ctcctccgcc accgggctca gctgttcctc cagttccgat ttgtaggcct tcaactcctt 840

catggtctca tccatcagcg tcgtcagttc ctgggtgacc tgggggctga gcagctcctc 900

ctgcacctgc tcagacagtg tctgcaccca gcgcaggtaa tcccaaaagc gacccagtgc 960

cagctcccag ggctggccgc tctgcccctc agcctgctgg cgaagctcgg gttccgtctc 1020

tggctccacc ggttgctcca ccttggcctg gcatcctgcc aggaatgtga ccagcaacgc 1080

agcccacaga accttcatct tcctgcctgt gattggccag tctggaggcc agggcttccc 1140

gaggtcccag ctacactttc tagaggcgcc tgagctcat 1179

<210> 11
<211> 16
<212> PRT
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
RFGF peptide"

<400> 11
Ala Ala Val Ala Leu Leu Pro Ala Val Leu Leu Ala Leu Leu Ala Pro
1 5 10 15

<210> 12
<211> 11
<212> PRT
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
RFGF analogue peptide"

<400> 12
Ala Ala Leu Leu Pro Val Leu Leu Ala Ala Pro
1 5 10

<210> 13
<211> 13
<212> PRT
<213> Human immunodeficiency virus

<400> 13
Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln
1 5 10

<210> 14

<211> 16
<212> PRT
<213> Drosophila sp.

<400> 14
Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys
1 5 10 15

<210> 15
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 15
ggccaaucac aggcaggaag u 21

<210> 16
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 16
caggcaggaa gaugaagguu u 21

<210> 17
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 17
aggaagauga agguucugug u 21

<210> 18
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 18
augaagguuc ugugggcugc u 21

<210> 19
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 19
uucugugggc ugcguugcug u 21

<210> 20
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 20
ugggcugcgu ugcuggucac u 21

<210> 21
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 21
gcguugcugg ucacauuccu u 21

<210> 22
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 22
gcuggucaca uuccuggcag u 21

<210> 23
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 23
ucacauuccu ggcaggaugc u 21

<210> 24
<211> 21
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 24
uggcaggaug ccaggccaag u 21

<210> 25
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 25
caggccaagg uggagcaagc u 21

<210> 26
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 26
aagguggagc aagcggugga u 21

<210> 27
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 27
caagcggugg agacagagcc u 21

<210> 28
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 28
gguggagaca gagccggagc u 21

<210> 29
<211> 21

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 29
cccgagcugc gccagcagac u 21

<210> 30
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 30
gcugcgccag cagaccgagu u 21

<210> 31
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 31
ccagcagacc gaguggcaga u 21

<210> 32
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 32
cagcgcuggg aacuggcacu u 21

<210> 33
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 33
cugggaacug gcacuggguc u 21

<210> 34

<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 34
aacuggcacu gggucgcuuu u 21

<210> 35
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 35
cacugggucg cuuuugggau u 21

<210> 36
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 36
gucgcuuuug ggauuaccug u 21

<210> 37
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 37
uuuugggauu accugcgcug u 21

<210> 38
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 38
cugcgcuggg ugcagacacu u 21

<210> 39
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 39
ugggugcaga cacugucuga u 21

<210> 40
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 40
cagacacugu cugagcaggu u 21

<210> 41
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 41
caggaggagc ugcucagcuc u 21

<210> 42
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 42
cugcucagcu cccaggucac u 21

<210> 43
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 43
ucccagguca cccaggaacu u 21

<210> 44
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 44
acccaggaac ugagggcgcu u 21

<210> 45
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 45
ugagggcgcu gauggacgag u 21

<210> 46
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 46
gcgcugaugg acgagaccau u 21

<210> 47
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 47
gacgagacca ugaaggaguu u 21

<210> 48
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 48
accaugaagg aguugaaggc u 21

<210> 49
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 49
ggaguugaag gccuacaaau u 21

<210> 50
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 50
aaggccuaca aaucggaacu u 21

<210> 51
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 51
acaaaucgga acuggaggaa u 21

<210> 52
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 52
ucggaacugg aggaacaacu u 21

<210> 53
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 53

gaggaacaac ugaccccggu u 21

<210> 54
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 54
cgcgggcacg gcuguccaag u 21

<210> 55
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 55
gcacggcugu ccaaggagcu u 21

<210> 56
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 56
gcuguccaag gagcugcagg u 21

<210> 57
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 57
gcccggcugg gcgcggacau u 21

<210> 58
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 58
cugggcgcgg acauggagga u 21

<210> 59
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 59
cgcggacaug gaggacgugc u 21

<210> 60
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 60
gcggacaugg aggacgugug u 21

<210> 61
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 61
gcggacaugg aggacgugcg u 21

<210> 62
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 62
cggacaugga ggacgugcgc u 21

<210> 63
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 63
ggacauggag gacgugcgcg u 21

<210> 64
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 64
gacauggagg acgugcgcgg u 21

<210> 65
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 65
acauggagga cgugcgcggc u 21

<210> 66
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 66
cauggaggac gugcgcggcc u 21

<210> 67
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 67
auggaggacg ugcgcggccg u 21

<210> 68
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 68
uggaggacgu gcgcggccgc u 21

<210> 69
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 69
ggaggacgug cgcggccgcc u 21

<210> 70
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 70
gaggacgugc gcggccgccu u 21

<210> 71
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 71
aggacgugcg cggccgccug u 21

<210> 72
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 72
ggacgugcgc ggccgccugg u 21

<210> 73
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 73
gacgugcgcg gccgccuggu u 21

<210> 74
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 74
acgugcgcgg ccgccuggug u 21

<210> 75
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 75
cgugcgcggc cgccuggugc u 21

<210> 76
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 76
gugcgcggcc gccuggugca u 21

<210> 77
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 77
gcggccgccu ggugcaguac u 21

<210> 78
<211> 21
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 78
cgccuggugc aguaccgcgg u 21

<210> 79
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 79
gaggugcagg ccaugcucgg u 21

<210> 80
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 80
gccaugcucg gccagagcac u 21

<210> 81
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 81
ggccagagca ccgaggagcu u 21

<210> 82
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 82
gcugcgggug cgccucgccu u 21

<210> 83
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 83
gugcgccucg ccucccaccu u 21

<210> 84
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 84
cgccucccac cugcgcaagc u 21

<210> 85
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 85
cccaccugcg caagcugcgu u 21

<210> 86
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 86
cugcgcaagc ugcguaagcg u 21

<210> 87
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 87
aagcugcgua agcggcuccu u 21

<210> 88
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 88
guaagcggcu ccuccgcgau u 21

<210> 89
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 89
ggcuccuccg cgaugccgau u 21

<210> 90
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 90
cuccgcgaug ccgaugaccu u 21

<210> 91
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 91
gaugccgaug accugcagaa u 21

<210> 92
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 92
gaugaccugc agaagcgccu u 21

<210> 93
<211> 21
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 93
cugcagaagc gccuggcagu u 21

<210> 94
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 94
aagcgccugg caguguacca u 21

<210> 95
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 95
cuggcagugu accaggccgg u 21

<210> 96
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 96
gagcgcggcc ucagcgccau u 21

<210> 97
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 97
cggccucagc gccauccgcg u 21

<210> 98
<211> 21

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 98
agcgccaucc gcgagcgccu u 21

<210> 99
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 99
uggggccccu gguggaacag u 21

<210> 100
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 100
ccccuggugg aacagggccg u 21

<210> 101
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 101
cgcgugcggg ccgccacugu u 21

<210> 102
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 102
gccgccacug ugggcucccu u 21

<210> 103

<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 103
ccuggccggc cagccgcuac u 21

<210> 104
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 104
ggccagccgc uacaggagcg u 21

<210> 105
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 105
gccgcuacag gagcgggccc u 21

<210> 106
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 106
gcgcggaugg aggagauggg u 21

<210> 107
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 107
cgcgaccgcc uggacgaggu u 21

<210> 108
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 108
gccuggacga ggugaaggag u 21

<210> 109
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 109
gacgagguga aggagcaggu u 21

<210> 110
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 110
ggaggugcgc gccaagcugg u 21

<210> 111
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 111
cgcgccaagc uggaggagca u 21

<210> 112
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 112
caggcccagc agauacgccu u 21

<210> 113
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 113
ccagcagaua cgccugcagg u 21

<210> 114
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 114
cgccugcagg ccgaggccuu u 21

<210> 115
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 115
gcaggccgag gccuuccagg u 21

<210> 116
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 116
ccgaggccuu ccaggcccgc u 21

<210> 117
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 117
gccuuccagg cccgccucaa u 21

<210> 118
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 118
ccaggcccgc cucaagagcu u 21

<210> 119
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 119
ccgccucaag agcugguucg u 21

<210> 120
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 120
caagagcugg uucgagcccc u 21

<210> 121
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 121
agccccuggu ggaagacaug u 21

<210> 122
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 122

cugguggaag acaugcagcg u 21

<210> 123
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 123
ggaagacaug cagcgccagu u 21

<210> 124
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 124
gccgggcugg uggagaaggu u 21

<210> 125
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 125
gcugguggag aaggugcagg u 21

<210> 126
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 126
accagcgccg ccccugugcc u 21

<210> 127
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 127
gccccugugc ccagcgacaa u 21

<210> 128
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 128
ugugcccagc gacaaucacu u 21

<210> 129
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 129
cagcgacaau cacugaacgc u 21

<210> 130
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 130
caaucacuga acgccgaagc u 21

<210> 131
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 131
acugaacgcc gaagccugca u 21

<210> 132
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 132
acgccgaagc cugcagccau u 21

<210> 133
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 133
gaagccugca gccaugcgac u 21

<210> 134
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 134
ugcagccaug cgaccccacg u 21

<210> 135
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 135
gcgaccccac gccaccccgu u 21

<210> 136
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 136
cccacgccac cccgugccuc u 21

<210> 137
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 137
ccaccccgug ccuccugccu u 21

<210> 138
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 138
cgugccuccu gccuccgcgc u 21

<210> 139
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 139
cuccugccuc cgcgcagccu u 21

<210> 140
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 140
gccuccgcgc agccugcagc u 21

<210> 141
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 141
ccuguccccg ccccagccgu u 21

<210> 142
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 142
cccgccccag ccguccuccu u 21

<210> 143
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 143
cccagccguc cuccuggggu u 21

<210> 144
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 144
uccuggggug gacccuaguu u 21

<210> 145
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 145
ggguggaccc uaguuuaaua u 21

<210> 146
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 146
gacccuaguu uaauaaagau u 21

<210> 147
<211> 21
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 147
uaguuuaaua aagauucacc u 21

<210> 148
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 148
uaauaaagau ucaccaaguu u 21

<210> 149
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 149
agauucacca aguuucacgc u 21

<210> 150
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 150
acuuccugcc ugugauuggc cag 23

<210> 151
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 151
aaaccuucau cuuccugccu gug 23

<210> 152
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 152
acacagaacc uucaucuucc ugc 23

<210> 153
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 153
agcagcccac agaaccuuca ucu 23

<210> 154
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 154
acagcaacgc agcccacaga acc 23

<210> 155
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 155
agugaccagc aacgcagccc aca 23

<210> 156
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 156
aaggaaugug accagcaacg cag 23

<210> 157
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 157
acugccagga augugaccag caa 23

<210> 158
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 158
agcauccugc caggaaugug acc 23

<210> 159
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 159
acuuggccug gcauccugcc agg 23

<210> 160
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 160
agcuugcucc accuuggccu ggc 23

<210> 161
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 161
auccaccgcu ugcuccaccu ugg 23

<210> 162
<211> 23
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 162
aggcucuguc uccaccgcuu gcu 23

<210> 163
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 163
agcuccggcu cugucuccac cgc 23

<210> 164
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 164
agucugcugg cgcagcucgg gcu 23

<210> 165
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 165
aacucggucu gcuggcgcag cuc 23

<210> 166
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 166
aucugccacu cggucugcug gcg 23

<210> 167
<211> 23

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 167
aagugccagu ucccagcgcu ggc 23

<210> 168
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 168
agacccagug ccaguuccca gcg 23

<210> 169
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 169
aaaagcgacc cagugccagu ucc 23

<210> 170
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 170
aaucccaaaa gcgacccagu gcc 23

<210> 171
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 171
acagguaauc ccaaaagcga ccc 23

<210> 172

<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 172
acagcgcagg uaaucccaaa agc 23

<210> 173
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 173
aagugucugc acccagcgca ggu 23

<210> 174
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 174
aucagacagu gucugcaccc agc 23

<210> 175
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 175
aaccugcuca gacagugucu gca 23

<210> 176
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 176
agagcugagc agcuccuccu gca 23

<210> 177
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 177
agugaccugg gagcugagca gcu 23

<210> 178
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 178
aaguuccugg gugaccuggg agc 23

<210> 179
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 179
aagcgcccuc aguuccuggg uga 23

<210> 180
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 180
acucguccau cagcgcccuc agu 23

<210> 181
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 181
aauggucucg uccaucagcg ccc 23

<210> 182
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 182
aaacuccuuc auggucucgu cca 23

<210> 183
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 183
agccuucaac uccuucaugg ucu 23

<210> 184
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 184
aauuuguagg ccuucaacuc cuu 23

<210> 185
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 185
aaguuccgau uuguaggccu uca 23

<210> 186
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 186
auuccuccag uuccgauuug uag 23

<210> 187
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 187
aaguuguucc uccaguuccg auu 23

<210> 188
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 188
aaccgggguc aguuguuccu cca 23

<210> 189
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 189
acuuggacag ccgugcccgc guc 23

<210> 190
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 190
aagcuccuug gacagccgug ccc 23

<210> 191
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 191

accugcagcu ccuuggacag ccg 23

<210> 192
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 192
aauguccgcg cccagccggg ccu 23

<210> 193
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 193
auccuccaug uccgcgccca gcc 23

<210> 194
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 194
agcacguccu ccauguccgc gcc 23

<210> 195
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 195
acacacgucc uccauguccg cgc 23

<210> 196
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 196
acgcacgucc uccauguccg cgc 23

<210> 197
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 197
agcgcacguc cuccaugucc gcg 23

<210> 198
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 198
acgcgcacgu ccuccauguc cgc 23

<210> 199
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 199
accgcgcacg uccuccaugu ccg 23

<210> 200
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 200
agccgcgcac guccuccaug ucc 23

<210> 201
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 201
aggccgcgca cguccuccau guc 23

<210> 202
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 202
acggccgcgc acguccucca ugu 23

<210> 203
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 203
agcggccgcg cacguccucc aug 23

<210> 204
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 204
aggcggccgc gcacguccuc cau 23

<210> 205
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 205
aaggcggccg cgcacguccu cca 23

<210> 206
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 206
acaggcggcc gcgcacgucc ucc 23

<210> 207
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 207
accaggcggc cgcgcacguc cuc 23

<210> 208
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 208
aaccaggcgg ccgcgcacgu ccu 23

<210> 209
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 209
acaccaggcg gccgcgcacg ucc 23

<210> 210
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 210
agcaccaggc ggccgcgcac guc 23

<210> 211
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 211
augcaccagg cggccgcgca cgu 23

<210> 212
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 212
aguacugcac caggcggccg cac 23

<210> 213
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 213
accgcgguac ugcaccaggc ggc 23

<210> 214
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 214
accgagcaug gccugcaccu cgc 23

<210> 215
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 215
agugcucugg ccgagcaugg ccu 23

<210> 216
<211> 23
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 216
aagcuccucg gugcucuggc cga 23

<210> 217
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 217
aaggcgaggc gcacccgcag cuc 23

<210> 218
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 218
aaggugggag gcgaggcgca ccc 23

<210> 219
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 219
agcuugcgca ggugggaggc gag 23

<210> 220
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 220
aacgcagcuu gcgcaggugg gag 23

<210> 221
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 221
acgcuuacgc agcuugcgca ggu 23

<210> 222
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 222
aaggagccgc uuacgcagcu ugc 23

<210> 223
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 223
aaucgcggag gagccgcuua cgc 23

<210> 224
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 224
aaucggcauc gcggaggagc cgc 23

<210> 225
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 225
aaggucaucg gcaucgcgga gga 23

<210> 226
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 226
auucugcagg ucaucggcau cgc 23

<210> 227
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 227
aaggcgcuuc ugcaggucau cgg 23

<210> 228
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 228
aacugccagg cgcuucugca ggu 23

<210> 229
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 229
augguacacu gccaggcgcu ucu 23

<210> 230
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 230
accggccugg uacacugcca ggc 23

<210> 231
<211> 23
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 231
aauggcgcug aggccgcgcu cgg 23

<210> 232
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 232
acgcggaugg cgcugaggcc gcg 23

<210> 233
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 233
aaggcgcucg cggauggcgc uga 23

<210> 234
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 234
acuguuccac caggggcccc agg 23

<210> 235
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 235
acggcccugu uccaccaggg gcc 23

<210> 236
<211> 23

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 236
aacaguggcg gcccgcacgc ggc 23

<210> 237
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 237
aagggagccc acaguggcgg ccc 23

<210> 238
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 238
aguagcggcu ggccggccag gga 23

<210> 239
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 239
acgcuccugu agcggcuggc cgg 23

<210> 240
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 240
agggcccgcu ccuguagcgg cug 23

<210> 241

<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 241
acccaucucc uccauccgcg cgc 23

<210> 242
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 242
aaccucgucc aggcggucgc ggg 23

<210> 243
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 243
acuccuucac cucguccagg cgg 23

<210> 244
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 244
aaccugcucc uucaccucgu cca 23

<210> 245
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 245
accagcuugg cgcgcaccuc cgc 23

<210> 246
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 246
augcuccucc agcuuggcgc gca 23

<210> 247
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 247
aaggcguauc ugcugggccu gcu 23

<210> 248
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 248
accugcaggc guaucugcug ggc 23

<210> 249
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 249
aaaggccucg gccugcaggc gua 23

<210> 250
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 250
accuggaagg ccucggccug cag 23

<210> 251
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 251
agcgggccug gaaggccucg gcc 23

<210> 252
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 252
auugaggcgg gccuggaagg ccu 23

<210> 253
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 253
aagcucuuga ggcgggccug gaa 23

<210> 254
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 254
acgaaccagc ucuugaggcg ggc 23

<210> 255
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 255
aggggcucga accagcucuu gag 23

<210> 256
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 256
acaugucuuc caccaggggc ucg 23

<210> 257
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 257
acgcugcaug ucuuccacca ggg 23

<210> 258
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 258
aacuggcgcu gcaugucuuc cac 23

<210> 259
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 259
aaccuucucc accagcccgg ccc 23

<210> 260
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 260

accugcaccu ucuccaccag ccc 23

<210> 261
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 261
aggcacaggg gcggcgcugg ugc 23

<210> 262
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 262
auugucgcug ggcacagggg cgg 23

<210> 263
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 263
aagugauugu cgcugggcac agg 23

<210> 264
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 264
agcguucagu gauugucgcu ggg 23

<210> 265
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 265
agcuucggcg uucagugauu guc 23

<210> 266
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 266
augcaggcuu cggcguucag uga 23

<210> 267
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 267
aauggcugca ggcuucggcg uuc 23

<210> 268
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 268
agucgcaugg cugcaggcuu cgg 23

<210> 269
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 269
acgugggguc gcauggcugc agg 23

<210> 270
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 270
aacggggugg cgugggggucg cau 23

<210> 271
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 271
agaggcacgg gguggcgugg ggu 23

<210> 272
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 272
aaggcaggag gcacggggug gcg 23

<210> 273
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 273
agcgcggagg caggaggcac ggg 23

<210> 274
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 274
aaggcugcgc ggaggcagga ggc 23

<210> 275
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 275
agcugcaggc ugcgcggagg cag 23

<210> 276
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 276
aacggcuggg gcggggacag ggu 23

<210> 277
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 277
aaggaggacg gcuggggcgg gga 23

<210> 278
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 278
aaccccagga ggacggcugg ggc 23

<210> 279
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 279
aaacuagggu ccaccccagg agg 23

<210> 280
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 280
auauuaaacu aggguccacc cca 23

<210> 281
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 281
aaucuuuauu aaacuagggu cca 23

<210> 282
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 282
aggugaaucu uuauuaaacu agg 23

<210> 283
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 283
aaacuuggug aaucuuuauu aaa 23

<210> 284
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 284
agcgugaaac uuggugaauc uuu 23

<210> 285
<211> 21
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 285
ggccaaucac aggcaggaag u 21

<210> 286
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 286
caggcaggaa gaugaagguu u 21

<210> 287
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 287
aggaagauga agguucugug u 21

<210> 288
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 288
augaagguuc ugugggcugc u 21

<210> 289
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 289
uucugugggc ugcguugcug u 21

<210> 290
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 290
ugggcugcgu ugcuggucac u 21

<210> 291
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 291
gcguugcugg ucacauuccu u 21

<210> 292
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 292
gcuggucaca uuccuggcag u 21

<210> 293
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 293
ucacauuccu ggcaggaugc u 21

<210> 294
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 294
uggcaggaug ccaggccaag u 21

<210> 295
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 295
caggccaagg uggagcaagc u 21

<210> 296
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 296
aagguggagc aagcggugga u 21

<210> 297
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 297
caagcggugg agacagagcc u 21

<210> 298
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 298
gguggagaca gagccggagc u 21

<210> 299
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 299
cccgagcugc gccagcagac u 21

<210> 300
<211> 21
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 300
gcugcgccag cagaccgagu u 21

<210> 301
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 301
ccagcagacc gaguggcaga u 21

<210> 302
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 302
cagcgcuggg aacuggcacu u 21

<210> 303
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 303
cugggaacug gcacuggguc u 21

<210> 304
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 304
aacuggcacu gggucgcuuu u 21

<210> 305
<211> 21

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 305
cacugggucg cuuuugggau u 21

<210> 306
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 306
gucgcuuuug ggauuaccug u 21

<210> 307
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 307
uuuugggauu accugcgcug u 21

<210> 308
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 308
cugcgcuggg ugcagacacu u 21

<210> 309
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 309
ugggugcaga cacugucuga u 21

<210> 310

<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 310
cagacacugu cugagcaggu u 21

<210> 311
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 311
caggaggagc ugcucagcuc u 21

<210> 312
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 312
cugcucagcu cccaggucac u 21

<210> 313
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 313
ucccagguca cccaggaacu u 21

<210> 314
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 314
acccaggaac ugagggcgcu u 21

<210> 315
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 315
ugagggcgcu gauggacgag u 21

<210> 316
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 316
gcgcugaugg acgagaccau u 21

<210> 317
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 317
gacgagacca ugaaggaguu u 21

<210> 318
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 318
accaugaagg aguugaaggc u 21

<210> 319
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 319
ggaguugaag gccuacaaau u 21

<210> 320
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 320
aaggccuaca aaucggaacu u 21

<210> 321
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 321
acaaaucgga acuggaggaa u 21

<210> 322
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 322
ucggaacugg aggaacaacu u 21

<210> 323
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 323
gaggaacaac ugaccccggu u 21

<210> 324
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 324
cgcgggcacg gcuguccaag u 21

<210> 325
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 325
gcacggcugu ccaaggagcu u 21

<210> 326
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 326
gcuguccaag gagcugcagg u 21

<210> 327
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 327
gcccggcugg gcgcggacau u 21

<210> 328
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 328
cugggcgcgg acauggagga u 21

<210> 329
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 329

cgcggacaug gaggacgugc u 21

<210> 330
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 330
gcggacaugg aggacgugug u 21

<210> 331
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 331
gcggacaugg aggacgugcg u 21

<210> 332
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 332
cggacaugga ggacgugcgc u 21

<210> 333
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 333
ggacauggag gacgugcgcg u 21

<210> 334
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 334
gacauggagg acgugcgcgg u 21

<210> 335
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 335
acauggagga cgugcgcggc u 21

<210> 336
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 336
cauggaggac gugcgcggcc u 21

<210> 337
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 337
auggaggacg ugcgcggccg u 21

<210> 338
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 338
uggaggacgu gcgcggccgc u 21

<210> 339
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 339
ggaggacgug cgcggccgcc u 21

<210> 340
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 340
gaggacgugc gcggccgccu u 21

<210> 341
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 341
aggacgugcg cggccgccug u 21

<210> 342
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 342
ggacgugcgc ggccgccugg u 21

<210> 343
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 343
gacgugcgcg gccgccuggu u 21

<210> 344
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 344
acgugcgcgg ccgccuggug u 21

<210> 345
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 345
cgugcgcggc cgccuggugc u 21

<210> 346
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 346
gugcgcggcc gccuggugca u 21

<210> 347
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 347
gcggccgccu ggugcaguac u 21

<210> 348
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 348
cgccuggugc aguaccgcgg u 21

<210> 349
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 349
gaggugcagg ccaugcucgg u 21

<210> 350
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 350
gccaugcucg gccagagcac u 21

<210> 351
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 351
ggccagagca ccgaggagcu u 21

<210> 352
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 352
gcugcgggug cgccucgccu u 21

<210> 353
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 353
gugcgccucg ccucccaccu u 21

<210> 354
<211> 21
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 354
cgccucccac cugcgcaagc u 21

<210> 355
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 355
cccaccugcg caagcugcgu u 21

<210> 356
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 356
cugcgcaagc ugcguaagcg u 21

<210> 357
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 357
aagcugcgua agcggcuccu u 21

<210> 358
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 358
guaagcggcu ccuccgcgau u 21

<210> 359
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 359
ggcuccuccg cgaugccgau u 21

<210> 360
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 360
cuccgcgaug ccgaugaccu u 21

<210> 361
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 361
gaugccgaug accugcagaa u 21

<210> 362
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 362
gaugaccugc agaagcgccu u 21

<210> 363
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 363
cugcagaagc gccuggcagu u 21

<210> 364
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 364
aagcgccugg caguguacca u 21

<210> 365
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 365
cuggcagugu accaggccgg u 21

<210> 366
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 366
gagcgcggcc ucagcgccau u 21

<210> 367
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 367
cggccucagc gccauccgcg u 21

<210> 368
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 368
agcgccaucc gcgagcgccu u 21

<210> 369
<211> 21
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 369
uggggccccu gguggaacag u 21

<210> 370
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 370
ccccuggugg aacagggccg u 21

<210> 371
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 371
cgcgugcggg ccgccacugu u 21

<210> 372
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 372
gccgccacug ugggcucccu u 21

<210> 373
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 373
ccuggccggc cagccgcuac u 21

<210> 374
<211> 21

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 374
ggccagccgc uacaggagcg u 21

<210> 375
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 375
gccgcuacag gagcgggccc u 21

<210> 376
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 376
gcgcggaugg aggagauggg u 21

<210> 377
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 377
cgcgaccgcc uggacgaggu u 21

<210> 378
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 378
gccuggacga ggugaaggag u 21

<210> 379

<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 379
gacgagguga aggagcaggu u 21

<210> 380
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 380
ggaggugcgc gccaagcugg u 21

<210> 381
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 381
cgcgccaagc uggaggagca u 21

<210> 382
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 382
caggcccagc agauacgccu u 21

<210> 383
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 383
ccagcagaua cgccugcagg u 21

<210> 384
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 384
cgccugcagg ccgaggccuu u 21

<210> 385
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 385
gcaggccgag gccuuccagg u 21

<210> 386
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 386
ccgaggccuu ccaggcccgc u 21

<210> 387
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 387
gccuuccagg cccgccucaa u 21

<210> 388
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 388
ccaggcccgc cucaagagcu u 21

<210> 389
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 389
ccgccucaag agcugguucg u 21

<210> 390
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 390
caagagcugg uucgagcccc u 21

<210> 391
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 391
agccccuggu ggaagacaug u 21

<210> 392
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 392
cugguggaag acaugcagcg u 21

<210> 393
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 393
ggaagacaug cagcgccagu u 21

<210> 394
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 394
gccgggcugg uggagaaggu u 21

<210> 395
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 395
gcugguggag aaggugcagg u 21

<210> 396
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 396
accagcgccg ccccugugcc u 21

<210> 397
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 397
gccccugugc ccagcgacaa u 21

<210> 398
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 398

ugugcccagc gacaaucacu u 21

<210> 399
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 399
cagcgacaau cacugaacgc u 21

<210> 400
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 400
caaucacuga acgccgaagc u 21

<210> 401
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 401
acugaacgcc gaagccugca u 21

<210> 402
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 402
acgccgaagc cugcagccau u 21

<210> 403
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 403
gaagccugca gccaugcgac u 21

<210> 404
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 404
ugcagccaug cgaccccacg u 21

<210> 405
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 405
gcgaccccac gccaccccgu u 21

<210> 406
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 406
cccacgccac cccgugccuc u 21

<210> 407
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 407
ccaccccgug ccuccugccu u 21

<210> 408
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 408
cgugccuccu gccuccgcgc u 21

<210> 409
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 409
cuccugccuc cgcgcagccu u 21

<210> 410
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 410
gccuccgcgc agccugcagc u 21

<210> 411
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 411
ccuguccccg ccccagccgu u 21

<210> 412
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 412
cccgccccag ccguccuccu u 21

<210> 413
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 413
cccagccguc cuccuggggu u 21

<210> 414
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 414
uccuggggug gacccuaguu u 21

<210> 415
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 415
ggguggaccc uaguuuaaua u 21

<210> 416
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 416
gacccuaguu uaauaaagau u 21

<210> 417
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 417
uaguuuaaua aagauucacc u 21

<210> 418
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 418
uaauaaagau ucaccaaguu u 21

<210> 419
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 419
agauucacca aguuucacgc u 21

<210> 420
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 420
acuuccugcc ugugauuggc cag 23

<210> 421
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 421
aaaccuucau cuuccugccu gug 23

<210> 422
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 422
acacagaacc uucaucuucc ugc 23

<210> 423
<211> 23
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 423
agcagcccac agaaccuuca ucu 23

<210> 424
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 424
acagcaacgc agcccacaga acc 23

<210> 425
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 425
agugaccagc aacgcagccc aca 23

<210> 426
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 426
aaggaaugug accagcaacg cag 23

<210> 427
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 427
acugccagga augugaccag caa 23

<210> 428
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 428
agcauccugc caggaaugug acc 23

<210> 429
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 429
acuuggccug gcauccugcc agg 23

<210> 430
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 430
agcuugcucc accuuggccu ggc 23

<210> 431
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 431
auccaccgcu ugcuccaccu ugg 23

<210> 432
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 432
aggcucuguc uccaccgcuu gcu 23

<210> 433
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 433
agcuccggcu cugucuccac cgc 23

<210> 434
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 434
agucugcugg cgcagcucgg gcu 23

<210> 435
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 435
aacucggucu gcuggcgcag cuc 23

<210> 436
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 436
aucugccacu cggucugcug gcg 23

<210> 437
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 437
aagugccagu ucccagcgcu ggc 23

<210> 438
<211> 23
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 438
agacccagug ccaguuccca gcg 23

<210> 439
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 439
aaaagcgacc cagugccagu ucc 23

<210> 440
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 440
aaucccaaaa gcgacccagu gcc 23

<210> 441
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 441
acagguaauc ccaaaagcga ccc 23

<210> 442
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 442
acagcgcagg uaaucccaaa agc 23

<210> 443
<211> 23

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 443
aagugucugc acccagcgca ggu 23

<210> 444
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 444
aucagacagu gucugcaccc agc 23

<210> 445
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 445
aaccugcuca gacagugucu gca 23

<210> 446
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 446
agagcugagc agcuccuccu gca 23

<210> 447
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 447
agugaccugg gagcugagca gcu 23

<210> 448

<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 448
aaguuccugg gugaccuggg agc 23

<210> 449
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 449
aagcgcccuc aguuccuggg uga 23

<210> 450
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 450
acucguccau cagcgcccuc agu 23

<210> 451
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 451
aauggucucg uccaucagcg ccc 23

<210> 452
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 452
aaacuccuuc auggucucgu cca 23

<210> 453
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 453
agccuucaac uccuucaugg ucu 23

<210> 454
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 454
aauuuguagg ccuucaacuc cuu 23

<210> 455
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 455
aaguuccgau uuguaggccu uca 23

<210> 456
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 456
auuccuccag uuccgauuug uag 23

<210> 457
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 457
aaguuguucc uccaguuccg auu 23

<210> 458
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 458
aaccgggguc aguuguuccu cca 23

<210> 459
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 459
acuuggacag ccgugcccgc guc 23

<210> 460
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 460
aagcuccuug gacagccgug ccc 23

<210> 461
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 461
accugcagcu ccuuggacag ccg 23

<210> 462
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 462
aauguccgcg cccagccggg ccu 23

<210> 463
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 463
auccuccaug uccgcgccca gcc 23

<210> 464
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 464
agcacguccu ccauguccgc gcc 23

<210> 465
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 465
acacacgucc uccauguccg cgc 23

<210> 466
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 466
acgcacgucc uccauguccg cgc 23

<210> 467
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 467

agcgcacguc cuccaugucc gcg 23

<210> 468
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 468
acgcgcacgu ccuccauguc cgc 23

<210> 469
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 469
accgcgcacg uccuccaugu ccg 23

<210> 470
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 470
agccgcgcac guccuccaug ucc 23

<210> 471
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 471
aggccgcgca cguccuccau guc 23

<210> 472
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 472
acggccgcgc acguccucca ugu 23

<210> 473
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 473
agcggccgcg cacguccucc aug 23

<210> 474
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 474
aggcggccgc gcacguccuc cau 23

<210> 475
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 475
aaggcggccg cgcacguccu cca 23

<210> 476
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 476
acaggcggcc gcgcacgucc ucc 23

<210> 477
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 477
accaggcggc cgcgcacguc cuc 23

<210> 478
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 478
aaccaggcgg ccgcgcacgu ccu 23

<210> 479
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 479
acaccaggcg gccgcgcacg ucc 23

<210> 480
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 480
agcaccaggc ggccgcgcac guc 23

<210> 481
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 481
augcaccagg cggccgcgca cgu 23

<210> 482
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 482
aguacugcac caggcggccg cac 23

<210> 483
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 483
accgcgguac ugcaccaggc ggc 23

<210> 484
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 484
accgagcaug gccugcaccu cgc 23

<210> 485
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 485
agugcucugg ccgagcaugg ccu 23

<210> 486
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 486
aagcuccucg gugcucuggc cga 23

<210> 487
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 487
aaggcgaggc gcacccgcag cuc 23

<210> 488
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 488
aaggugggag gcgaggcgca ccc 23

<210> 489
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 489
agcuugcgca ggugggaggc gag 23

<210> 490
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 490
aacgcagcuu gcgcaggugg gag 23

<210> 491
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 491
acgcuuacgc agcuugcgca ggu 23

<210> 492
<211> 23
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 492
aaggagccgc uuacgcagcu ugc 23

<210> 493
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 493
aaucgcggag gagccgcuua cgc 23

<210> 494
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 494
aaucggcauc gcggaggagc cgc 23

<210> 495
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 495
aaggucaucg gcaucgcgga gga 23

<210> 496
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 496
auucugcagg ucaucggcau cgc 23

<210> 497
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 497
aaggcgcuuc ugcaggucau cgg 23

<210> 498
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 498
aacugccagg cgcuucugca ggu 23

<210> 499
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 499
augguacacu gccaggcgcu ucu 23

<210> 500
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 500
accggccugg uacacugcca ggc 23

<210> 501
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 501
aauggcgcug aggccgcgcu cgg 23

<210> 502
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 502
acgcggaugg cgcugaggcc gcg 23

<210> 503
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 503
aaggcgcucg cggauggcgc uga 23

<210> 504
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 504
acuguuccac caggggcccc agg 23

<210> 505
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 505
acggcccugu uccaccaggg gcc 23

<210> 506
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 506
aacaguggcg gcccgcacgc ggc 23

<210> 507
<211> 23
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 507
aagggagccc acaguggcgg ccc 23

<210> 508
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 508
aguagcggcu ggccggccag gga 23

<210> 509
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 509
acgcuccugu agcggcuggc cgg 23

<210> 510
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 510
agggcccgcu ccuguagcgg cug 23

<210> 511
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 511
acccaucucc uccauccgcg cgc 23

<210> 512
<211> 23

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 512
aaccucgucc aggcggucgc ggg 23

<210> 513
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 513
acuccuucac cucguccagg cgg 23

<210> 514
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 514
aaccugcucc uucaccucgu cca 23

<210> 515
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 515
accagcuugg cgcgcaccuc cgc 23

<210> 516
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 516
augcuccucc agcuuggcgc gca 23

<210> 517

<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 517
aaggcguauc ugcugggccu gcu 23

<210> 518
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 518
accugcaggc guaucugcug ggc 23

<210> 519
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 519
aaaggccucg gccugcaggc gua 23

<210> 520
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 520
accuggaagg ccucggccug cag 23

<210> 521
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 521
agcgggccug gaaggccucg gcc 23

<210> 522
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 522
auugaggcgg gccuggaagg ccu 23

<210> 523
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 523
aagcucuuga ggcgggccug gaa 23

<210> 524
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 524
acgaaccagc ucuugaggcg ggc 23

<210> 525
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 525
aggggcucga accagcucuu gag 23

<210> 526
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 526
acaugucuuc caccaggggc ucg 23

<210> 527
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 527
acgcugcaug ucuuccacca ggg 23

<210> 528
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 528
aacuggcgcu gcaugucuuc cac 23

<210> 529
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 529
aaccuucucc accagcccgg ccc 23

<210> 530
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 530
accugcaccu ucuccaccag ccc 23

<210> 531
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 531
aggcacaggg gcggcgcugg ugc 23

<210> 532
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 532
auugucgcug ggcacagggg cgg 23

<210> 533
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 533
aagugauugu cgcugggcac agg 23

<210> 534
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 534
agcguucagu gauugucgcu ggg 23

<210> 535
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 535
agcuucggcg uucagugauu guc 23

<210> 536
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 536

augcaggcuu cggcguucag uga 23

<210> 537
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 537
aauggcugca ggcuucggcg uuc 23

<210> 538
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 538
agucgcaugg cugcaggcuu cgg 23

<210> 539
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 539
acgugggguc gcauggcugc agg 23

<210> 540
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 540
aacggggugg cgugggucg cau 23

<210> 541
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 541
agaggcacgg gguggcgugg ggu 23

<210> 542
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 542
aaggcaggag gcacggggug gcg 23

<210> 543
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 543
agcgcggagg caggaggcac ggg 23

<210> 544
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 544
aaggcugcgc ggaggcagga ggc 23

<210> 545
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 545
agcugcaggc ugcgcggagg cag 23

<210> 546
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 546
aacggcuggg gcggggacag ggu 23

<210> 547
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 547
aaggaggacg gcuggggcgg gga 23

<210> 548
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 548
aaccccagga ggacggcugg ggc 23

<210> 549
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 549
aaacuagggu ccaccccagg agg 23

<210> 550
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 550
auauuaaacu aggguccacc cca 23

<210> 551
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

oligonucleotide"

<400> 551
aaucuuuauu aaacuagggu cca 23

<210> 552
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 552
aggugaaucu uuauuaaacu agg 23

<210> 553
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 553
aaacuuggug aaucuuuauu aaa 23

<210> 554
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 554
agcgugaaac uuggugaauc uuu 23

<210> 555
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 555
ctggccaatc acaggcagga aga 23

<210> 556
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source

<223> /note="Description of Unknown:
Target sequence"

<400> 556
cacaggcagg aagatgaagg ttc 23

<210> 557
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 557
gcaggaagat gaaggttctg tgg 23

<210> 558
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 558
agatgaaggt tctgtgggct gcg 23

<210> 559
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 559
ggttctgtgg gctgcgttgc tgg 23

<210> 560
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 560
tgtgggctgc gttgctggtc aca 23

<210> 561
<211> 23
<212> DNA
<213> Unknown

<220>

<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 561
ctgcgttgct ggtcacattc ctg 23

<210> 562
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 562
ttgctggtca cattcctggc agg 23

<210> 563
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 563
ggtcacattc ctggcaggat gcc 23

<210> 564
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 564
cctggcagga tgccaggcca agg 23

<210> 565
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 565
gccaggccaa ggtggagcaa gcg 23

<210> 566
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 566
ccaaggtgga gcaagcggtg gag 23

<210> 567
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 567
agcaagcggt ggagacagag ccg 23

<210> 568
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 568
gcggtggaga cagagccgga gcc 23

<210> 569
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 569
agcccgagct gcgccagcag acc 23

<210> 570
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 570
gagctgcgcc agcagaccga gtg 23

<210> 571
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 571
cgccagcaga ccgagtggca gag 23

<210> 572
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 572
gccagcgctg ggaactggca ctg 23

<210> 573
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 573
cgctgggaac tggcactggg tcg 23

<210> 574
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 574
ggaactggca ctgggtcgct ttt 23

<210> 575
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 575
ggcactgggt cgcttttggg att 23

<210> 576
<211> 23
<212> DNA

<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 576
gggtcgcttt tgggattacc tgc 23

<210> 577
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 577
gcttttggga ttacctgcgc tgg 23

<210> 578
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 578
acctgcgctg ggtgcagaca ctg 23

<210> 579
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 579
gctgggtgca gacactgtct gag 23

<210> 580
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 580
tgcagacact gtctgagcag gtg 23

<210> 581
<211> 23

<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 581
tgcaggagga gctgctcagc tcc 23

<210> 582
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 582
agctgctcag ctcccaggtc acc 23

<210> 583
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 583
gctcccaggt cacccaggaa ctg 23

<210> 584
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 584
tcacccagga actgagggcg ctg 23

<210> 585
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 585
actgagggcg ctgatggacg aga 23

<210> 586

<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 586
gggcgctgat ggacgagacc atg 23

<210> 587
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 587
tggacgagac catgaaggag ttg 23

<210> 588
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 588
agaccatgaa ggagttgaag gcc 23

<210> 589
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 589
aaggagttga aggcctacaa atc 23

<210> 590
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 590
tgaaggccta caaatcggaa ctg 23

<210> 591
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 591
ctacaaatcg gaactggagg aac 23

<210> 592
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 592
aatcggaact ggaggaacaa ctg 23

<210> 593
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 593
tggaggaaca actgaccccg gtg 23

<210> 594
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 594
gacgcgggca cggctgtcca agg 23

<210> 595
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 595
gggcacggct gtccaaggag ctg 23

<210> 596
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 596
cggctgtcca aggagctgca ggc 23

<210> 597
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 597
aggcccggct gggcgcggac atg 23

<210> 598
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 598
ggctgggcgc ggacatggag gac 23

<210> 599
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 599
ggcgcggaca tggaggacgt gcg 23

<210> 600
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 600
gcgcggacat ggaggacgtg tgc 23

<210> 601
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 601
gcgcggacat ggaggacgtg cgc 23

<210> 602
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 602
cgcggacatg gaggacgtgc gcg 23

<210> 603
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 603
gcggacatgg aggacgtgcg cgg 23

<210> 604
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 604
cggacatgga ggacgtgcgc ggc 23

<210> 605
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 605

ggacatggag gacgtgcgcg gcc 23

<210> 606
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 606
gacatggagg acgtgcgcgg ccg 23

<210> 607
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 607
acatggagga cgtgcgcggc cgc 23

<210> 608
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 608
catggaggac gtgcgcggcc gcc 23

<210> 609
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 609
atggaggacg tgcgcggccg cct 23

<210> 610
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 610
tggaggacgt gcgcggccgc ctg 23

<210> 611
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 611
ggaggacgtg cgcggccgcc tgg 23

<210> 612
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 612
gaggacgtgc gcggccgcct ggt 23

<210> 613
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 613
aggacgtgcg cggccgcctg gtg 23

<210> 614
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 614
ggacgtgcgc ggccgcctgg tgc 23

<210> 615
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 615
gacgtgcgcg gccgcctggt gca 23

<210> 616
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 616
acgtgcgcgg ccgcctggtg cag 23

<210> 617
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 617
gtgcggccgc ctggtgcagt acc 23

<210> 618
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 618
gccgcctggt gcagtaccgc ggc 23

<210> 619
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 619
gcgaggtgca ggccatgctc ggc 23

<210> 620
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:

Target sequence"

<400> 620
aggccatgct cggccagagc acc 23

<210> 621
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 621
tcggccagag caccgaggag ctg 23

<210> 622
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 622
gagctgcggg tgcgcctcgc ctc 23

<210> 623
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 623
gggtgcgcct cgcctcccac ctg 23

<210> 624
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 624
ctcgcctccc acctgcgcaa gct 23

<210> 625
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source

<223> /note="Description of Unknown:
Target sequence"

<400> 625
ctcccacctg cgcaagctgc gta 23

<210> 626
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 626
acctgcgcaa gctgcgtaag cgg 23

<210> 627
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 627
gcaagctgcg taagcggctc ctc 23

<210> 628
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 628
gcgtaagcgg ctcctccgcg atg 23

<210> 629
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 629
gcggctcctc cgcgatgccg atg 23

<210> 630
<211> 23
<212> DNA
<213> Unknown

<220>

<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 630
tcctccgcga tgccgatgac ctg 23

<210> 631
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 631
gcgatgccga tgacctgcag aag 23

<210> 632
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 632
ccgatgacct gcagaagcgc ctg 23

<210> 633
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 633
acctgcagaa gcgcctggca gtg 23

<210> 634
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 634
agaagcgcct ggcagtgtac cag 23

<210> 635
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 635
gcctggcagt gtaccaggcc ggg 23

<210> 636
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 636
ccgagcgcgg cctcagcgcc atc 23

<210> 637
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 637
cgcggcctca gcgccatccg cga 23

<210> 638
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 638
tcagcgccat ccgcgagcgc ctg 23

<210> 639
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 639
cctggggccc ctggtggaac agg 23

<210> 640
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 640
ggcccctggt ggaacagggc cgc 23

<210> 641
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 641
gccgcgtgcg ggccgccact gtg 23

<210> 642
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 642
gggccgccac tgtgggctcc ctg 23

<210> 643
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 643
tccctggccg gccagccgct aca 23

<210> 644
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 644
ccggccagcc gctacaggag cgg 23

<210> 645
<211> 23
<212> DNA

<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 645
cagccgctac aggagcgggc cca 23

<210> 646
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 646
gcgcgcggat ggaggagatg ggc 23

<210> 647
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 647
cccgcgaccg cctggacgag gtg 23

<210> 648
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 648
ccgcctggac gaggtgaagg agc 23

<210> 649
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 649
tggacgaggt gaaggagcag gtg 23

<210> 650
<211> 23

<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 650
gcggaggtgc gcgccaagct gga 23

<210> 651
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 651
tgcgcgccaa gctggaggag cag 23

<210> 652
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 652
agcaggccca gcagatacgc ctg 23

<210> 653
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 653
gcccagcaga tacgcctgca ggc 23

<210> 654
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 654
tacgcctgca ggccgaggcc ttc 23

<210> 655

<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 655
ctgcaggccg aggccttcca ggc 23

<210> 656
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 656
ggccgaggcc ttccaggccc gcc 23

<210> 657
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 657
aggccttcca ggcccgcctc aag 23

<210> 658
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 658
ttccaggccc gcctcaagag ctg 23

<210> 659
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 659
gcccgcctca agagctggtt cga 23

<210> 660
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 660
ctcaagagct ggttcgagcc cct 23

<210> 661
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 661
cgagcccctg gtggaagaca tgc 23

<210> 662
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 662
ccctggtgga agacatgcag cgc 23

<210> 663
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 663
gtggaagaca tgcagcgcca gtg 23

<210> 664
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 664
gggccgggct ggtggagaag gtg 23

<210> 665
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 665
gggctggtgg agaaggtgca ggc 23

<210> 666
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 666
gcaccagcgc cgcccctgtg ccc 23

<210> 667
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 667
ccgcccctgt gcccagcgac aat 23

<210> 668
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 668
cctgtgccca gcgacaatca ctg 23

<210> 669
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 669
cccagcgaca atcactgaac gcc 23

<210> 670
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 670
gacaatcact gaacgccgaa gcc 23

<210> 671
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 671
tcactgaacg ccgaagcctg cag 23

<210> 672
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 672
gaacgccgaa gcctgcagcc atg 23

<210> 673
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 673
ccgaagcctg cagccatgcg acc 23

<210> 674
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 674

cctgcagcca tgcgacccca cgc 23

<210> 675
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 675
atgcgacccc acgccacccc gtg 23

<210> 676
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 676
accccacgcc accccgtgcc tcc 23

<210> 677
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 677
cgccaccccg tgcctcctgc ctc 23

<210> 678
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 678
cccgtgcctc ctgcctccgc gca 23

<210> 679
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 679
gcctcctgcc tccgcgcagc ctg 23

<210> 680
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 680
ctgcctccgc gcagcctgca gcg 23

<210> 681
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 681
accctgtccc cgccccagcc gtc 23

<210> 682
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 682
tccccgcccc agccgtcctc ctg 23

<210> 683
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 683
gccccagccg tcctcctggg gtg 23

<210> 684
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 684
cctcctgggg tggaccctag ttt 23

<210> 685
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 685
tggggtggac cctagtttaa taa 23

<210> 686
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 686
tggaccctag tttaataaag att 23

<210> 687
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 687
cctagtttaa taaagattca cca 23

<210> 688
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 688
tttaataaag attcaccaag ttt 23

<210> 689
<211> 23
<212> DNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:

Target sequence"

<400> 689
aaagattcac caagtttcac gca 23

<210> 690
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 690
caggcaggaa gaugaagguu a 21

<210> 691
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 691
aggaagauga agguucugug a 21

<210> 692
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 692
uucugugggc ugcguugcug a 21

<210> 693
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 693
gcguugcugg ucacauuccu a 21

<210> 694
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 694
cacugggucg cuuuugggau a 21

<210> 695
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 695
gucgcuuuug ggauuaccug a 21

<210> 696
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 696
uuuugggauu accugcgcug a 21

<210> 697
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 697
ccgccucaag agcugguucg a 21

<210> 698
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 698
gacccuaguu uaauaaagau a 21

<210> 699
<211> 21
<212> RNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 699
cggccucagc gccauccgcg a 21

<210> 700
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 700
uaaccuucau cuuccugccu gug 23

<210> 701
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 701
ucacagaacc uucaucuucc ugc 23

<210> 702
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 702
ucagcaacgc agcccacaga acc 23

<210> 703
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 703
uaggaaugug accagcaacg cag 23

<210> 704
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 704
uaucccaaaa gcgacccagu gcc 23

<210> 705
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 705
ucagguaauc ccaaaagcga ccc 23

<210> 706
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 706
ucagcgcagg uaaucccaaa agc 23

<210> 707
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 707
ucgaaccagc ucuugaggcg ggc 23

<210> 708
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 708
uaucuuuauu aaacuagggu cca 23

<210> 709
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 709
ucgcggaugg cgcugaggcc gcg 23

<210> 710
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 710
caggcaggaa gaugaagguu a 21

<210> 711
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 711
aggaagauga agguucugug a 21

<210> 712
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 712
uucugugggc ugcguugcug a 21

<210> 713
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 713
gcguugcugg ucacauuccu a 21

<210> 714
<211> 21
<212> RNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 714
cacugggucg cuuuugggau a 21

<210> 715
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 715
gucgcuuuug ggauuaccug a 21

<210> 716
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 716
uuuugggauu accugcgcug a 21

<210> 717
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 717
ccgccucaag agcugguucg a 21

<210> 718
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 718
gacccuaguu uaauaaagau a 21

<210> 719
<211> 21

<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 719
cggccucagc gccauccgcg a 21

<210> 720
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 720
uaaccuucau cuuccugccu gug 23

<210> 721
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 721
ucacagaacc uucaucuucc ugc 23

<210> 722
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 722
ucagcaacgc agcccacaga acc 23

<210> 723
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 723
uaggaaugug accagcaacg cag 23

<210> 724

<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 724
uaucccaaaa gcgacccagu gcc 23

<210> 725
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 725
ucagguaauc ccaaaagcga ccc 23

<210> 726
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 726
ucagcgcagg uaaucccaaa agc 23

<210> 727
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 727
ucgaaccagc ucuugaggcg ggc 23

<210> 728
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic oligonucleotide"

<400> 728
uaucuuuauu aaacuagggu cca 23

<210> 729
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 729
ucgcggaugg cgcugaggcc gcg 23

<210> 730
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 730
cacaggcagg aagaugaagg uuc 23

<210> 731
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 731
gcaggaagau gaagguucug ugg 23

<210> 732
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 732
gguucugugg gcugcguugc ugg 23

<210> 733
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 733
cugcguugcu ggucacauuc cug 23

<210> 734
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 734
ggcacugggu cgcuuuuggg auu 23

<210> 735
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 735
gggucgcuuu ugggauuacc ugc 23

<210> 736
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 736
gcuuuuggga uuaccugcgc ugg 23

<210> 737
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 737
gcccgccuca agagcugguu cga 23

<210> 738
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 738
uggacccuag uuuaauaaag auu 23

<210> 739
<211> 23
<212> RNA
<213> Unknown

<220>
<221> source
<223> /note="Description of Unknown:
Target sequence"

<400> 739
cgcggccuca gcgccauccg cga 23