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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 November 2021 (18.11.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/231204 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *A61K 31/7125* (2006.01)
A61K 31/712 (2006.01) *A61K 31/7115* (2006.01)

(21) International Application Number:
PCT/US2021/031265

(22) International Filing Date:
07 May 2021 (07.05.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/023,103 11 May 2020 (11.05.2020) US

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(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))*

(54) **Title:** COMPLEMENT COMPONENT 4 INHIBITORS FOR TREATING NEUROLOGICAL DISEASES, AND RELATED COMPOSITONS, SYSTEMS AND METHODS OF USING SAME

(57) **Abstract:** The present invention relates to complement component 4 (C4) inhibitors for use in treatment of a neurological disease. The invention in particular relates to the use of C4 inhibitors for down-regulation of C4 expression. The invention also relates to nucleic acid molecules, which are complementary to C4A and/or C4B and capable of reducing the level of an C4A and/or C4B mRNA. Also comprised in the present invention is a pharmaceutical composition and its use in the treatment of a neurological disease.

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COMPLEMENT COMPONENT 4 INHIBITORS FOR TREATING NEUROLOGICAL DISEASES, AND RELATED COMPOSITIONS, SYSTEMS AND METHODS OF USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application relates to U.S. Provisional Application filed May 11, 2020, entitled "Complement Component C1R Inhibitors For Treating A Neurological Disease, And Related Compositions, Systems And Methods Of Using Same" and US Provisional Application filed May 11, 2020, entitled "Complement Component C1S Inhibitors For Treating A Neurological Disease, And Related Compositions, Systems And Methods Of Using Same," the contents of which are both incorporated herein by reference in their entireties. This application claims priority to U.S. Provisional Application No. 63/023103, filed May 11, 2020, entitled "Complement Component C4 Inhibitors For Treating A Neurological Disease, And Related Compositions, Systems And Methods Of Using Same," the contents of which are incorporated herein by reference in its entirety.

SEQUENCE LISTING

This 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 May 6, 2021, is named P36089-WO_C4_SequenceList_ST25.txt and is 218,918 bytes in size.

FIELD OF INVENTION

The present invention relates to complement component 4 (C4) inhibitors for use in treatment of neurological diseases. The invention in particular relates to the use of C4 inhibitors for down-regulation of C4 expression. The invention also relates to nucleic acid molecules, which are complementary to C4A and/or C4B and capable of reducing the level of an C4A and/or C4B mRNA. Also comprised in the present invention is a pharmaceutical composition and its use in the treatment of neurological diseases.

BACKGROUND

The complement system is a part of the innate immune system that enhances the clearance of microbes or damaged cells by phagocytes and promotes inflammation. The complement system also participates in synaptic pruning in the brain, with the classical pathway of the complement

system mediating synapse removal. This process involves initiation of the classical pathway by the complement component 1 (C1) complex (consisting of C1Q, C1S and C1R), leading to cleavage of complement component 2 (C2) and complement component 4 (C4), which in turn lead to cleavage of complement component 3 (C3) followed by engulfment of synapses by microglia cells. Beyond roles in normal brain circuitry refinement during early development, it is well established that aberrant activity of the classical complement pathway can mediate synapse loss and neurodegeneration in various neurological diseases. Observations of elevated complement levels in patient samples and beneficial effects of reducing or eliminating complement components in mouse models have identified a damaging role for complement in conditions including, Alzheimer's disease, frontotemporal dementia, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, virus-induced cognitive impairment, glaucoma, macular degeneration, myasthenia gravis, Guillain-Barré syndrome, neuromyelitis optica, central nervous system lupus erythematosus and schizophrenia.

There remains a need in the art for therapeutic and prognostic agents to address such conditions. The present invention meets these and other needs.

OBJECTIVE OF THE INVENTION

The present invention provides nucleic acid inhibitors of complement component 4 (C4) which may be used both *in vivo* and *in vitro* for down-regulation of C4 expression and for the prophylactic and therapeutic intervention in neurological diseases. The present invention further identifies novel nucleic acid molecules, such as antisense oligonucleotides, which are capable of inhibiting the expression of C4 *in vitro* and *in vivo*.

SUMMARY OF INVENTION

The present invention relates to oligonucleotides targeting a nucleic acid and capable of modulating the expression of C4, useful, for example, to treat or prevent diseases related to the functioning of C4.

Accordingly, in a first aspect, the invention provides a C4 inhibitor for use in the treatment and/or prevention of neurological diseases, such as tauopathies or schizophrenia, in particular, a C4 inhibitor is capable of reducing the amount of C4, such as C4 mRNA and/or C4 protein. Such an inhibitor is advantageously a nucleic acid molecule of 12 to 60 nucleotides in length, which is capable of reducing C4 mRNA levels. In some embodiments, C4 is C4A and/or C4B.

In a further aspect, the invention relates to a nucleic acid molecule of 12-60 nucleotides, such as of 12-30 nucleotides, comprising a contiguous nucleotide sequence of at least 10 nucleotides, in particular of 16 to 20 nucleotides, which is at least 90% complementary, such as 90-95%, 95-98%, or fully complementary to a mammalian C4, e.g. a human C4A and/or C4B, a mouse C4b and/or C4a or a cynomolgus monkey C4. Such a nucleic acid molecule is capable of inhibiting the expression of C4A and/or C4B in a cell expressing C4A and/or C4B. The inhibition of C4A and/or C4B expression allows for a reduction of the amount of C4A and/or C4B protein present in the cell. The nucleic acid molecule can be selected from a single stranded antisense oligonucleotide, a double stranded siRNA molecule or a shRNA nucleic acid molecule (in particular chemically produced shRNA molecules).

A further aspect of the present invention relates to single stranded antisense oligonucleotides or siRNAs that inhibit the expression and/or activity of C4A and/or C4B. In particular, modified antisense oligonucleotides or modified siRNAs comprising one or more 2' sugar modified nucleoside(s) and one or more phosphorothioate linkage(s), which reduce C4A and/or C4B mRNA are advantageous.

In a further aspect, the invention provides pharmaceutical compositions comprising the C4 inhibitor of the present invention, such as the antisense oligonucleotide or siRNA of the invention and a pharmaceutically acceptable excipient.

In some embodiments, the C4 inhibitor is selected from the group consisting of a C4A inhibitor, a C4B inhibitor and a pan-C4 inhibitor.

In a further aspect, the invention provides methods for *in vivo* or *in vitro* modulation of C4A and/or C4B expression in a target cell, which is expressing C4A and/or C4B, by administering a C4 inhibitor of the present invention, such as an antisense oligonucleotide or composition of the invention in an effective amount to said cell. In some embodiments, the C4A and/or C4B expression is reduced by at least 50%, e.g., 50-60%; or at least 60%, e.g., 60-70%; or at least 70%, e.g., 70-80%; or at least 80%, e.g., 80-90%; or at least 90%, e.g., 90-95%, in the target cell compared to the level without any treatment or treated with a control.

In a further aspect, the invention provides methods for treating or preventing a disease, disorder or dysfunction associated with *in vivo* activity of C4 comprising administering a therapeutically or prophylactically effective amount of the C4 inhibitor of the present invention, such as the antisense oligonucleotide or siRNA of the invention to a subject suffering from or susceptible to the disease, disorder or dysfunction.

In some embodiments, the C4 inhibitor is selected from the group consisting of a C4A inhibitor, a C4B inhibitor and a pan-C4 inhibitor.

DEFINITIONS

Compound

Herein, the term "compound", with respect to a compound of the invention, means any molecule capable of inhibition C4 expression or activity. Particular compounds of the invention are nucleic acid molecules, such as RNAi molecules or antisense oligonucleotides according to the invention or any conjugate comprising such a nucleic acid molecule. For example, herein the compound may be a nucleic acid molecule targeting C4A and/or C4B, in particular an antisense oligonucleotide or a siRNA. In some embodiments, the compound is herein also referred to as an "inhibitor" or a "C4 inhibitor". In some embodiments, the C4 inhibitor is selected from the group consisting of a C4A inhibitor, a C4B inhibitor and a pan-C4 inhibitor. The term "C4A inhibitor" as used herein designates a molecule capable of specifically inhibiting C4A expression or activity. The term "C4B inhibitor" as used herein designates a molecule capable of specifically inhibiting C4B expression or activity. The term "pan-C4 inhibitor" as used herein designates a molecule capable of inhibiting both, C4A and C4B expression or activity.

Oligonucleotide

The term "oligonucleotide" as used herein is defined as it is generally understood by the skilled person, such as, as a molecule comprising two or more covalently linked nucleosides. An oligonucleotide is also referred to herein as a "nucleic acid" or "nucleic acid molecule". Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers. The oligonucleotides referred to in the description and claims are generally therapeutic oligonucleotides below 70 nucleotides in length. The oligonucleotide may be or comprise a single stranded antisense oligonucleotide, or may be another nucleic acid molecule, such as a CRISPR RNA, an siRNA, an shRNA, an aptamer, or a ribozyme. Therapeutic oligonucleotide molecules are commonly made in the laboratory by solid-phase chemical synthesis followed by purification and isolation. shRNA's are often delivered to cells using lentiviral vectors from which they are then transcribed to produce single stranded RNA that will form a stem loop (hairpin) RNA structure capable of interacting with RNA interference machinery (including the RNA-induced silencing complex (RISC)). In an embodiment of the present invention, the shRNA is a chemically produced shRNA molecule (not relying on cell-based expression from plasmids or viruses). When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. Generally, the oligonucleotide of the invention is man-made, and is chemically synthesized, and is typically

purified or isolated. Although in some embodiments, the oligonucleotide of the invention is an shRNA transcribed from a vector upon entry into the target cell. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides.

In some embodiments, the term oligonucleotide of the invention also includes pharmaceutically acceptable salts, esters, solvates and prodrugs thereof.

In some embodiments, the oligonucleotide of the invention comprises or consists of 10 to 70 nucleotides in length, such as from 12 to 60, such as from 13 to 50, such as from 14 to 40, such as from 15 to 30, such as from 16 to 25, such as from 16 to 22, such as from 16 to 20 contiguous nucleotides in length. Accordingly, the oligonucleotide of the present invention, in some embodiments, may have a length of 12 to 25 nucleotides. Alternatively, the oligonucleotide of the present invention, in some embodiments, may have a length of 15 to 21 nucleotides.

In some embodiments, the oligonucleotide, or a contiguous nucleotide sequence thereof, comprises or consists of 24 or less nucleotides, such as 22, such as 20 or less nucleotides, such as 14, 15, 16, 17, 18, 19, 20 or 21 nucleotides. It is to be understood that any range given herein includes the range endpoints. Accordingly, if a nucleic acid molecule is said to include from 15 to 20 nucleotides, both 15 and 20 nucleotide lengths are included.

In some embodiments, the contiguous nucleotide sequence comprises or consists of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 contiguous nucleotides in length.

The oligonucleotide(s) can modulate the expression of a target nucleic acid in a mammal or in a mammalian cell. In some embodiments, the nucleic acid molecules, such as for siRNAs, shRNAs and antisense oligonucleotides inhibit expression of a target nucleic acid(s).

In one embodiment of the invention, the oligonucleotide is selected from an RNAi agent, such as an siRNA or shRNA. In another embodiment, the oligonucleotide is a single stranded antisense oligonucleotide, such as a high affinity modified antisense oligonucleotide interacting with RNase H.

In some embodiments, the oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides, such as 2' sugar modified nucleosides.

In some embodiments, the oligonucleotide comprises phosphorothioate internucleoside linkages.

A library of oligonucleotides is to be understood as a collection of different oligonucleotides. The purpose of the library of oligonucleotides can vary. In some embodiments, the library of oligonucleotides is composed of oligonucleotides with overlapping nucleobase sequence targeting one or more mammalian C4A and/or C4B target nucleic acids, designed for the purpose of

identifying potent sequences, e.g., the most potent sequence, within the library of oligonucleotides. In some embodiments, the library of oligonucleotides is a library of oligonucleotide design variants (child nucleic acid molecules) of a parent or ancestral oligonucleotide, wherein the oligonucleotide design variants retain a core nucleobase sequence of the parent nucleic acid molecule, e.g., a conserved sequence of the parent.

Antisense oligonucleotides

The term "antisense oligonucleotide" or "ASO" as used herein is defined as oligonucleotides capable of hybridizing to a target nucleic acid, in particular to a contiguous sequence on a target nucleic acid, e.g., to modulate expression of the corresponding target gene. Generally, nucleic acid molecules of the invention are antisense nucleic acids. The antisense oligonucleotides are not essentially double stranded and need not be siRNAs or shRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded. It is understood that single stranded oligonucleotides of the present invention can form hairpins or intermolecular duplex structures (duplex between two molecules of the same oligonucleotide), e.g., where the degree of intra or inter self-complementarity is less than 50% across of the full length of the oligonucleotide.

Advantageously, in some embodiments, the single stranded antisense oligonucleotide of the invention does not contain RNA nucleosides, since this will decrease nuclease resistance.

Advantageously, in some embodiments, the oligonucleotide of the invention comprises one or more modified nucleosides or nucleotides, such as 2' sugar modified nucleosides. Furthermore, it is advantageous that, some, most, or all of the nucleosides, which are not modified, are DNA nucleosides, e.g., 50%, 75%, 95%, or 100% of the nucleosides which are not modified are DNA nucleosides.

RNAi molecules

Herein, the term "RNA interference (RNAi) molecule" refers to short double-stranded oligonucleotide containing RNA nucleosides and which mediates targeted cleavage of an RNA transcript, e.g., via the RNA-induced silencing complex (RISC), where they interact with the catalytic RISC component argonaute. The RNAi molecule modulates, e g., inhibits, the expression of the target nucleic acid in a cell, e.g. a cell within a subject, such as a mammalian subject. RNAi molecules includes single stranded RNAi molecules (Lima at al 2012 Cell 150: 883) and double stranded molecules, e.g., siRNAs or partially double-stranded molecules, as well as short hairpin RNAs (shRNAs). In some embodiments of the invention, the oligonucleotide of the invention or contiguous nucleotide sequence thereof is a RNAi agent, such as a siRNA.

siRNA

The term "small interfering ribonucleic acid" or "siRNA" refers to a small interfering ribonucleic acid RNAi molecule that generally interferes with the expression of an mRNA. The term refers to a class of double-stranded RNA molecules, also known in the art as short interfering RNA or silencing RNA. siRNAs typically comprise a sense strand (also referred to as a passenger strand) and an antisense strand (also referred to as the guide strand), wherein one or both strands are of 17 to 30 nucleotides in length, typically 19 to 25 nucleosides in length, wherein the antisense strand is complementary, such as at least 90%, e.g., 90-95% complementary, or such as fully complementary, to the target nucleic acid (suitably a mature mRNA sequence), and the sense strand is complementary to the antisense strand so that the sense strand and antisense strand form a duplex or duplex region. siRNA strands may form a blunt ended duplex, or advantageously the sense and/or antisense strand 3' end may form a 3' overhang of, e.g. 1, 2, or 3 nucleosides (e.g., to resemble the product produced by Dicer, which forms the RISC substrate *in vivo*. Effective extended forms of Dicer substrates have been described in US 8,349,809 and US 8,513,207, hereby incorporated by reference. In some embodiments, both the sense strand and antisense strand have a 2nt 3' overhang. The duplex region may therefore be, for example 17 to 25 nucleotides in length, such as 21 to 23 nucleotide in length.

Once inside a cell the antisense strand can be incorporated into the RISC complex, which mediate target degradation or target inhibition of the target nucleic acid. siRNAs typically comprise modified nucleosides in addition to RNA nucleosides. In one embodiment, the siRNA molecule may be chemically modified using modified internucleotide linkages and 2' sugar modified nucleosides, such as 2'-4' bicyclic ribose modified nucleosides, including LNA and cET or 2' substituted modifications like of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA. In particular, 2'fluoro, 2'-O-methyl or 2'-O-methoxyethyl may be incorporated into siRNAs.

In some embodiments, some, most, or all (e.g., 75-90%, 80-95%, 90-99%, or 100%) of the nucleotides of an siRNA sense (passenger) strand may be modified with 2' sugar modified nucleosides such as LNA (see WO2004/083430 and WO2007/085485, for example). In some embodiments, the passenger stand of the siRNA may be discontinuous (see WO2007/107162 for example). In some embodiments, thermally destabilizing nucleotides at a seed region of the antisense strand of siRNAs are useful in reducing off-target activity of the siRNAs (see WO2018/098328 for example). In some embodiments, the siRNA comprises a 5' phosphate group or a 5'-phosphate mimic at the 5' end of the antisense strand. In some embodiments, the 5' end of the antisense strand is a RNA nucleoside.

In one embodiment, the siRNA molecule further comprises at least one phosphorothioate or methylphosphonate internucleoside linkage. The phosphorothioaie or methylphosphonate internucleoside linkage may be at the 3'- terminus of one or both strands (e.g., the antisense strand and/or the sense strand); or the phosphorothioate or methylphosphonate internucleoside linkage may be at the 5'-terminus of one or both strands (e.g., the antisense strand and/or the sense strand); or the phosphorothioate or methylphosphonate internucleoside linkage may be at both the 5'- and 3'-termini of one or both strands (e.g., the antisense strand and/or the sense strand). In some embodiments, the remaining internucleoside linkages are phosphodiester linkages. In some embodiments, the siRNA molecule comprises one or more phosphorothioate internucleoside linkages. In siRNA molecules, phosphorothioate internucleoside linkages may reduce or inhibit nuclease cleavage in RICS. Accordingly, in some embodiments, not all internucleoside linkages in the antisense strand are modified, e.g., in some embodiments, 10-90%, 20-80%, 30-70%, or 40-60% of internucleoside linkages in the antisense strand are modified.

The siRNA molecule may further comprise a ligand. In some embodiments, the ligand is conjugated to the 3' end of the sense strand.

For biological distribution, siRNAs may be conjugated to a targeting ligand, and/or be formulated into lipid nanoparticles. In a particular example, the nucleic acid molecule is conjugated to a moiety that targets a brain cell or other cell of the CNS. Thus, the nucleic acid molecule may be conjugated to a moiety that facilitates delivery across the blood brain barrier. For example, the nucleic acid molecule may be conjugated to an antibody or antibody fragment targeting the transferrin receptor.

Other aspects of the invention relate to pharmaceutical compositions, in particular, pharmaceutical compositions comprising dsRNA, such as siRNA molecules suitable for therapeutic use, and methods of inhibiting the expression of a target gene by administering the dsRNA molecules such as siRNAs of the invention, e.g., for the treatment of various disease conditions as disclosed herein.

shRNA

The term "short hairpin RNA" or "shRNA" refers to molecules that are generally between 40 and 70 nucleotides in length, such as between 45 and 65 nucleotides in length, such as 50 and 60 nucleotides in length and form a stem loop (hairpin) RNA structure which can interact with the endonuclease known as Dicer (believed to processes dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs which then can be incorporated into an RNA-induced silencing complex (RISC)). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing. shRNA oligonucleotides may

be chemically modified using modified internucleotide linkages and 2' sugar modified nucleosides, such as 2'-4' bicyclic ribose modified nucleosides, including LNA and cET or 2' substituted modifications like of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA. In some embodiments, an shRNA molecule comprises one or more phosphorothioate internucleoside linkages. In RNAi molecules, phosphorothioate internucleoside linkages may reduce or inhibit nuclease cleavage in RICS. Accordingly, not all internucleoside linkages in the stem loop of the shRNA molecule are modified, e.g., in some embodiments, 10-90%, 20-80%, 30-70%, or 40-60% of internucleoside linkages in the antisense strand are modified. Phosphorothioate internucleoside linkages can advantageously be placed in the 3' and/or 5' end of the stem loop of the shRNA molecule, in particular, in the part of the molecule that is not complementary to the target nucleic acid. The region of the shRNA molecule that is complementary to the target nucleic acid may however also be modified, e.g., in the first 2 to 3 internucleoside linkages in the part that is predicted to become the 3' and/or 5' terminal following cleavage by Dicer.

Contiguous Nucleotide Sequence

The term "contiguous nucleotide sequence" refers to the region of the nucleic acid molecule, which is complementary to the target nucleic acid. The term is used interchangeably herein with the term "contiguous nucleobase sequence" and the term "oligonucleotide motif sequence". In some embodiments, all the nucleotides of the oligonucleotide constitute the contiguous nucleotide sequence. In some embodiments, the contiguous nucleotide sequence is included in the guide strand of an siRNA molecule. In some embodiments, the contiguous nucleotide sequence is the part of an shRNA molecule, which is 95%, 98%, 99%, or 100% complementary to the target nucleic acid. In some embodiments, the oligonucleotide comprises the contiguous nucleotide sequence, such as a F-G-F' gapmer region, and may optionally comprise further nucleotide(s), for example, a nucleotide linker region which may be used to attach a functional group (e.g. a conjugate group for targeting) to the contiguous nucleotide sequence. The nucleotide linker region may or may not be complementary to the target nucleic acid. In some embodiments, the nucleobase sequence of the antisense oligonucleotide is the contiguous nucleotide sequence. In some embodiments, the contiguous nucleotide sequence is 100% complementary to the target nucleic acid.

Nucleotides and nucleosides

Nucleotides and nucleosides are the building blocks of oligonucleotides and polynucleotides, and for the purposes of the present invention include both naturally occurring and non-naturally occurring nucleotides and nucleosides. In nature, nucleotides, such as DNA and RNA nucleotides

comprise a ribose sugar moiety, a nucleobase moiety and one or more phosphate groups (which is absent in nucleosides). Nucleosides and nucleotides may also interchangeably be referred to as "units" or "monomers".

Modified nucleoside

The term "modified nucleoside" or "nucleoside modification" as used herein refers to nucleosides modified as compared to the equivalent DNA or RNA nucleoside by the introduction of one or more modifications of the sugar moiety or the (nucleo)base moiety. Advantageously, in some embodiments, one or more of the modified nucleoside comprises a modified sugar moiety. The term "modified nucleoside" may also be used herein interchangeably with the term "nucleoside analogue" or "modified unit" or "modified monomer". Nucleosides with an unmodified DNA or RNA sugar moiety are termed DNA or RNA nucleosides herein. Nucleosides with modifications in the base region of the DNA or RNA nucleoside are still generally termed DNA or RNA if they allow Watson Crick base pairing.

Modified internucleoside linkage

The term "modified internucleoside linkage" is defined as generally understood by the skilled person, such as, as being a linkage other than phosphodiester (PO) linkages, that covalently couples two nucleosides together. The oligonucleotides of the invention may therefore comprise one or more modified internucleoside linkages, such as a one or more phosphorothioate internucleoside linkages, or one or more phosphorodithioate internucleoside linkages.

With the oligonucleotide of the invention, it can be advantageous to use phosphorothioate internucleoside linkages, e.g., for 10-90%, 20-80%, 30-70%, or 40-60% of internucleoside linkages.

Phosphorothioate internucleoside linkages are particularly useful due to nuclease resistance, beneficial pharmacokinetics, and ease of manufacture. In some embodiments, at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate, such as at least 60%, e.g., 60-80%; such as at least 70%, e.g., 70-85%; such as at least 75%, e.g., 75-90%; such as at least 80%, e.g.80-95%; or such as at least 90%, e.g., 90-99%, of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate. In some embodiments, all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate.

In some advantageous embodiments, all the internucleoside linkages of the contiguous nucleotide sequence of the oligonucleotide are phosphorothioate, or all the internucleoside linkages of the oligonucleotide are phosphorothioate linkages.

In some embodiments, the antisense oligonucleotides may comprise other internucleoside linkages (other than phosphodiester and phosphorothioate), for example alkyl phosphonate/methyl phosphonate internucleoside linkages, which may be tolerated in an otherwise DNA phosphorothioate gap region (e.g., as in EP 2 742 135).

Nucleobase

The term "nucleobase" includes the purine (e.g. adenine and guanine) and pyrimidine (e.g. uracil, thymine and cytosine) moiety present in nucleosides and nucleotides, which form hydrogen bonds in nucleic acid hybridization. In the context of the present invention, the term nucleobase also encompasses modified nucleobases, which may differ from naturally occurring nucleobases, but are functional during nucleic acid hybridization. In this context, "nucleobase" refers to both naturally occurring nucleobases such as adenine, guanine, cytosine, thymidine, uracil, xanthine and hypoxanthine, as well as non-naturally occurring variants. Such variants are for example described in Hirao et al (2012) Accounts of Chemical Research vol 45 page 2055 and Bergstrom (2009) Current Protocols in Nucleic Acid Chemistry Suppl. 37 1.4.1.

In some embodiments, the nucleobase moiety is modified by changing the purine or pyrimidine into a modified purine or pyrimidine, such as substituted purine or substituted pyrimidine, such as a nucleobase selected from isocytosine, pseudoisocytosine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uracil, 5-bromouracil 5-thiazolo-uracil, 2-thio-uracil, 2'thio-thymine, inosine, diaminopurine, 6-aminopurine, 2-aminopurine, 2,6-diaminopurine and 2-chloro-6-aminopurine.

The nucleobase moieties may be indicated by the letter code for each corresponding nucleobase, e.g. A, T, G, C or U, wherein each letter may optionally include modified nucleobases of equivalent function. For example, in the exemplified oligonucleotides, the nucleobase moieties are selected from A, T, G, C, and 5-methyl cytosine. Optionally, for LNA gapmers, 5-methyl cytosine LNA nucleosides may be used.

Modified oligonucleotide

The term "modified oligonucleotide" describes an oligonucleotide comprising one or more sugar-modified nucleosides and/or modified internucleoside linkages and/or modified nucleobases. The term "chimeric oligonucleotide" is a term that has been used in the literature to describe oligonucleotides comprising modified nucleosides and DNA nucleosides. The antisense oligonucleotide of the invention is advantageously a chimeric oligonucleotide.

Complementarity

The term "complementarity" or "complementary" describes the capacity for Watson-Crick base-pairing of nucleosides/nucleotides. Watson-Crick base pairs are guanine (G)-cytosine (C) and adenine (A) - thymine (T)/uracil (U). It will be understood that oligonucleotides may comprise nucleosides with modified nucleobases, for example 5-methyl cytosine is often used in place of cytosine, and as such the term complementarity encompasses Watson Crick base-paring between non-modified and modified nucleobases (see for example Hirao et al (2012) Accounts of Chemical Research vol 45 page 2055 and Bergstrom (2009) Current Protocols in Nucleic Acid Chemistry Suppl. 37 1.4.1).

The term "% complementary" as used herein, refers to the proportion of nucleotides (in percent) of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which across the contiguous nucleotide sequence, are complementary to a reference sequence (e.g. a target sequence or sequence motif). The percentage of complementarity is thus calculated by counting the number of aligned nucleobases that are complementary (from Watson Crick base pair) between the two sequences (when aligned with the target sequence 5'-3' and the oligonucleotide sequence from 3'-5'), dividing that number by the total number of nucleotides in the oligonucleotide and multiplying by 100. In such a comparison, a nucleobase/nucleotide, which does not align (form a base pair), is termed a mismatch. Insertions and deletions are not allowed in the calculation of % complementarity of a contiguous nucleotide sequence. It will be understood that in determining complementarity, chemical modifications of the nucleobases are disregarded as long as the functional capacity of the nucleobase to form Watson Crick base pairing is retained (e.g. 5'-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

The term "fully complementary", refers to 100% complementarity.

Identity

The term "Identity" as used herein, refers to the proportion of nucleotides (expressed in percent) of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which across the contiguous nucleotide sequence, are identical to a reference sequence (e.g. a sequence motif). The percentage of identity is thus calculated by counting the number of aligned nucleobases that are identical (a Match) between two sequences (in the contiguous nucleotide sequence of the compound of the invention and in the reference sequence), dividing that number by the total number of nucleotides in the oligonucleotide and multiplying by 100. Therefore, Percentage of Identity = (Matches x 100)/Length of aligned region (e.g. the contiguous nucleotide sequence). Insertions and deletions are not allowed in the calculation the percentage of identity of a contiguous nucleotide sequence. It will be understood that in determining identity, chemical

modifications of the nucleobases are disregarded as long as the functional capacity of the nucleobase to form Watson Crick base pairing is retained (e.g. 5-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

Hybridization

The term "hybridizing" or "hybridizes" as used herein is to be understood as referring to two nucleic acid strands (e.g. an oligonucleotide and a target nucleic acid) forming hydrogen bonds between base pairs on opposite strands, thereby forming a duplex. The affinity of the binding between two nucleic acid strands is the strength of the hybridization. It is often described in terms of the melting temperature (Tm) defined as the temperature at which half of the oligonucleotides are duplexed with the target nucleic acid. At physiological conditions, Tm is not strictly proportional to the affinity (Mergny and Lacroix, 2003, Oligonucleotides 13:515–537). The standard state Gibbs free energy ΔG° is a more accurate representation of binding affinity and is related to the dissociation constant (Kd) of the reaction by ΔG°=-RTln(Kd), where R is the gas constant and T is the absolute temperature. Therefore, a very low ΔG° of the reaction between an oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. ΔG° is the energy associated with a reaction where aqueous concentrations are 1M, the pH is 7, and the temperature is 37°C. The hybridization of oligonucleotides to a target nucleic acid is a spontaneous reaction and for spontaneous reactions, ΔG° is less than zero. ΔG° can be measured experimentally, for example, by use of the isothermal titration calorimetry (ITC) method as described in Hansen et al., 1965, Chem. Comm. 36–38 and Holdgate et al., 2005, Drug Discov Today. The skilled person will know that commercial equipment is available for ΔG° measurements. ΔG° can also be estimated numerically by using the nearest neighbor model as described by SantaLucia, 1998, Proc Natl Acad Sci USA. 95: 1460–1465 using appropriately derived thermodynamic parameters described by Sugimoto et al., 1995, Biochemistry 34:11211–11216 and McTigue et al., 2004, Biochemistry 43:5388–5405. In order to have the possibility of modulating a nucleic acid target by hybridization, oligonucleotides of the present invention hybridize to a target nucleic acid with estimated ΔG° values below -10 kcal/mol for oligonucleotides that are 10 to 30 nucleotides in length. In some embodiments, the degree or strength of hybridization is measured by the standard state Gibbs free energy ΔG°. The oligonucleotides may hybridize to a target nucleic acid with estimated ΔG° values below -10 kcal/mol, such as below -15 kcal/mol, such as below -20 kcal/mol and such as below -25 kcal/mol for oligonucleotides that are 8 to 30 nucleotides in length. In some embodiments, the oligonucleotides hybridize to a target nucleic acid with an estimated ΔG° value in the range of -10 to -60 kcal/mol, such as -12 to -40, such as from -15 to -30 kcal/mol or -16 to -27 kcal/mol such as -18 to -25 kcal/mol.

Target nucleic acid

According to the present invention, the target nucleic acid is a nucleic acid, which encodes a mammalian C4A or a mammalian C4B and may for example be a gene, a RNA, a mRNA, and pre-mRNA, a mature mRNA or a cDNA sequence. The target may therefore be referred to as C4A target nucleic acid or C4B target nucleic acid.

The therapeutic oligonucleotides of the invention may for example target exon regions of a mammalian C4A and/or C4B (in particular siRNA and shRNA, but also antisense oligonucleotides), or may for example target any intron region in the C4A and/or C4B pre-mRNA (in particular antisense oligonucleotides).

Table 1a and 1b list predicted exon and intron regions of SEQ ID NO: 3 and 4, i.e. of the human C4A and C4B pre-mRNA sequence.

Table 1a. Exons and introns in the human C4A pre-mRNA.

Exemplary exonic regions in the human C4A premRNA (SEQ ID NO 3)			Exemplary intronic regions in the human C4A premRNA (SEQ ID NO 3)		
ID	start	end	ID	start	end
Ea1	1	149	Ia1	150	281
Ea2	282	480	Ia2	481	694
Ea3	695	896	Ia3	897	1105
Ea4	1106	1176	Ia4	1177	1254
Ea5	1255	1343	Ia5	1344	1561
Ea6	1562	1644	Ia6	1645	1814
Ea7	1815	1911	Ia7	1912	2049
Ea8	2050	2155	Ia8	2156	2252
Ea9	2253	2385	Ia9	2386	9168
Ea10	9169	9284	Ia10	9285	9383
Ea11	9384	9563	Ia11	9564	9708
Ea12	9709	9891	Ia12	9892	10023

Ea13	10024	10209	Ia13	10210	10362
Ea14	10363	10521	Ia14	10522	10772
Ea15	10773	10899	Ia15	10900	11066
Ea16	11067	11141	Ia16	11142	11401
Ea17	11402	11599	Ia17	11600	11690
Ea18	11691	11802	Ia18	11803	11892
Ea19	11893	11963	Ia19	11964	12221
Ea20	12222	12361	Ia20	12362	12474
Ea21	12475	12684	Ia21	12685	12928
Ea22	12929	12980	Ia22	12981	13081
Ea23	13082	13171	Ia23	13172	13306
Ea24	13307	13516	Ia24	13517	13695
Ea25	13696	13771	Ia25	13772	13931
Ea26	13932	14088	Ia26	14089	14183
Ea27	14184	14300	Ia27	14301	14405
Ea28	14406	14577	Ia28	14578	14805
Ea29	14806	15038	Ia29	15039	15120
Ea30	15121	15288	Ia30	15289	15681
Ea31	15682	15741	Ia31	15742	16790
Ea32	16791	16884	Ia32	16885	16981
Ea33	16982	17168	Ia33	17169	17282
Ea34	17283	17373	Ia34	17374	17463
Ea35	17464	17538	Ia35	17539	19029
Ea36	19030	19132	Ia36	19133	19297
Ea37	19298	19387	Ia37	19388	19472

Ea38	19473	19571	Ia38	19572	19753
Ea39	19754	19837	Ia39	19838	20099
Ea40	20100	20232	Ia40	20233	20375
Ea41	20376	20658			

Table 1b. Exons and introns in the human C4B pre-mRNA.

Exemplary exonic regions in the human C4B premRNA (SEQ ID NO 4)			Exemplary intronic regions in the human C4B premRNA (SEQ ID NO 4)		
ID	start	end	ID	start	end
Eb1	1	116	Ib1	117	248
Eb2	249	447	Ib2	448	661
Eb3	662	863	Ib3	864	1072
Eb4	1073	1143	Ib4	1144	1221
Eb5	1222	1310	Ib5	1311	1528
Eb6	1529	1611	Ib6	1612	1781
Eb7	1782	1878	Ib7	1879	2016
Eb8	2017	2122	Ib8	2123	2219
Eb9	2220	2352	Ib9	2353	9135
Eb10	9136	9251	Ib10	9252	9350
Eb11	9351	9530	Ib11	9531	9675
Eb12	9676	9858	Ib12	9859	9990
Eb13	9991	10176	Ib13	10177	10329
Eb14	10330	10488	Ib14	10489	10739
Eb15	10740	10866	Ib15	10867	11033
Eb16	11034	11108	Ib16	11109	11368

Eb17	11369	11566	Ib17	11567	11657
Eb18	11658	11769	Ib18	11770	11859
Eb19	11860	11930	Ib19	11931	12188
Eb20	12189	12328	Ib20	12329	12441
Eb21	12442	12651	Ib21	12652	12895
Eb22	12896	12947	Ib22	12948	13048
Eb23	13049	13138	Ib23	13139	13273
Eb24	13274	13483	Ib24	13484	13662
Eb25	13663	13738	Ib25	13739	13898
Eb26	13899	14055	Ib26	14056	14150
Eb27	14151	14267	Ib27	14268	14372
Eb28	14373	14544	Ib28	14545	14771
Eb29	14772	15004	Ib29	15005	15086
Eb30	15087	15254	Ib30	15255	15647
Eb31	15648	15707	Ib31	15708	16756
Eb32	16757	16850	Ib32	16851	16947
Eb33	16948	17134	Ib33	17135	17248
Eb34	17249	17339	Ib34	17340	17429
Eb35	17430	17504	Ib35	17505	18995
Eb36	18996	19098	Ib36	19099	19263
Eb37	19264	19353	Ib37	19354	19438
Eb38	19439	19537	Ib38	19538	19719
Eb39	19720	19803	Ib39	19804	20065
Eb40	20066	20198	Ib40	20199	20341
Eb41	20342	20624			

In some embodiments, the target nucleic acid encodes a C4A protein, in particular a mammalian C4A protein, such as a human C4A protein. In some embodiments, the target nucleic acid encodes a C4B protein, in particular a mammalian C4B protein, such as a human C4B protein. See for example Table 2 and Table 3, which provides an overview on the genomic sequences of human, cyno monkey and mouse C4 (Table 2) and on pre-mRNA sequences for human, monkey and mouse C4 and for the mature mRNAs for human C4 (Table 3).

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, and 7, or naturally occurring variants thereof (e.g. sequences encoding a mammalian C4A and/or C4B).

Table 2. Genome and assembly information for C4A and C4B across species.

Species	Chr	Strand	Genomic coordinates		Assembly	NCBI reference sequence* accession number for mRNA
			Start	End		
Mouse	17	Rev	34728380	34743882	GRCm38.p6	NM_009780.2
Mouse	17	Rev	34809092	34823464	GRCm38.p6	NM_011413.2
Human	6	Fwd	31982057	32002681	GRCh38.p12	NM_007293.3 and NM_001252204.1
Human	6	Fwd	32014795	32035418	GRCh38.p12	NM_001002029.3
Cyno	4	Rev	138859330	138873551	Macaca_fascicularis_5	

Fwd = forward strand. Rev = reverse strand. The genome coordinates provide the pre-mRNA sequence (genomic sequence).

If employing the nucleic acid molecule of the invention in research or diagnostics, the target nucleic acid may be a cDNA or a synthetic nucleic acid derived from DNA or RNA.

For *in vivo* or *in vitro* application, the therapeutic nucleic acid molecule of the invention is typically capable of inhibiting the expression of the C4A and/or C4B target nucleic acid in a cell, which is expressing the C4A and C4B target nucleic acid. The contiguous sequence of nucleobases of the nucleic acid molecule of the invention is typically complementary to a conserved region of the C4A and/or C4B target nucleic acid, as measured across the length of the nucleic acid molecule, optionally with the exception of one or two mismatches. In some embodiments, the target nucleic acid is a messenger RNA, such as a pre-mRNA which encodes mammalian C4A protein, such as

mouse C4a, e.g. the mouse C4a pre-mRNA sequence, such as that disclosed as SEQ ID NO: 2, the human C4A pre-mRNA sequence, such as that disclosed as SEQ ID NO: 3, or the cyno monkey C4 pre-mRNA sequence, such as that disclosed as SEQ ID NO: 5, or a mature C4A mRNA, such as that of a human mature mRNA disclosed as SEQ ID NO: 6. In some embodiments, the target nucleic acid is a messenger RNA, such as a pre-mRNA which encodes mammalian C4B protein, such as mouse C4b, e.g. the mouse C4b pre-mRNA sequence, such as that disclosed as SEQ ID NO: 1, the human C4B pre-mRNA sequence, such as that disclosed as SEQ ID NO: 4, or the cyno monkey C4 pre-mRNA sequence, such as that disclosed as SEQ ID NO: 5, or a mature C4B mRNA, such as that of a human mature mRNA disclosed as SEQ ID NO:7. SEQ ID NOs: 1, 2, 3, 4, 5, 6, and 7, are DNA sequences – it will be understood that target RNA sequences have uracil (U) bases in place of the thymidine bases (T).

It is known that different, i.e. shorter, annotated mRNA isoforms of the above sequences exist. The isoforms are well-known in the art and can be derived from the known sequence databases.

Further information on exemplary target nucleic acids is provided in Table 3.

Table 3. Overview on target nucleic acids.

Target Nucleic Acid, Species, Reference	Sequence ID
C4b Mus musculus pre-mRNA	SEQ ID NO: 1
C4a Mus musculus pre-mRNA	SEQ ID NO: 2
C4A Homo sapiens pre-mRNA	SEQ ID NO: 3
C4B Homo sapiens pre-mRNA	SEQ ID NO: 4
C4 Macaca fascicularis pre-mRNA	SEQ ID NO: 5
C4A Homo sapiens mature mRNA	SEQ ID NO: 6
C4B Homo sapiens mature mRNA	SEQ ID NO: 7

In some embodiments, the target nucleic acid is SEQ ID NO: 1.

In some embodiments, the target nucleic acid is SEQ ID NO: 2.

In some embodiments, the target nucleic acid is SEQ ID NO: 3.

In some embodiments, the target nucleic acid is SEQ ID NO: 4.

In some embodiments, the target nucleic acid is SEQ ID NO: 5.

In some embodiments, the target nucleic acid is SEQ ID NO: 6.

In some embodiments, the target nucleic acid is SEQ ID NO: 7.

Target

The term "target" as used herein refers to the complement component 4 (C4), which can in the context of this disclosure be C4A and/or C4B. Further, the term "target" can refer to the C4A target nucleic acid and/or C4B target nucleic acid, as well as the C4A protein and/or C4B protein. For example, part of the antisense oligonucleotides described herein target both C4A target nucleic acid and C4B target nucleic acid, i.e. such antisense oligonucleotides target both C4A target nucleic acid and C4B target nucleic acid by binding C4A/C4B homologous regions (pan-C4 antisense oligonucleotides). As known in the art, the terms "C4A" and "C4B" (uppercase A/B) relate to the human target. The terms "C4a" and "C4b" (lower case a/b) relate to the mouse target.

Target Sequence

The term "target sequence" as used herein refers to a sequence of nucleotides present in the target nucleic acid, which comprises the nucleobase sequence, which is complementary to the oligonucleotide or nucleic acid molecule of the invention. In some embodiments, the target sequence comprises or consists of a region on the target nucleic acid with a nucleobase sequence that is complementary to the contiguous nucleotide sequence of the oligonucleotide of the invention. This region of the target nucleic acid may interchangeably be referred to as the target nucleotide sequence, target sequence or target region. In some embodiments, the target sequence is longer than the complementary sequence of a nucleic acid molecule of the invention, and may, for example represent a preferred region of the target nucleic acid, which may be targeted by several nucleic acid molecules of the invention. It is well known in the art that C4A and C4B genes display high level of variability between individuals. The term "target sequence" encompasses all publicly annotated variants of C4A and C4B.

In some embodiments, the target sequence is a sequence selected from the group consisting of a human C4A mRNA exon, such as a human C4A mRNA exon selected from the group consisting of Ea1 - Ea41 (see for example Table 1a above). In some embodiments, the target sequence is a sequence selected from the group consisting of a human C4B mRNA exon, such as a human C4B mRNA exon selected from the group consisting of Eb1 - Eb41 (see for example Table 1b above).

Accordingly, the invention provides for an oligonucleotide, wherein said oligonucleotide comprises a contiguous sequence which is at least 90% complementary, such as 90-95% or fully

complementary, to an exon region of SEQ ID NO: 3 and 4, selected from the group consisting of Ea1 - Ea41 and Eb1 - Eb41 (see Table 1a and 1b).

In some embodiments, the target sequence is a sequence selected from the group consisting of a human C4A mRNA intron, such as a human C4A mRNA intron selected from the group consisting of Ia1 - Ia40 (see for example Table 1a above). In some embodiments, the target sequence is a sequence selected from the group consisting of a human C4B mRNA exon, such as a human C4B mRNA intron selected from the group consisting of Ib1 - Ib40 (see for example Table 1b above).

Accordingly, the invention provides an oligonucleotide, wherein said oligonucleotide comprises a contiguous sequence which is at least 90% complementary, such as 90-95% or fully complementary, to an intron region of SEQ ID NO: 3 and 4, selected from the group consisting of Ia1 - Ia40 and Ib1 - Ib40 (see Table(s) 1a and 1b).

In some embodiments, the target sequence is selected from the group consisting of SEQ ID NO: 6, and 7. In some embodiments, the contiguous nucleotide sequence as referred to herein is at least 90% (e.g., 90-95%) complementary, such as at least 95% (e.g., 95-98) complementary to a target sequence selected from the group consisting of SEQ ID NO: 6, and 7. In some embodiments, the contiguous nucleotide sequence is fully complementary to a target sequence selected from the group consisting of SEQ ID NO: 6, and 7.

The oligonucleotide of the invention comprises a contiguous nucleotide sequence, which is complementary to or hybridizes to a region on the target nucleic acid, such as a target sequence described herein.

The target nucleic acid sequence to which the oligonucleotide is complementary or hybridizes to generally comprises a stretch of contiguous nucleobases of at least 10 nucleotides. The contiguous nucleotide sequence is between 12 to 70 nucleotides, such as 12 to 50, such as 13 to 30, such as 14 to 25, such as 15 to 21 contiguous nucleotides.

In some embodiments, the oligonucleotide of the present invention targets a region shown in Table 4a and 4b.

Table 4a: Exemplary target regions on SEQ ID NO: 3

Target region	start SEQ ID NO: 3	end SEQ ID NO: 3	Target region	start SEQ ID NO: 3	end SEQ ID NO: 3	Target region	start SEQ ID NO: 3	end SEQ ID NO: 3
1A	22	65	100A	11012	11054	199A	15857	15897

2A	74	92	101A	11056	11070	200A	15915	15942
3A	94	131	102A	11073	11099	201A	15966	15995
4A	133	196	103A	11114	11149	202A	16003	16024
5A	200	230	104A	11166	11181	203A	16026	16043
6A	248	390	105A	11200	11219	204A	16045	16064
7A	405	452	106A	11238	11277	205A	16068	16086
8A	454	485	107A	11284	11303	206A	16126	16178
9A	496	516	108A	11305	11337	207A	16180	16212
10A	567	591	109A	11339	11375	208A	16244	16268
11A	612	640	110A	11377	11420	209A	16280	16327
12A	648	670	111A	11422	11467	210A	16329	16363
13A	678	702	112A	11469	11495	211A	16385	16405
14A	712	768	113A	11497	11566	212A	16456	16486
15A	770	800	114A	11586	11634	213A	16497	16584
16A	819	852	115A	11637	11655	214A	16589	16603
17A	854	922	116A	11657	11671	215A	16674	16699
18A	945	980	117A	11673	11812	216A	16724	16752
19A	982	997	118A	11870	11925	217A	16762	16800
20A	1031	1046	119A	11939	11967	218A	16802	16843
21A	1056	1072	120A	11972	11996	219A	16845	16908
22A	1074	1091	121A	12017	12054	220A	16941	17022
23A	1099	1133	122A	12086	12116	221A	17049	17185
24A	1135	1168	123A	12118	12142	222A	17187	17265
25A	1170	1236	124A	12155	12195	223A	17274	17325
26A	1238	1286	125A	12213	12235	224A	17327	17366

27A	1295	1349	126A	12251	12347	225A	17368	17383
28A	1351	1368	127A	12352	12367	226A	17387	17405
29A	1370	1384	128A	12375	12392	227A	17415	17445
30A	1386	1431	129A	12394	12409	228A	17448	17552
31A	1444	1485	130A	12411	12428	229A	17558	17579
32A	1487	1501	131A	12454	12572	230A	17595	17631
33A	1504	1524	132A	12574	12593	231A	17690	17724
34A	1541	1612	133A	12595	12611	232A	17789	17837
35A	1614	1673	134A	12613	12641	233A	17841	17861
36A	1675	1701	135A	12643	12659	234A	17874	17896
37A	1719	1737	136A	12667	12706	235A	17901	17918
38A	1739	1756	137A	12719	12748	236A	17930	17953
39A	1780	1890	138A	12750	12784	237A	17960	17977
40A	1892	1938	139A	12807	12855	238A	17970	17984
41A	1950	1966	140A	12875	12902	239A	17987	18008
42A	1968	1989	141A	12904	12947	240A	18038	18053
43A	1998	2040	142A	12949	12976	241A	18055	18074
44A	2042	2100	143A	12989	13006	242A	18076	18095
45A	2102	2128	144A	13008	13106	243A	18121	18170
46A	2130	2173	145A	13108	13160	244A	18172	18190
47A	2175	2204	146A	13162	13188	245A	18207	18269
48A	2226	2302	147A	13190	13210	246A	18358	18376
49A	2312	2329	148A	13227	13267	247A	18389	18423
50A	2342	2418	149A	13284	13328	248A	18418	18455
51A	2422	2452	150A	13330	13349	249A	18462	18495

52A	2487	2522	151A	13351	13375	250A	18515	18552
53A	2538	2562	152A	13386	13467	251A	18582	18596
54A	2606	2638	153A	13477	13496	252A	18606	18621
55A	2649	2668	154A	13498	13526	253A	18638	18652
56A	3229	3243	155A	13530	13546	254A	18654	18668
57A	3231	3245	156A	13548	13566	255A	18680	18713
58A	9028	9045	157A	13568	13594	256A	18715	18729
59A	9047	9070	158A	13621	13642	257A	18731	18761
60A	9072	9099	159A	13644	13692	258A	18776	18807
61A	9108	9130	160A	13694	13758	259A	18824	18875
62A	9142	9161	161A	13799	13813	260A	18877	18895
63A	9163	9204	162A	13857	13874	261A	18901	18933
64A	9206	9317	163A	13892	13918	262A	18972	19022
65A	9320	9348	164A	13924	13947	263A	19024	19076
66A	9350	9445	165A	13949	14009	264A	19081	19107
67A	9447	9477	166A	14017	14042	265A	19109	19130
68A	9501	9526	167A	14056	14070	266A	19171	19188
69A	9528	9562	168A	14082	14105	267A	19230	19259
70A	9564	9618	169A	14145	14168	268A	19288	19344
71A	9595	9611	170A	14201	14269	269A	19346	19423
72A	9665	9687	171A	14285	14305	270A	19438	19458
73A	9701	9725	172A	14308	14326	271A	19466	19616
74A	9736	9911	173A	14394	14449	272A	19630	19648
75A	9921	9945	174A	14457	14515	273A	19650	19667
76A	9956	9971	175A	14532	14584	274A	19677	19698

77A	9973	10010	176A	14608	14642	275A	19713	19728
78A	10019	10074	177A	14644	14676	276A	19742	19763
79A	10090	10153	178A	14749	14771	277A	19765	19790
80A	10155	10214	179A	14803	14831	278A	19792	19846
81A	10226	10241	180A	14833	14860	279A	19853	19870
82A	10250	10295	181A	14881	14965	280A	19872	19893
83A	10309	10327	182A	14967	14981	281A	19900	19934
84A	10352	10389	183A	14988	15048	282A	19966	19990
85A	10393	10430	184A	15064	15079	283A	19992	20019
86A	10440	10540	185A	15109	15176	284A	20048	20065
87A	10562	10577	186A	15190	15218	285A	20067	20081
88A	10589	10613	187A	15244	15301	286A	20083	20116
89A	10635	10662	188A	15323	15355	287A	20132	20151
90A	10665	10685	189A	15381	15414	288A	20156	20180
91A	10693	10712	190A	15418	15437	289A	20182	20239
92A	10741	10763	191A	15439	15478	290A	20246	20260
93A	10764	10782	192A	15514	15540	291A	20264	20294
94A	10784	10798	193A	15554	15608	292A	20296	20320
95A	10800	10876	194A	15610	15630	293A	20330	20420
96A	10890	10931	195A	15643	15668	294A	20422	20462
97A	10933	10963	196A	15679	15742	295A	20474	20530
98A	10987	11008	197A	15794	15817	296A	20554	20585
99A	11001	11017	198A	15819	15855	297A	20597	20629

Table 4b: Exemplary target regions on SEQ ID NO: 4

Target region	start SEQ ID NO: 4	end SEQ ID NO: 4	Target region	start SEQ ID NO: 4	end SEQ ID NO: 4	Target region	start SEQ ID NO: 4	end SEQ ID NO: 4
1B	1	32	100B	10979	11021	199B	15823	15863
2B	41	59	101B	11023	11037	200B	15881	15908
3B	61	98	102B	11040	11066	201B	15932	15961
4B	100	163	103B	11081	11116	202B	15969	15990
5B	167	197	104B	11133	11148	203B	15992	16009
6B	215	357	105B	11167	11186	204B	16011	16030
7B	372	419	106B	11205	11244	205B	16034	16052
8B	421	452	107B	11251	11270	206B	16092	16144
9B	463	483	108B	11272	11304	207B	16146	16178
10B	534	558	109B	11306	11342	208B	16210	16234
11B	579	607	110B	11344	11387	209B	16246	16293
12B	615	637	111B	11389	11434	210B	16295	16329
13B	645	669	112B	11436	11462	211B	16351	16371
14B	679	735	113B	11464	11533	212B	16422	16452
15B	737	767	114B	11553	11601	213B	16463	16550
16B	786	819	115B	11604	11622	214B	16555	16569
17B	821	889	116B	11624	11638	215B	16640	16665
18B	912	947	117B	11640	11779	216B	16690	16718
19B	949	964	118B	11837	11892	217B	16728	16766
20B	998	1013	119B	11906	11934	218B	16768	16809
21B	1023	1039	120B	11939	11963	219B	16811	16874
22B	1041	1058	121B	11984	12021	220B	16907	16988

23B	1066	1100	122B	12053	12083	221B	17015	17151
24B	1102	1135	123B	12085	12109	222B	17153	17231
25B	1137	1203	124B	12122	12162	223B	17240	17291
26B	1205	1253	125B	12180	12202	224B	17293	17332
27B	1262	1316	126B	12218	12314	225B	17334	17349
28B	1318	1335	127B	12319	12334	226B	17353	17371
29B	1337	1351	128B	12341	12359	227B	17381	17411
30B	1353	1398	129B	12361	12376	228B	17414	17518
31B	1411	1452	130B	12378	12395	229B	17524	17545
32B	1454	1468	131B	12421	12539	230B	17561	17597
33B	1471	1491	132B	12541	12560	231B	17656	17690
34B	1508	1579	133B	12580	12608	232B	17755	17803
35B	1581	1640	134B	12610	12626	233B	17807	17827
36B	1642	1668	135B	12634	12673	234B	17840	17862
37B	1686	1704	136B	12686	12715	235B	17867	17884
38B	1706	1723	137B	12717	12751	236B	17896	17919
39B	1747	1857	138B	12774	12822	237B	17926	17943
40B	1859	1905	139B	12842	12869	238B	17936	17950
41B	1917	1933	140B	12871	12914	239B	17953	17974
42B	1935	1956	141B	12916	12943	240B	18004	18019
43B	1965	2007	142B	12956	12973	241B	18021	18040
44B	2009	2067	143B	12975	13073	242B	18042	18061
45B	2069	2095	144B	13075	13127	243B	18087	18136
46B	2097	2140	145B	13129	13155	244B	18138	18156
47B	2142	2171	146B	13157	13177	245B	18173	18235

48B	2193	2269	147B	13194	13234	246B	18324	18342
49B	2279	2296	148B	13251	13295	247B	18355	18389
50B	2309	2385	149B	13297	13316	248B	18384	18421
51B	2389	2419	150B	13318	13342	249B	18428	18461
52B	2454	2489	151B	13353	13434	250B	18481	18518
53B	2505	2529	152B	13444	13463	251B	18548	18562
54B	2573	2605	153B	13465	13493	252B	18572	18587
55B	2616	2635	154B	13497	13513	253B	18604	18618
56B	3196	3210	155B	13515	13533	254B	18620	18634
57B	3198	3212	156B	13535	13561	255B	18646	18679
58B	8995	9012	157B	13588	13609	256B	18681	18695
59B	9014	9037	158B	13611	13659	257B	18697	18727
60B	9039	9066	159B	13661	13735	258B	18742	18773
61B	9075	9097	160B	13766	13780	259B	18790	18841
62B	9109	9128	161B	13824	13841	260B	18843	18861
63B	9130	9171	162B	13859	13885	261B	18867	18899
64B	9173	9284	163B	13891	13914	262B	18938	18988
65B	9287	9315	164B	13916	13976	263B	18990	19042
66B	9317	9412	165B	13984	14009	264B	19047	19073
67B	9414	9444	166B	14049	14072	265B	19075	19096
68B	9468	9493	167B	14112	14135	266B	19137	19154
69B	9495	9529	168B	14168	14236	267B	19196	19225
70B	9531	9585	169B	14252	14272	268B	19254	19310
71B	9562	9578	170B	14275	14293	269B	19312	19389
72B	9632	9654	171B	14361	14394	270B	19404	19424

73B	9668	9692	172B	14396	14416	271B	19432	19582
74B	9703	9878	173B	14424	14482	272B	19596	19614
75B	9888	9912	174B	14489	14551	273B	19616	19633
76B	9923	9938	175B	14574	14608	274B	19643	19664
77B	9940	9977	176B	14610	14642	275B	19679	19694
78B	9986	10041	177B	14715	14737	276B	19708	19729
79B	10057	10120	178B	14769	14797	277B	19731	19756
80B	10122	10181	179B	14799	14826	278B	19758	19812
81B	10193	10208	180B	14847	14931	279B	19819	19836
82B	10217	10262	181B	14933	14947	280B	19838	19859
83B	10276	10294	182B	14954	15014	281B	19866	19900
84B	10319	10356	183B	15030	15045	282B	19932	19956
85B	10360	10397	184B	15075	15142	283B	19958	19985
86B	10407	10507	185B	15156	15184	284B	20014	20031
87B	10529	10544	186B	15210	15267	285B	20033	20047
88B	10556	10580	187B	15289	15321	286B	20049	20082
89B	10602	10629	188B	15347	15380	287B	20098	20117
90B	10632	10652	189B	15384	15403	288B	20122	20146
91B	10660	10679	190B	15409	15444	289B	20148	20205
92B	10708	10730	191B	15480	15506	290B	20212	20226
93B	10731	10749	192B	15520	15548	291B	20230	20260
94B	10751	10765	193B	15550	15574	292B	20262	20286
95B	10767	10843	194B	15576	15596	293B	20296	20386
96B	10857	10898	195B	15609	15634	294B	20388	20428
97B	10900	10930	196B	15645	15708	295B	20440	20496

98B	10954	10975	197B	15760	15783	296B	20520	20551
99B	10968	10984	198B	15785	15821	297B	20563	20595

Target Cell

The term "target cell" as used herein refers to a cell expressing the target nucleic acid. For the therapeutic use of the present invention, it is advantageous if the target cell is a brain cell. In some embodiments, the brain cell is selected from the group consisting of a neuron, an astrocyte, an oligodendrocyte, and a microglia cell. In some embodiments, the target cell may be *in vivo* or *in vitro*. In some embodiments, the target cell is a mammalian cell such as a rodent cell, such as a mouse cell or a rat cell, or a woodchuck cell, or a primate cell such as a monkey cell (e.g. a cynomolgus monkey cell) or a human cell.

In some embodiments, the target cell expresses C4A mRNA, such as the C4A pre-mRNA or C4A mature mRNA. In some embodiments, the target cell expresses C4B mRNA, such as the C4B pre-mRNA or C4B mature mRNA. The poly A tail of the C4A mRNA or the C4B mRNA is typically disregarded for antisense oligonucleotide targeting.

Naturally occurring variant

The term "naturally occurring variant" refers to variants of the C4A and/or C4B gene or transcripts which originate from the same genetic loci as the target nucleic acid, but may differ, for example, by virtue of degeneracy of the genetic code causing a multiplicity of codons encoding the same amino acid, or due to alternative splicing of pre-mRNA, or the presence of polymorphisms, such as single nucleotide polymorphisms (SNPs), and allelic variants. Based on the presence of the sufficiently complementary sequence of the oligonucleotide, the oligonucleotide of the invention may therefore target the target nucleic acid and naturally occurring variants thereof.

In some embodiments, the naturally occurring variants have at least 95% (e.g., 95-98%), such as at least 98% (e.g., 99-99%), or at least 99% (e.g., 99-100%) homology to a mammalian C4A target nucleic acid, such as a target nucleic acid of SEQ ID NO: 3 and/or SEQ ID NO: 5. In some embodiments, the naturally occurring variants have at least 99% (e.g., 99-100%) homology to the human C4A target nucleic acid of SEQ ID NO: 3. In some embodiments, the naturally occurring variants have at least 95% (e.g., 95-98%), such as at least 98% (e.g., 98-99%), or at least 99% (e.g., 99-100%) homology to a mammalian C4B target nucleic acid, such as a target nucleic acid of SEQ ID NO: 4 and/or SEQ ID NO: 5. In some embodiments, the naturally occurring variants have at least 99% (e.g., 99-100%) homology to the human C4B target nucleic acid of SEQ ID NO: 4. In some embodiments, the naturally occurring variants are known polymorphisms.

Inhibition of expression

The term "inhibition of expression" as used herein is to be understood as an overall term for a C4 inhibitor's ability to inhibit an amount or the activity of C4 in a target cell. Inhibition of expression or activity may be determined by measuring the level of C4 pre-mRNA or C4 mRNA, or by measuring the level of C4 protein or activity in a cell. Inhibition of expression may be determined *in vitro* or *in vivo*. Inhibition is determined by reference to a control. It is generally understood that the control is an individual or target cell treated with a saline composition. In some embodiments, C4 is C4A and/or C4B.

The term "inhibitor," "inhibition" or "inhibit" may also be referred to as down-regulate, reduce, suppress, lessen, lower, or decrease the amount, expression, and/or activity of C4.

The inhibition of expression of C4A and/or C4B may occur e.g. by degradation of pre-mRNA or mRNA e.g. using RNase H recruiting oligonucleotides, such as gapmers, or nucleic acid molecules that function via the RNA interference pathway, such as siRNA or shRNA. Alternatively, the inhibitor of the present invention may bind to C4A and/or C4B mRNA or polypeptide and inhibit the activity of C4A and/or C4B or prevent its binding to other molecules.

In some embodiments, the inhibition of expression of the C4A and/or C4B target nucleic acid results in a decreased amount of C4A and/or C4B protein in the target cell. Preferably, the amount of C4A and/or C4B protein is decreased as compared to a control. In some embodiments, the decrease in amount of C4A and/or C4B protein is at least 20%, at least 30%, as compared to a control. In some embodiments, the amount of C4A and/or C4B protein in the target cell is reduced by at least 50%, e.g., 50-60%, or at least 60%, e.g., 60-70%, or at least 70%, e.g., 70-80%, at least 80%, e.g., 80-90%, or at least 90%, e.g., 90-95%, when compared to a control.

Sugar modifications

The oligonucleotide of the invention may comprise one or more nucleosides, which have a modified sugar moiety, i.e. a modification of the sugar moiety when compared to the ribose sugar moiety found in DNA and RNA.

Numerous nucleosides with modification of the ribose sugar moiety have been made, primarily with the aim of improving certain properties of oligonucleotides, such as affinity and/or nuclease resistance.

Such modifications include those where the ribose ring structure is modified, e.g. by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradical bridge between the C2 and C4 carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g. UNA). Other sugar-modified nucleosides include, for

example, bicyclohexose nucleic acids (WO2011/017521) or tricyclic nucleic acids (WO2013/154798). Modified nucleosides also include nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

Sugar modifications also include modifications made via altering one or more substituent groups on the ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in DNA and RNA nucleosides. Substituents may, for example, be introduced at the 2', 3', 4' or 5' positions.

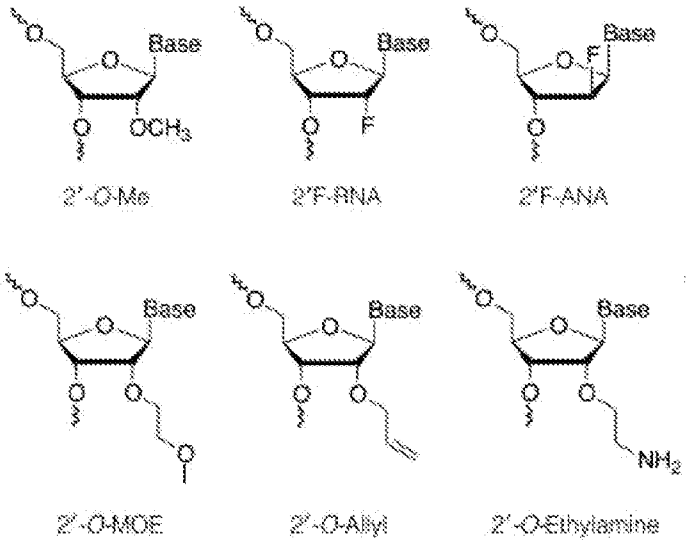
High affinity modified nucleosides

A "high affinity modified nucleoside" is a modified nucleotide which, when incorporated into the oligonucleotide, enhances the affinity of the oligonucleotide for its complementary target, for example as measured by the melting temperature (Tm). A high affinity modified nucleoside of the present invention preferably results in an increase in melting temperature in the range of +0.5 to +12C, more preferably in the range of +1.5 to +10°C and most preferably in the range of +3 to +8°C per modified nucleoside. Numerous high affinity modified nucleosides are known in the art and include for example, many 2' substituted nucleosides as well as locked nucleic acids (LNA) (see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213).

2' sugar modified nucleosides

A 2' sugar modified nucleoside is a nucleoside which has a substituent other than H or –OH at the 2' position (2' substituted nucleoside) or comprises a 2' linked biradical capable of forming a bridge between the 2' carbon and a second carbon in the ribose ring, such as LNA (2' – 4' biradical bridged) nucleosides.

Indeed, much focus has been spent on developing 2' sugar substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial properties when incorporated into oligonucleotides. For example, the 2' modified sugar may provide enhanced binding affinity and/or increased nuclease resistance to the oligonucleotide. Examples of 2' substituted modified nucleosides are 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, and 2'-F-ANA nucleoside. For further examples, please see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213, and Deleavey and Damha, Chemistry and Biology 2012, 19, 937. Below are illustrations of some 2' substituted modified nucleosides.



In relation to the present invention, a 2' substituted sugar modified nucleoside does not include 2' bridged nucleosides like LNA.

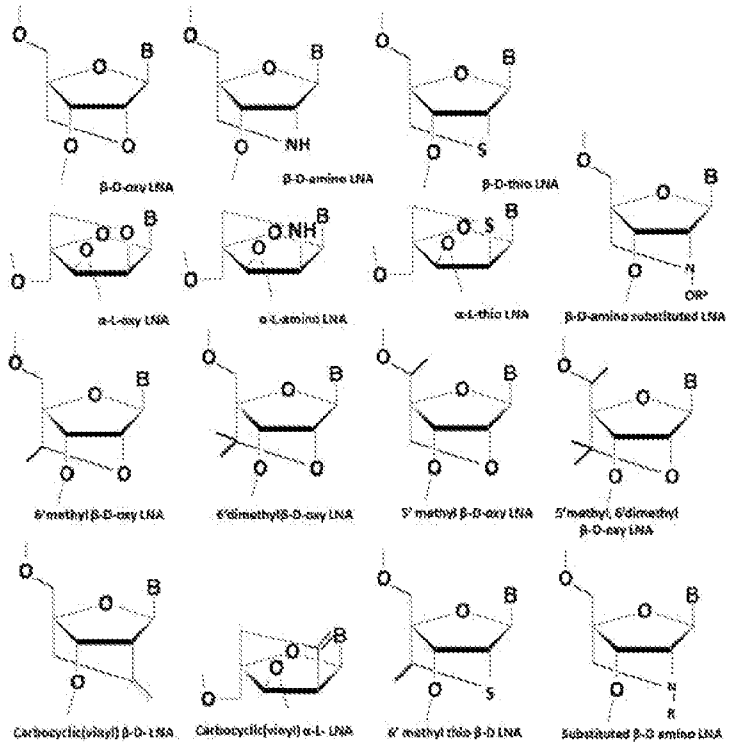
Locked Nucleic Acid Nucleosides (LNA nucleoside)

A "LNA nucleoside" is a 2'- modified nucleoside which comprises a biradical linking the C2' and C4' of the ribose sugar ring of said nucleoside (also referred to as a "2'- 4' bridge"), which restricts or locks the conformation of the ribose ring. These nucleosides are also termed bridged nucleic acids or bicyclic nucleic acids (BNAs) in the literature. The locking of the conformation of the ribose is associated with an enhanced affinity of hybridization (duplex stabilization) when the LNA is incorporated into an oligonucleotide for a complementary RNA or DNA molecule. This can be routinely determined by measuring the melting temperature of the oligonucleotide/complement duplex.

Non limiting, exemplary LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352 , WO 2004/046160, WO 00/047599, WO 2007/134181, WO 2010/077578, WO 2010/036698, WO 2007/090071, WO 2009/006478, WO 2011/156202, WO 2008/154401, WO 2009/067647, WO 2008/150729, Morita et al., Bioorganic & Med.Chem. Lett. 12, 73-76, Seth et al. J. Org. Chem. 2010, Vol 75(5) pp. 1569-81, and Mitsuoka et al., Nucleic Acids Research 2009, 37(4), 1225-1238, and Wan and Seth, J. Medical Chemistry 2016, 59, 9645−9667.

Particular examples of LNA nucleosides of the invention are presented in Scheme 1 (wherein B is as defined above).

Scheme 1:



Particular LNA nucleosides for use in molecules of the invention are beta-D-oxy-LNA, 6'-methyl-beta-D-oxy LNA such as (S)-6'-methyl-beta-D-oxy-LNA (ScET) and ENA. A particularly advantageous LNA is beta-D-oxy-LNA.

RNase H Activity and Recruitment

The RNase H activity of an antisense oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule. WO01/23613, for example, provides *in vitro* methods for determining RNase H activity, which may be used to determine ability to recruit RNase H. Typically, an oligonucleotide is deemed capable of recruiting RNase H if it, when provided with a complementary target nucleic acid sequence, has an initial rate, as measured in pmol/l/min, of at least 5%, such as at least 10%-15% or more than 20%, e.g., 20-25%, or 20-30%, of the of the initial rate determined when using a oligonucleotide having the same base sequence as the modified oligonucleotide being tested, but containing only DNA monomers with phosphorothioate linkages between all monomers in the oligonucleotide, and using the methodology provided by Example 91 - 95 of WO 01/23613 (hereby incorporated by reference). For use in determining RNase H activity, recombinant human RNase H1 is available from Creative Biomart® (Recombinant Human RNase H1 fused with His tag expressed in E. coli).

Gapmer

The antisense oligonucleotide of the invention, or contiguous nucleotide sequence thereof, may be a gapmer, also termed gapmer oligonucleotide or gapmer designs. Antisense gapmers are commonly used to inhibit a target nucleic acid via RNase H mediated degradation. A gapmer oligonucleotide comprises at least three distinct structural regions: a 5'-flank, a gap, and a 3'-flank, F-G-F' in the '5 -> 3' orientation. The "gap" region (G) comprises a stretch of contiguous DNA nucleotides, which enable the oligonucleotide to recruit RNase H. The gap region is flanked by a 5' flanking region (F) comprising one or more sugar modified nucleosides, advantageously high affinity sugar modified nucleosides, and by a 3' flanking region (F') comprising one or more sugar modified nucleosides, advantageously high affinity sugar modified nucleosides. The one or more sugar modified nucleosides in region F and F' enhance the affinity of the oligonucleotide for the target nucleic acid (i.e. are affinity enhancing sugar modified nucleosides). In some embodiments, the one or more sugar modified nucleosides in region F and F' are 2' sugar modified nucleosides, such as high affinity 2' sugar modifications, such as independently selected from LNA and 2'-MOE.

In a gapmer design, the 5' and 3' most nucleosides of the gap region are DNA nucleosides, and are positioned adjacent to a sugar modified nucleoside of the 5' (F) and/or 3' (F') region respectively. The flanks may further be defined by having at least one sugar modified nucleoside at the end most distant from the gap region, i.e. at the 5' end of the 5' flank and at the 3' end of the 3' flank.

Regions F-G-F' form a contiguous nucleotide sequence. Antisense oligonucleotides of the invention, or the contiguous nucleotide sequence thereof, may comprise a gapmer region of formula F-G-F'.

The overall length of the gapmer design F-G-F' may be, for example 12 to 32 nucleosides, such as 13 to 24, such as 14 to 22 nucleosides, such as 15 to 21 nucleosides.

By way of example, the gapmer oligonucleotide of the present invention can be represented by the following formulae:

F_{1-8}-G_{5-16}-F'_{1-8}, such as

F_{1-8}-G_{7-16}-F'_{2-8}

with the proviso that the overall length of the gapmer regions F-G-F' is at least 12 (e.g., 12-15 nucleotides), such as at least 14 nucleotides (e.g., 14-20 nucleotides) in length.

In an aspect of the invention, the antisense oligonucleotide or contiguous nucleotide sequence thereof consists of or comprises a gapmer of formula 5'-F-G-F'-3', where region F and F'

independently comprise or consist of 1- 8 nucleosides, of which 1-4 are 2' sugar modified and define the 5' and 3' ends of the F and F' region, respectively, and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH.

In an aspect of the invention, the antisense oligonucleotide or contiguous nucleotide sequence thereof consists of or comprises a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise or consist of 1-8 nucleosides, of which 1-4 are 2' sugar modified and define the 5' and 3' end of the F and F' region, respectively, and G is a region between 6 and 18 nucleosides which are capable of recruiting RNase H. In some embodiments, the G region consists of DNA nucleosides.

In some embodiments, region F and F' independently consists of or comprises a contiguous sequence of sugar-modified nucleosides. In some embodiments, the sugar modified nucleosides of region F may be independently selected from 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, LNA units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units.

In some embodiments, region F and F' independently comprises both LNA and a 2'-substituted sugar modified nucleotide (mixed wing design). In some embodiments, the 2'-substituted sugar modified nucleotide is independently selected from the group consisting of 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units.

In some embodiments, all the modified nucleosides of region F and F' are LNA nucleosides, such as independently selected from beta-D-oxy LNA, ENA or ScET nucleosides, wherein region F or F', or F and F' may optionally comprise DNA nucleosides. In some embodiments, all the modified nucleosides of region F and F' are beta-D-oxy LNA nucleosides, wherein region F or F', or F and F' may optionally comprise DNA nucleosides. In such embodiments, the flanking region F or F', or both F and F' comprise at least three nucleosides, wherein the 5' and 3' most nucleosides of the F and/or F' region are LNA nucleosides.

LNA Gapmer

An "LNA gapmer" is a gapmer wherein either one or both of region F and F' comprises or consists of LNA nucleosides. A beta-D-oxy gapmer is a gapmer wherein either one or both of region F and F' comprises or consists of beta-D-oxy LNA nucleosides.

In some embodiments, the LNA gapmer is of formula: $[LNA]_{1-5}$-[region $G]_{6-18}$-$[LNA]_{1-5}$, wherein region G is as defined in the Gapmer region G definition.

MOE Gapmers

An "MOE gapmer" is a gapmer wherein regions F and F' consist of MOE (methoxyethy) nucleosides. In some embodiments, the MOE gapmer is of design $[MOE]_{1-8}$-[Region G]$_{5-16}$-$[MOE]_{1-8}$, such as $[MOE]_{2-7}$-[Region G]$_{6-14}$-$[MOE]_{2-7}$, such as $[MOE]_{3-6}$-[Region G]$_{8-12}$-$[MOE]_{3-6}$, such as $[MOE]_5$-[Region G]$_{10}$-$[MOE]_5$ wherein region G is as defined in the Gapmer definition. MOE gapmers with a 5-10-5 design (MOE-DNA-MOE) have been widely used in the art.

Region D' or D'' in an oligonucleotide

The oligonucleotide of the invention may in some embodiments comprise or consist of the contiguous nucleotide sequence of the oligonucleotide which is complementary to the target nucleic acid, such as a gapmer region F-G-F', and may further comprise 5' and/or 3' nucleosides. The further 5' and/or 3' nucleosides may or may not be fully complementary to the target nucleic acid. Such further 5' and/or 3' nucleosides may be referred to as region D' and D'' herein.

The addition of region D' or D'' may be used for the purpose of joining the contiguous nucleotide sequence, such as the gapmer, to a conjugate moiety or another functional group. When used for joining the contiguous nucleotide sequence with a conjugate moiety is can serve as a biocleavable linker. Alternatively, it may be used to provide exonucleoase protection or for ease of synthesis or manufacture.

Region D' and D'' can be attached to the 5' end of region F or the 3' end of region F', respectively, to generate designs of the following formulas D'-F-G-F', F-G-F'-D'' or D'-F-G-F'-D''. In this instance, the F-G-F' is the gapmer portion of the oligonucleotide and region D' or D'' constitute a separate part of the oligonucleotide.

Region D' or D'' may independently comprise or consist of 1, 2, 3, 4 or 5 additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. In some embodiment, the nucleotide adjacent to the F or F' region is not a sugar-modified nucleotide, such as a DNA or RNA or base modified versions of these. The D' or D' region may serve as a nuclease susceptible biocleavable linker (see definition of linkers). In some embodiments, the additional 5' and/or 3' end nucleotides are linked with phosphodiester linkages, and are DNA or RNA. Nucleotide based biocleavable linkers suitable for use as region D' or D'' are disclosed, for example, in WO2014/076195, which include by way of example a phosphodiester linked DNA dinucleotide. The use of biocleavable linkers in poly-oligonucleotide constructs is disclosed, for example, in WO2015/113922, where they are used to link multiple antisense constructs (e.g. gapmer regions) within a single oligonucleotide.

In one embodiment, the oligonucleotide of the invention comprises a region D' and/or D'' in addition to the contiguous nucleotide sequence which constitutes the gapmer.

In some embodiments, the oligonucleotide of the present invention can be represented by one or more of the following formulae:

F-G-F'; in particular F_{1-8}-G_{5-18}-F'_{2-8}

D'-F-G-F', in particular D'_{1-3}-F_{1-8}-G_{5-18}-F'_{2-8}

F-G-F'-D'', in particular F_{1-8}-G_{5-18}-F'_{2-8}-D''_{1-3}

D'-F-G-F'-D'', in particular D'_{1-3}- F_{1-8}-G_{5-18}-F'_{2-8}-D''_{1-3}

In some embodiments the internucleoside linkage positioned between region D' and region F is a phosphodiester linkage. In some embodiments the internucleoside linkage positioned between region F' and region D'' is a phosphodiester linkage.

Treatment

The term "treatment" as used herein refers to both treatment of an existing disease (e.g. a disease or disorder as herein referred to), or prevention of a disease, i.e. prophylaxis. Prophylaxis also includes delaying or reducing the likelihood of disease occurrence, delaying or reducing frequency of relapse of the disease, and/or reducing severity or duration of the disease if the subject eventually succumbs to the disease. It will therefore be recognized that treatment as referred to herein may, in some embodiments, be prophylactic. In some embodiments, treatment is performed on a patient who has been diagnosed with a complement mediated neurological disease, such as a neurological disease selected from the group consisting of Alzheimer's disease, frontotemporal dementia, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, virus-induced cognitive impairment, glaucoma, macular degeneration, myasthenia gravis, Guillain-Barré syndrome, neuromyelitis optica, central nervous system lupus erythematosus, and schizophrenia. In some embodiments, the compounds of the invention are for use in the treatment of a tauopathy, such as Alzheimer's disease. In some embodiments, the compounds of the invention are for use in the treatment of schizophrenia.

Patient

For the purposes of the present invention, the "subject" (or "patient") may be a vertebrate. In context of the present invention, the term "subject" includes both humans and other animals, particularly mammals, and other organisms. Thus, the herein provided means and methods are applicable to both human therapy and veterinary applications. Preferably, the subject is a mammal. More preferably, the subject is human.

As described elsewhere herein, the patient to be treated may suffer from or be susceptible to a neurological disease or neurodegenerative disorder. A patient "susceptible to" a disease or disorder is one who is pre-disposed thereto and/or otherwise at risk of developing or having a recurrence of the disease or disorder. A susceptible patient can be understood a patent likely to develop the disease or disorder, to the extent that the patient would benefit from prophylactic treatment or intervention.

By " neurological disease" is meant a disease or disorder of the nervous system including, but not limited to, neurological conditions associated with cancer, and neurodegenerative disease.

By "neurodegenerative disease" is meant diseases including, but not limited to Alzheimer's disease, frontotemporal dementia, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, virus-induced cognitive impairment, glaucoma, macular degeneration, myasthenia gravis, Guillain-Barré syndrome, neuromyelitis optica, central nervous system lupus erythematosus, and schizophrenia. In some embodiments, the patient to be treated suffers from a tauopathy, such as Alzheimer's disease. In some embodiments, the patient to be treated suffers from schizophrenia.

Alzheimer's disease (AD), also referred to as Alzheimer disease or "Alzheimer's," is a chronic neurodegenerative disorder typically characterized by progressive cognitive deterioration, as well as increasing memory loss, problems with language, judgment, and/or problem solving, and that can lead to inability to perform daily tasks, and eventually dementia.

DETAILED DESCRIPTION OF THE INVENTION

Synapse removal and neuronal damage can be mediated by the classical pathway of the complement system, which is initiated by activation of the C1 complex (consisting of C1Q, C1S and C1R), leading to cleavage of C2 and C4, which in turn lead to cleavage of C3 which can trigger phagocytosis as well as inflammation and further downstream complement activation. In the context of the present invention, the present inventors have shown that nucleic acid molecules, such as antisense oligonucleotides, inhibit the expression of C4A and/or C4B. Reduced expression of C4 can lead to reduced cleavage of C3 and thereby to reduced engulfment of synapses by microglia cells and other harmful effects of complement activation.

One aspect of the present invention is a C4 inhibitor for use in the treatment and/or prevention of a neurological disease, in particular a neurological disease selected from a tauopathy and schizophrenia. In some embodiments, the tauopathy is Alzheimer's disease. The C4 inhibitor can for example be a small molecule that specifically binds to a C4 protein, wherein said inhibitor prevents or reduces cleavage of the C4 protein.

An embodiment of the invention is a C4 inhibitor, which is capable of preventing or reducing expression of C4A protein and/or C4B protein thereby leading to reduced cleavage of C3. In some embodiments, the C4 inhibitor leads to inhibition of engulfment of synapses by microglia cells.

C4 inhibitors for use in treatment of Neurological diseases

Without being bound by theory, it is believed that C4 is involved in the in the cleavage of C3 and thereby in the engulfment of synapses by microglia cells.

In some embodiments of the present invention, the inhibitor is small molecule compound. In some embodiments, the inhibitor may be a small molecule that specifically binds to the C4A and/or C4B protein. In some embodiments, the C4A protein is encoded by a sequence selected from SEQ ID NO: 3, 5, and 6. In some embodiments, the C4B protein is encoded by a sequence selected from SEQ ID NO: 4, 5, and 7.

Nucleic acid molecules of the Invention

Therapeutic nucleic acid molecules find use as C4 inhibitors since they can target C4 transcripts and promote their degradation, e.g., either via the RNA interference pathway or via RNase H cleavage. Alternatively, oligonucleotides such as aptamers can also act as inhibitors of C4 proteins.

One aspect of the present invention is a C4 targeting nucleic acid molecule for use in treatment and/or prevention of Neurological diseases. Such a nucleic acid molecule can be selected from the group consisting of a single stranded antisense oligonucleotide, an siRNA, and a shRNA.

The present section describes novel nucleic acid molecules suitable for use in treatment and/or prevention of a neurological disease. In some embodiments, the neurological disease is selected from the group consisting of Alzheimer's disease, frontotemporal dementia, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, virus-induced cognitive impairment, glaucoma, macular degeneration, myasthenia gravis, Guillain-Barré syndrome, neuromyelitis optica, central nervous system lupus erythematosus, and schizophrenia. In some embodiments, the neurological disease is a tauopathy, such as Alzheimer's disease. In some embodiments, the neurological disease is schizophrenia.

The nucleic acid molecules of the present invention are capable of inhibiting C4 mRNA and/or expression of C4 protein *in vitro* and *in vivo*. The inhibition can be achieved by hybridizing an oligonucleotide to a target nucleic acid encoding a C4A and/or C4B protein. In some embodiments, the target nucleic acid may be a mammalian C4A sequence. In some embodiments, the target nucleic acid may be a human C4A pre-mRNA sequence such as the sequence of SEQ ID NO: 3 or a human mature C4A mRNA sequence such as the sequence of SEQ ID NO: 6. In some

embodiments, the target nucleic acid may be a mammalian C4B sequence. In some embodiments, the target nucleic acid may be a human C4B pre-mRNA sequence such as the sequence of SEQ ID NO: 4 or a human mature C4B mRNA sequence such as the sequence of SEQ ID NO: 7. In some embodiments, the target nucleic acid may be a cynomolgus monkey C4 sequence such as the sequence of SEQ ID NO: 5.

In some embodiments, the nucleic acid molecule of the invention is capable of modulating the expression of the target by inhibiting or down-regulating it. Preferably, such modulation produces an inhibition of expression of at least 20% (e.g., 20-30%) compared to the normal expression level of the target, more preferably at least 30% (e.g., 30-40%), at least 40% (e.g., 40-50%), or at least 50% (e.g., 50-60%), inhibition compared to the normal expression level of the target. In some embodiments, the nucleic acid molecule of the invention may be capable of inhibiting expression levels of C4 mRNA by at least 50% (e.g., 50-60%) or 60% (e.g., 50-60%) *in vitro* by using 20-50 nM nucleic acid molecule for transfection. In some embodiments, the nucleic acid molecule of the invention may be capable of inhibiting expression levels of C4 mRNA by at least 50% (e.g., 50-60%) or 60% (e.g., 50-60%) *in vitro* by using 50-350 nM nucleic acid molecule for gymnosis. Suitably, the examples provide assays, which may be used to measure C4 mRNA inhibition (e.g. Example 1 and the "Materials and Methods" section). C4 inhibition is triggered by the hybridization between a contiguous nucleotide sequence of the oligonucleotide, such as the guide strand of a siRNA or gapmer region of an antisense oligonucleotide, and the target nucleic acid. In some embodiments, the nucleic acid molecule of the invention comprises mismatches between the oligonucleotide and the target nucleic acid. Despite mismatches, hybridization to the target nucleic acid may still be sufficient to show a desired inhibition of C4 expression. Reduced binding affinity resulting from mismatches may advantageously be compensated by increased number of nucleotides in the oligonucleotide complementary to the target nucleic acid and/or an increased number of modified nucleosides capable of increasing the binding affinity to the target, such as 2' sugar modified nucleosides, including LNA, present within the oligonucleotide sequence.

An aspect of the present invention relates to a nucleic acid molecule of 12 to 60 nucleotides in length, which comprises a contiguous nucleotide sequence of at least 12 nucleotides in length, such as at least 12 to 30 nucleotides in length, which is at least 95% complementary, such as fully complementary, to a mammalian C4 target nucleic acid, in particular a human C4 mRNA. These nucleic acid molecules are capable of inhibiting the expression of C4 mRNA and/or C4 protein.

An aspect of the invention relates to a nucleic acid molecule of 12 to 30 nucleotides in length, comprising a contiguous nucleotide sequence of at least 12 nucleotides, such as 12 to 30, or such

as 15 to 21 nucleotides in length, which is at least 90% complementary, such as fully complementary, to a mammalian C4 target sequence.

A further aspect of the present invention relates to a nucleic acid molecule according to the invention comprising a contiguous nucleotide sequence of 14 to 22, such as 15 to 21 nucleotides in length with at least 90% complementary, such as fully complementary, to the target sequence of SEQ ID NO: 3 and/or 4.

In some embodiments, the nucleic acid molecule comprises a contiguous sequence of 12 to 30 nucleotides in length, which is at least 90% complementary, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or 100% complementary with a region of the target nucleic acid or a target sequence.

It is advantageous if the oligonucleotide, or contiguous nucleotide sequence thereof, is fully complementary (100% complementary) to a region of the target sequence, or in some embodiments may comprise one or two mismatches between the oligonucleotide and the target sequence.

In some embodiments, the oligonucleotide sequence is 100% complementary to a region of the target sequence of SEQ ID NO: 3 and/or 4. In some embodiments, the oligonucleotide sequence is 100% complementary to a region of the target sequence of SEQ ID NO: 6 and/or 7.

In some embodiments, the nucleic acid molecule or the contiguous nucleotide sequence of the invention is at least 90% or 95% complementary, such as fully (or 100%) complementary, to the target nucleic acid of SEQ ID NO: 3 and/or 4.

In some embodiments, the oligonucleotide or the contiguous nucleotide sequence of the invention is at least 90% or 95% complementary, such as fully (or 100%) complementary, to the target nucleic acid of SEQ ID NO: 5 and/or SEQ ID NO: 6 and 7.

In some embodiments, the oligonucleotide or the contiguous nucleotide sequence of the invention is at least 90% or 95% complementary, such as fully (or 100%) complementary, to the target nucleic acid of SEQ ID NO: 1 and 2, and/or SEQ ID NO: 3 and 4, and/or SEQ ID NO: 5.

In some embodiments, the contiguous sequence of the nucleic acid molecule of the present invention is least 90% complementary, such as fully complementary to a region of SEQ ID NO: 3 and/or 4, selected from the group consisting of target regions 1A to 297A as shown in Table 4a and/or regions 1B to 297B as shown in Table 4b.

In some embodiments, the nucleic acid molecule of the invention comprises or consists of 12 to 60 nucleotides in length, such as from 13 to 50, such as from 14 to 35, such as 15 to 30, such as from 15 to 21 contiguous nucleotides in length. In a preferred embodiment, the nucleic acid molecule comprises or consists of 15, 16, 17, 18, 19, 20 or 21 nucleotides in length.

In some embodiments, the contiguous nucleotide sequence of the nucleic acid molecule, which is complementary to the target nucleic acids, comprises or consists of 12 to 30, such as from 13 to 25, such as from 15 to 21 contiguous nucleotides in length.

In some embodiments, the oligonucleotide is selected from the group consisting of an antisense oligonucleotide, an siRNA and a shRNA.

In some embodiments, the contiguous nucleotide sequence of the siRNA or shRNA, which is complementary to the target sequence, comprises or consists of 18 to 28, such as from 19 to 26, such as from 20 to 24, such as from 21 to 23, contiguous nucleotides in length.

In some embodiments, the contiguous nucleotide sequence of the antisense oligonucleotide, which is complementary to the target nucleic acids, comprises or consists of 12 to 22, such as from 14 to 21, such as from 15 to 21such as from 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides in length.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting of sequences listed in Table 7.

It is understood that the contiguous oligonucleotide sequence (motif sequence) can be modified to, for example, increase nuclease resistance and/or binding affinity to the target nucleic acid.

The pattern in which the modified nucleosides (such as high affinity modified nucleosides) are incorporated into the oligonucleotide sequence is generally termed oligonucleotide design.

The nucleic acid molecule of the invention may be designed with modified nucleosides and RNA nucleosides (in particular for siRNA and shRNA molecules) or DNA nucleosides (in particular for single stranded antisense oligonucleotides).

In advantageous embodiments, the nucleic acid molecule or contiguous nucleotide sequence comprises one or more sugar modified nucleosides, such as 2' sugar modified nucleosides, such as comprise one or more 2' sugar modified nucleoside independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA and LNA nucleosides. It is advantageous if one or more of the modified nucleoside(s) is a locked nucleic acid (LNA).

In some embodiments, the contiguous nucleotide sequence comprises LNA nucleosides.

In some embodiments, the contiguous nucleotide sequence comprises LNA nucleosides and DNA nucleosides.

In some embodiments, the contiguous nucleotide sequence comprises 2'-O-methoxyethyl (2'MOE) nucleosides.

In some embodiments, the contiguous nucleotide sequence comprises 2'-O-methoxyethyl (2'MOE) nucleosides and DNA nucleosides.

Advantageously, the 3' most nucleoside of the antisense oligonucleotide, or contiguous nucleotide sequence thereof, is a 2'sugar modified nucleoside.

In a further embodiment, the nucleic acid molecule comprises at least one modified internucleoside linkage. Suitable internucleoside modifications are described in the "Definitions" section under "Modified internucleoside linkage".

Advantageously, the oligonucleotide comprises at least one modified internucleoside linkage, such as phosphorothioate or phosphorodithioate.

In some embodiments, at least one internucleoside linkage in the contiguous nucleotide sequence is a phosphodiester internucleoside linkage.

It is advantageous if at least 2 to 3 internucleoside linkages at the 5' or 3' end of the oligonucleotide are phosphorothioate internucleoside linkages.

For single stranded antisense oligonucleotides, it is advantageous if at least 75%, such as 70-80%, at least 90%, such as 90-95%, or all, the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages. In some embodiments, all the internucleotide linkages in the contiguous sequence of the single stranded antisense oligonucleotide are phosphorothioate linkages.

In an advantageous embodiment of the invention, the antisense oligonucleotide of the invention is capable of recruiting RNase H, such as RNase H1. An advantageous structural design is a gapmer design as described in the "Definitions" section under for example "Gapmer", "LNA Gapmer" and "MOE gapmer". In the present invention, it is advantageous if the antisense oligonucleotide of the invention is a gapmer with an F-G-F' design.

In some embodiments, the F-G-F' design may further include region D' and/or D'' as described in the "Definitions" section under "Region D' or D'' in an oligonucleotide".

In some embodiments, the inhibitor of the present invention is a nucleic acid capable of inducing the process of RNA interference (as described, e.g., in WO 2014/089121).

Method of manufacture

In a further aspect, the invention provides methods for manufacturing the oligonucleotide of the invention. In some embodiments, the method comprises reacting nucleotide units and thereby forming covalently linked contiguous nucleotide units comprised in the oligonucleotide in a sequence according to a nucleic acid molecule of the present invention. Preferably, the method uses phophoramidite chemistry (see for example Caruthers et al, 1987, Methods in Enzymology vol. 154, pages 287-313).

The manufactured oligonucleotides may comprise one or more modifications as described herein. For example, the manufactured oligonucleotides may comprise one or more sugar-modified nucleosides, one or more modified internucleoside linkages and/or one or more modified nucleobases. Accordingly, the method for manufacturing the oligonucleotide of the invention may further comprise the introduction of such modifications into the oligonucleotide.

In some embodiments, one or more modified internucleoside linkages, such as phosphorothioate internucleoside linkages, may be introduced into the oligonucleotide. In some embodiments, one or more sugar-modified nucleosides, such as 2' sugar modified nucleosides, may be introduced. In some embodiments, one or more high affinity modified nucleosides and/or one or more LNA nucleosides may be introduced into the oligonucleotide. In some embodiments, region D' and/or D'' as described elsewhere herein are added to the oligonucleotide.

In a further aspect, a method is provided for manufacturing the pharmaceutical composition of the invention, comprising mixing the oligonucleotides of the invention with a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

As described elsewhere herein in more detail, the oligonucleotide of the invention may exist in the form of its pharmaceutically acceptable salts, esters, solvates or in the form of prodrugs. Accordingly, methods are provided for manufacturing the oligonucleotide of the invention in such forms.

Pharmaceutically salts

The compounds according to the present invention may exist in the form of their pharmaceutically acceptable salts. The term "pharmaceutically acceptable salt" refers to conventional acid-addition salts or base-addition salts that retain, or substantially retain, the biological effectiveness and properties of the compounds of the present invention. By way of example, the following salts may be mentioned: Alkaline metal salts such as sodium salts, potassium salts or lithium salts; alkaline earth metal salts such as calcium salts or magnesium salts; metal salts such as aluminum salts,

iron salts, zinc salts, copper salts; amine salts including inorganic salts such as ammonium salts and organic salts such as t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycine alkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanolamine salts, N-benzyl-phenethylamine salts, piperazine salts, tetramethylammonium salts or tris(hydroxymethyl)aminomethane salts; inorganic acid salts including hydrohalogenic acid salts such as hydrofluorides, hydrochlorides, hydrobromides or hydroiodides, sulfates or phosphates; organic acid salts including lower alkane sulfonic acid salts such as methanesulfonates, trifluoromethanesulfonates or ethanesulfonates, arylsulfonic acid salts such as benzenesulfonates or p-toluenesulfonates, acetates, malates, fumarates, succinates, citrates, tartrates, oxalates or maleates; and amino acid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamic acid salts or aspartic acid salts. These salts may be prepared by known methods.

In a further aspect, the invention provides a pharmaceutically acceptable salt of the nucleic acid molecule of the invention, such as a pharmaceutically acceptable sodium salt, ammonium salt or potassium salt.

Solvates

The compounds according to the present invention may exist in the form of solvates. The term 'solvate' is used herein to describe a molecular complex comprising the oligonucleotide of the invention and one or more pharmaceutically acceptable solvent molecules, for example, ethanol or water. If the solvent is water, the solvate is a "hydrate". Pharmaceutically acceptable solvates within the meaning of the present invention include hydrates and other solvates.

Prodrugs

Further, the compounds according to the present invention may be administered in the form of a prodrug. A prodrug is defined as a compound that undergoes transformations *in vivo* to yield the parent active drug. Because cell membranes are lipophilic in nature, cellular uptake of oligonucleotides is often reduced compared to neutral or lipophilic equivalents. One solution is to use a prodrug approach (see e.g. Crooke, R. M. (1998) in Crooke, S. T. Antisense research and Application. Springer-Verlag, Berlin, Germany, vol. 131, pp. 103-140). Examples of such prodrugs include, but are not limited to, amides, esters, carbamates, carbonates, ureides and phosphates. These prodrugs may be prepared by known methods.

Pharmaceutical Composition

In a further aspect, the invention provides pharmaceutical compositions comprising any of the compounds of the invention, in particular the aforementioned nucleic acid molecules or salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes, but is not limited to, phosphate-buffered saline (PBS). Pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. In some embodiments the pharmaceutically acceptable diluent is sterile phosphate buffered saline. In some embodiments, the nucleic acid molecule is used in the pharmaceutically acceptable diluent at a concentration of 50 to 300 μM solution. Suitable formulations for use in the present invention are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (Science 249:1527-1533, 1990). WO 2007/031091, e.g., provides further suitable and preferred examples of pharmaceutically acceptable diluents, carriers and adjuvants (hereby incorporated by reference). Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations, and the like, are also provided, e.g., in WO2007/031091. In some embodiments, the nucleic acid molecule of the invention, or pharmaceutically acceptable salt thereof is in a solid form, such as a powder, such as a lyophilized powder. Compounds or nucleic acid molecules of the invention may be mixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

Administration

The oligonucleotides or pharmaceutical compositions of the present invention may be administered via parenteral (such as, intravenous, subcutaneous, intra-muscular, intranasal, intracerebral, intracerebroventricular intraocular, or intrathecal administration).

In some embodiments, the administration is via intrathecal administration, e.g., by lumbar puncture.

Advantageously, e.g. for treatment of neurological disorders, the oligonucleotide or pharmaceutical compositions of the present invention are administered intrathecally or intracranially, e.g. via intracerebral or intraventricular administration.

The invention also provides for the use of the oligonucleotide or conjugate thereof, such as pharmaceutical salts or compositions of the invention, for the manufacture of a medicament wherein the medicament is in a dosage form for subcutaneous administration.

The invention also provides for the use of the oligonucleotide of the invention, or conjugate thereof, such as pharmaceutical salts or compositions of the invention, for the manufacture of a medicament wherein the medicament is in a dosage form for intrathecal administration.

In some embodiments, a therapeutically or prophylactically effective amount of the oligonucleotide or pharmaceutical composition of the present invention is administered.

Delivery platforms

Delivery of the oligonucleotides to the target tissue may be enhanced by carrier-mediated delivery including, but not limited to, cationic liposomes, cyclodextrins, porphyrin derivatives, branched chain dendrimers, polyethylenimine polymers, nanoparticles, cell-penetrating peptides, and microspheres (see e.g. Dass, C R. J Pharm Pharmacol 2002; 54(1):3-27).

In some embodiments, the inhibitors of the present invention, such as the oligonucleotides of the present invention, are targeted to the brain. For example, delivery to the brain might be achieved by conjugating said inhibitor to a moiety that facilitates delivery across the blood brain barrier, such as an antibody or antibody fragment targeting the transferrin receptor.

Combination therapies

In some embodiments, the inhibitor of the present invention such as the nucleic acid molecule, nucleic acid molecule conjugate, pharmaceutically acceptable salt, or pharmaceutical composition of the invention is for use in a combination treatment with another therapeutic agent. The

therapeutic agent can for example be the standard of care for the diseases or disorders described above.

By way of example, the inhibitor of the present invention may be used in combination with other actives, such as oligonucleotide-based therapeutic agents – such as sequence specific oligonucleotide-based therapeutic agents - acting through nucleotide sequence-dependent mode of action.

By way of further example, the inhibitor of the present invention may be used in combination with one or more acetylcholinesterase inhibitors and/or one or more NMDA receptor antagonists. A cholinesterase inhibitor may be, for example, donepezil, tacrine, galantamine or rivastigmine. A NMDA receptor antagonist may be, for example, memantine.

By way of further example, the inhibitor of the present invention may be used in combination with one or more typical antipsychotics and/or one or more atypical antipsychotics. A typical antipsychotic may be, for example, chlorpromazine, fluphenazine, haloperidol, perphenazine, thioridazine, thiothixene, or trifluoperazine. An atypical antipsychotic may be, for example, aripiprazole, aripiprazole lauroxil, asenapine, brexpiprazole, cariprazine, clozapine, lloperidone, lumateperone tosylate, lurasidone, olanzapine, paliperidone, aliperidone palmitate, or ziprasidone.

In some embodiments, the inhibitor of the present invention is used in combination with an antibody that binds to complement C4 or the C4b portion of C4 (e.g., as described in WO 2017/196969).

In some embodiments, the inhibitor of the present invention is used in combination with one or more of the following: an antisense compound that targets C9ORT72 (e.g., as described in WO 2014/062736); an antisense oligonucleotide, aptamer, miRNA, ribozyme, or siRNA that blocks expression of one or more of C3 convertase, C5, C6, C7, C8, and C9 (e.g., as described in WO 2008/044928); an antibody that blocks the activity of one or more of C3 convertase, C5, C6, C7, C8, and C9 (e.g., as described in WO 2008/044928); an antisense or double stranded RNA that decreases activity of the complement cascade (e.g., as described in WO 2005/060667); and an antibody that binds C1s protein, e.g., to inhibit proteolytic activity of C1s (e.g., as described in WO 2014/066744).

In some embodiments, the inhibitor of the present invention is used in combination with one or more nucleic acid molecules disclosed in U.S. Provisional Application filed May 11, 2020, entitled "Complement Component C1R Inhibitors For Treating A Neurological Disease, And Related Compositions, Systems And Methods Of Using Same" and US Provisional Application filed May

11, 2020, entitled "Complement Component C1S Inhibitors For Treating A Neurological Disease, And Related Compositions, Systems And Methods Of Using Same,"

Applications

The nucleic acid molecules of the invention may be utilized as research reagents for, for example, diagnostics, as well as for therapeutics and prophylaxis.

In research, such nucleic acid molecules may be used to specifically modulate the synthesis of a C4 protein in cells (e.g. *in vitro* cell cultures) and animal models thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention. Typically, the target modulation is achieved by degrading or inhibiting the mRNA corresponding to the protein, thereby preventing protein formation or by degrading or inhibiting a modulator of the gene or mRNA producing the protein.

If employing the nucleic acid molecules of the invention in research or diagnostics, the target nucleic acid may be a cDNA or a synthetic nucleic acid derived from DNA or RNA.

Methods of detection or diagnosis

Further encompassed by the present invention is a method for diagnosing a neurological disease in a patient suspected of a having a neurological disease, said method comprising the step of

a) determining the amount of one or more C4 nucleic acids, such as C4 mRNA or cDNA derived from C4 mRNA, in a sample from the subject, wherein the determination comprises contacting the sample with one or more oligonucleotides of the present invention,
b) comparing the amount determined in step a) to a reference amount, and
c) diagnosing whether the subject suffers from the neurological disease, or not, based on the results of step b).

In some embodiments, the method of diagnosing a neurological disease is an *in vitro* method.

The term "neurological disease" has been defined elsewhere herein. The definition applies accordingly. In some embodiments, the neurological disease to be diagnosed is a tauopathy, such as Alzheimer's disease. In some embodiments, the neurological disease to be diagnosed is schizophrenia.

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ. Samples of body fluids can be obtained by well-known techniques and include samples of blood, plasma, serum, urine, lymphatic fluid, sputum, ascites, saliva, and lacrimal fluid. In some embodiments, the sample is a cerebrospinal fluid sample.

Tissue or organ samples may be obtained from any tissue or organ by, e.g., biopsy. In some embodiments, the sample is a neural tissue sample, such as a brain tissue sample or spinal cord sample.

In some embodiments, the sample comprises neuron, astrocytes, oligodendrocytes, and/or microglia cells.

The subject may be a mammal. In some embodiments, the subject is a human. In some embodiments, the subject is a human. In some embodiments, the subject is a cynomolgus monkey.

In step a) of the aforementioned method, the amount of C4 nucleic acid present in the sample shall be determined. The C4 nucleic acid to be determined shall be a nucleic acid encoding a C4 protein, such as a C4A or C4B protein. In some embodiments, the C4 nucleic acid is mammalian C4 nucleic acid. In some embodiments, the C4 nucleic acid is a human C4 nucleic acid, such as a human C4A or C4B nucleic acid.

The C4 nucleic acid may for example be a gene, a RNA, a mRNA, and pre-mRNA, a mature mRNA or a cDNA sequence. In an embodiment, the nucleic acid is a C4 mRNA, such as a C4A or C4B mRNA. In another embodiment, the C4 nucleic acid is cDNA derived from a C4 mRNA.

In step b) of aforementioned method, the amount of the C4 nucleic acid shall be compared to a reference, i.e. to a reference amount. The terms “reference amount” or “reference” are well understood by the skilled person. Suitable reference amounts can, in principle, be calculated for a cohort of subjects based on the average or mean values for a given biomarker by applying standard methods of statistics. A suitable reference shall allow for the diagnosis of the neurological disease. Accordingly, the reference shall allow for differentiating between a patient suffering from a neurological disease and a subject who is not suffering from a neurological disease. In some embodiments, the reference is a predetermined value.

In some embodiments, an amount of the one or more C4 nucleic acids larger than the reference amount is indicative for a patient suffering from a neurological disease, whereas an amount of the one or more C4 nucleic acids lower than the reference amount is indicative for a patient not suffering from neurological disease.

The determination of the amount of the one or more nucleic acids in step a) shall comprise contacting the sample with one or more oligonucleotides of the present invention. For example, the sample is contacted with said one or more oligonucleotides under conditions, which allow for the hybridization of said one or more oligonucleotides to the one or more C4 nucleic acids present in the sample (such as the C4 mRNA), thereby forming duplexes of said oligonucleotides and said

C4 nucleic acids. In some embodiments, the amount of the one or more C4 nucleic acids is determined by determining the amount of the formed duplexes, e.g. via a detectable label. Accordingly, the one or more oligonucleotides to be used may comprise a detectable label.

Further encompassed by the present invention is a method for detecting one or more C4 nucleic acids in a sample, for example, in a sample as defined above. The method may comprise contacting the sample with one or more oligonucleotides of the present invention as described above. In some embodiments, the sample is from a patient having or suspected of a having a neurological disease.

Also encompassed by the present invention is an *in vivo* or *in vitro* method for modulating C4 expression in a target cell, which is expressing C4, said method comprising administering a nucleic acid molecule, conjugate compound, or pharmaceutical composition of the invention in an effective amount to said cell.

In some embodiments, the target cell is a mammalian cell, in particular a human cell. The target cell may be an *in vitro* cell culture or an *in vivo* cell forming part of a tissue in a mammal. In preferred embodiments, the target cell is present in the brain. The target cell may be a brain cell. In some embodiments, the brain cell is selected from the group consisting of a neuron, an astrocyte, an oligodendrocyte, and a microglia cell.

One aspect of the present invention is related to the nucleic acid molecules or pharmaceutical compositions of the invention for use as a medicament.

In an aspect of the invention, the C4 inhibitor, such as a nucleic acid molecule or pharmaceutical composition of the invention, is capable of reducing the amount of C4 in a cell expressing C4.

For example, a nucleic acid molecule that inhibits C4 expression may reduce the C4 protein in an affected cell by at least 50% (e.g., 50-60%), or at least 60% (e.g., 60-70%), or at least 70% (e.g., 70-80%), at least 80% (e.g., 80-90%), or at least 90% (e.g., 90-95%) reduction compared to controls. The controls may be untreated cells or animals, or cells or animals treated with an appropriate control.

Inhibition of C4 expression may be measured by RT-qPCR, e.g. as described in the Materials and Methods section.

Due to the decrease of C4 levels, the nucleic acid molecules or pharmaceutical compositions of the present invention can be used to inhibit development of or in the treatment of Neurological diseases.

Accordingly, one aspect of the present invention is related to use of an C4 inhibitor, such as the nucleic acid molecule or pharmaceutical compositions of the invention to decrease C4 protein in an individual having or susceptible to a neurological disease.

The subject to be treated with the C4 inhibitor, such as the nucleic acid molecules or pharmaceutical compositions of the invention (or who prophylactically receives nucleic acid molecules or pharmaceutical compositions of the present invention) is preferably a human, more preferably a human patient who has a neurological diseases, even more preferably a human patient having a tauopathy, even more preferably a human patient having Alzheimer's disease. In some embodiments, the human patient has schizophrenia.

Accordingly, the present invention relates to a method of treating Neurological diseases, wherein the method comprises administering an effective amount of a C4 inhibitor, such as a nucleic acid molecule or pharmaceutical composition of the invention. The present invention further relates to a method of preventing Neurological diseases. In one embodiment, the C4 inhibitors of the present invention is not intended for the treatment of Neurological diseases, only its prevention.

The invention also provides for the use of a C4 inhibitor, such as nucleic acid molecule or a pharmaceutical composition of the invention, for the manufacture of a medicament, in particular a medicament for use in the treatment of Neurological diseases. In preferred embodiments, the medicament is manufactured in a dosage form for intrathecal or intracranial administration.

In some embodiments, the subject to be treated does not have a cardiovascular disorder or disease (e.g., as described in WO 2014/089121). In some embodiments, the subject to be treated does not require treatment for pain (e.g., as described in WO 2005/060667).

The invention also provides for the use of the nucleic acid molecule or the pharmaceutical composition of the invention for the manufacture of a medicament wherein the medicament is in a dosage form for intravenous administration.

In some embodiments, C4 is C4A and/or C4B.

Kits

The invention also provides a kit containing the C4 inhibitor of the present invention, such as the nucleic acid molecule or pharmaceutical composition of the present invention, and instructions for administering the C4 inhibitor. The instructions may indicate that the C4 inhibitor may be used for the treatment of a neurological disease or neurodegenerative disorder as referred to herein, such as Alzheimer's disease or Schizophrenia.

The term "kit" as used herein refers to a packaged product comprising components with which to administer the C4 inhibitor of the present invention. The kit may comprise a box or container that holds the components of the kit. The kit can also include instructions for administering the C4 inhibitor of the present invention of the invention.

EXAMPLES

Materials and Methods

Example 1: Testing *in vitro* efficacy of antisense oligonucleotides targeting C4 in primary mouse hepatocytes

Cells were maintained in a humidified incubator as recommended by the supplier. The vendor and recommended culture conditions are reported in Table 5.

Table 5. Cell culture details.

Cell Line	Vendor	Culture Condition	Seeding density (cells/well)	Incub. time before oligo (hrs)	Incub. time with oligo (hrs)
mouse hepatocytes	Minerva Imaging	WME (Sigma #W1878) w/FBS: complemented with 1x Pen/Strep/Glutamine (freshly added), 10% (v/v) FBS (Sigma F7524), non-heat inactivated.	25000	24	72

For assays, cells were seeded in a 96-multi well plate in culture media and incubated as reported in Table 5 before addition of oligonucleotides dissolved in PBS. The seeding density of the cells is reported in Table 5.

Oligonucleotides were added at the concentrations reported in Table 8. The cells were harvested 72 hours after the addition of oligonucleotides (see Table 5). RNA was extracted using the RNeasy 96 kit (Qiagen) according to the manufacturer's instructions and eluted in 200 µL of water. The RNA was subsequently heated to 90°C for one minute.

For gene expressions analysis, One Step RT-qPCR was performed using qScript™ XLT One-Step RT-qPCR ToughMix®, Low ROX™ (Quantabio) in a duplex set up. The primer assays used for qPCR are collated in Table 6 for both target and endogenous control.

Table 6. qPCR primer-probe details.

Endogen contr. assay	Endog. contr. vendor	Endogen. contr. fluorophore	Target assay	Target vendor	Target fluorophore
RPLP0: Rplp0_MmPT5 843894205	IDT	HEX-ZEN	C4: C4_Mm00550309_m1	Thermo Scientific	FAM-MGB
RPLP0: Rplp0_MmPT5 843894205	IDT	HEX-ZEN	C4B: C4B_Mm00437890_m1	Thermo Scientific	FAM-MGB

Provided herein are the following oligonucleotide compounds (Table 7):

Table 7. Oligonucleotide compounds

SEQ ID NO	Motif	Design	Compound	CMP ID NO	ΔG°	start_ C4a	start_ C4b
42	CCAAATTAACCACAGAA	4-10-3	CCAAattaaccacaGAA	264_1	-19.8	243	240
43	GTCCCCAAATTAACCACA	2-14-2	GTccccaaattaaccaCA	265_1	-22.9	246	243
44	GTCCCCAAATTAACCAC	2-12-3	GTccccaaattaacCAC	266_1	-22.1	247	244
45	TGATCCTTTTACCTCCT	2-13-2	TGatccttttacctcCT	267_1	-22.2	308	305
46	ACTGATCCTTTTACCTC	2-12-3	ACtgatccttttacCTC	268_1	-21.5	310	307
47	CACTGATCCTTTTACCTC	2-14-2	CActgatccttttaccTC	269_1	-21.8	310	307
48	ACACTGATCCTTTTACCT	2-14-2	ACactgatccttttacCT	270_1	-21.3	311	308
49	CACTGATCCTTTTACCT	2-13-2	CActgatccttttacCT	271_1	-20.9	311	308
50	AACACTGATCCTTTTACCT	2-15-2	AAcactgatccttttacCT	272_1	-21	311	308
51	CACTGATCCTTTTACC	3-11-2	CACtgatcctttaCC	273_1	-21.2	312	309
52	AACACTGATCCTTTTACC	2-14-2	AAcactgatcctttaCC	274_1	-19.9	312	309

53	ACACTGATCCTTTTACC	2-13-2	ACactgatccttttaCC	275_1	-20.2	312	309
54	AGCACAAAGTCATCTCC	2-13-2	AGcacaaagtcatctCC	276_1	-20.8	388	385
55	TCACATAACAAGCTCC	4-10-2	TCACataacaagctCC	277_1	-20.4	495	492
56	CCTATGTCACATAACAA	4-9-4	CCTAtgtcacataACAA	278_1	-22.3	500	497
57	GCCTATGTCACATAACA	2-13-2	GCctatgtcacataaCA	279_1	-20.5	501	498
58	AGCCTATGTCACATAAC	2-11-4	AGcctatgtcacaTAAC	280_1	-20.4	502	499
59	AGCAAAACTAAACAATAAAAC	4-13-4	AGCAaaactaaacaataAAAC	281_1	-17.7	603	600
60	AGCAAAACTAAACAATAAA	4-11-4	AGCAaaactaaacaaTAAA	282_1	-17.2	605	602
61	AATCTAGGTTACACCC	2-11-3	AAtctaggttacaCCC	283_1	-20.5	641	638
62	CCGATAACGAACTAA	4-7-4	CCGAtaaegaaCTAA	284_1	-19.6	1281	1277
63	ACCCGATAACGAACT	3-8-4	ACCegataaegAACT	285_1	-20.2	1283	1279
64	CGCATCTTTTGATCC	3-10-2	CGCatcttttgatCC	286_1	-21	1307	1303
65	AAGTAAATATCTCCTTCTT	3-12-4	AAGtaaatatctcctTCTT	287_1	-21	1459	1455
66	AAGTAAATATCTCCTTCT	3-11-4	AAGtaaatatctccTTCT	288_1	-20.2	1460	1456
67	AAGTAAATATCTCCTTC	3-10-4	AAGtaaatatctcCTTC	289_1	-18.3	1461	1457
68	GAAGTAAATATCTCCTTC	4-11-3	GAAGtaaatatctccTTC	290_1	-20	1461	1457
69	ACCCATAGACAACTTTA	3-12-2	ACCcatagacaacttTA	291_1	-20	1726	1722
70	CACCCATAGACAACTTTA	2-14-2	CAcccatagacaacttTA	292_1	-19.9	1726	1722
71	CACCCATAGACAACTTT	4-11-2	CACCcatagacaactTT	293_1	-21.9	1727	1723
72	CCACCCATAGACAACTT	2-13-2	CCacccatagacaacTT	294_1	-21.3	1728	1724
73	CACCCATAGACAACTT	3-11-2	CACccatagacaacTT	295_1	-18.8	1728	1724
74	CCACCCATAGACAACT	2-12-2	CCacccatagacaaCT	296_1	-21.1	1729	1725
75	ACCCACCCATAGACAAC	2-12-3	ACccacccatagacAAC	297_1	-21.5	1730	1726
76	AGCTACCCACCGACA	2-11-2	AGctacccacegaCA	298_1	-22.3	1776	1772
77	CATACTTCTTCACTTCAAA	2-13-4	CAtacttcttcacttCAAA	299_1	-20	1881	1877

78	ACCATACTTCTTCACTTCAAA	2-15-4	ACcatacttcttcacttCAAA	300_1	-23.6	1881	1877
79	ATACTTCTTCACTTCAAA	3-11-4	ATActtcttcacttCAAA	301_1	-19.6	1881	1877
80	CACCATACTTCTTCACTTCAA	2-17-2	CAccatacttcttcacttcAA	302_1	-22.8	1882	1878
81	CATACTTCTTCACTTCAA	4-11-3	CATActtcttcacttCAA	303_1	-22.1	1882	1878
82	ACCATACTTCTTCACTTCAA	2-16-2	ACcatacttcttcacttcAA	304_1	-20.7	1882	1878
83	CCATACTTCTTCACTTCAA	2-15-2	CCatacttcttcacttcAA	305_1	-20.9	1882	1878
84	ACCATACTTCTTCACTTCA	2-15-2	ACcatacttcttcacttCA	306_1	-22.1	1883	1879
85	CATACTTCTTCACTTCA	3-12-2	CATacttcttcacttCA	307_1	-20.2	1883	1879
86	CCATACTTCTTCACTTCA	2-14-2	CCatacttcttcacttCA	308_1	-22.2	1883	1879
87	ACCATACTTCTTCACTTC	3-13-2	ACCatacttcttcactTC	309_1	-21.9	1884	1880
88	CACCATACTTCTTCACTTC	2-15-2	CAccatacttcttcactTC	310_1	-21.9	1884	1880
89	ACCATACTTCTTCACTT	4-11-2	ACCAtacttcttcacTT	311_1	-21.9	1885	1881
90	TCACCATACTTCTTCACTT	3-14-2	TCAccatacttcttcacTT	312_1	-22.9	1885	1881
91	CACCATACTTCTTCACTT	2-14-2	CAccatacttcttcacTT	313_1	-20.4	1885	1881
92	CACCATACTTCTTCACT	2-13-2	CAccatacttcttcaCT	314_1	-20.2	1886	1882
93	TCACCATACTTCTTCACT	3-13-2	TCAccatacttcttcaCT	315_1	-22.7	1886	1882
94	CACTCACCATACTTCTTCAC	2-16-2	CActcaccatacttcttcAC	316_1	-23.1	1887	1883
95	TCACCATACTTCTTCAC	4-11-2	TCACcatacttcttcAC	317_1	-21	1887	1883
96	ACTCACCATACTTCTTCA	2-13-3	ACtcaccatacttctTCA	318_1	-22.3	1888	1884
97	CACTCACCATACTTCTTCA	2-15-2	CActcaccatacttcttCA	319_1	-23.2	1888	1884
98	CTCACCATACTTCTTCA	3-12-2	CTCaccatacttcttCA	320_1	-21.8	1888	1884
99	CACTCACCATACTTCTTC	2-14-2	CActcaccatacttctTC	321_1	-20.9	1889	1885
100	CACTCACCATACTTCTT	2-13-2	CActcaccatacttcTT	322_1	-19.4	1890	1886
101	GCACTCACCATACTTCT	2-13-2	GCactcaccatacttCT	323_1	-22.7	1891	1887
102	TCAGCACTCACCATACTTC	2-15-2	TCagcactcaccatactTC	324_1	-22.8	1892	1888

103	GCACTCACCATACTTC	2-12-2	GCactcaccatactTC	325_1	-20.2	1892	1888
104	AGCACTCACCATACTTC	2-13-2	AGcactcaccatactTC	326_1	-20.4	1892	1888
105	TCAGCACTCACCATACTT	2-14-2	TCagcactcaccatacTT	327_1	-21.4	1893	1889
106	CAGCACTCACCATACTT	2-13-2	CAgcactcaccatacTT	328_1	-20.8	1893	1889
107	AGCACTCACCATACTT	2-12-2	AGcactcaccatacTT	329_1	-19	1893	1889
108	ACTTCAGCACTCACCAT	2-12-3	ACttcagcactcacCAT	330_1	-22.8	1897	1893
109	GAGTAATCTTCACCTC	2-11-3	GAgtaatcttcacCTC	331_1	-19.6	2014	2010
110	TAATTGGATTTCATCAC	4-9-4	TAATtggatttcaTCAC	332_1	-19.3	2066	2062
111	GACGCTACCCTACCTT	2-12-2	GAegctaccctaccTT	333_1	-22.5	2733	2711
112	TGACGCTACCCTACCT	2-12-2	TGaegctaccctacCT	334_1	-22.9	2734	2712
113	GACGCTACCCTACCT	2-11-2	GAegctaccctacCT	335_1	-22.2	2734	2712
114	TTGACGCTACCCTACC	2-12-2	TTgaegctaccctaCC	336_1	-22.5	2735	2713
115	TTGACGCTACCCTAC	2-9-4	TTgaegctaccCTAC	337_1	-21.5	2736	2714
116	CTTGACGCTACCCTAC	2-12-2	CTtgaegctaccctAC	338_1	-20.1	2736	2714
117	CTTGACGCTACCCTA	2-11-2	CTtgaegctacccTA	339_1	-20.1	2737	2715
118	CCTTCCACCAACTAAG	2-12-2	CCttccaccaactaAG	340_1	-20.6	2806	2784
119	AATATTGATTTTATCCA	4-9-4	AATAttgattttaTCCA	341_1	-19.9	2866	2844
120	CAATATTGATTTTATCC	3-10-4	CAAtattgattttATCC	342_1	-18	2867	2845
121	CCAATATTGATTTTATCC	2-13-3	CCaatattgattttaTCC	343_1	-20.3	2867	2845
122	TCTCTGACCCCAATATT	2-13-2	TCtctgaccccaataTT	344_1	-20.4	2877	2855
123	CATTTGCATTTTTAAACT	4-10-4	CATTtgcatttttaAACT	345_1	-19.6	3005	2981
124	CCATTTGCATTTTTAAAC	4-12-2	CCATttgcatttttaaAC	346_1	-19.9	3006	2982
125	AACAACTCATGCACCC	3-11-2	AACaactcatgcacCC	347_1	-20	3723	3659
126	CCCAGGAAACAACTCAT	2-12-3	CCcaggaaacaactCAT	348_1	-21.6	3729	3665
127	CACCCAGGAAACAACTC	3-12-2	CACccaggaaacaacTC	349_1	-20.5	3731	3667

128	CAAGTCCCACATACCAT	2-13-2	CAagtcccacataccAT	350_1	-20.9	4004	3945
129	TATACCCCAAGTCCCAC	2-13-2	TAtaccccaagtcccAC	351_1	-22.9	4011	3952
130	ACTCCTATACCCCAAG	2-12-2	ACtcctataccccaAG	352_1	-20.9	4017	3958
131	CTAAACCAGTCATCCT	3-11-2	CTAaaccagtcatcCT	353_1	-20.4	4502	4453
132	GCTAAACCAGTCATCC	2-12-2	GCtaaaccagtcatCC	354_1	-21.5	4503	4454
133	CTGTTGATTACTTCAAA	4-9-4	CTGTtgattacttCAAA	355_1	-19.9	5514	6127
134	TAGTCGATCACCATCA	2-11-3	TAgtegatcaccaTCA	356_1	-20.4	5606	6219
135	TTAGTCGATCACCATC	2-10-4	TTagtegatcacCATC	357_1	-20.2	5607	6220
136	TAGTCGATCACCATC	2-10-3	TAgtegatcaccATC	358_1	-18	5607	6220
137	TTAGTCGATCACCAT	3-8-4	TTAgtegatcaCCAT	359_1	-21.7	5608	6221
138	CTACGTTCTTACCCTCT	2-13-2	CTaegttcttaccctCT	360_1	-22.9	5633	6246
139	TACGTTCTTACCCTCT	2-11-3	TAegttcttacccTCT	361_1	-21.9	5633	6246
140	CACTACGTTCTTACCCTC	2-14-2	CActaegttcttacccTC	362_1	-23	5634	6247
141	CTACGTTCTTACCCTC	2-12-2	CTaegttcttacccTC	363_1	-20.7	5634	6247
142	ACTACGTTCTTACCCTC	2-12-3	ACtaegttcttaccCTC	364_1	-22.6	5634	6247
143	CACTACGTTCTTACCCT	2-13-2	CActaegttcttaccCT	365_1	-22.1	5635	6248
144	ACTACGTTCTTACCCT	2-12-2	ACtaegttcttaccCT	366_1	-20.1	5635	6248
145	ACTACGTTCTTACCC	3-10-2	ACTaegttcttacCC	367_1	-20.6	5636	6249
146	TCACTACGTTCTTACCC	2-13-2	TCactaegttcttacCC	368_1	-21.8	5636	6249
147	CACTACGTTCTTACCC	3-11-2	CACtaegttcttacCC	369_1	-22.2	5636	6249
148	TCACTACGTTCTTACC	2-11-3	TCactaegttcttACC	370_1	-19.6	5637	6250
149	CACTACGTTCTTACC	2-11-2	CActaegttcttaCC	371_1	-18	5637	6250
150	CTCACTACGTTCTTACC	2-13-2	CTcactaegttcttaCC	372_1	-21.2	5637	6250
151	CACTCACTACGTTCTTAC	3-13-2	CACtcactaegttcttAC	373_1	-20.7	5638	6251
152	ACTCACTACGTTCTTAC	4-10-3	ACTCactaegttctTAC	374_1	-21.7	5638	6251

153	TCACTACGTTCTTAC	3-9-3	TCActaegttctTAC	375_1	-17.7	5638	6251
154	CTCACTACGTTCTTAC	3-10-3	CTCactaegttctTAC	376_1	-19.6	5638	6251
155	CCACTCACTACGTTCTTA	2-14-2	CCactcactaegttctTA	377_1	-22.6	5639	6252
156	CTCACTACGTTCTTA	4-7-4	CTCActaegttCTTA	378_1	-21.8	5639	6252
157	ACTCACTACGTTCTTA	2-11-3	ACtcactaegttcTTA	379_1	-18.1	5639	6252
158	CACTCACTACGTTCTT	2-10-4	CActcactaegtTCTT	380_1	-20.4	5640	6253
159	CCACTCACTACGTTCT	2-11-3	CCactcactaegtTCT	381_1	-22.2	5641	6254
160	CCCACTCACTACGTTC	2-12-2	CCcactcactaegtTC	382_1	-21.8	5642	6255
161	CCACTCACTACGTTC	4-9-2	CCACtcactaegtTC	383_1	-21.7	5642	6255
162	CCCACTCACTACGTT	2-11-2	CCcactcactaegTT	384_1	-20.4	5643	6256
163	AGTTAACATTTCTCTTTT	3-11-4	AGTtaacatttctcTTTT	385_1	-19.8	5883	6947
164	AAGTTAACATTTCTCTTT	4-10-4	AAGTtaacatttctCTTT	386_1	-20.4	5884	6948
165	CAACATACACTTCTCACT	2-14-2	CAacatacacttctcaCT	387_1	-19.2	5915	6979
166	CACAACATACACTTCTCACT	2-16-2	CAcaacatacacttctcaCT	388_1	-21.9	5915	6979
167	AACATACACTTCTCACT	3-10-4	AACatacacttctCACT	389_1	-20.2	5915	6979
168	ACAACATACACTTCTCACT	2-15-2	ACaacatacacttctcaCT	390_1	-19.7	5915	6979
169	CCACAACATACACTTCTCAC	2-16-2	CCacaacatacacttctcAC	391_1	-22.6	5916	6980
170	CACAACATACACTTCTCAC	2-15-2	CAcaacatacacttctcAC	392_1	-19	5916	6980
171	CACAACATACACTTCTCA	3-13-2	CACaacatacacttctCA	393_1	-20.1	5917	6981
172	CCACAACATACACTTCTCA	2-15-2	CCacaacatacacttctCA	394_1	-22.7	5917	6981
173	CACAACATACACTTCTC	4-9-4	CACAacatacactTCTC	395_1	-22.2	5918	6982
174	CCACAACATACACTTCTC	2-14-2	CCacaacatacacttcTC	396_1	-20.6	5918	6982
175	CCCACAACATACACTTCT	2-14-2	CCcacaacatacacttCT	397_1	-22.7	5919	6983
176	ACCCACAACATACACTTCT	2-15-2	ACccacaacatacacttCT	398_1	-22.5	5919	6983
177	CACCCACAACATACACTTC	2-15-2	CAcccacaacatacactTC	399_1	-22.1	5920	6984

178	ACCCACAACATACACTTC	2-14-2	ACccacaacatacactTC	400_1	-20	5920	6984
179	CCCACAACATACACTTC	2-13-2	CCcacaacatacactTC	401_1	-20.2	5920	6984
180	CACCCACAACATACACTT	3-13-2	CACccacaacatacacTT	402_1	-21.8	5921	6985
181	ACCCACAACATACACTT	3-11-3	ACCcacaacatacaCTT	403_1	-22.5	5921	6985
182	CACCCACAACATACACT	2-13-2	CAcccacaacatacaCT	404_1	-20.5	5922	6986
183	GGCACCCACAACATACA	2-13-2	GGcacccacaacataCA	405_1	-22.8	5924	6988
184	CTCCATACCACAACTG	3-10-3	CTCcataccacaaCTG	406_1	-22.1	6012	7076
185	CCTCCATACCACAACT	2-12-2	CCtccataccacaaCT	407_1	-22.2	6013	7077
186	ACCCTCCATACCACAAC	2-13-2	ACcctccataccacaAC	408_1	-22	6014	7078
187	CCCTCCATACCACAAC	2-12-2	CCctccataccacaAC	409_1	-22.2	6014	7078
188	CACCCTCCATACCACAA	2-13-2	CAccctccataccacAA	410_1	-22.3	6015	7079
189	CAAACCTAGAACCACCA	2-13-2	CAaacctagaaccacCA	411_1	-19.8	6105	7170
190	CAAACCTAGAACCACC	4-10-2	CAAAcctagaaccaCC	412_1	-20	6106	7171
191	TCAAACCTAGAACCACC	3-11-3	TCAaacctagaaccACC	413_1	-21.9	6106	7171
192	ATCAAACCTAGAACCAC	3-10-4	ATCaaacctagaaCCAC	414_1	-21.7	6107	7172
193	CATCAAACCTAGAACCAC	2-13-3	CAtcaaacctagaacCAC	415_1	-20	6107	7172
194	TCAAACCTAGAACCAC	3-10-3	TCAaacctagaacCAC	416_1	-18.7	6107	7172
195	CATCAAACCTAGAACC	3-9-4	CATcaaacctagAACC	417_1	-19.9	6109	7174
196	CCATCAAACCTAGAAC	3-10-3	CCAtcaaacctagAAC	418_1	-18.6	6110	7175
197	AGCATCCATCAAACCTA	2-12-3	AGcatccatcaaacCTA	419_1	-21.9	6114	7179
198	GCATCCATCAAACCTA	2-11-3	GCatccatcaaacCTA	420_1	-21.6	6114	7179
199	CAGCATCCATCAAACCT	2-13-2	CAgcatccatcaaacCT	421_1	-21.5	6115	7180
200	TCAGCATCCATCAAACC	2-13-2	TCagcatccatcaaaCC	422_1	-20.9	6116	7181
201	CAGCAAACTTGCAACA	3-9-4	CAGcaaacttgcAACA	423_1	-19.9	6308	7373
202	TCTACAGGTTCCACTC	2-12-2	TCtacaggttccacTC	424_1	-19.7	6542	7616

203	AACACTCGAACACGA	4-7-4	AACActegaacACGA	425_1	-18.8	6956	8030
204	CCCCTACGGTCTCAC	2-11-2	CCcctaeggtctcAC	426_1	-22.6	7074	8148
205	TCCCCTACGGTCTCAC	2-12-2	TCccctaeggtctcAC	427_1	-22.9	7074	8148
206	TCACATGGACACTCACC	2-13-2	TCacatggacactcaCC	428_1	-21.4	7201	8275
207	ATTGTAAGTTTATCCA	4-9-3	ATTGtaagtttatCCA	429_1	-20	7506	8580
208	CAATTGAGTCACAATA	4-8-4	CAATtgagtcacAATA	430_1	-17	7526	8600
209	CTCAATATAAGTAAACAA	4-10-4	CTCAatataagtaaACAA	431_1	-16.9	7539	8627
210	GCCTCTGTCTCAATATA	2-13-2	GCctctgtctcaataTA	432_1	-21.2	7548	8636
211	AGACTAGTAATTCCAAA	4-9-4	AGACtagtaattcCAAA	433_1	-19.7	7648	8711
212	TTCTCTACCATGTACAA	3-11-3	TTCtctaccatgtaCAA	434_1	-19.9	7758	8821
213	CTTAACCCAGAACCTTT	2-12-3	CTtaacccagaaccTTT	435_1	-20.2	8436	9514
214	CCTTAACCCAGAACCTT	2-13-2	CCttaacccagaaccTT	436_1	-22.3	8437	9515
215	TTCCCTTAACCCAGAAC	3-12-2	TTCccttaacccagaAC	437_1	-21.4	8440	9518
216	CAAGGTTCCTACTCTC	3-11-2	CAAggttcctactcTC	438_1	-20	8564	9642
217	CCAAACTCAGAATCTTCA	2-14-2	CCaaactcagaatcttCA	439_1	-19.7	8794	9872
218	TGCTGACATCCTACAC	2-11-3	TGctgacatcctaCAC	440_1	-20.5	8955	10033
219	CTGCTGACATCCTACAC	2-13-2	CTgctgacatcctacAC	441_1	-20.6	8955	10033
220	CACGGCTTCCTTACCAC	2-13-2	CAeggcttccttaccAC	442_1	-22.9	9141	10221
221	CACGGCTTCCTTACCA	2-12-2	CAeggcttccttacCA	443_1	-22.9	9142	10222
222	CAACACAACTTTGTCCT	3-12-2	CAAcacaactttgtcCT	444_1	-19.5	9631	10711
223	AATACCCCAGCAACCC	2-12-2	AAtaccccagcaacCC	445_1	-22.4	10098	11182
224	CAATACCCCAGCAACC	2-12-2	CAataccccagcaaCC	446_1	-22.3	10099	11183
225	CCAATACCCCAGCAAC	2-12-2	CCaataccccagcaAC	447_1	-21.7	10100	11184
226	CCCAATACCCCAGCAA	2-12-2	CCcaataccccagcAA	448_1	-22.9	10101	11185
227	AACAATGAAATATATTCAT	4-11-4	AACAatgaaatatatTCAT	449_1	-16.8	10342	11425

228	ACAATGAAATATATTCAT	4-10-4	ACAAtgaaatatatTCAT	450_1	-16.6	10342	11425
229	GATATTACAATTCATAACTA	3-13-4	GATattacaattcataACTA	451_1	-20.2	10382	11465
230	CAGATATTACAATTCATAACT	3-16-2	CAGatattacaattcataaCT	452_1	-19.6	10383	11466
231	GATATTACAATTCATAACT	3-13-3	GATattacaattcataACT	453_1	-18	10383	11466
232	AGATATTACAATTCATAACT	4-12-4	AGATattacaattcatAACT	454_1	-20.9	10383	11466
233	GTCAGATATTACAATTCA	3-11-4	GTCagatattacaaTTCA	455_1	-20	10388	11471
234	AATCTCCAGAACAACTA	4-10-3	AATCtccagaacaaCTA	456_1	-20	10462	11545
235	CAATCTCCAGAACAACT	2-11-4	CAatctccagaacAACT	457_1	-18.8	10463	11546
236	TCAATCTCCAGAACAAC	4-9-4	TCAAtctccagaaCAAC	458_1	-20.4	10464	11547
237	TACAGTGTCAATCTCCA	2-13-2	TAcagtgtcaatctcCA	459_1	-19.8	10471	11554
238	CTACAGTGTCAATCTC	3-10-3	CTAcagtgtcaatCTC	460_1	-20	10473	11556
239	TAGTAGCCCCACACAC	2-12-2	TAgtagccccacacAC	461_1	-21.5	10517	11588
240	CCCCTGTACACTTTAC	2-12-2	CCcctgtacactttAC	462_1	-20.9	10763	11879
241	CTACCAAGACATCTAT	2-10-4	CTaccaagacatCTAT	463_1	-19.7	11188	12300
242	GCTACCAAGACATCTA	2-12-2	GCtaccaagacatcTA	464_1	-19.3	11189	12301
243	TGACCTTCACTTCTATC	4-11-2	TGACcttcacttctaTC	465_1	-21.9	11658	12770
244	CACTCACCAGATACACACA	2-15-2	CActcaccagatacacaCA	466_1	-22.7	11951	13063
245	ACTCACCAGATACACACA	2-14-2	ACtcaccagatacacaCA	467_1	-20.8	11951	13063
246	TCTCTTTACTCACCGA	2-11-3	TCtctttactcacCGA	468_1	-21	12349	13461
247	GAAACTTCTCTTTACTCACC	2-16-2	GAaacttctctttactcaCC	469_1	-22.8	12351	13463
248	ACTTCTCTTTACTCACC	3-12-2	ACTtctctttactcaCC	470_1	-21.8	12351	13463
249	AAACTTCTCTTTACTCACC	2-15-2	AAacttctctttactcaCC	471_1	-20.1	12351	13463
250	AACTTCTCTTTACTCACC	2-14-2	AActtctctttactcaCC	472_1	-20	12351	13463
251	GAAACTTCTCTTTACTCAC	3-12-4	GAAacttctctttacTCAC	473_1	-22	12352	13464
252	AACTTCTCTTTACTCAC	4-9-4	AACTtctctttacTCAC	474_1	-21.2	12352	13464

253	AAACTTCTCTTTACTCAC	3-11-4	AAActtctctttacTCAC	475_1	-19.4	12352	13464
254	TGAAACTTCTCTTTACTCAC	2-16-2	TGaaacttctctttactcAC	476_1	-18.9	12352	13464
255	AAACTTCTCTTTACTCA	4-9-4	AAACttctctttaCTCA	477_1	-20.4	12353	13465
256	GAAACTTCTCTTTACTCA	2-14-2	GAaacttctctttactCA	478_1	-18.2	12353	13465
257	TGAAACTTCTCTTTACTCA	3-13-3	TGAaacttctctttacTCA	479_1	-21.9	12353	13465
258	GAAACTTCTCTTTACTC	4-10-3	GAAActtctctttaCTC	480_1	-18.9	12354	13466
259	TGAAACTTCTCTTTACTC	3-12-3	TGAaacttctctttaCTC	481_1	-20.3	12354	13466
260	TGAAACTTCTCTTTACT	3-12-2	TGAaacttctctttaCT	482_1	-17.8	12355	13467
261	GTGAAACTTCTCTTTACT	3-13-2	GTGaaacttctctttaCT	483_1	-19.8	12355	13467
262	TACAGACTCAAAAACCCA	3-12-3	TACagactcaaaaacCCA	484_1	-21.8	12385	13497
263	ACAGACTCAAAAACCCA	3-12-2	ACAgactcaaaaaccCA	485_1	-19.2	12385	13497
264	ATACAGACTCAAAAACCCA	3-14-2	ATAcagactcaaaaaccCA	486_1	-20.5	12385	13497
265	CATACAGACTCAAAAACCC	2-14-3	CAtacagactcaaaaaCCC	487_1	-22.2	12386	13498
266	ATACAGACTCAAAAACCC	3-13-2	ATAcagactcaaaaacCC	488_1	-19.4	12386	13498
267	TACAGACTCAAAAACCC	2-12-3	TAcagactcaaaaaCCC	489_1	-19.8	12386	13498
268	CATACAGACTCAAAAACC	2-12-4	CAtacagactcaaaAACC	490_1	-18.4	12387	13499
269	ATACAGACTCAAAAACC	4-9-4	ATACagactcaaaAACC	491_1	-19.1	12387	13499
270	TCATACAGACTCAAAAAC	4-10-4	TCATacagactcaaAAAC	492_1	-17.9	12388	13500
271	ATCATACAGACTCAAAAAC	4-11-4	ATCAtacagactcaaAAAC	493_1	-18.3	12388	13500
272	ACCCTTATCATACAGA	3-11-2	ACCcttatcatacaGA	494_1	-20.5	12397	13509
273	TGACCCTTATCATACA	2-12-2	TGacccttatcataCA	495_1	-18.3	12399	13511
274	ACTAGACTCTAAAATCT	4-9-4	ACTAgactctaaaATCT	496_1	-20.1	12725	13837
275	CCACTAGACTCTAAAATCT	2-15-2	CCactagactctaaaatCT	497_1	-20.1	12725	13837
276	CACTAGACTCTAAAATCT	3-13-2	CACtagactctaaaatCT	498_1	-17.9	12725	13837
277	CACTAGACTCTAAAATC	4-9-4	CACTagactctaaAATC	499_1	-18.8	12726	13838

278	CCACTAGACTCTAAAATC	2-14-2	CCactagactctaaaaTC	500_1	-17.7	12726	13838
279	CCACTAGACTCTAAAAT	4-9-4	CCACtagactctaAAAT	501_1	-20.4	12727	13839
280	CACTGGCATACATCTCC	2-13-2	CActggcatacatctCC	502_1	-22.5	13154	14283
281	ACTGGCATACATCTCC	2-12-2	ACtggcatacatctCC	503_1	-20.6	13154	14283
282	AGGCGAACCTCATCC	2-11-2	AGgegaacctcatCC	504_1	-21.8	13316	14445
283	ACTGACCATACTCCACT	2-13-2	ACtgaccatactccaCT	505_1	-21.4	13344	14473
284	GACTGACCATACTCCAC	2-13-2	GActgaccatactccAC	506_1	-20.4	13345	14474
285	GACTGACCATACTCCA	3-11-2	GACtgaccatactcCA	507_1	-21.7	13346	14475
286	AACCTTAACCGTGAA	4-7-4	AACCttaacegTGAA	508_1	-20.8	13491	14620
287	CGAAGAACCTTAACC	4-7-4	CGAAgaaccttAACC	509_1	-19.6	13496	14625
288	TCGAAGAACCTTAACC	2-10-4	TCgaagaaccttAACC	510_1	-18.1	13496	14625
289	TCGAAGAACCTTAAC	4-7-4	TCGAagaacctTAAC	511_1	-17.6	13497	14626
290	TTCTCGAAGAACCTTA	3-9-4	TTCtegaagaacCTTA	512_1	-19.7	13499	14628
291	AGATTTCCCATTTCCAA	3-12-2	AGAtttcccatttccAA	513_1	-20.8	13643	14772
292	GAGATTTCCCATTTCCAA	2-14-2	GAgatttcccatttccAA	514_1	-21.1	13643	14772
293	GAGATTTCCCATTTCCA	2-13-2	GAgatttcccatttcCA	515_1	-22.3	13644	14773
294	ACTTGTTGCTCACTAT	2-11-3	ACttgttgctcacTAT	516_1	-19.2	13732	14861
295	CCATCCCCATGATCAA	3-11-2	CCAtccccatgatcAA	517_1	-22.7	13946	15075
296	CTTTTCTTTTATTTACCCT	3-14-2	CTTttcttttatttaccCT	518_1	-22.2	14340	15469
297	TTTTCTTTTATTTACCCT	3-13-2	TTTtcttttatttaccCT	519_1	-19.5	14340	15469
298	GCTTTTCTTTTATTTACCC	2-15-2	GCttttcttttatttacCC	520_1	-24	14341	15470
299	CTTTTCTTTTATTTACCC	2-14-2	CTtttcttttatttacCC	521_1	-20.2	14341	15470
300	AAGCTTTTCTTTTATTTACC	2-16-2	AAgcttttcttttatttaCC	522_1	-20.4	14342	15471
301	GCTTTTCTTTTATTTACC	2-14-2	GCttttcttttatttaCC	523_1	-21.2	14342	15471
302	AGCTTTTCTTTTATTTACC	2-14-3	AGcttttcttttatttACC	524_1	-21.7	14342	15471

In the table, capital letters are beta-D-oxy LNA nucleosides, lowercase letters are DNA nucleosides, all LNA C are 5-methyl cytosine, and all internucleoside linkages are phosphorothioate internucleoside linkages.

The relative mouse C4b and mouse C4a mRNA expression level in Table 8 is shown as percent of control (PBS-treated cells). The values in the columns designated with underlined C4b are based on detection of C4b transcripts only. The values in the columns designated with underlined C4a and C4b are based on detection of C4a and C4b transcripts.

Table 8.

CMP ID NO	Conc	C4 mRNA qPCR SP probe1 mouse hepatocytes C4b: AP015278	C4 mRNA qPCR SP probe1 mouse hepatocytes C4a and C4b: AP015277	CMP ID NO	Conc	C4 mRNA qPCR SP probe1 mouse hepatocytes C4b: AP015278	C4 mRNA qPCR SP probe1 mouse hepatocytes C4a and C4b: AP015277
264_1	0.3	33.2	24.4	395_1	0.3	87.4	87.7
265_1	0.06	89.1	78.6	396_1	0.06	83.7	75.5
265_1	0.3	45.6	39.5	396_1	0.3	80.2	70
266_1	0.06	109	96	397_1	0.06	95.7	94.4
266_1	0.3	52.8	50.4	397_1	0.3	78.3	71.8
267_1	0.06	85	73	398_1	0.06	101	87.2
267_1	0.3	44.8	38.6	398_1	0.3	91.2	82.9
268_1	0.06	82	83.7	399_1	0.06	105	88.7
268_1	0.3	42.1	34.5	399_1	0.3	109	99.7
269_1	0.06	96.6	92.6	400_1	0.06	154	155
269_1	0.3	53.1	47.1	400_1	0.3	96.4	105
270_1	0.06	107	91.9	401_1	0.06	90.3	86.5
270_1	0.3	44.7	34.8	401_1	0.3	100	86.3

271_1	0.06	83.1	72.9	402_1	0.06	81.2	72.8
271_1	0.3	55.9	46.2	402_1	0.3	36.6	31.2
272_1	0.06	104	106	403_1	0.06	114	109
272_1	0.3	63	54.7	403_1	0.3	118	114
273_1	0.06	90	71.7	404_1	0.06	74	58.9
273_1	0.3	43.7	35.9	404_1	0.3	69.7	63.7
274_1	0.06	68.1	52.3	405_1	0.06	106	103
274_1	0.3	44.1	37.1	405_1	0.3	111	112
275_1	0.06	118	107	406_1	0.06	92	72.5
275_1	0.3	48.3	36	406_1	0.3	60.9	53.6
276_1	0.06	104	102	407_1	0.06	103	102
276_1	0.3	50	44.8	407_1	0.3	113	105
277_1	0.06	73.7	59.2	408_1	0.06	83	80.2
277_1	0.3	40.9	33.6	408_1	0.3	86.5	77.6
278_1	0.06	104	92.4	409_1	0.06	117	119
278_1	0.3	33.7	29.3	409_1	0.3	61.1	53.2
279_1	0.06	78.8	75.4	410_1	0.06	107	95.8
279_1	0.3	41.3	34.8	410_1	0.3	86.8	88.9
280_1	0.06	48.6	39.3	411_1	0.06	80.9	81.1
280_1	0.3	47.5	45.8	411_1	0.3	56.4	52.6
281_1	0.06	73.2	70.7	412_1	0.06	68.8	63.1
281_1	0.3	36.4	31.7	412_1	0.3	66.5	60
282_1	0.06	59.4	42.4	413_1	0.06	105	93.7
282_1	0.3	60.5	52.8	413_1	0.3	85.5	82.1
283_1	0.06	164	183	414_1	0.06	110	111

283_1	0.3	75.9	64.3	414_1	0.3	73.9	73.2
284_1	0.06	118	120	415_1	0.06	94.7	93.3
284_1	0.3	81.4	69.3	415_1	0.3	68.8	62
285_1	0.06	63	49.6	416_1	0.06	104	90.2
285_1	0.3	72.4	65	416_1	0.3	64.6	58
286_1	0.06	40.9	35.1	417_1	0.06	95.3	85.2
286_1	0.3	20.9	17.3	417_1	0.3	64.7	54.8
287_1	0.06	80	63.4	418_1	0.06	69	61.3
287_1	0.3	52.2	43.9	418_1	0.3	68.9	60.8
288_1	0.06	83.9	73.2	419_1	0.06	105	107
288_1	0.3	44	43.5	419_1	0.3	67.9	66.8
289_1	0.06	99.9	93.3	420_1	0.06	115	92.4
289_1	0.3	52.5	51.5	420_1	0.3	64	57.1
290_1	0.06	129	132	421_1	0.06	100	104
290_1	0.3	108	104	421_1	0.3	77	74.7
291_1	0.06	83	72.7	422_1	0.06	105	98.4
291_1	0.3	84.9	86.3	422_1	0.3	68.2	56.2
292_1	0.06	124	119	423_1	0.06	73.8	60.5
292_1	0.3	98.1	93.3	423_1	0.3	66.2	63.2
293_1	0.06	161	170	424_1	0.06	84.1	72.7
293_1	0.3	74.2	68.2	424_1	0.3	73.1	72.9
294_1	0.06	113	117	425_1	0.06	96.8	93.6
294_1	0.3	126	129	425_1	0.3	72	73.8
295_1	0.06	74.5	67.9	426_1	0.06	120	109
295_1	0.3	105	102	426_1	0.3	96.5	85.2

296_1	0.06	96.9	91.4	427_1	0.06	102	104
296_1	0.3	71.7	72.3	427_1	0.3	79.7	72.8
297_1	0.06	129	122	428_1	0.06	88.9	77.2
297_1	0.3	84.8	81.8	428_1	0.3	53.5	49.8
298_1	0.06	170	177	429_1	0.06	41.2	34.4
298_1	0.3	103	108	429_1	0.3	17.3	13.2
299_1	0.06	61.8	54.2	430_1	0.06	72.7	64.2
299_1	0.3	41.9	38.2	430_1	0.3	48.2	41.6
300_1	0.06	95.6	81.9	431_1	0.06	61.7	53.2
300_1	0.3	64.5	57	431_1	0.3	28.1	23.7
301_1	0.06	79.7	59.5	432_1	0.06	61	64.7
301_1	0.3	25.1	16.9	432_1	0.3	26	26.1
302_1	0.06	172	136	433_1	0.06	42	32.4
302_1	0.3	102	104	433_1	0.3	26.1	23.7
303_1	0.06	69.5	56	434_1	0.06	49.6	41.6
303_1	0.3	23.6	22.9	434_1	0.3	13.7	10.8
304_1	0.06	86.2	79	435_1	0.06	102	105
304_1	0.3	80.1	84.7	435_1	0.3	134	159
305_1	0.06	121	112	436_1	0.06	135	145
305_1	0.3	84.1	77.5	436_1	0.3	93.3	94.9
306_1	0.06	85	83.3	437_1	0.06	79.7	74.6
306_1	0.3	76.5	75.2	437_1	0.3	44.9	42.5
307_1	0.06	85.3	75.7	438_1	0.06	85.8	79.5
307_1	0.3	42.5	35.8	438_1	0.3	79.2	75.9
308_1	0.06	80.1	66.8	439_1	0.06	71.6	72

308_1	0.3	63.2	51.6	439_1	0.3	80.6	74.6
309_1	0.06	105	87	440_1	0.06	96.1	89
309_1	0.3	71.2	56.6	440_1	0.3	97	94.4
310_1	0.06	93.1	90.9	441_1	0.06	98.6	84.8
310_1	0.3	75.2	72.2	441_1	0.3	71.9	63.2
311_1	0.06	73.9	64.9	442_1	0.06	137	125
311_1	0.3	108	111	442_1	0.3	81.8	84.2
312_1	0.06	108	93	443_1	0.06	117	106
312_1	0.3	73.9	78.4	443_1	0.3	67.3	58.9
313_1	0.06	114	107	444_1	0.06	70.8	58.6
313_1	0.3	88.9	78	444_1	0.3	38.4	34.8
314_1	0.06	118	115	445_1	0.06	115	123
314_1	0.3	83.4	76.9	445_1	0.3	83.5	82.1
315_1	0.06	128	120	446_1	0.06	99.8	88.6
315_1	0.3	87.8	92.1	446_1	0.3	77.2	70.8
316_1	0.06	117	128	447_1	0.06	92.7	95
316_1	0.3	115	132	447_1	0.3	52.7	55.1
317_1	0.06	81.9	77	448_1	0.06	59.7	48.1
317_1	0.3	116	115	448_1	0.3	75.3	65
318_1	0.06	108	93.2	449_1	0.06	75.2	76.2
318_1	0.3	108	96.6	449_1	0.3	24.6	25.2
319_1	0.06	127	124	450_1	0.06	91.1	77.4
319_1	0.3	71.8	68.4	450_1	0.3	68.8	68
320_1	0.06	55.5	50.1	451_1	0.06	117	121
320_1	0.3	81.5	86.6	451_1	0.3	37.6	34.4

321_1	0.06	131	123	452_1	0.06	74.4	74.6
321_1	0.3	93.8	89.3	452_1	0.3	43.3	39.5
322_1	0.06	92.8	90.8	453_1	0.06	67.4	60.2
322_1	0.3	75.5	82.5	453_1	0.3	55.9	53.3
323_1	0.06	60.7	56.5	454_1	0.06	73.9	63.4
323_1	0.3	76.9	71.5	454_1	0.3	30.6	22.8
324_1	0.06	89.4	75.8	455_1	0.06	48.4	40.7
324_1	0.3	67.2	63.5	455_1	0.3	20.7	17.1
325_1	0.06	75.7	66.8	456_1	0.06	72.5	57.7
325_1	0.3	64.4	53.6	456_1	0.3	54.8	44.3
326_1	0.06	90.4	85.1	457_1	0.06	134	125
326_1	0.3	83	79.4	457_1	0.3	72.1	73.9
327_1	0.06	84	76.3	458_1	0.06	107	98.7
327_1	0.3	92.8	99.5	458_1	0.3	66	56.2
328_1	0.06	77.3	76.4	459_1	0.06	87	72.9
328_1	0.3	61.6	67.3	459_1	0.3	87.9	83
329_1	0.06	136	137	460_1	0.06	106	102
329_1	0.3	106	90.2	460_1	0.3	79.8	75.8
330_1	0.06	196	193	461_1	0.06	91.5	76.5
330_1	0.3	111	115	461_1	0.3	95.3	88.6
331_1	0.06	108	112	462_1	0.06	102	88.5
331_1	0.3	86.9	82.1	462_1	0.3	75.7	69.7
332_1	0.06	123	126	463_1	0.06	74.1	71.3
332_1	0.3	126	135	463_1	0.3	64.8	64.6
333_1	0.06	108	106	464_1	0.06	109	98.8

333_1	0.3	91.5	91.2	464_1	0.3	56.2	51.8
334_1	0.06	138	137	465_1	0.06	83.8	79.2
334_1	0.3	83.5	87.7	465_1	0.3	65.5	64.2
335_1	0.06	96.5	87.7	466_1	0.06	120	108
335_1	0.3	90.5	75	466_1	0.3	78.9	92.1
336_1	0.06	153	144	467_1	0.06	97.2	91
336_1	0.3	103	86.6	467_1	0.3	87.6	84.6
337_1	0.06	71.8	62.8	468_1	0.06	101	92.6
337_1	0.3	77.9	66.3	468_1	0.3	100	104
338_1	0.06	81.4	68.4	469_1	0.06	158	152
338_1	0.3	117	110	469_1	0.3	93	90
339_1	0.06	73.5	54.9	470_1	0.06	103	102
339_1	0.3	133	124	470_1	0.3	61.3	56
340_1	0.06	53.2	44.4	471_1	0.06	151	131
340_1	0.3	27	22	471_1	0.3	105	102
341_1	0.06	116	115	472_1	0.06	108	107
341_1	0.3	72.5	65.9	472_1	0.3	118	114
342_1	0.06	63.4	57.7	473_1	0.06	126	126
342_1	0.3	90.9	92.1	473_1	0.3	120	114
343_1	0.06	62.6	67	474_1	0.06	88.2	76.5
343_1	0.3	57.9	58.9	474_1	0.3	62.2	50.1
344_1	0.06	104	99.5	475_1	0.06	125	97
344_1	0.3	65.2	56	475_1	0.3	94.8	91.8
345_1	0.06	112	123	476_1	0.06	111	121
345_1	0.3	79.8	77.9	476_1	0.3	89.3	89.3

346_1	0.06	64.2	62.5	477_1	0.06	93.7	94
346_1	0.3	26	20.8	477_1	0.3	90.2	89.3
347_1	0.06	82.8	84.2	478_1	0.06	84.2	87.6
347_1	0.3	53.3	53.8	478_1	0.3	84.2	88.7
348_1	0.06	124	134	479_1	0.06	91.1	84.3
348_1	0.3	71.5	71.3	479_1	0.3	99.6	100
349_1	0.06	102	104	480_1	0.06	82	83.9
349_1	0.3	89.8	90.3	480_1	0.3	69.5	59.7
350_1	0.06	122	103	481_1	0.06	95.3	86.8
350_1	0.3	79.3	70.3	481_1	0.3	60.3	48.9
351_1	0.06	137	136	482_1	0.06	96	100
351_1	0.3	129	130	482_1	0.3	102	102
352_1	0.06	106	91.7	483_1	0.06	78.7	73.8
352_1	0.3	90.6	79	483_1	0.3	43.8	36.4
353_1	0.06	102	97.2	484_1	0.06	80.1	72.5
353_1	0.3	83.3	83.2	484_1	0.3	70.1	64.2
354_1	0.06	85.6	73.4	485_1	0.06	86.2	83.4
354_1	0.3	97.8	90.8	485_1	0.3	46.7	41.3
355_1	0.06	62.8	55.3	486_1	0.06	101	96.1
355_1	0.3	18	15.1	486_1	0.3	59.9	59.1
356_1	0.06	123	113	487_1	0.06	111	112
356_1	0.3	124	122	487_1	0.3	88.3	87.3
357_1	0.06	89.7	76	488_1	0.06	125	124
357_1	0.3	72.1	63	488_1	0.3	113	111
358_1	0.06	78.4	64.9	489_1	0.06	114	105

358_1	0.3	73.5	75.7	489_1	0.3	88.2	87.2
359_1	0.06	105	103	490_1	0.06	89.8	85
359_1	0.3	86.2	82.1	490_1	0.3	50.1	45.1
360_1	0.06	121	114	491_1	0.06	117	104
360_1	0.3	105	97.3	491_1	0.3	57.3	55.3
361_1	0.06	98.5	101	492_1	0.06	88.1	76.2
361_1	0.3	73.4	67.2	492_1	0.3	68	65.1
362_1	0.06	152	145	493_1	0.06	61.8	51.2
362_1	0.3	136	132	493_1	0.3	52	47.1
363_1	0.06	121	129	494_1	0.06	153	135
363_1	0.3	90.2	88.1	494_1	0.3	109	101
364_1	0.06	105	122	495_1	0.06	103	108
364_1	0.3	123	122	495_1	0.3	106	104
365_1	0.06	88.2	96.4	496_1	0.06	127	122
365_1	0.3	62.1	54.9	496_1	0.3	82.3	79.5
366_1	0.06	99	89	497_1	0.06	66.8	60.2
366_1	0.3	72.5	72.7	497_1	0.3	66.6	66.5
367_1	0.06	94.9	94.5	498_1	0.06	97.7	95.1
367_1	0.3	102	106	498_1	0.3	84.9	75.7
368_1	0.06	102	92.1	499_1	0.06	94.2	87.9
368_1	0.3	128	107	499_1	0.3	73.4	69.6
369_1	0.06	121	140	500_1	0.06	147	129
369_1	0.3	94.1	105	500_1	0.3	79.3	73.6
370_1	0.06	85.5	77.2	501_1	0.06	106	99.9
370_1	0.3	154	176	501_1	0.3	71.8	64.3

371_1	0.06	118	102	502_1	0.06	67.9	66.1
371_1	0.3	115	101	502_1	0.3	53.3	52.3
372_1	0.06	94.9	93.3	503_1	0.06	123	121
372_1	0.3	62.6	58.4	503_1	0.3	88.4	84.7
373_1	0.06	118	108	504_1	0.06	85.7	72.8
373_1	0.3	95.1	98	504_1	0.3	41.4	36.7
374_1	0.06	97.1	106	505_1	0.06	190	180
374_1	0.3	99.4	102	505_1	0.3	117	119
375_1	0.06	91.4	86.9	506_1	0.06	124	113
375_1	0.3	73.5	69.7	506_1	0.3	116	102
376_1	0.06	126	109	507_1	0.06	90.7	84.9
376_1	0.3	97.9	93.4	507_1	0.3	94.8	102
377_1	0.06	89.1	77.2	508_1	0.06	73.2	73.6
377_1	0.3	77.2	66.7	508_1	0.3	49.4	48.1
378_1	0.06	76.1	64.2	509_1	0.06	74.7	73.4
378_1	0.3	82.1	68.2	509_1	0.3	50.7	41.7
379_1	0.06	89.6	81.4	510_1	0.06	83.7	66.8
379_1	0.3	75.4	74.5	510_1	0.3	28	23.9
380_1	0.06	115	116	511_1	0.06	71.5	72.7
380_1	0.3	90.5	99	511_1	0.3	43.6	41.1
381_1	0.06	121	107	512_1	0.06	54.7	48.2
381_1	0.3	86.6	94.2	512_1	0.3	30.1	23.8
382_1	0.06	95.5	96.9	513_1	0.06	96.9	98.2
382_1	0.3	84.3	91.5	513_1	0.3	45.4	41
383_1	0.06	95.5	97.9	514_1	0.06	70.5	63.9

383_1	0.3	70.6	69.1	514_1	0.3	21.6	16.9
384_1	0.06	142	145	515_1	0.06	65.5	55.1
384_1	0.3	212	220	515_1	0.3	27.1	23.5
385_1	0.06	73.3	67.1	516_1	0.06	107	82
385_1	0.3	69.7	70.6	516_1	0.3	68.7	59.9
386_1	0.06	109	107	517_1	0.06	49.5	38.6
386_1	0.3	137	136	517_1	0.3	18.6	12.8
387_1	0.06	90	77.1	518_1	0.06	33.1	25.6
387_1	0.3	70.5	69.5	518_1	0.3	9.6	5.9
388_1	0.06	152	153	519_1	0.06	35.9	31.6
388_1	0.3	116	124	519_1	0.3	10.2	7.3
389_1	0.06	130	130	520_1	0.06	29.6	22.4
389_1	0.3	103	102	520_1	0.3	-	7.1
390_1	0.06	85.8	128	521_1	0.06	84.8	80.2
390_1	0.3	83.8	85.2	521_1	0.3	24.1	17.7
391_1	0.06	58.9	55.1	522_1	0.06	88.6	89.8
391_1	0.3	63.4	54.9	522_1	0.3	41.4	38.4
392_1	0.06	114	95.2	523_1	0.06	45.2	34.4
392_1	0.3	100	81.2	523_1	0.3	14.8	11.6
393_1	0.06	121	119	524_1	0.06	65.2	61.4
393_1	0.3	87.5	75.6	524_1	0.3	32.1	27.3
394_1	0.06	108	108	-	0.06	103	95.2
394_1	0.3	59.8	58	-	0.3	66.5	60.9

From Table 8 it can be taken that the C4 pool is capable of reducing C4a mRNA and C4b mRNA efficiently at different concentrations.

CLAIMS

1. An oligonucleotide C4 inhibitor for use in the treatment of a neurological disease.
2. The C4 inhibitor for use according to claim 1, wherein Neurological diseases is selected from a tauopathy or schizophrenia.
3. The C4 inhibitor for use according to claim 1 or 2, wherein the C4 inhibitor is capable of reducing the amount of C4A and/or C4B.
4. The C4 inhibitor for use according to any one of claims 1 to 3, wherein said inhibitor is a nucleic acid molecule of 12 to 30 nucleotides in length comprising a contiguous nucleotide sequence of at least 12 nucleotides in length which is at least 95% complementary, such as fully complementary, to a mammalian C4 target sequence, in particular a human C4 target sequence, and is capable of reducing the expression of C4 mRNA in a cell which expresses the C4 mRNA.
5. The C4 inhibitor for use according to any one of claims 1 to 4, wherein said inhibitor is selected from the group consisting of a single stranded antisense oligonucleotide, an siRNA and a shRNA.
6. The C4 inhibitor for use according to any one of claim 1 to 5, wherein the mammalian C4 target sequence is selected from the group consisting of SEQ ID NOs: 3 and/or 4, and 6 and/or 7.
7. The C4 inhibitor for use according to any one of claims 4 to 6, wherein the contiguous nucleotide sequence is at least 98% complementary, such as fully complementary, to the target sequence of SEQ ID NO: SEQ ID NO: 3 and/or 4.
8. The C4 inhibitor for use according to any one of claims 4 to 7, wherein the C4 mRNA is reduced by at least 60%, e.g. 60-70%.
9. A nucleic acid molecule of 12 to 30 nucleotides in length comprising a contiguous nucleotides sequence of at least 12 nucleotides which is 95% complementary, such as fully complementary, to a mammalian C4 target sequence, in particular a human C4 target sequence, wherein the nucleic acid molecule is capable of inhibiting the expression of a C4 mRNA.
10. The nucleic acid molecule according to claim 9, wherein the contiguous nucleotide sequence is fully complementary to a sequence selected from one or more of SEQ ID NOs: 3, 4, 6 and 7.

11. The nucleic acid molecule according to claim 9 or 10, wherein the nucleic acid molecule comprises a contiguous nucleotide sequence of 12 to 25, such as 16 to 20 nucleotides in length.

12. The nucleic acid molecule of any one of claims 9 to 11, wherein the nucleic acid molecule is a RNAi molecule, such as a guide strand of a double stranded siRNA or a shRNA.

13. The nucleic acid molecule of any one of claims 9 to 11, wherein the nucleic acid molecule is a single stranded antisense oligonucleotide.

14. The nucleic acid molecule according to 13, wherein the single stranded antisense oligonucleotide is capable of recruiting RNase H.

15. The nucleic acid molecule according to any one of claims 9 to 14, wherein the nucleic acid molecule comprises one or more 2' sugar modified nucleosides.

16. The nucleic acid molecule according to claim 15, wherein the one or more 2' sugar modified nucleosides are independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA and LNA nucleosides.

17. The nucleic acid molecule according to any one of claims 15 or 16, wherein the one or more 2' sugar modified nucleosides are LNA nucleosides.

18. The nucleic acid molecule according to any one of claims 9 to 17, where the contiguous nucleotide sequence comprises at least one phosphorothioate internucleoside linkage.

19. The nucleic acid molecule according to claim 18, wherein at least 90% or 90-95% of the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

20. The nucleic acid molecule according to any one of claims 9 to 19, wherein the nucleic acid molecule, or contiguous nucleotide sequence thereof, comprises a gapmer of formula 5'-F-G-F'-3', wherein regions F and F' independently comprise 1-4 2' sugar modified nucleosides and G is a region between 6 and 18 nucleosides which are capable of recruiting RNase H, such as a region comprising between 6 and 18 DNA nucleosides.

21. A pharmaceutically acceptable salt of a nucleic acid molecule according to any one of claims 9 to 20.

22. A pharmaceutical composition comprising a nucleic acid molecule according to any one of claims 9 to 20, or a pharmaceutically acceptable salt according to claim 21 and a pharmaceutically acceptable excipient.

23. An in vivo or in vitro method for inhibiting C4 expression in a target cell which is expressing C4, said method comprising administering a nucleic acid molecule according to any one of claims 9 to 20, a pharmaceutically acceptable salt according to claim 21, or a pharmaceutical composition according to claim 22 in an effective amount to said cell.

24. A method for treating a disease comprising administering a therapeutically or prophylactically effective amount of a nucleic acid molecule according to any one of claims 9 to 20, a pharmaceutically acceptable salt according to claim 21, or a pharmaceutical composition according to claim 22, to a subject suffering from or susceptible to a neurological disease.

25. A method according to claim 24, wherein the neurological disease is selected from the group consisting of a tauopathy and schizophrenia.

26. A nucleic acid molecule according any one of claims 9 to 20, a pharmaceutically acceptable salt according to claim 21, or a pharmaceutical composition according to claim 22 for use as a therapeutic or diagnostic agent.

27. A nucleic acid molecule according any one of claims 9 to 20, a pharmaceutically acceptable salt according to claim 21, or a pharmaceutical composition according to claim 22, for use in the treatment of a neurological disease, such as a tauopathy or schizophrenia.

28. Use of a nucleic acid molecule according any one of claims 9 to 20, a pharmaceutically acceptable salt according to claim 21, or a pharmaceutical composition according to claim 22, for the preparation of a medicament for the treatment of a neurological disease, such as a tauopathy or schizophrenia.

29. A C4 inhibitor for use according to any one of claims 1 to 8, a nucleic acid molecule according any one of claims 9 to 20, and 26 to 28, a pharmaceutically acceptable salt according to claim 21, a pharmaceutical composition according to claim 22, or a method according to any one of claims 23 to 25, wherein the C4 target sequence is C4A target sequence and/or C4B target sequence.

30. A kit comprising a C4 inhibitor according to any one of claims 1 to 8, a nucleic acid molecule according any one of claims 9 to 20, and 26 to 28, a pharmaceutically acceptable salt according to claim 21, or a pharmaceutical composition according to claim 22, and instructions for administering said C4 inhibitor, said nucleic acid molecule, said pharmaceutically acceptable salt or said pharmaceutical composition.

31. A method for diagnosing a neurological disease in a patient suspected of a having a neurological disease, said method comprising the steps of

a) determining the amount of one or more C4 nucleic acids, such as C4 mRNA or cDNA derived from C4 mRNA, in a sample from the subject, wherein the determination comprises contacting the sample with one or more nucleic acid molecules as defined in any one of claims 9 to 20,

b) comparing the amount determined in step a) to a reference amount, and

c) diagnosing whether the subject suffers from the neurological disease, or not, based on the results of step b).

32. The method of claim 31, wherein the sample is contacted in step a) with said one or more nucleic acid molecules under conditions which allow for the hybridization of said one or more nucleic acid molecules to said one or more C4 nucleic acids present in the sample (such as the C4 mRNA), thereby forming duplexes of said nucleic acid molecules and said C4 nucleic acids.

33. A method for manufacturing a nucleic acid molecule as defined in any one of claims 9 to 20, comprising reacting nucleotide units and thereby forming covalently linked contiguous nucleotide units comprised in the nucleic acid molecule.

34. The method of claim 33, wherein the method comprises the introduction of one or more sugar-modified nucleosides, of one or more modified internucleoside linkages, and/or of one or more modified nucleobases into the nucleic acid molecule.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/031265

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712 A61K31/7125 A61K31/7115
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/081862 A1 (HARVARD COLLEGE [US]) 23 April 2020 (2020-04-23) paragraphs [0023], [0025]; claims 1,83,84; figures 12,14,15,18; example 2 -----	1-34
X	HAMER RIZWAN ET AL: "Human Leukocyte Antigen-Specific Antibodies and Gamma-Interferon Stimulate Human Microvascular and Glomerular Endothelial Cells to Produce Complement Factor C4", TRANSPLANTATION, vol. 93, no. 9, 15 May 2012 (2012-05-15), pages 867-873, XP55826586, GB ISSN: 0041-1337, DOI: 10.1097/TP.0b013e31824b3762 the whole document -----	9,11,12, 23
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 July 2021	02/08/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ASWIN SEKAR ET AL: "Schizophrenia risk from complex variation of complement component 4", NATURE, vol. 530, no. 7589, 27 January 2016 (2016-01-27), pages 177-183, XP055601143, London ISSN: 0028-0836, DOI: 10.1038/nature16549 figures 3,5	26,31,32
A	WO 2019/113535 A1 (HARVARD COLLEGE [US]) 13 June 2019 (2019-06-13) figures 3,23,29-32	1-34
A	NIMGAONKAR V L ET AL: "The complement system: a gateway to gene-environment interactions in schizophrenia pathogenesis", MOLECULAR PSYCHIATRY, vol. 22, no. 11, 1 November 2017 (2017-11-01), pages 1554-1561, XP55826906, GB ISSN: 1359-4184, DOI: 10.1038/mp.2017.151 the whole document	1-34
A	WOO JULIA J ET AL: "The complement system in schizophrenia: where are we now and what's next?", MOLECULAR PSYCHIATRY, BASINGSTOKE, GB, vol. 25, no. 1, 22 August 2019 (2019-08-22), pages 114-130, XP036953775, ISSN: 1359-4184, DOI: 10.1038/S41380-019-0479-0 [retrieved on 2019-08-22] the whole document	1-34

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/031265

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/031265

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020081862 A1	23-04-2020	NONE	
WO 2019113535 A1	13-06-2019	NONE	