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(54) Title: ANTISENSE OLIGONUCLEOTIDES FOR MODULATING REL EXPRESSION

(57) Abstract: The present invention relates to antisense oligonucleotides that are capable of modulating expression of c-Rel in a target cell. The present invention further relates to conjugates of the oligonucleotide and pharmaceutical compositions and methods for treatment of cancer, inflammation or autoimmune diseases using the oligonucleotide.

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ANTISENSE OLIGONUCLEOTIDES FOR MODULATING REL EXPRESSION

FIELD OF INVENTION

The present invention relates to oligonucleotides (oligomers) complementary to REL pre-mRNA sequences, which are capable inhibiting the expression of c-Rel. Inhibition of REL expression is beneficial for a range of medical disorders including autoimmunity and cancer.

BACKGROUND

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a key regulator of processes such as immunity, inflammation, gene expression, cancer cell migration, invasion, apoptosis, and proliferation. NF-κB subunits share a Rel homology domain in their N-terminus. REL (REL proto-oncogene, NF-kB subunit) codes for a protein (c-Rel) that as homo- or heterodimer can form some members of the NF-κB family of transcription factors. The most common c-Rel containing NF-κB proteins are c-Rel homodimers and p50/c-Rel heterodimers but c-Rel can also form heterodimers with other NF-κB subunits.

NF-KB subunit expression can be altered in disease, and dysfunctional NF-κB activation contributes to disorders including rheumatoid arthritis, atherosclerosis, inflammatory bowel diseases, multiple sclerosis and malignant tumors (Park and Hong, 2016, Cells 5:15), as well as asthma and chronic inflammatory airway disease (Schuliga, 2015, Biomolecules, 5-1266).

There are >700 compounds described in literature to have NF-κB inhibitory effect, most of them with broad effect on NF-κB signaling, but a narrow therapeutic index, poor specificity, short *in vivo* half-life of molecules, and only minor effects on signaling, and have therefore limited the therapeutic use of described NF-κB inhibitors to date.

Some NF-κB homo- and heterodimers are found in many different tissues and cell types. A special characteristic of c-Rel is a high expression level in B and T cells with a central role in B and T cell differentiation and function. Specific reduction of c-Rel expression and activity has been suggested to give therapeutic benefits for human hematopoietic cancers as well as chronic inflammatory or autoimmune diseases; REL is a susceptibility locus for certain autoimmune diseases such as arthritis, psoriasis, and celiac disease (Gilmore & Gerondakis, 201 1, Genes & Cancer 2:695). The REL locus is also frequently altered (amplified, mutated, or rearranged), and expression of REL is increased in a variety of B and T cell malignancies and, to a lesser extent, in other cancer types. Thus, agents that modulate c-Rel activity may have therapeutic benefits for certain human cancers and chronic inflammatory diseases.

WO00/17400 provides DNA phosphorothioate and MOE gapmer oligonucleotides targeting REL.

Pizzi *et ai,* 2002, JBC 227: 20717 discloses an oligonucleotide targeting REL of sequence 5-tacgcaccggaggccatggct-3 ', and reports that the balance between cell death and survival in neurons may rely on the distinct activation of NF-KB/Rel proteins.

Ishige *et al.,* 2005, Neurochem. Int. 47:545 reports on the effect of an antisense oligodeoxynucleotide targeted against REL on glutamate induced cell death of HT22 cells. Ishige *et al* discloses a phosphorothioate DNA antisense targeting human REL (c-Rel) transcript of sequence 5-TATCCACTCGAGGCCATGGCT-3.

OBJECTIVE OF THE INVENTION

The present invention identifies novel oligonucleotides which inhibit human REL (c-Rel) which are useful in the treatment of a range of medical disorders including autoimmunity, inflammation and cancer.

SUMMARY OF INVENTION

The present invention relates to oligonucleotides targeting a nucleic acid capable of modulating, such as inhibiting the expression of c-Rel.

The invention provides for LNA oligonucleotides targeting human REL.

The invention provides for an antisense oligonucleotide targeting human REL, 10 to 30 contiguous nucleotides in length, wherein the contiguous sequence of the oligonucleotide comprises at least 10 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NO 3, 4, 1, 2, 5, 6, 7, 8, 9 and 10.

The invention provides for an LNA antisense oligonucleotide targeting human REL, 10 to 30 contiguous nucleotides in length, wherein the contiguous sequence of the oligonucleotide comprises at least 10 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NO 3, 4, 1, 2, 5, 6, 7, 8, 9 and 10.

The invention provides for an LNA antisense oligonucleotide targeting human REL, 12 to 30 contiguous nucleotides in length, wherein the contiguous sequence of the oligonucleotide comprises at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NO 3, 4, 1, 2, 5, 6, 7, 8, 9 and 10.

The invention provides for an antisense oligonucleotide targeting human REL, 12 to 30 contiguous nucleotides in length, wherein the contiguous sequence of the oligonucleotide comprises at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NO 3, 4, 1, 2, 5, 6, 7, 8, 9 and 10.

The invention provides for a conjugate comprising the oligonucleotide according to the invention.

In a further aspect, the invention provides pharmaceutical compositions comprising the oligonucleotide or conjugate of the invention and pharmaceutically acceptable diluents, carriers, salts and/or adjuvants.

In a further aspect, the invention provides methods for *in vivo* or *in vitro* method for modulation of Rel expression in a target cell which is expressing c-Rel by administering an oligonucleotide, conjugate, or composition of the invention in an effective amount to said cell.

In a further aspect the invention provides methods for treating or preventing a disease, disorder or dysfunction associated with *in vivo* activity of c-Rel comprising administering a therapeutically or prophylactically effective amount of the oligonucleotide, conjugate or composition of the invention to a subject suffering from or susceptible to the disease, disorder or dysfunction.

In a further aspect the oligonucleotide, conjugate or composition of the invention is used for the treatment or prevention of cancer, autoimmune diseases, and inflammation or an inflammatory disease.

In some embodiments, the oligonucleotide, conjugate or composition of the invention is an antisense oligonucleotide, preferably a gapmer antisense oligonucleotide.

BRIEF DESCRIPTION OF FIGURES

Figure 1A, 1B and 1C: Mouse *in vivo* efficacy, 16 days of treatment, Intravenous injection (tail vein).

Figure 2: Testing *in vitro* efficacy of various antisense oligonucleotides targeting human REL mRNA in HEK293 and HeLa cell lines at single dose concentration.

Figure 3: Testing *in vitro* efficacy of antisense oligonucleotides targeting human REL mRNA in HEK293 and HeLa cell lines at single dose concentration.

Figure 4: Testing *in vitro* efficacy of antisense oligonucleotides targeting human REL mRNA in HEK293 and HeLa cell lines at single dose concentration. Zoom in illustrating the data for compounds targeting the hot spot regions.

Figure 5A, 5B & 5C: Testing *in vitro* potency and efficacy of selected oligonucleotides targeting human REL mRNA in HEK-293 and HeLa cell lines in a dose response curve.

Figure 6: Human REL pre-mRNA sequence (SEQ ID NO 21) derived from the human genomic sequence NC_000002.12 (60881495..60928171). See sequence listing.

DEFINITIONS

Oligonucleotide

The term "oligonucleotide" as used herein is defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers. Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucieobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The oligonucleotide of the invention is man-made, and is chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides.

Antisense oligonucleotides

The term "Antisense oligonucleotide" as used herein is defined as oligonucleotides capable of modulating expression of a target gene by hybridizing to a target nucleic acid, in particular to a contiguous sequence (a sub-sequence) on a target nucleic acid. The antisense oligonucleotides are not essentially double stranded and are therefore not siRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded.

An LNA antisense oligonucleotide is an antisense oligonucleotide which comprises at least one LNA nucleoside. In some embodiments the LNA antisense oligonucleotide is a LNA gapmer oligonucleotide. In some embodiments, the oligonucleotide of the invention is an LNA antisense oligonucleotide.

Targeting

The oligonucleotides of the invention are capable of targeting the human REL transcript. Targeting refers to the ability of the oligonucleotide to form a functional complementary hybridization accross the contiguous nucleotide sequence of the oligonucleotide with the human REL transcript, such as a fully complementary hybridization, and inhibit the expression of the human REL transcript in a cell.

Contiguous Nucleotide Sequence

The term "contiguous nucleotide sequence" refers to the region of the oligonucleotide which is complementary to the target nucleic acid. The term is used interchangeably herein with the term "contiguous nucieobase 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 oligonucleotide comprises the contiguous nucleotide sequence and may optionally comprise further nucleotide(s), for example a nucleotide linker

region which may be used to attach a functional group to the contiguous nucleotide sequence. The nucleotide linker region may or may not be complementary to the target nucleic acid.

Nucleotides

Nucleotides 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. 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. In some embodiments the modified nucleoside comprise a modified sugar moiety. The term modified nucleoside may also be used herein interchangeably with the term "nucleoside analogue" or modified "units" or modified "monomers".

Modified internucleoside linkage

The term "modified internucleoside linkage" is defined as generally understood by the skilled person as linkages other than phosphodiester (PO) linkages, that covalently couples two nucleosides together. Nucleotides with modified internucleoside linkage are also termed "modified nucleotides". In some embodiments, the modified internucleoside linkage increases the nuclease resistance of the oligonucleotide compared to a phosphodiester linkage. For naturally occurring oligonucleotides, the internucleoside linkage includes phosphate groups creating a phosphodiester bond between adjacent nucleosides. Modified internucleoside linkages are particularly useful in stabilizing oligonucleotides for in vivo use, and may serve to protect against nuclease cleavage at regions of DNA or RNA nucleosides in the oligonucleotide of the invention, for example within the gap region of a gapmer oligonucleotide, as well as in regions of modified nucleosides.

In an embodiment, the oligonucleotide comprises one or more internucleoside linkages modified from the natural phosphodiester to a linkage that is for example more resistant to nuclease attack. Nuclease resistance may be determined by incubating the oligonucleotide in blood serum or by using a nuclease resistance assay (e.g. snake venom phosphodiesterase (SVPD)), both are well known in the art. Internucleoside linkages which are capable of enhancing the nuclease resistance of an oligonucleotide are referred to as nuclease resistant internucleoside linkages. In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are modified, such as at least 60%,

such as at least 70%, such as at least 80 or such as at least 90% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are modified. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are modified. It will be recognized that, in some embodiments the nucleosides which link the oligonucleotide of the invention to a non-nucleotide functional group, such as a conjugate, may be phosphodiester. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are nuclease resistant internucleoside linkages.

Modified internucleoside linkages may be selected from the group comprising phosphorothioate, diphosphorothioate and boranophosphate. In some embodiments, the modified internucleoside linkages are compatible with the RNaseH recruitment of the oligonucleotide of the invention, for example phosphorothioate, diphosphorothioate or boranophosphate.

In some embodiments the internucleoside linkage comprises sulphur (S), such as a phosphorothioate internucleoside linkage.

A phosphorothioate internucleoside linkage is particularly useful due to nuclease resistance, beneficial pharmakokinetics 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%, such as at least 70%, such as at least 80 or such as at least 90% 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 embodiments, the oligonucleotide comprises one or more neutral internucleoside linkage, particularly a internucleoside linkage selected from phosphotriester, methylphosphonate, MMI, amide-3, formacetal or thioformacetal.

Further internucleoside linkages are disclosed in WO2009/124238 (incorporated herein by reference). In an embodiment the internucleoside linkage is selected from linkers disclosed in WO2007/031091 (incorporated herein by reference). Particularly, the internucleoside linkage may be selected from -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -S-P(O)$_2$-O-, -S-P(O,S)-O-, -S-P(S)$_2$-O-, -O-P(O)$_2$-S-, -O-P(O,S)-S-, -S-P(O)$_2$-S-, -O-PO(R^H)-O-, O-PO(OCH$_3$)-O-, -O-PO(NR^H)-O-, -O-PO(OCH$_2$CH$_2$S-R)-O-, -O-PO(BH$_3$)-O-, -O-PO(NHR^H)-O-, -O-P(O)$_2$-NR^H-, -NR^H-P(O)$_2$-O-, -NR^H-CO-O-, -NR^H-CO-NR^H-, and/or the internucleoside linker may be selected form the group consisting of: -O-CO-O-, -O-CO-NR^H-, -NR^H-CO-CH$_2$-, -O-CH$_2$-CO-NR^H-, -O-CH$_2$-CH$_2$-NR^H-, -CO-NR^H-CH$_2$-, -CH$_2$-NR^{H}CO-, -O-CH$_2$-CH$_2$-S-, -S-CH$_2$-CH$_2$-O-, -S-CH$_2$-CH$_2$-S-, -CH$_2$-SO$_2$-CH$_2$-, -CH$_2$-CO-NR^H-, -O-CH$_2$-CH$_2$-NR^H-CO-, -CH$_2$-NCH$_3$-O-CH$_2$-, where R^H is selected from hydrogen and C$_{1-4}$ alkyl.

Nuclease resistant linkages, such as phosphothioate linkages, are particularly useful in oligonucleotide regions capable of recruiting nuclease when forming a duplex with the target nucleic acid, such as region G for gapmers, or the non-modified nucleoside region of headmers and tailmers. Phosphorothioate linkages may, however, also be useful in non-nuclease recruiting regions and/or affinity enhancing regions such as regions F and F' for gapmers, or the modified nucleoside region of headmers and tailmers.

Each of the design regions may however comprise internucleoside linkages other than phosphorothioate, such as phosphodiester linkages, in particularly in regions where modified nucleosides, such as LNA, protect the linkage against nuclease degradation. Inclusion of phosphodiester linkages, such as one or two linkages, particularly between or adjacent to modified nucleoside units (typically in the non-nuclease recruiting regions) can modify the bioavailability and/or bio-distribution of an oligonucleotide - see WO2008/1 13832, incorporated herein by reference.

In an embodiment all the internucleoside linkages in the oligonucleotide are phosphorothioate and/or boranophosphate linkages. In some embodiments, all the internucleoside linkages in the oligonucleotide are phosphorothioate linkages.

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 a 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 nucleobased 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. The term chimeric" oligonucleotide is a term that has been used in the literature to describe oligonucleotides with modified nucleosides.

Complementarity

The term "complementarity" 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 number of nucleotides in percent of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which, at a given position, are complementary to *(i.e.* form Watson Crick base pairs with) a contiguous nucleotide sequence, at a given position of a separate nucleic acid molecule (e.g. the target nucleic acid). The percentage is calculated by counting the number of aligned bases that form pairs between the two sequences, dividing 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.

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

Identity

The term "Identity" as used herein, refers to the number of nucleotides in percent of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which, at a given position, are identical to *(i.e.* in their ability to form Watson Crick base pairs with the complementary nucleoside) a contiguous nucleotide sequence, at a given position of a separate nucleic acid molecule (e.g. the target nucleic acid). The percentage is calculated by counting the number of aligned bases that are identical between the two sequences, including gaps, dividing by the total number of nucleotides in the oligonucleotide and multiplying by 100. Percent Identity = (Matches x 100)/Length of aligned region (with gaps).

Hybridization

The term "hybridizing" or "hybridizes" as used herein is to be understood as 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 (T_m) defined as the temperature at which half of the oligonucleotides are duplexed with the target nucleic acid. At physiological conditions T_m is not strictly proportional to the affinity (Mergny and Lacroix, *2003.Oligonucleotides* 13:515-537). The standard state Gibbs free energy $\Delta G°$ is a more accurate representation of binding affinity and is related to the dissociation constant (K_d) of the reaction by $AG°=-RTIn(K_d)$, where R is the gas constant and T is the absolute temperature. Therefore, a very low $AG°$ of the reaction between an oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. $\Delta G°$ 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 $\Delta G°$ is less than zero. $AG°$ 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 $\Delta G°$ measurements. $AG°$ 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* 34:1 121 1-1 1216 and McTigue et al., 2004, *Biochemistry* 43:5388-5405. In order to have the possibility of modulating its intended nucleic acid target by hybridization, oligonucleotides of the present invention hybridize to a target nucleic acid with estimated $\Delta G°$ values below -10 kcal for oligonucleotides that are 10-30 nucleotides in length. In some embodiments the degree or strength of hybridization is measured by the standard state Gibbs free energy $\Delta G°$. The oligonucleotides may hybridize to a target nucleic acid with estimated $\Delta G°$ values below the range of -10 kcal, such as below -15 kcal, such as below -20 kcal and such as below -25 kcal for oligonucleotides that are 8-30 nucleotides in length. In some embodiments the oligonucleotides hybridize to a target nucleic acid with an estimated $\Delta G°$ value of -10 to -60 kcal, such as -12 to -40, such as from -15 to -30 kcal or -16 to -27 kcal such as -18 to -25 kcal.

Target nucleic acid

According to the present invention, the target nucleic acid is a nucleic acid which encodes mammalian c-Rel 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 an REL target nucleic acid.

The oligonucleotide of the invention targets intron regions of a mammalian REL pre-mRNA, such as SEQ ID NO 21 (the human REL pre-mRNA sequence). For *in vivo* or *in vitro* application, the oligonucleotide of the invention is typically capable of inhibiting the expression of the REL target nucleic acid in a cell which is expressing the REL target nucleic acid. The contiguous sequence of nucleobases of the oligonucleotide of the invention is typically complementary to the REL target nucleic acid, as measured across the length of the oligonucleotide, optionally with the exception of one or two mismatches, and optionally excluding nucleotide based linker regions which may link the oligonucleotide to an optional functional group such as a conjugate, or other non-complementary terminal nucleotides (e.g. region D' or D"). The target nucleic acid may, in some embodiments, be a REL pre-mRNA

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 of the invention. In some embodiments, the target sequence consists of a region on the target nucleic acid which is complementary to the contiguous nucleotide sequence of the oligonucleotide of the invention. In some embodiments the target sequence is longer than the complementary sequence of a single oligonucleotide, and may, for example represent a preferred region of the target nucleic acid which may be targeted by several oligonucleotides of the invention.

The target sequence may be a sub-sequence of the target nucleic acid.

In some embodiments the sub-sequence is a sequence present in a human REL mRNA intron, such as a REL human pre-mRNA selected from the group consisting of SEQ ID NO 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22 and 23.

The oligonucleotide of the invention comprises a contiguous nucleotide sequence which is complementary to or hybridizes to the target nucleic acid, such as a sub-sequence of the target nucleic acid, such as a target sequence described herein. In some embodiments, the oligonucleotide consists of the contiguous nucleotide sequence.

The oligonucleotide comprises a contiguous nucleotide sequence of at least 10 nucleotides which is complementary to or hybridizes to a target sequence present in the target nucleic acid molecule. The contiguous nucleotide sequence (and therefore the target sequence) comprises of at least 10 contiguous nucleotides, such as 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides, such as from 12-25, such as from 14-18 contiguous nucleotides.

Target Cell

The term a "target cell" as used herein refers to a cell which is expressing the target nucleic acid. 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 primate cell such as a monkey cell or a human cell.

In preferred embodiments the target cell expresses REL pre-mRNA.

In some embodiments the oligonucleotides, conjugates or compositions, of the invention are capable to inhibiting the expression of human REL in a cell selected from the group consisting of HEK293 and HeLa cells.

Naturally occurring variant

The term "naturally occurring variant" refers to variants of REL 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, and allelic variants. Based on the presence of the sufficient complementary sequence to 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% such as at least 98% or at least 99% homology to a mammalian REL target nucleic acid, such SEQ ID NO 21.

Modulation of expression

The term "modulation of expression" as used herein is to be understood as an overall term for an oligonucleotide's ability to alter the amount of REL when compared to the amount of REL before administration of the oligonucleotide. Alternatively modulation of expression may be determined by reference to a control experiment. It is generally understood that the control is an individual or target cell treated with a saline composition or an individual or target cell treated with a non-targeting oligonucleotide (mock). It may however also be an individual treated with the standard of care.

One type of modulation is an oligonucleotide's ability to inhibit, down-regulate, reduce, suppress, remove, stop, block, prevent, lessen, lower, avoid or terminate expression of c-Rel e.g. by degradation of rrsRNA or blockage of transcription.

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 (T^m). A high affinity modified nucleoside of the present invention preferably result in an increase in melting temperature between +0.5 to

+12°C, more preferably between +1.5 to +10°C and most preferably between +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).

Sugar modifications

The oligomer 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.

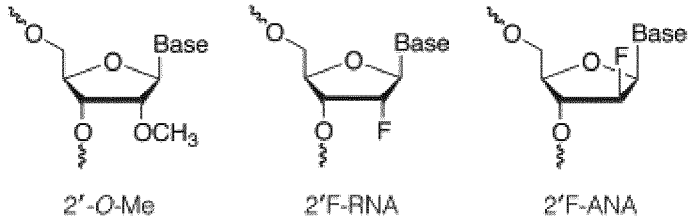
Such modifications include those where the ribose ring structure is modified, *e.g.* by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradicle bridge between the C2 and C4 carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g. UNA). Other sugar modified nucleosides include, for example, bicyclohexose nucleic acids (WO201 1/01 7521) or tricyclic nucleic acids (WO201 3/1 54798). 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 the 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. Nucleosides with modified sugar moieties also include 2' modified nucleosides, such as 2' substituted nucleosides. Indeed, much focus has been spent on developing 2' substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial properties when incorporated into oligonucleotides, such as enhanced nucleoside resistance and enhanced affinity.

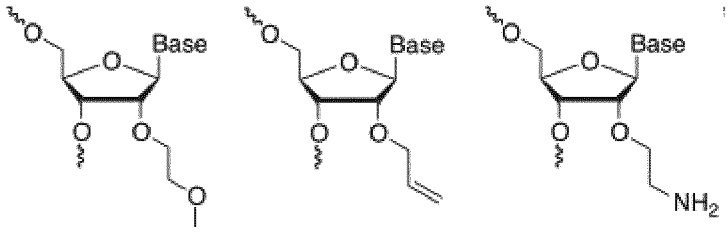
2' modified nucleosides.

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 biradicle, and includes 2' substituted nucleosides and LNA (2' - 4' biradicle bridged) nucleosides. 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.



2′-*O*-Me 2′F-RNA 2′F-ANA

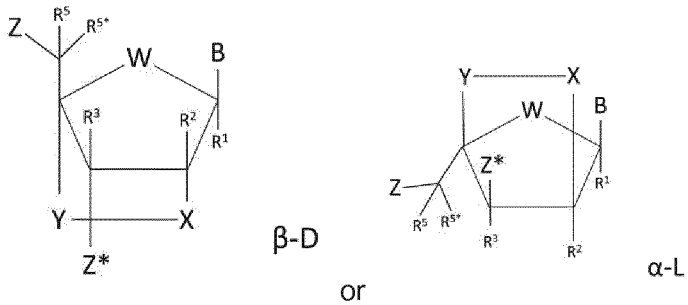


2′-*O*-MOE 2′-*O*-Allyl 2′-*O*-Ethylamine

Locked Nucleic Acid Nucleosides (LNA).

LNA nucleosides are modified nucleosides which comprise a linker group (referred to as a biradicle or a bridge) between C2' and C4' of the ribose sugar ring of a nucleotide. These nucleosides are also termed bridged nucleic acid or bicyclic nucleic acid (BNA) in the literature.

In some embodiments, the modified nucleoside or the LNA nucleosides of the oligomer of the invention has a general structure of the formula I or II:



Formula I Formula II

wherein W is selected from -O-, -S-, -N(R^a)-, -C(R^aR^b)-, such as, in some embodiments -O-;

B designates a nucleobase or modified nucleobase moiety;

Z designates an internucleoside linkage to an adjacent nucleoside, or a 5'-terminal group;

Z^* designates an internucleoside linkage to an adjacent nucleoside, or a 3'-terminal group;

X designates a group selected from the list consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -S0 $_2$-, -N(R^a)-, and >C=Z

In some embodiments, X is selected from the group consisting of: -O-, -S-, NH-, NR^aR^b, $-CH_2-$, CR^aR^b, $-C(=CH_2)-$, and $-C(=CR^aR^b)-$

In some embodiments, X is -O-

Y designates a group selected from the group consisting of $-C(R^aR^b)-$, $-C(R^a)=C(R^b)-$, $-C(R^a)=N-$, -O-, $-Si(R^a)_2-$, -S-, $-SO_2-$, $-N(R^a)-$, and >C=Z

In some embodiments, Y is selected from the group consisting of: $-CH_2-$, $-C(R^aR^b)-$, $-CH_2CH_2-$, $-C(R^aR^b)-C(R^aR^b)-$, $-CH_2CH_2CH_2-$, $-C(R^aR^b)C(R^aR^b)C(R^aR^b)-$, $-C(R^a)=C(R^b)-$, and $-C(R^a)=N-$

In some embodiments, Y is selected from the group consisting of: $-CH_2-$, $-CHR^a-$, $-CHCH_3-$, CR^aR^b-

or -X-Y- together designate a bivalent linker group (also referred to as a radicle) together designate a bivalent linker group consisting of 1, 2, 3 or 4 groups/atoms selected from the group consisting of $-C(R^aR^b)-$, $-C(R^a)=C(R^b)-$, $-C(R^a)=N-$, -O-, $-Si(R^a)_2-$, -S-, $-SO_2-$, $-N(R^a)-$, and >C=Z,

In some embodiments, -X-Y- designates a biradicle selected from the groups consisting of: $-X-CH_2-$, $-X-CR^aR^b-$, $-X-CHR^a-$, $-X-C(HCH_3)\ -O-Y-$, $-O-CH_2-$, $-S-CH_2-$, $-NH-CH_2-$, $-O-CHCH_3-$, $-CH_2-O-CH_2$, $-O-CH(CH_3CH_3)-$, $-O-CH_2-CH_2-$, $OCH_2-CH_2-CH_2-$.$-O-CH_2OCH_2-$, $-O-NCH_2-$, $-C(=CH_2)-CH_2-$, $-NR^a-CH_2-$, $N-O-CH_2$, $-S-CR^aR^b-$ and $-S-CHR^3-$.

In some embodiments -X-Y- designates $-O-CH_2-$ or $-O-CH(CH_3)-$.

wherein Z is selected from -O-, -S-, and $-N(R^a)-$,

and R^a and, when present R^b, each is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, optionally substituted C_{1-6}-alkoxy, C_{2-6}-alkoxyalkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamide C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene ($=CH_2$), wherein for all chiral centers, asymmetric groups may be found in either *R* or S orientation.

wherein R^1, R^2, R^3, R^5 and R^{5*} are independently selected from the group consisting of: hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkoxyalkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-

alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(Ci-6-alkyl)amino-Ci-6-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene.

In some embodiments R^1, R^2, R^3, R^5 and R^{5*} are independently selected from C_{1-6} alkyl, such as methyl, and hydrogen.

In some embodiments R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen.

In some embodiments R^1, R^2, R^3, are all hydrogen, and either R^5 and R^{5*} is also hydrogen and the other of R^5 and R^{5*} is other than hydrogen, such as C_{1-6} alkyl such as methyl.

In some embodiments, R^a is either hydrogen or methyl. In some embodiments, when present, R^b is either hydrogen or methyl.

In some embodiments, one or both of R^a and R^b is hydrogen

In some embodiments, one of R^a and R^b is hydrogen and the other is other than hydrogen

In some embodiments, one of R^a and R^b is methyl and the other is hydrogen

In some embodiments, both of R^a and R^b are methyl.

In some embodiments, the biradicle -X-Y- is -O-CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such LNA nucleosides are disclosed in WO99/014226, WO00/66604, WO98/039352 and WO2004/046160 which are all hereby incorporated by reference, and include what are commonly known as beta-D-oxy LNA and alpha-L-oxy LNA nucleosides.

In some embodiments, the biradicle -X-Y- is -S-CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such thio LNA nucleosides are disclosed in WO99/0 14226 and WO2004/046160 which are hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -NH-CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such amino LNA nucleosides are disclosed in WO99/014226 and WO2004/046160 which are hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -O-CH_2-CH_2- or -O-CH_2-CH_2- CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such LNA nucleosides are disclosed in WO00/047599 and Morita et al, Bioorganic & Med.Chem. Lett. 12 73-76, which are hereby incorporated by reference, and include what are commonly known as 2'-O-4'C-ethylene bridged nucleic acids (ENA).

In some embodiments, the biradicle -X-Y- is -O-CH_2-, W is O, and all of R^1, R^2, R^3, and one of R^5 and R^{5*} are hydrogen, and the other of R^5 and R^{5*} is other than hydrogen such as C_{1-6} alkyl, such as methyl. Such 5' substituted LNA nucleosides are disclosed in WO2007/134181 which is hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -O-CR^aR^b-, wherein one or both of R^a and R^b are other than hydrogen, such as methyl, W is O, and all of R^1, R^2, R^3, and one of R^5 and R^{5*} are hydrogen, and the other of R^5 and R^{5*} is other than hydrogen such as C_{1-6} alkyl, such as methyl. Such bis modified LNA nucleosides are disclosed in WO201 0/077578 which is hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- designate the bivalent linker group -O-$CH(CH_2OCH_3)$- (2' O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem. Vol 75(5) pp. 1569-81). In some embodiments, the biradicle -X-Y- designate the bivalent linker group - O-$CH(CH_2CH_3)$- (2'O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem. Vol 75(5) pp. 1569-81). In some embodiments, the biradicle -X-Y- is -O-CHR^a-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such 6' substituted LNA nucleosides are disclosed in W01 0036698 and WO07090071 which are both hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -O-$CH(CH_2OCH_3)$-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such LNA nucleosides are also known as cyclic MOEs in the art (cMOE) and are disclosed in WO07090071 .

In some embodiments, the biradicle -X-Y- designate the bivalent linker group - O-$CH(CH_3)$-. - in either the R- or S- configuration. In some embodiments, the biradicle -X-Y- together designate the bivalent linker group - O-CH_2-O-CH_2- (Seth at al., 2010, J. Org. Chem). In some embodiments, the biradicle -X-Y- is - O-$CH(CH_3)$-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such 6' methyl LNA nucleosides are also known as cET nucleosides in the art, and may be either (S)cET or (R)cET stereoisomers, as disclosed in WO07090071 (beta-D) and WO201 0/036698 (alpha-L) which are both hereby incorporated by reference).

In some embodiments, the biradicle -X-Y- is -O-CR^aR^b-, wherein in neither R^a or R^b is hydrogen, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. In some embodiments, R^a and R^b are both methyl. Such 6' di-substituted LNA nucleosides are disclosed in WO 2009006478 which is hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -S-CHR^a-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such 6' substituted thio LNA nucleosides are disclosed in W01 1156202 which is hereby incorporated by reference. In some 6' substituted thio LNA embodiments R^a is methyl.

In some embodiments, the biradicle -X-Y- is -C(=CH2)-C(R aRb)-, such as -C(=CH 2)-CH 2- , or -C(=CH 2)-CH(CH 3)-W is O, and all of R1, R2, R3, R5 and R5" are all hydrogen. Such vinyl carbo LNA nucleosides are disclosed in WO081 5440 1 and WO09067647 which are both hereby incorporated by reference.

In some embodiments the biradicle -X-Y- is -N(-OR a)-, W is O, and all of R1, R2, R3, R5 and R5* are all hydrogen. In some embodiments Ra is C1-6 alkyl such as methyl. Such LNA nucleosides are also known as N substituted LNAs and are disclosed in WO2008/1 50729 which is hereby incorporated by reference. In some embodiments, the biradicle -X-Y- together designate the bivalent linker group - O-NRa-CH 3- (Seth at al., 201 0, J. Org. Chem). In some embodiments the biradicle -X-Y- is -N(R a)-, W is O, and all of R1, R2, R3, R5 and R5* are all hydrogen. In some embodiments Ra is C1-6 alkyl such as methyl.

In some embodiments, one or both of R5 and R5* is hydrogen and, when substituted the other of R5 and R5* is C1-6 alkyl such as methyl. In such an embodiment, R1, R2, R3, may all be hydrogen, and the biradicle -X-Y- may be selected from - O-CH2- or - O-C(HCRa)-, such as -O-C(HCH3)-.

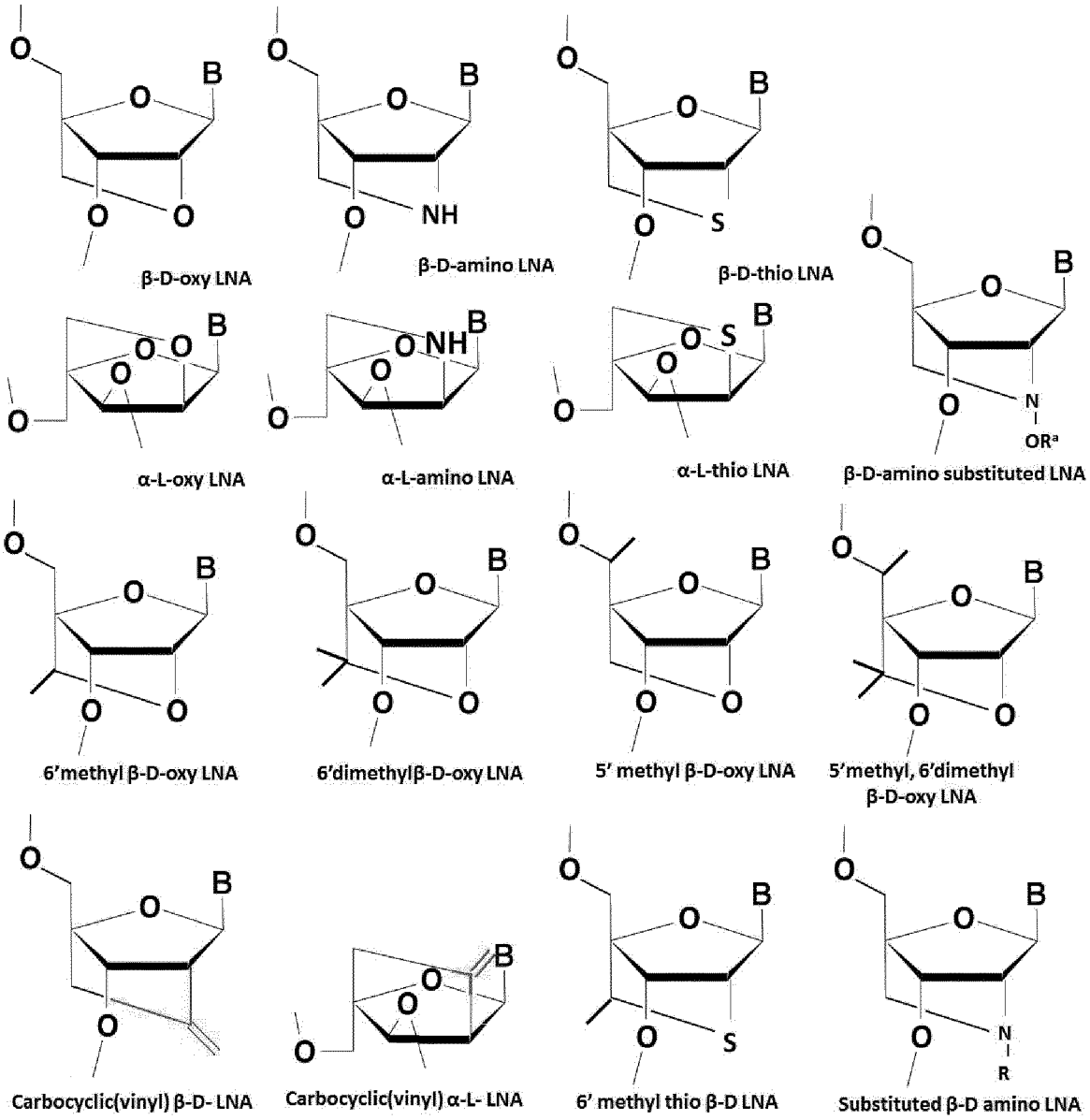
In some embodiments, the biradicle is -CR aR"-O-CRaRb-, such as CH 2-O-CH 2-, W is O and all of R1, R2, R3, R5 and R5* are all hydrogen. In some embodiments Ra is d-e alkyl such as methyl. Such LNA nucleosides are also known as conformationally restricted nucleotides (CRNs) and are disclosed in WO201 3036868 which is hereby incorporated by reference.

In some embodiments, the biradicle is - O-CRaRb-O-CRaRb-, such as O-CH 2-O-CH 2-, W is O and all of R1, R2, R3, R5 and R5* are all hydrogen. In some embodiments Ra is C 1-6 alkyl such as methyl. Such LNA nucleosides are also known as COC nucleotides and are disclosed in Mitsuoka et al., Nucleic Acids Research 2009 37(4), 1225-1 238, which is hereby incorporated by reference.

It will be recognized than, unless specified, the LNA nucleosides may be in the beta-D or alpha-L stereoisoform.

Certain examples of LNA nucleosides are presented in Scheme 1.

Scheme 1



As illustrated in the examples, in some embodiments of the invention the LNA nucleosides in the oligonucleotides are beta-D-oxy-LNA nucleosides.

Nuclease mediated degradation

Nuclease mediated degradation refers to an oligonucleotide capable of mediating degradation of a complementary nucleotide sequence when forming a duplex with such a sequence.

In some embodiments, the oligonucleotide may function via nuclease mediated degradation of the target nucleic acid, where the oligonucleotides of the invention are capable of recruiting a nuclease, particularly and endonuclease, preferably endoribonuclease (RNase), such as RNase H. Examples of oligonucleotide designs which operate via nuclease mediated mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 DNA nucleosides and are

flanked on one side or both sides by affinity enhancing nucleosides, for example gapmers, headmers and tailmers.

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 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. 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% or more than 20% 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 WO01/23613 (hereby incorporated by reference).

Gapmer

The term gapmer as used herein refers to an antisense oligonucleotide which comprises a region of RNase H recruiting oligonucleotides (gap) which is flanked 5' and 3' by regions which comprise one or more affinity enhancing modified nucleosides (flanks or wings). Various gapmer designs are described herein. Headmers and tailmers are oligonucleotides capable of recruiting RNase H where one of the flanks is missing, i.e. only one of the ends of the oligonucleotide comprises affinity enhancing modified nucleosides. For headmers the 3' flank is missing (i.e. the 5' flank comprises affinity enhancing modified nucleosides) and for tailmers the 5' flank is missing (i.e. the 3' flank comprises affinity enhancing modified nucleosides).

LNA Gapmer

The term LNA gapmer is a gapmer oligonucleotide wherein at least one of the affinity enhancing modified nucleosides is an LNA nucleoside. In some embodiments the LNA gapmer oligonucleotide comprises one or more LNA nucleoside within both of the the wing (or flank regions). In some embodiments, the LNA gapmer is a mixed wing gapmer. In some embodiments the wing (flank) regions of the LNA gapmer comprise of only LNA nucleosides.

Mixed Wing Gapmer

The term mixed wing gapmer or mixed flank gapmer refers to a LNA gapmer wherein at least one of the flank regions comprise at least one LNA nucleoside and at least one non-LNA modified nucleoside, such as at least one 2' substituted modified nucleoside, such as, for example, 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(s). In some embodiments the mixed wing gapmer has one flank which comprises only LNA nucleosides (e.g. 5' or 3') and the other flank

(3' or 5' respectfully) comprises 2' substituted modified nucleoside(s) and optionally LNA nucleosides.

Gapbreaker

The term "gapbreaker oligonucleotide" is used in relation to a gapmer capable of maintaining RNAseH recruitment even though the gap region is disrupted by a non-RNaseH recruiting nucleoside (a gap-breaker nucleoside, E) such that the gap region comprise less than 5 consecutive DNA nucleosides. Non-RNaseH recruiting nucleosides are for example nucleosides in the 3' endo conformation, such as LNA's where the bridge between C2' and C4' of the ribose sugar ring of a nucleoside is in the beta conformation, such as beta-D-oxy LNA or ScET nucleoside. The ability of gapbreaker oligonucleotide to recruit RNaseH is typically sequence or even compound specific - see Rukov et al. 2015 Nucl. Acids Res. Vol. 43 pp. 8476-8487, which discloses "gapbreaker" oligonucleotides which recruit RNaseH which in some instances provide a more specific cleavage of the target RNA.

In some embodiments, the oligonucleotide of the invention is a gapbreaker oligonucleotide. In some embodiments the gapbreaker oligonucleotide comprise a 5'-flank (F), a gap (G) and a 3'-flank (F'), wherein the gap is disrupted by a non-RNaseH recruiting nucleoside (a gap-breaker nucleoside, E) such that the gap contain at least 3 or 4 consecutive DNA nucleosides. In some embodiments the gapbreaker nucleoside (E) is an LNA nucleoside where the bridge between C2' and C4' of the ribose sugar ring of a nucleoside is in the beta conformation and is placed within the gap region such that the gap-breaker LNA nucleoside is flanked 5' and 3' by at least 3 (5') and 3 (3') or at least 3 (5') and 4 (3') or at least 4(5') and 3(3') DNA nucleosides, and wherein the oligonucleotide is capable of recruiting RNaseH.

The gapbreaker oligonucleotide can be represented by the following formulae:

F-G-E-G-F'; in particular F_{1-7}-G_{3-4}-E_1-G_{3-4}-F'_{1-7}

D'-F-G-F', in particular D'_{1-3}-F_{1-7}- G_{3-4}-E_1-G_{3-4}-F'_{1-7}

F-G-F'-D'', in particular F_{1-7}- G_{3-4}-E_1-G_{3-4}-F'_{1-7}-D''_{1-3}

D'-F-G-F'-D'', in particular D'_{1-3}-F_{1-7}- G_{3-4}-E_1-G_{3-4}-F'_{1-7}-D''_{1-3}

Where region D' and D" are as described in the section "Gapmer design".

In some embodiments the gapbreaker nucleoside (E) is a beta-D-oxy LNA or ScET or another beta-LNA nucleosides shown in Scheme 1).

Conjugate

The term conjugate as used herein refers to an oligonucleotide which is covalently linked to a non-nucleotide moiety (conjugate moiety or region C or third region).

Conjugation of the oligonucleotide of the invention to one or more non-nucleotide moieties may improve the pharmacology of the oligonucleotide, *e.g.* by affecting the activity, cellular

distribution, cellular uptake or stability of the oligonucleotide. In some embodiments the conjugate moiety modify or enhance the pharmacokinetic properties of the oligonucleotide by improving cellular distribution, bioavailability, metabolism, excretion, permeability, and/or cellular uptake of the oligonucleotide. In particular the conjugate may target the oligonucleotide to a specific organ, tissue or cell type and thereby enhance the effectiveness of the oligonucleotide in that organ, tissue or cell type. A the same time the conjugate may serve to reduce activity of the oligonucleotide in non-target cell types, tissues or organs, e.g. off target activity or activity in non-target cell types, tissues or organs. WO 93/07883 and WO201 3/033230 provides suitable conjugate moieties, which are hereby incorporated by reference. Further suitable conjugate moieties are those capable of binding to the asialoglycoprotein receptor (ASGPr). In particular tri-valent N-acetylgalactosamine conjugate moieties are suitable for binding to the the ASGPr, see for example WO 2014/076196, WO 2014/207232 and WO 2014/179620 (hereby incorporated by reference).

Oligonucleotide conjugates and their synthesis has also been reported in comprehensive reviews by Manoharan in Antisense Drug Technology, Principles, Strategies, and Applications, S.T. Crooke, ed., Ch. 16, Marcel Dekker, Inc., 2001 and Manoharan, Antisense and Nucleic Acid Drug Development, 2002, 12, 103, each of which is incorporated herein by reference in its entirety.

In an embodiment, the non-nucleotide moiety (conjugate moiety) is selected from the group consisting of carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins (e.g. bacterial toxins), vitamins, viral proteins (e.g. capsids) or combinations thereof.

Linkers

A linkage or linker is a connection between two atoms that links one chemical group or segment of interest to another chemical group or segment of interest via one or more covalent bonds. Conjugate moieties can be attached to the oligonucleotide directly or through a linking moiety (e.g. linker or tether). Linkers serve to covalently connect a third region, e.g. a conjugate moiety (Region C), to a first region, e.g. an oligonucleotide or contiguous nucleotide sequence complementary to the target nucleic acid (region A).

In some embodiments of the invention the conjugate or oligonucleotide conjugate of the invention may optionally, comprise a linker region (second region or region B and/or region Y) which is positioned between the oligonucleotide or contiguous nucleotide sequence complementary to the target nucleic acid (region A or first region) and the conjugate moiety (region C or third region).

Region B refers to biocleavable linkers comprising or consisting of a physiologically labile bond that is cleavable under conditions normally encountered or analogous to those encountered

within a mammalian body. Conditions under which physiologically labile linkers undergo chemical transformation (e.g., cleavage) include chemical conditions such as pH, temperature, oxidative or reductive conditions or agents, and salt concentration found in or analogous to those encountered in mammalian cells. Mammalian intracellular conditions also include the presence of enzymatic activity normally present in a mammalian cell such as from proteolytic enzymes or hydrolytic enzymes or nucleases. In one embodiment the biocleavable linker is susceptible to S1 nuclease cleavage. In some embodiments the nuclease susceptible linker comprises between 1 and 10 nucleosides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleosides, more preferably between 2 and 6 nucleosides and most preferably between 2 and 4 linked nucleosides comprising at least two consecutive phosphodiester linkages, such as at least 3 or 4 or 5 consecutive phosphodiester linkages. Preferably the nucleosides are DNA or RNA. Phosphodiester containing biocleavable linkers are described in more detail in WO 2014/076195 (hereby incorporated by reference).

Region Y refers to linkers that are not necessarily biocleavable but primarily serve to covalently connect a conjugate moiety (region C or third region), to an oligonucleotide (region A or first region). The region Y linkers may comprise a chain structure or an oligomer of repeating units such as ethylene glycol, amino acid units or amino alkyl groups The oligonucleotide conjugates of the present invention can be constructed of the following regional elements A-C, A-B-C, A-B-Y-C, A-Y-B-C or A-Y-C. In some embodiments the linker (region Y) is an amino alkyl, such as a C2 - C36 amino alkyl group, including, for example C6 to C12 amino alkyl groups. In some embodiments the linker (region Y) is a C6 amino alkyl group.

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. It will therefore be recognized that treatment as referred to herein may, in some embodiments, be prophylactic.

DETAILED DESCRIPTION OF THE INVENTION

The Oligonucleotides of the Invention

The invention relates to oligonucleotides capable of inhibiting the expression of c-Rel. The modulation is may achieved by hybridizing to a target nucleic acid encoding REL or which is involved in the regulation of REL. The target nucleic acid may be a mammalian REL sequence, such as SEQ ID NO 21.

The oligonucleotide of the invention is an antisense oligonucleotide which targets a REL sequence.

In some embodiments the antisense oligonucleotide 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% compared to the normal expression level of the target, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% inhibition compared to the normal expression level of the target. In some embodiments oligonucleotides of the invention may be capable of inhibiting expression levels of REL mRNA by at least 60% or 70% *in vitro* using HEK-293 or HeLa cells. In some embodiments compounds of the invention may be capable of inhibiting expression levels of Rel protein by at least 50% *in vitro* using HEK-293 or HeLa cells. Suitably, the examples provide assays which may be used to measure reduction of REL RNA and subsequently the protein. The target modulation is triggered by the hybridization between a contiguous nucleotide sequence of the oligonucleotide and the target nucleic acid. In some embodiments the oligonucleotide 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 modulation of c-Rel expression. Reduced binding affinity resulting from mismatches may advantageously be compensated by increased number of nucleotides in the oligonucleotide and/or an increased number of modified nucleosides capable of increasing the binding affinity to the target, such as *2'* modified nucleosides, including LNA, present within the oligonucleotide sequence.

An aspect of the present invention relates to an antisense oligonucleotide which consists or comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity to a human REL sequence.

In some embodiments, the oligonucleotide comprises a contiguous sequence 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.

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

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 10 to 15 or 10 to 16 nucleotides in length with at least 90% complementary, such as fully (or 100%) complementary, to a region of a sequence selected from SEQ ID NOs 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22 and 23.

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 12 to 16 nucleotides in length which is fully (or 100%) complementary, to a region of a sequence selected from SEQ ID NOs 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22 and 23.

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 14 nucleotides in length which is fully (or 100%) complementary, to a region of a sequence selected from SEQ ID NOs 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22 and 23.

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 15 nucleotides in length which is fully (or 100%) complementary, to a region of a sequence selected from SEQ ID NOs 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22 and 23.

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 16 nucleotides in length which is fully (or 100%) complementary, to a region of a sequence selected from SEQ ID NOs 11, 12, 13, 14, 15, 17, 18, 19, 20, 22 and 23.

In some embodiments, the oligonucleotide of the invention comprises or consists of 10 to 35 nucleotides in length, such as from 10 to 30, such as 11 to 22, such as from 12 to 18, such as from 13 to 17 or 14 to 16 contiguous nucleotides in length. In some embodiments the oligonucleotide comprises or consists of 13, 14, 15, 16 or 17 nucleotides in length.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence thereof comprises or consists of 22 or less nucleotides, such as 20 or less nucleotides, such as 18 or less nucleotides, such as 14, 15, 16 or 17 nucleotides. It is to be understood that any range given herein includes the range endpoints. Accordingly, if an oligonucleotide is said to include from 10 to 30 nucleotides, both 10 and 30 nucleotides are included.

In some embodiments, the contiguous nucleotide sequence comprises or consists of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides in length. In some embodiments, the oligonucleotide comprises or consists of 14, 15 or 16 nucleotides in length.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or at least 12 contiguous nucleotides thereof.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or at least 13 contiguous nucleotides thereof.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or at least 14 contiguous nucleotides thereof.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or at least 15 contiguous nucleotides thereof.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, or at least 16 contiguous nucleotides thereof.

Oligonucleotide design

Oligonucleotide design refers to the pattern of nucleoside sugar modifications in the oligonucleotide sequence. The oligonucleotides of the invention comprise sugar-modified nucleosides and may also comprise DNA or RNA nucleosides. In some embodiments, the oligonucleotide comprises sugar-modified nucleosides and DNA nucleosides. Incorporation of modified nucleosides into the oligonucleotide of the invention may enhance the affinity of the oligonucleotide for the target nucleic acid. In that case, the modified nucleosides can be referred to as affinity enhancing modified nucleotides, the modified nucleosides may also be termed units.

In an embodiment, the oligonucleotide comprises at least 1 modified nucleoside, such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 modified nucleosides. In an embodiment the oligonucleotide comprises from 1 to 10 modified nucleosides, such as from 2 to 9 modified nucleosides, such as from 3 to 8 modified nucleosides, such as from 4 to 7 modified nucleosides, such as 6 or 7 modified nucleosides.

In an embodiment, the oligonucleotide comprises one or more sugar modified nucleosides, such as 2' sugar modified nucleosides. Preferably the oligonucleotide of the invention comprise the 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. Even more preferably the one or more modified nucleoside is a locked nucleic acid (LNA).

In a further embodiment the oligonucleotide comprises at least one modified internucleoside linkage. In some embodiments all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate or boranophosphate internucleoside linkages. In some embodiments all the internucleotide linkages in the contiguous sequence of the oligonucleotide are phosphorothioate linkages.

In some embodiments, the oligonucleotide of the invention comprises at least one LNA nucleoside, such as 1, 2, 3, 4, 5, 6, 7, or 8 LNA nucleosides, such as from 2 to 6 LNA nucleosides, such as from 3 to 7 LNA nucleosides, 4 to 8 LNA nucleosides or 3, 4, 5, 6, 7 or 8 LNA nucleosides. In some embodiments, at least 75% of the modified nucleosides in the oligonucleotide are LNA nucleosides, such as 80%, such as 85%, such as 90% of the modified nucleosides are LNA nucleosides. In a still further embodiment all the modified nucleosides in the oligonucleotide are LNA nucleosides. In a further embodiment, the oligonucleotide may

comprise both beta-D-oxy-LNA, and one or more of the following LNA nucleosides: thio-LNA, amino-LNA, oxy-LNA, and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In a further embodiment, all LNA cytosine units are 5-methyl-cytosine. In some embodiments the oligonucleotide or contiguous nucleotide sequence has at least 1 LNA nucleoside at the 5' end and at least 2 LNA nucleosides at the 3' end of the nucleotide sequence.

In some embodiments, the oligonucleotide of the invention comprises at least one modified nucleoside which is a 2'-MOE-RNA nucleoside, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-MOE-RNA nucleosides. In some embodiments, at least one of said modified nucleoside is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-fluoro-DNA nucleosides.

In some embodiments, the oligonucleotide of the invention comprises at least one LNA nucleoside and at least one 2' substituted modified nucleoside.

In some embodiments of the invention, the oligonucleotide comprise both 2' sugar modified nucleosides and DNA units. Preferably the oligonucleotide comprises both LNA and DNA nucleosides (units). Preferably, the combined total of LNA and DNA units is 8-30, such as 10 - 25, preferably 12-22, such as 12 - 18, even more preferably 11-16. In some embodiments of the invention, the nucleotide sequence of the oligonucleotide, such as the contiguous nucleotide sequence consists of at least one or two LNA nucleosides and the remaining nucleosides are DNA units. In some embodiments the oligonucleotide comprises only LNA nucleosides and naturally occurring nucleosides (such as RNA or DNA, most preferably DNA nucleosides), optionally with modified internucleoside linkages such as phosphorothioate.

In an embodiment of the invention the oligonucleotide of the invention is capable of recruiting RNase H.

The structural design of the oligonucleotide of the invention may be selected from gapmers, gapbreakers, headmers and tailmers. In some embodiments the oligonucleotide of the invention is a gapmer.

Gapmer design

In some embodiments the oligonucleotide of the invention has a gapmer design or structure also referred herein merely as "Gapmer". In a gapmer structure the oligonucleotide comprises at least three distinct structural regions a 5'-flank, a gap and a 3'-flank, F-G-F' in '5 -> 3' orientation. In this design, flanking regions F and F' (also termed wing regions) comprise a contiguous stretch of modified nucleosides, which are complementary to the REL target nucleic acid, while the gap region, G, comprises a contiguous stretch of nucleotides which are capable of recruiting a nuclease, preferably an endonuclease such as RNase, for example RNase H, when the oligonucleotide is in duplex with the target nucleic acid. Nucleosides which are

capable of recruiting a nuclease, in particular RNase H, can be selected from the group consisting of DNA, alpha-L-oxy-LNA, 2'-Flouro-ANA and UNA. Regions F and F', flanking the 5' and 3' ends of region G, preferably comprise non-nuclease recruiting nucleosides (nucleosides with a 3' endo structure), more preferably one or more affinity enhancing modified nucleosides. In some embodiments, the 3' flank comprises at least one LNA nucleoside, preferably at least 2 LNA nucleosides. In some embodiments, the 5' flank comprises at least one LNA nucleoside. In some embodiments both the 5' and 3' flanking regions comprise a LNA nucleoside. In some embodiments all the nucleosides in the flanking regions are LNA nucleosides. In other embodiments, the flanking regions may comprise both LNA nucleosides and other nucleosides (mixed flanks), such as DNA nucleosides and/or non-LNA modified nucleosides, such as 2' substituted nucleosides. In this case the gap is defined as a contiguous sequence of at least 5 RNase H recruiting nucleosides (nucleosides with a 2' endo structure, preferably DNA) flanked at the 5' and 3' end by an affinity enhancing modified nucleoside, preferably LNA, such as beta-D-oxy-LNA. Consequently, the nucleosides of the 5' flanking region and the 3' flanking region which are adjacent to the gap region are modified nucleosides, preferably non-nuclease recruiting nucleosides.

Region F

Region F (5' flank or 5' wing) attached to the '5 end of region G comprises, contains or consists of at least one modified nucleoside such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 modified nucleosides. In an embodiment region F comprises or consists of from 1 to 7 modified nucleosides, such as from 2 to 6 modified nucleosides, such as from 2 to 5 modified nucleosides, such as from 2 to 4 modified nucleosides, such as from 1 to 3 modified nucleosides, such as 1, 2, 3 or 4 modified nucleosides. The F region is defined by having at least on modified nucleoside at the 5' end and at the 3' end of the region.

In some embodiments, the modified nucleosides in region F have a 3' endo structure.

In an embodiment, one or more of the modified nucleosides in region F are 2' modified nucleosides. In one embodiment all the nucleosides in Region F are 2' modified nucleosides.

In another embodiment region F comprises DNA and/or RNA in addition to the 2' modified nucleosides. Flanks comprising DNA and/or RNA are characterized by having a *2'* modified nucleoside in the 5' end and the 3'end (adjacent to the G region) of the F region. In one embodiment the region F comprise DNA nucleosides, such as from 1 to 3 contiguous DNA nucleosides, such as 1 to 3 or 1 to 2 contiguous DNA nucleosides. The DNA nucleosides in the flanks should preferably not be able to recruit RNase H. In some embodiments the 2' modified nucleosides and DNA and/or RNA nucleosides in the F region alternate with 1 to 3 2' modified nucleosides and 1 to 3 DNA and/or RNA nucleosides. Such flanks can also be termed alternating flanks. The length of the 5' flank (region F) in oligonucleotides with alternating flanks

may be 4 to 10 nucleosides, such as 4 to 8, such as 4 to 6 nucleosides, such as 4, 5, 6 or 7 modified nucleosides. In some embodiments only the 5' flank of the oligonucleotide is alternating. Specific examples of region F with alternating nucleosides are

$2'_{1-3}$-N'_{1-4}-$2'_{1-3}$

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$- N'_{1-2}-$2'_{1-2}$

Where 2' indicates a modified nucleoside and NT is a RNA or DNA. In some embodiments all the modified nucleosides in the alternating flanks are LNA and the N' is DNA. In a further embodiment one or more of the 2' modified nucleosides in region F are 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 the F region comprises both LNA and a 2' substituted modified nucleoside. These are often termed mixed wing or mixed flank oligonucleotides.

In one embodiment of the invention all the modified nucleosides in region F are LNA nucleosides. In a further embodiment all the nucleosides in Region F are LNA nucleosides. In a further embodiment the LNA nucleosides in region F are independently selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA, cET, and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments region F comprise at least 1 beta-D-oxy LNA unit, at the 5' end of the contiguous sequence.

Region G

Region G (gap region) preferably comprise, contain or consist of at least 4, such as at least 5, such as at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 consecutive nucleosides capable of recruiting the aforementioned nuclease, in particular RNaseH. In a further embodiment region G comprise, contain or consist of from 5 to 12, or from 6 to 10 or from 7 to 9, such as 8 consecutive nucleotide units capable of recruiting aforementioned nuclease.

The nucleoside units in region G, which are capable of recruiting nuclease are in an embodiment selected from the group consisting of DNA, alpha-L-LNA, C4' alkylated DNA (as described in PCT/EP2009/050349 and Vester *et al.,* Bioorg. Med. Chem. Lett. 18 (2008) 2296 - 2300, both incorporated herein by reference), arabinose derived nucleosides like ANA and 2'F-ANA (Mangos et al. 2003 J. AM. CHEM. SOC. 125, 654-661), UNA (unlocked nucleic acid) (as described in Fluiter *et al.,* Mol. Biosyst., 2009, 10, 1039 incorporated herein by reference). UNA is unlocked nucleic acid, typically where the bond between C2 and C3 of the ribose has been removed, forming an unlocked "sugar" residue.

In a still further embodiment at least one nucleoside unit in region G is a DNA nucleoside unit, such as from 1 to 12 DNA units, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 DNA units, preferably

from 2 to 12 DNA units, such as from 4 to 12 DNA units, more preferably from 5 to 11, or from 2 to 10, 4 to 10 or 6 to 10 DNA units, such as from 7 to 10 DNA units, such as 8, 9 or 10 DNA units. In some embodiments, region G consists of 100% DNA units. In some embodiment G consists of 8 - 12 DNA units.

In further embodiments the region G may consist of a mixture of DNA and other nucleosides capable of mediating RNase H cleavage. Region G may consist of at least 50% DNA, more preferably 60 %, 70% or 80 % DNA, and even more preferred 90% or 95% DNA.

In a still further embodiment at least one nucleoside unit in region G is an alpha-L-LNA nucleoside unit, such as at least one alpha-L-LNA, such as 2, 3, 4, 5, 6, 7, 8 or 9 alpha-L-LNA. In a further embodiment, region G comprises the least one alpha-L-LNA is alpha-L-oxy-LNA. In a further embodiment region G comprises a combination of DNA and alpha-L-LNA nucleoside units.

In some embodiments the size of the contiguous sequence in region G may be longer, such as 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleoside units.

In some embodiments, nucleosides in region G have a 2' endo structure.

In some embodiments region G may comprise a gapbreaker nucleoside, leading to a gapbreaker oligonucleotide, which is capable of recruiting RNase H.

Region F

Region F' (3' flank or 3' wing) attached to the '3 end of region G comprises, contains or consists of at least one modified nucleoside such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 modified nucleosides. In an embodiment region F' comprise or consist of from 1 to 7 modified nucleosides, such as from 2 to 6 modified nucleside, such as from 2 to 4 modified nucleosides, such as from 1 to 3 modified nucleosides, such as 1, 2, 3 or 4 modified nucleosides. The F' region is defined by having at least on modified nucleoside at the 5' end and at the 3' end of the region.

In some embodiments, the modified nucleosides in region F' have a 3' endo structure.

In an embodiment, one or more of the modified nucleosides in region F' are 2' modified nucleosides. In one embodiment all the nucleosides in Region F' are 2' modified nucleosides.

In an embodiment, one or more of the modified nucleosides in region F' are 2' modified nucleosides.

In one embodiment all the nucleosides in Region F' are 2' modified nucleosides. In another embodiment region F' comprises DNA or RNA in addition to the 2' modified nucleosides. Flanks comprising DNA or RNA are characterized by having a 2' modified nucleoside in the 5' end (adjacent to the G region) and the 3'end of the F' region. In one embodiment the region F'

comprises DNA nucleosides, such as from 1 to 4 contiguous DNA nucleosides, such as 1 to 3 or 1 to 2 contiguous DNA nucleosides. The DNA nucleosides in the flanks should preferably not be able to recruit RNase H. In some embodiments the 2' modified nucleosides and DNA and/or RNA nucleosides in the F' region alternate with 1 to 3 2' modified nucleosides and 1 to 3 DNA and/or RNA nucleosides, such flanks can also be termed alternating flanks. The length of the 3' flank (region F') in oligonucleotides with alternating flanks may be 4 to 10 nucleosides, such as 4 to 8, such as 4 to 6 nucleosides, such as 4, 5, 6 or 7 modified nucleosides. In some embodiments only the 3' flank of the oligonucleotide is alternating. Specific examples of region F' with alternating nucleosides are

$2'_{1-2}$-N'_{1-4}-$2'_{1-4}$

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$- N'_{1-2}-$2'_{1-2}$

Where 2' indicates a modified nucleoside and N' is a RNA or DNA. In some embodiments all the modified nucleosides in the alternating flanks are LNA and the N' is DNA. In a further embodiment modified nucleosides in region F' are 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 the F' region comprises both LNA and a 2' substituted modified nucleoside. These are often termed mixed wing or mixed flank oligonucleotides.

In one embodiment of the invention all the modified nucleosides in region F' are LNA nucleosides. In a further embodiment all the nucleosides in Region F' are LNA nucleosides. In a further embodiment the LNA nucleosides in region F' are independently selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA, cET and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments region F' has at least 2 beta-D-oxy LNA unit, at the 3' end of the contiguous sequence.

Region D' and D"

Region D' and D" can be attached to the 5' end of region F or the 3' end of region F', respectively.

Region D' or D" may independently comprise 1, 2, 3, 4 or 5 additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. In this respect the oligonucleotide of the invention, may in some embodiments comprise a contiguous nucleotide sequence capable of modulating the target which is flanked at the 5' and/or 3' end by additional nucleotides. Such additional nucleotides 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 may be DNA or RNA. In another embodiment, the additional 5' and/or 3' end nucleotides are modified nucleotides which may for

example be included to enhance nuclease stability or for ease of synthesis. In an embodiment of the oligonucleotide, the invention comprises a region D' and/or D" in addition to the contiguous nucleotide sequence.

In some embodiments the oligonucleotide of the invention may consist of the contiguous nucleotide sequence and region D' and/or D", and a conjugation group covalently attached to region D' or D".

The gapmer oligonucleotide of the present invention can be represented by the following formulae:

F-G-F'; in particular F_{1-7}-G_{4-12}-F'_{1-7}

D'-F-G-F', in particular D'_{1-3}-F_{1-7}-G_{4-12}-F'_{1-7}

F-G-F'-D'', in particular F_{1-7}-G_{4-12}-F'_{1-7}-D''_{1-3}

D'-F-G-F'-D'', in particular D'_{1-3}-F_{1-7}-G_{4-12}-F'_{1-7}-D''_{1-3}

The preferred number and types of nucleosides in regions F, G and F', D' and D" have been described above.

The oligonucleotide conjugates of the present invention have a region C covalently attached to either the 5' or 3' end of the oligonucleotide, in particular the gapmer oligonucleotides presented above.

In one embodiment the oligonucleotide conjugate of the invention comprises a oligonucleotide with the formula 5'-D'-F-G-F'-3' or 5'-F-G-F'-D"-3', where region F and F' independently comprise 1 - 7 modified nucleosides, G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH and region D' or D" comprise 1 - 5 phosphodiester linked nucleosides. Preferably region D' or D" is present in the end of the oligonucleotide where conjugation to a conjugate moiety is contemplated.

Examples of oligonucleotides with alternating flanks can be represented by the following formulae:

$2'_{1-3}$-N'_{1-4}-$2'_{1-3}$-G_{6-12}-$2'_{1-2}$-N'_{1-4}-$2'_{1-4}$

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-G_{6-12}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$- N'_{1-2}-$2'_{1-2}$

F-G_{6-12}-$2'_{1-2}$-N'_{1-4}-$2'_{1-4}$

F-G_{6-12}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$

$2'_{1-3}$-N'_{1-4}-$2'_{1-3}$-G_{6-12}-F'

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-N_{1-2}-$2'_{1-2}$-G_{6-12}-F'

Where a flank is indicated by F or F' it only contains 2' modified nucleosides, such as LNA nucleosides. The preferred number and types of nucleosides in the alternating regions, and region F, G and F', D' and D" have been described above.

In some embodiments the oligonucleotide is a gapmer consisting of 10, 11, 12, 13, 14, 15 or 16 nucleotides in length, wherein each of regions F and F' independently consists of 1, 2, 3 or 4 modified nucleoside units complementary to the REL target nucleic acid and region G consists of 7, 8, 9, or 10 nucleoside units, capable of recruiting nuclease when in duplex with the REL target nucleic acid.

In a further embodiments, the oligonucleotide is a gapmer wherein each of regions F and F' independently consists of 3, 4, 5 or 6 modified nucleoside units, such as nucleoside units containing a 2'-O-methoxyethyl-ribose sugar (2'-MOE) or nucleoside units containing a 2'-fluoro-deoxyribose sugar and/or LNA units, and region G consists of 8, 9, 10, 11 or 12 nucleoside units, such as DNA units or other nuclease recruiting nucleosides such as alpha-L-LNA or a mixture of DNA and nuclease recruiting nucleosides.

In a further specific embodiment, the oligonucleotide is a gapmer wherein each of regions F and F' region consists of two LNA units each, and region G consists of 8, 9 or 10 nucleoside units, preferably DNA units. Specific gapmer designs of this nature include 2-8-2, 2-9-2 and 2-10-2.

In a further specific embodiment, the oligonucleotide is a gapmer wherein each of regions F and F' independently consists of three LNA units, and region G consists of 8, 9 or 10 nucleoside units, preferably DNA units. Specific gapmer designs of this nature include 3-8-3, 3-9-3 and 3-10-3.

In a further specific embodiment, the oligonucleotide is a gapmer wherein each of regions F and F' consists of four LNA units each, and region G consists of 8 or 9 or 10 nucleoside units, preferably DNA units. Specific gapmer designs of this nature include 4-8-4, 4-9-4 and 4-10-4

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 6 nucleosides and independently 1 to 4 modified nucleosides in the wings including 1-6-1 , 1-6-2, 2-6-1 , 1-6-3, 3-6-1 , 1-6-4, 4-6-1 , 2-6-2, 2-6-3, 3-6-2 2-6-4, 4-6-2, 3-6-3, 3-6-4 and 4-6-3 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 7 nucleosides and independently 1 to 4 modified nucleosides in the wings including 1-7-1 , 2-7-1 , 1-7-2, 1-7-3, 3-7-1 , 1-7-4, 4-7-1 , 2-7-2, 2-7-3, 3-7-2, 2-7-4, 4-7-2, 3-7-3, 3-7-4, 4-7-3 and 4-7-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 8 nucleosides and independently 1 to 4 modified nucleosides in the wings

including including 1-8-1 , 1-8-2, 1-8-3, 3-8-1 , 1-8-4, 4-8-1 ,2-8-1 , 2-8-2, 2-8-3, 3-8-2, 2-8-4, , 4-8-2 , 3-8-3, 3-8-4, 4-8-3, and 4-8-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 9 nucleosides and independently 1 to 4 modified nucleosides in the wings including, 1-9-1 , 2-9-1 , 1-9-2, 1-9-3, 3-9-1 , 1-9-4, 4-9-1 , 2-9-2, 2-9-3, 3-9-2, 2-9-4, 4-9-2, 3-9-3, 3-9-4, 4-9-3 and 4-9-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 10 nucleosides including, 1-10-1 , 2-10-1 , 1-10-2, 1-10-3, 3-10-1 , 1-10-4, 4-10-1 , 2-10-2, 2-10-3, 3-10-2, 2-10-4, 4-10-2, 3-10-3, 3-10-4, 4-10-3 and 4-10-4 gapmers.

In some embodiments the F-G-F' design is selected from 3-1 1-2, 2-10-3, 4-9-2, 2-10-4, 4-10-2, 3-10-3, 4-10-2, 3-9-3, 4-9-2, and 3-10-3.

In some embodiments, the F-G-F' design may, optionally, further include region D' and/or D", which may have 1, 2 or 3 nucleoside units, such as DNA units. In some embodiments, the nucleosides in region F and F' are modified nucleosides, while nucleotides in region G are preferably unmodified nucleosides, such as DNA nucleosides.

In each design, in some embodiments the modified nucleoside is LNA.

In another embodiment all the internucleoside linkages in the gap in a gapmer are phosphorothioate and/or boranophosphate linkages. In another embodiment all the internucleoside linkages in the flanks (F and F' region) in a gapmer are phosphorothioate and/or boranophosphate linkages. In another preferred embodiment all the internucleoside linkages in the D' and D" region in a gapmer are phosphodiester linkages.

For specific gapmers as disclosed herein, when the cytosine (C) residues are annotated as 5-methyl-cytosine, in various embodiments, one or more of the Cs present in the oligonucleotide may be unmodified C residues.

In a particular embodiment, the gapmer is a so-called shortmer as described in WO2008/1 13832 incorporated herein by reference.

Further gapmer designs are disclosed in WO2004/046160, WO2007/14651 1 and incorporated by reference.

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 1.1 ; 2.1 ; 3,1 ; 4.1 ; 5,1 ; 6,1 ; 7,1 ; 8,1 ; 9,1 ; and 10.1 .

Method of manufacture

In a further aspect, the invention provides methods for manufacturing the oligonucleotides of the invention comprising reacting nucleotide units and thereby forming covalently linked contiguous nucleotide units comprised in the oligonucleotide. Preferably, the method uses phophoramidite

chemistry (see for example Caruthers et al, 1987, Methods in Enzymology vol. 154, pages 287-313). In a further embodiment the method further comprises reacting the contiguous nucleotide sequence with a conjugating moiety (ligand). In a further aspect a method is provided for manufacturing the composition of the invention, comprising mixing the oligonucleotide or conjugated oligonucleotide of the invention with a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

Pharmaceutical Composition

In a further aspect, the invention provides pharmaceutical compositions comprising any of the aforementioned oligonucleotides and/or oligonucleotide conjugates or salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS) and 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 oligonucleotide is used in the pharmaceutically acceptable diluent at a concentration of 50 - 300 μM solution. The invention provides a sodium or potassium salt of the oligonucleotide or conjugate of the invention.

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 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 are also provided in WO2007/031091 .

Oligonucleotides or oligonucleotide conjugates 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.

In some embodiments, the oligonucleotide or oligonucleotide conjugate of the invention is a prodrug. In particular with respect to oligonucleotide conjugates the conjugate moiety is cleaved of the oligonucleotide once the prodrug is delivered to the site of action, e.g. the target cell.

Applications

The oligonucleotides of the invention may be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

In research, such oligonucleotides may be used to specifically modulate the synthesis of c-Rel protein in cells (e.g. *in vitro* cell cultures) and experimental animals 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 producing the protein, thereby prevent protein formation or by degrading or inhibiting a modulator of the gene or mRNA producing the protein.

If employing the oligonucleotide 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.

The present invention provides an *in vivo* or *in vitro* method for modulating REL expression in a target cell which is expressing c-Rel, said method comprising administering an oligonucleotide 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 diagnostics the oligonucleotides may be used to detect and quantitate REL expression in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics, an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the expression of c-Rel, such as cancer, inflammation or an inflammatory disease, or an autoimmune disease.

The invention provides methods for treating or preventing a disease, comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide, an oligonucleotide conjugate or a pharmaceutical composition of the invention to a subject suffering from or susceptible to the disease.

The invention also relates to an oligonucleotide, a composition or a conjugate as defined herein for use as a medicament.

The oligonucleotide, oligonucleotide conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

The invention also provides for the use of the oligonucleotide or oligonucleotide conjugate of the invention as described for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of as a disorder as referred to herein.

The disease or disorder, as referred to herein, is associated with expression of REL. In some embodiments disease or disorder may be associated with a mutation in the REL gene or a gene whose protein product is associated with or interacts with REL. Therefore, in some embodiments, the target nucleic acid is a mutated form of the REL sequence and in other embodiments, the target nucleic acid is a regulator of the REL sequence.

The methods of the invention are preferably employed for treatment or prophylaxis against diseases caused by abnormal levels and/or activity of c-Rel.

The invention further relates to use of an oligonucleotide, oligonucleotide conjugate or a pharmaceutical composition as defined herein for the manufacture of a medicament for the treatment of abnormal levels and/or activity of c-Rel.

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of diseases or disorders selected from the group consisting of cancer, inflammation and inflammatory disorders, and autoimmune diseases.

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of diseases or disorders selected from the group consisting of atherosclerosis, multiple sclerosis, Crohn's disease, inflammatory bowel disease, and rheumatoid arthritis.

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of cancer, such as hematopoietic cancer, such as lymphoma or leukemia, or lung cancer or breast cancer

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the reducing inflammation in a patient who is in need to reduced inflammation.

In some embodiments, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the reducing cytokine levels in a patient who is in need to reduced cytokines.

Administration

The oligonucleotides or pharmaceutical compositions of the present invention may be administered by any suitable means, such as via parenteral administration (such as, intravenous, subcutaneous, or intra-muscular.

In some embodiments the active oligonucleotide or oligonucleotide conjugate is administered intravenously. In another embodiment the active oligonucleotide or oligonucleotide conjugate is administered subcutaneously.

In some embodiments, the oligonucleotide, oligonucleotide conjugate or pharmaceutical composition of the invention is administered at a dose of 0.1 - 15 mg/kg, such as from 0.2 - 10 mg/kg, such as from 0.25 - 5 mg/kg. The administration can be once a week, every 2nd week, every third week or even once a month.

The invention also provides for the use of the oligonucleotide or oligonucleotide conjugate of the invention as described for the manufacture of a medicament wherein the medicament is in a dosage form for subcutaneous administration.

Combination therapies

In some embodiments the oligonucleotide, oligonucleotide conjugate or pharmaceutical composition of the invention is for use in a combination treatment with another therapeutic agent.

EMBODIMENTS

1. An LNA antisense oligonucleotide targeting human REL, 10 to 30 contiguous nucleotides in length, wherein the contiguous sequence of the oligonucleotide comprises at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NO 3, 4, 1, 2, 5, 6, 7, 8, 9 and 10.
2. The oligonucleotide according to embodiment 1, where the oligonucleotide comprises at least one modified internucleoside linkage.
3. The oligonucleotide of embodiment 2, wherein the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.
4. The oligonucleotide of any one of embodiments 1 - 3, wherein the oligonucleotide is capable of recruiting RNase H.
5. The oligonucleotide of embodiment 4, wherein the oligonucleotide is a gapmer.
6. The oligonucleotide of embodiment 4 or 5, wherein the oligonucleotide is a gapmer of formula 5'-F-G-F'-3\ where region F and F' independently comprise 1 - 7 modified

nucleosides and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH.

7. The oligonucleotide according to any one of embodiments 1 - 6, wherein said oligonucleotide consists or comprises of an oligonucleotide selected from the group consisting of: CGTCagattaggaaCC (SEQ ID NO 3), AGtattggaattgGCG (SEQ ID NO 4), GCAgaaacaactagGA (SEQ ID NO 1), CACAtcgaataccCA (SEQ ID NO 2), ACAcatcgaatacCC (SEQ ID NO 5), CTAtttcgtaggcTG (SEQ ID NO 6), ACAcatcgaataccCA (SEQ ID NO 7), CAGgaaattgtaggGA (SEQ ID NO 8), TAGtattggaattgGC (SEQ ID NO 9), and TTAagtttctacgGCA (SEQ ID NO 10), wherein capital letters represent LNA nucleosides and lower case letters represent DNA nucleosides, and cytosines are optionally 5-methyl cytosine.

8. The oligonucleotide according to embodiment 7, wherein all LNA nucleotides are beta-D-oxy LNA.

9. The oligonucleotide according to embodiments 7 or 8, wherein all LNA cytosines are 5-methyl cytosine.

10. The oligonucleotide according to any one of embodiments 7 - 9, wherein all internucleoside linkages present in the indicated sequence are phosphorothioate internucleoside linkages.

11. The oligonucleotide according to any one of embodiments 1 - 10, wherein the compound is selected from the group consisting of, CGTCagattaggaaCC (SEQ ID NO 3), AGtattggaattgGCG (SEQ ID NO 4), GCAgaaacaactagGA (SEQ ID NO 1), CACAfcgaataccCA (SEQ ID NO 2), ACAcafcgaatacCC (SEQ ID NO 5), CTAttfcgtaggcTG (SEQ ID NO 6), ACAcat^{m}cgaataccCA (SEQ ID NO 7), CAGgaaattgtaggGA (SEQ ID NO 8), TAGtattggaattgGC (SEQ ID NO 9), and TTAagtttcta^{m}cgGCA (SEQ ID NO 10), wherein capital letters represent beta-D-oxy LNA nucleosides, all LNA cytosines are 5-methyl cytosine, lower case letters are DNA nucleosides, ^{m}c indicates a 5-methyl cytosinse DNA nucleoside, and all internucleoside linkages are phosphorothioate internucleoside linkages.

12. A conjugate comprising the oligonucleotide according to any one of embodiments 1 - 11, and at least one conjugate moiety covalently attached to said oligonucleotide.

13. A pharmaceutical composition comprising the oligonucleotide of embodiment 1 - 11 or the conjugate of embodiment 12 and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

14. An *in vivo* or *in vitro* method for modulating REL expression in a target cell which is expressing REL said method comprising administering an oligonucleotide of any one of

embodiments 1 - 11, the conjugate according to embodiment 12, or the pharmaceutical composition of embodiment 13 in an effective amount to said cell.

15. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide of any one of embodiments 1 - 11 or the conjugate according to embodiment 12 or the pharmaceutical composition of embodiment 13 to a subject suffering from or susceptible to the disease.

16. The method of embodiment 14, wherein the disease is selected from the group consisting of cancer, inflammation and inflammatory disorders, and autoimmune diseases.

17. The method according to embodiment 15, wherein the disease is selected from the group consisting of multiple sclerosis, psoriasis, celiac disease, Crohn's disease and rheumatoid arthritis.

18. The method according to embodiment 15, wherein the disease is selected from the group consisting of lymphoma, leukemia, breast cancer and lung cancer.

19. The oligonucleotide of any one of embodiments 1 - 11 or the conjugate according to embodiment 12 or the pharmaceutical composition of embodiment 13 for use in medicine.

20. The oligonucleotide of any one of embodiments 1 - 11 or the conjugate according to embodiment 12 or the pharmaceutical composition of embodiment 13 for use in the treatment or prevention of cancer, inflammation and inflammatory disorders, and autoimmune diseases.

21. The use of the oligonucleotide of embodiment 1 - 11 or the conjugate according to embodiment 12 or the pharmaceutical composition of embodiment 13, for the preparation of a medicament for treatment or prevention of cancer, inflammation and inflammatory disorders, and autoimmune diseases.

22. The oligonucleotide or use according to any one of embodiments 1 - 20, wherein the oligonucleotide is for use in the treatment of a disease selected from the group consisting of multiple sclerosis, psoriasis, celiac disease, Crohn's disease and rheumatoid arthritis.

23. The oligonucleotide or use according to any one of embodiments 1 - 20, wherein the oligonucleotide is for use in the treatment of a disease selected from the group consisting of hematopoietic cancer, such as lymphoma or leukemia, lung cancer and breast cancer.

EXAMPLES

The work reported herein has received funding from the European Union Seventh Framework Programme [FP7-2007-2013] under grant agreement "HEALTH-F2-201 3-6021l 14" (Athero-B-Cell).

Materials and methods

Oligonucleotide synthesis

Oligonucleotide synthesis is generally known in the art. Below is a protocol which may be applied. The oligonucleotides of the present invention may have been produced by slightly varying methods in terms of apparatus, support and concentrations used.

Oligonucleotides are synthesized on uridine universal supports using the phosphoramidite approach on an Oligomaker 48 at 1 µmol scale. At the end of the synthesis, the oligonucleotides are cleaved from the solid support using aqueous ammonia for 5-16hours at 60°C. The oligonucleotides are purified by reverse phase HPLC (RP-HPLC) or by solid phase extractions and characterized by UPLC, and the molecular mass is further confirmed by ESI-MS.

Elongation of the oligonucleotide:

The coupling of β-cyanoethyl- phosphoramidites (DNA-A(Bz), DNA- G(ibu), DNA- C(Bz), DNA-T, LNA-5-methyl-C(Bz), LNA-A(Bz), LNA- G(dmf), or LNA-T) is performed by using a solution of 0.1 M of the 5'-O-DMT-protected amidite in acetonitrile and DCI (4,5-dicyanoimidazole) in acetonitrile (0.25 M) as activator. For the final cycle a phosphoramidite with desired modifications can be used, e.g. a C6 linker for attaching a conjugate group or a conjugate group as such. Thiolation for introduction of phosphorthioate linkages is carried out by using xanthane hydride (0.01 M in acetonitrile/pyridine 9:1). Phosphordiester linkages can be introduced using 0.02 M iodine in THF/Pyridine/water 7:2:1 . The rest of the reagents are the ones typically used for oligonucleotide synthesis.

For post solid phase synthesis conjugation a commercially available C6 aminolinker phorphoramidite can be used in the last cycle of the solid phase synthesis and after deprotection and cleavage from the solid support the aminolinked deprotected oligonucleotide is isolated. The conjugates are introduced via activation of the functional group using standard synthesis methods.

Purification by RP-HPLC:

The crude compounds are purified by preparative RP-HPLC on a Phenomenex Jupiter C18 10µ 150x10 mm column. 0.1 M ammonium acetate pH 8 and acetonitrile is used as buffers at a flow rate of 5 mL/min. The collected fractions are lyophilized to give the purified compound typically as a white solid.

Abbreviations:

DCI: 4,5-Dicyanoimidazole

DCM: Dichloromethane

DMF: Dimethylformamide

DMT: 4.4'-Dimethoxytrityl

THF: Tetrahydrofurane

Bz: Benzoyl

Ibu: Isobutyryl

RP-HPLC: Reverse phase high performance liquid chromatography

T_m Assay:

Oligonucleotide and RNA target (phosphate linked, PO) duplexes are diluted to 3 mM in 500 ml RNase-free water and mixed with 500 ml 2x T_m-buffer (200mM NaCl, 0.2mM EDTA, 20mM Naphosphate, pH 7.0). The solution is heated to 95°C for 3 min and then allowed to anneal in room temperature for 30 min. The duplex melting temperatures (T_m) is measured on a Lambda 40 UV/VIS Spectrophotometer equipped with a Peltier temperature programmer PTP6 using PE Templab software (Perkin Elmer). The temperature is ramped up from 20°C to 95°C and then down to 25°C, recording absorption at 260 nm. First derivative and the local maximums of both the melting and annealing are used to assess the duplex T_m.

Example 1: Testing *in vitro* potency and efficacy of selected oligonucleotides targeting mouse Nfkb-subunit mRNA in RAW264.7 cells in a dose response curve. RAW 264.7 cell line was purchased from ATCC and maintained as recommended by the supplier in a humidified incubator at 37°C with 5% C02. For assays, 2500 cells/well were seeded in a 96 multi well plate in culture media. Cells were incubated for 24 hours before addition of oligonucleotides dissolved in PBS. Concentration of oligonucleotides: from 50 µM, 1:1 dilution in eight steps. Three days after addition of oligonucleotides, the cells were harvested. RNA was extracted using the PureLink Pro 96 RNA Purification kit (Thermo Fisher Scientific) according to the manufacturer's instructions and eluated in 50µl water. The RNA was subsequently diluted 10 times with DNase/RNase free Water (Gibco) and heated to 90°C for one minute.

For gene expressions analysis, One Step RT-qPCR was performed using qScript™ XLT One-Step RT-qPCR Tough Mix®, Low ROX™ (Quantabio) in a duplex set up. The following TaqMan primer assays were used for qPCR: Nfkbl , Mm00476361_m1 ; Nfkb2, Mm00479810_g1 ; Rela Mm00501346_m1 ; Relb, Mm00485664_m1 ; or Rel, Mm01239661_m1 (FAM-MGB); each combined with endogenous control Gapdh, Mm99999915_g1 (VIC-MGB). All primer sets were purchased from Thermo Fisher Scientific. IC_{50} determinations were performed in GraphPad Prism6. The relative mRNA levels at treatment with 50 µM oligonucleotide is shown in the table as % of control (PBS).

SEQ ID NO	Target	Motif	CMP ID NO	Compound	IC50 [µM]	mRNA level at Max KD
24	Nfkb2	agatttcgattagac	M1,1	AGATttcgattagAC	2,5	38
25	Relb	tagaattgaagttaaa	M2,1	TAGAattgaagtTAAA	1,1	13
26	Rela	ataactgtgttttc	M3,1	ATaactgtgttTTC	2,7	41
	Rel				3,5	65

Example 2: Mouse *in vivo* efficacy and tolerance study, 16 days of treatment, intravenous injection (tail vein).

Animals

Experiment was performed on female C57BL/6JBom mice. Five animals were included in each group of the study, including a saline control group.

Compounds and dosing procedures

Animals were injected intravenously (tail vein) with 15mg/kg compound at day 0, 3, 7, 10, 14 until the study was terminated at day 16.

Euthanasia

At the end of the study (day 16) all mice were euthanized with CO_2 before tissue samples of liver, kidney and mesenteric lymphnode were dissected and snap frozen.

Quantification of Nfkb-subunit RNA expression (Figure 1A, 1B and 1C)

Tissue samples were kept frozen until lysed in MagNA Pure LC RNA Isolation Tissue Lysis Buffer (Product No. 03604721001 , Roche) and RNA extraction continued using the MagNA Pure 96 Cellular RNA Large Volume Kit (Product No. 05467535001 , Roche) on a MagNA Pure 96 Instrument (Roche) according to the user's manual and RNA diluted to 5ng/µl in water.

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 following TaqMan primer assays were used for qPCR: Nfkbl , Mm00476361_m1 ; Nfkb2, Mm00479810_g1 ; Rela Mm00501346_m1 ; Relb, Mm00485664_m1 ; or Rel, Mm01239661_m1 (FAM-MGB); each combined with endogenous control Gapdh, Mm99999915_g1 (VIC-MGB). All primer sets were purchased from Thermo Fisher Scientific. The relative mRNA expression levels are shown as % of control (PBS-treated animals).

Example 3: Testing *in vitro* efficacy of antisense oligonucleotides targeting human REL mRNA in HEK293 and HeLa cell lines at single dose concentration.

REL proto-oncogene, NF-kB subunit [Homo sapiens (human)] Also known as: p65; NFKB3

Assembly	Chr	Location
GRCh38.p7 (GCF_000001405.33)	11	NC_000011.10 (65653596..65662972, complement)

The Human REL pre-mRNA sequence is provided as SEQ ID NO 21 (Figure 6).

HEK-293 and HeLa cell lines were purchased from ATCC and maintained as recommended by the supplier in a humidified incubator at 37°C with 5% $C0_2$. For assays, 3500 cells/well (HEK-293) or 3000 cells/well (HeLa) were seeded in a 96 multi-well plate in culture media. Cells were incubated for 24 hours before addition of oligonucleotides dissolved in PBS. Final concentration of oligonucleotides: 25 μM. Three days after addition of oligonucleotides, the cells were harvested. RNA was extracted using the PureLink Pro 96 RNA Purification kit (Thermo Fisher Scientific) according to the manufacturer 's instructions and eiuated in 50μI water. The RNA was subsequently diluted 10 times with DNase/RNase free Water (Gibco) and 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 following TaqMan primer assays were used for qPCR: REL, Hs00968436_m1 (FAM-MGB) and endogenous control GAPDH, Hs99999905_m1 (VIC-MGB). All primer sets were purchased from Thermo Fisher Scientific. All primer sets were purchased from Life Technologies. The relative REL mRNA expression level in Table 1 is shown as percent of control (PBS-treated cells) .

A total of 77 oligos were designed at a length of 15-16 nucleotides with varying LNA patterns (3x3; 2x4; 4x2; 3x2; 2x3) to target REL across SEQ ID NO 21. A waterfall plot of relative REL expression in the two cell lines in shown in Figure 2.

Table 1:

SEQ ID NO	Motif	CMP ID NO	Compound	Rel. mRNA level HEK-293 at 25μM	Rel. mRNA level HeLa at 25μM
1	gcagaaacaactagga	1,1	GCAgaaacaactagGA	9	8

2	cacatcgaataccca	2,1	CACAt^{m}cgaataccCA	9	11
3	cgtcagattaggaacc	3,1	CGTCagattaggaaCC	17	9
4	agtattggaattggcg	4,1	AGtattggaattgGCG	14	13
5	acacatcgaataccc	5,1	ACAcat^{m}cgaatacCC	15	15
6	ctatttcgtaggctg	6,1	CTAttt^{m}cgtaggcTG	22	11
7	acacatcgaataccca	7,1	ACAcat^{m}cgaataccCA	18	16
8	caggaaattgtaggga	8,1	CAGgaaattgtaggGA	26	10
9	tagtattggaattggc	9,1	TAGtattggaattgGC	28	14
10	ttaagtttctacggca	10,1	TTAagtttcta^{m}cgGCA	21	24

For Compounds: Capital letters represent LNA nucleosides (beta-D-oxy LNA nucleosides were used), all LNA cytosines are 5-methyl cytosine, lower case letters represent DNA nucleosides, DNA cytosines preceded with a superscript ^{m} represents a 5-methyl C-DNA nucleoside. All internucleoside linkages are phosphorothioate internucleoside linkages.

The data obtained from the two cell lines is shown in Figure 3, which illustrates that the above compounds were particularly effective in both cell lines in targeting human REL, as compared to a library of other compounds targeting human REL (Figure 2). Each of the 10 sequences aligned to 7 regions of the REL transcript, illustrated in Figure 4, referred to as hotspot regions A, B, C, D, E, F & G:

Hotspot Region	Hotspot Region – REL pre-mRNA position (SEQ ID NO 21)	Sequences	Target Sequence	Target SEQ ID NO
A	43336-43351	1	TCCTAGTTGTTTCTGC	11
B	19483-19497	2, 7, 5	TGGGTATTCGATGTGT	22
C	1203-1218	3	GGTTCCTAATCTGACG	13
D	40797-40812	4, 9	CGCCAATTCCAATACTA	23
E	22546-22569	6	CAGCCTACGAAATAG	16
F	5539-5554	8	TCCCTACAATTTCCTG	18
G	30972-30987	10	TGCCGTAGAAACTTAA	20

SEQ ID NO 11 - 20 are the reverse complement of SEQ ID NO 1 - 10 respectively. SEQ ID NO 12 = TGGGTATTCGATGTG; SEQ ID NO 14 = CGCCAATTCCAATACT

Example 4: Testing *in vitro* potency and efficacy of selected oligonucleotides targeting human REL mRNA in HEK-293 and HeLa cell lines in a dose response curve.

HEK-293 cell line and HeLa cell line was described in Example 1. The assay was performed as described in Example 1. Concentration of oligonucleotides: from 50 µM, 1:1 dilution in eight steps. Three days after addition of oligonucleotides, the cells were harvested. RNA extraction and duplex One Step RT-qPCR were performed as described in Example 3. n=2 biological replicates per each cell line. IC_{50} determinations were performed in GraphPad Prism. The

relative REL mRNA level at treatment with 50 μM oligonucleotide is shown in the table as % of controll (PBS). See figures 5A, 5B & 5C.

SEQ ID NO	CMP ID NO	IC_{50} HeLa	mRNA level at Max KD in HeLa	IC_{50} HEK-293	mRNA level at Max KD in HEK-293
1	1,1	2,9	10	1,7	7
2	2,1	3,5	6	2,8	5
3	3,1	1,3	12	1,3	15
4	4,1	1,7	15	1,9	13
5	5,1	3,9	9	3,5	8
6	6,1	2,6	10	3,8	11
7	7,1	4,2	10	3,3	7
8	8,1	2,2	13	2,8	23
9	9,1	2,5	15	3,5	20
10	10,1	1,6	27	1,5	19

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CLAIMS

1. An LNA gapmer antisense oligonucleotide targeting human REL, 10 to 30 contiguous nucleotides in length, wherein the contiguous sequence of the oligonucleotide comprises at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NO 3, 4, 1, 2, 5, 6, 7, 8, 9 and 10, wherein the oligonucleotide is capable of recruiting RNase H, or a pharmaceutically acceptable salt thereof.

2. The LNA gapmer antisense oligonucleotide of claim 1, wherein the oligonucleotide comprises a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise 1 - 7 modified nucleosides and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH.

3. The LNA gapmer antisense oligonucleotide according to any one of claims 1 or 2, wherein said oligonucleotide consists or comprises of an oligonucleotide selected from the group consisting of: CGTCagattaggaaCC (SEQ ID NO 3), AGtattggaattgGCG (SEQ ID NO 4), GCAgaaacaactagGA (SEQ ID NO 1), CACAtcgaataccCA (SEQ ID NO 2), ACAcatcgaatacCC (SEQ ID NO 5), CTAtttcgtaggcTG (SEQ ID NO 6), ACAcatcgaataccCA (SEQ ID NO 7), CAGgaaattgtaggGA (SEQ ID NO 8), TAGtattggaattgGC (SEQ ID NO 9), and TTAagtttctacgGCA (SEQ ID NO 10), wherein capital letters represent LNA nucleosides and lower case letters represent DNA nucleosides, and cytosines are optionally 5-methyl cytosine.

4. The LNA gapmer antisense oligonucleotide according to claim 3, wherein all LNA nucleotides are beta-D-oxy LNA.

5. The LNA gapmer antisense oligonucleotide according to claims 3 or 4, wherein all LNA cytosines are 5-methyl cytosine.

6. The LNA gapmer antisense oligonucleotide according to any one of claims 1 - 5, wherein all internucleoside linkages present in the gapmer are phosphorothioate internucleoside linkages.

7. The LNA gapmer antisense oligonucleotide according to any one of claims 1 - 6, wherein the gapmer region (F-G-F') or the compound is selected from the group consisting of, CGTCagattaggaaCC (SEQ ID NO 3), AGtattggaattgGCG (SEQ ID NO 4), GCAgaaacaactagGA (SEQ ID NO 1), CACAt^{m}cgaataccCA (SEQ ID NO 2), ACAcat^{m}cgaatacCC (SEQ ID NO 5), CTAttt^{m}cgtaggcTG (SEQ ID NO 6), ACAcat^{m}cgaataccCA (SEQ ID NO 7), CAGgaaattgtaggGA (SEQ ID NO 8), TAGtattggaattgGC (SEQ ID NO 9), and TTAagtttcta^{m}cgGCA (SEQ ID NO 10), wherein capital letters represent beta-D-oxy LNA nucleosides, all LNA cytosines are 5-methyl

cytosine, lower case letters are DNA nucleosides, ^{m}c indicates a 5-methyl cytosinse DNA nucleoside, and all internucleoside linkages are phosphorothioate internucleoside linkages.

8. A conjugate comprising the LNA gapmer antisense oligonucleotide according to any one of claims 1 - 7, and at least one conjugate moiety covalently attached to said oligonucleotide.

9. A pharmaceutical composition comprising the LNA gapmer antisense oligonucleotide of claim 1 - 7 or the conjugate of claim 8 and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

10. An *in vitro* method for modulating REL expression in a target cell which is expressing REL said method comprising administering an LNA gapmer antisense oligonucleotide of any one of claims 1 - 7, the conjugate according to claim 8, or the pharmaceutical composition of claim 9 in an effective amount to said cell.

11. The LNA gapmer antisense oligonucleotide of any one of claims 1 - 7 or the conjugate according to claim 8 or the pharmaceutical composition of claim 9 for use in medicine.

12. The LNA gapmer antisense oligonucleotide of any one of claims 1 - 7 or the conjugate according to claim 8 or the pharmaceutical composition of claim 9 for use in the treatment or prevention of cancer, inflammation and inflammatory disorders, and autoimmune diseases.

13. The use of the LNA gapmer antisense oligonucleotide of claim 1 - 7 or the conjugate according to claim 8 or the pharmaceutical composition of claim 9, for the preparation of a medicament for treatment or prevention of cancer, inflammation and inflammatory disorders, and autoimmune diseases.

14. The LNA gapmer antisense oligonucleotide or use according to any one of claims 1 - 13, wherein the oligonucleotide is for use in the treatment of a disease selected from the group consisting of multiple sclerosis, psoriasis, celiac disease, Crohn's disease and rheumatoid arthritis.

15. The LNA gapmer antisense oligonucleotide or use according to any one of claims 1 - 13, wherein the oligonucleotide is for use in the treatment of a disease selected from the group consisting of hematopoietic cancer, such as lymphoma or leukemia, lung cancer and breast cancer.

FIGURES

Figure 1A

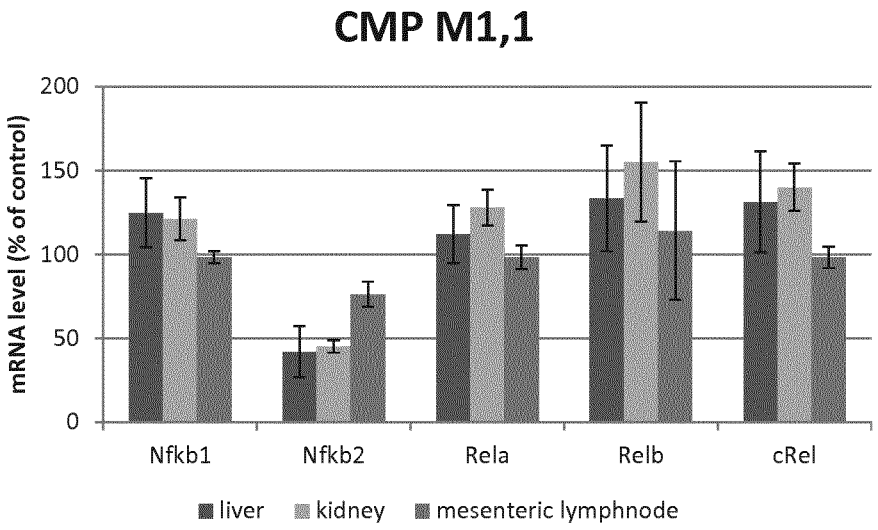


Figure 1B

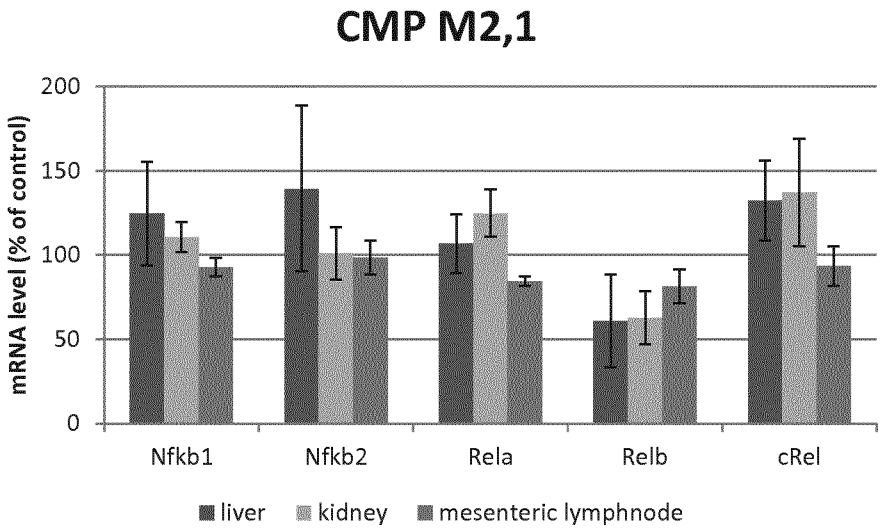
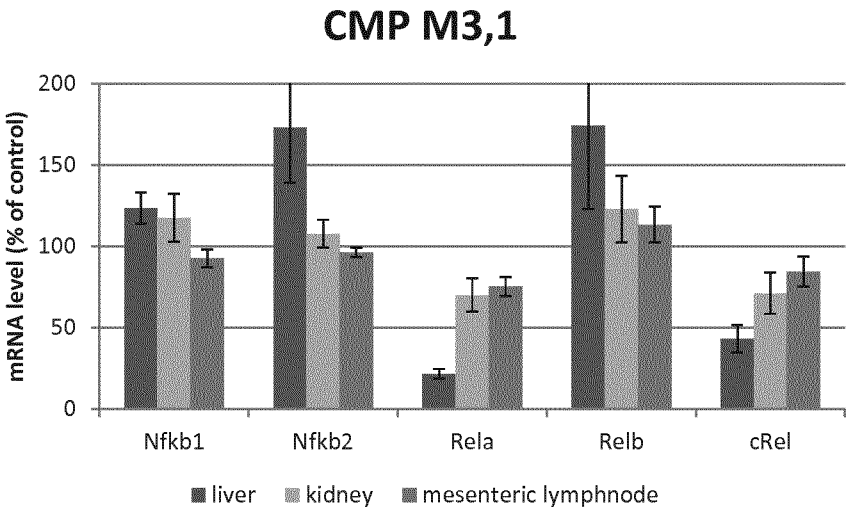


Figure 1C



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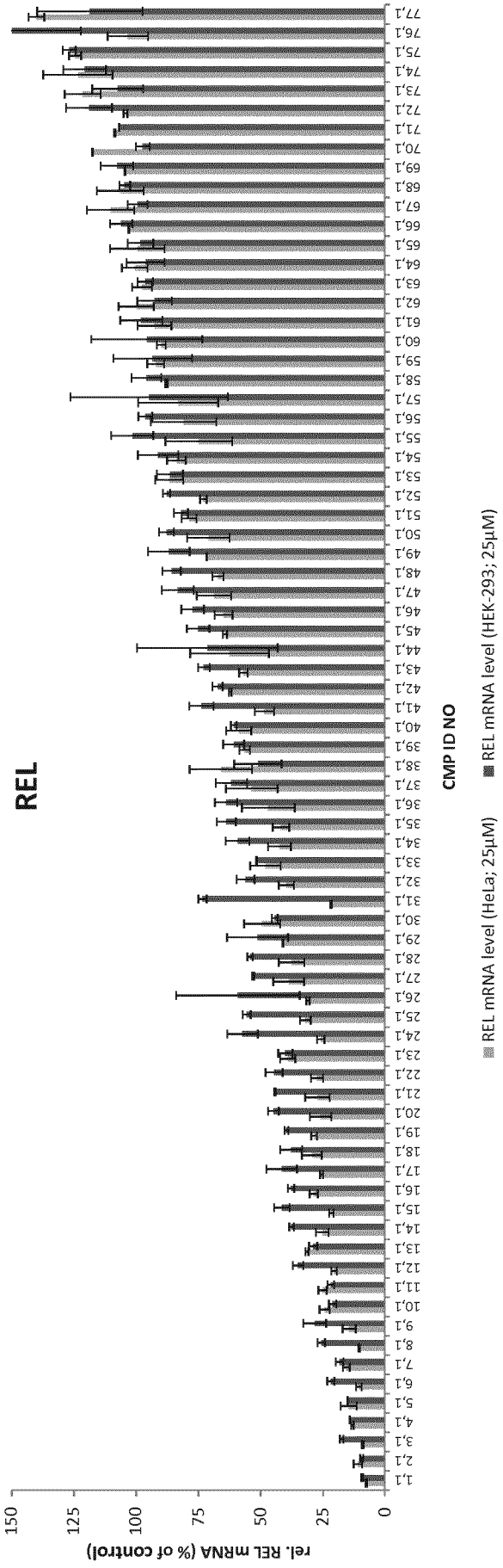
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rel. REL mRNA (% of control)
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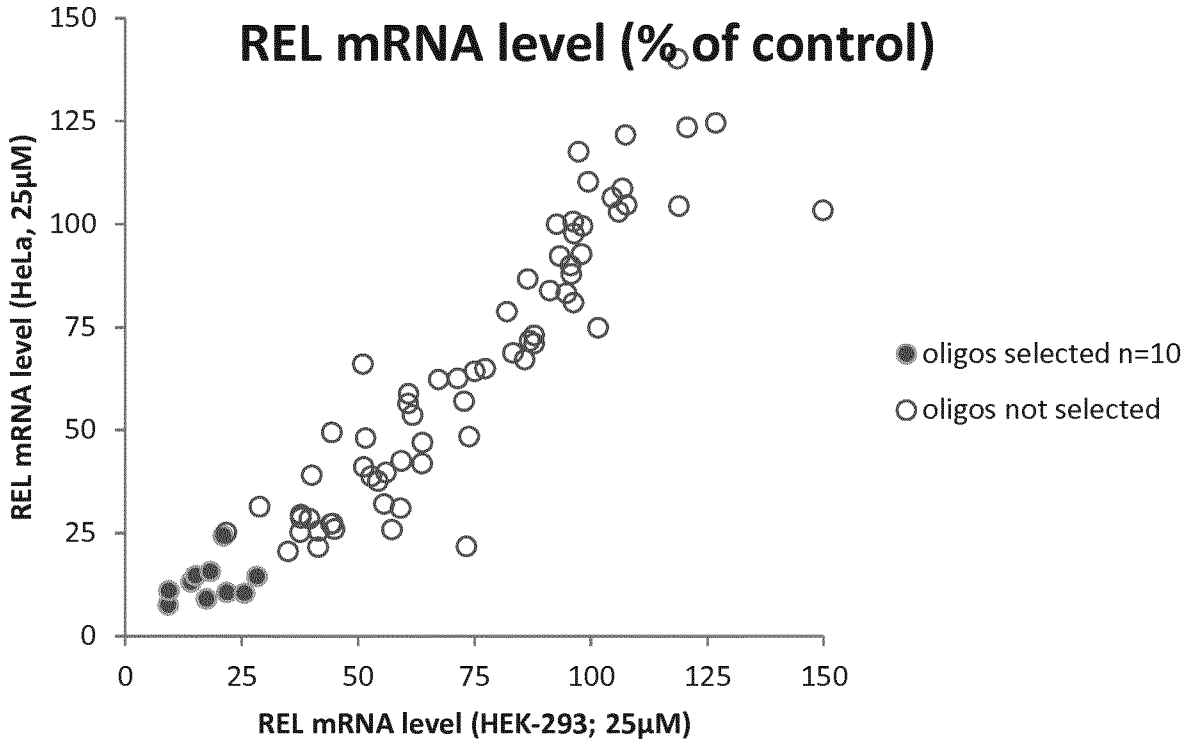

Figure 4

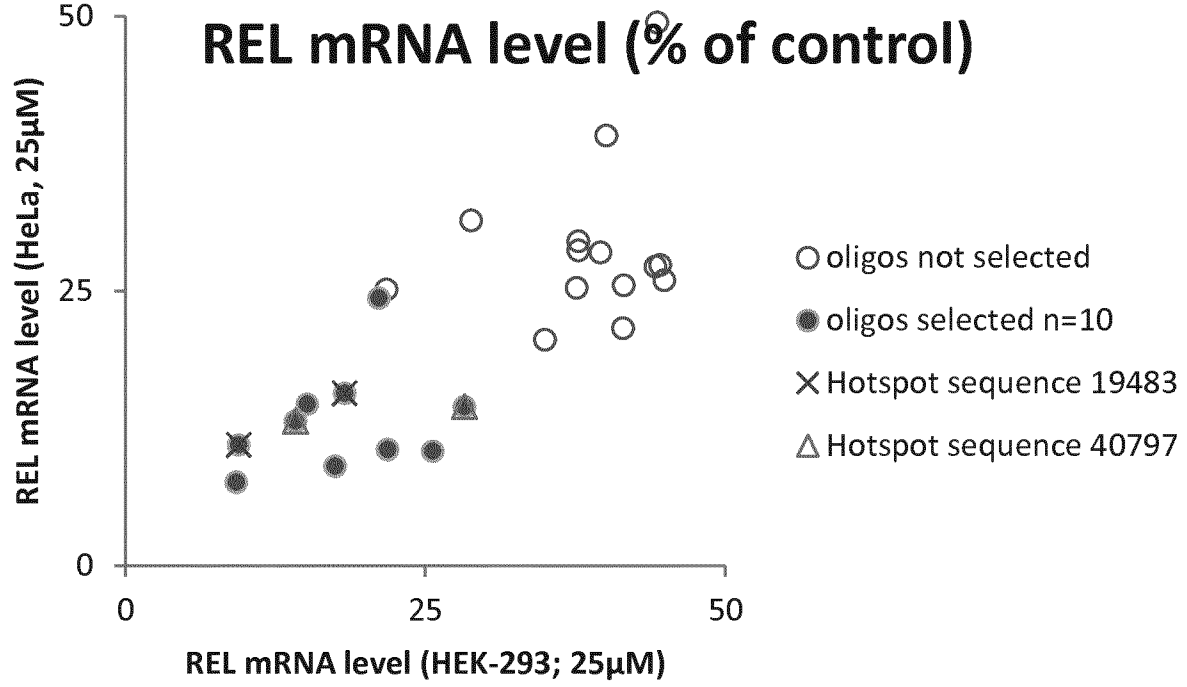

Figure 5A

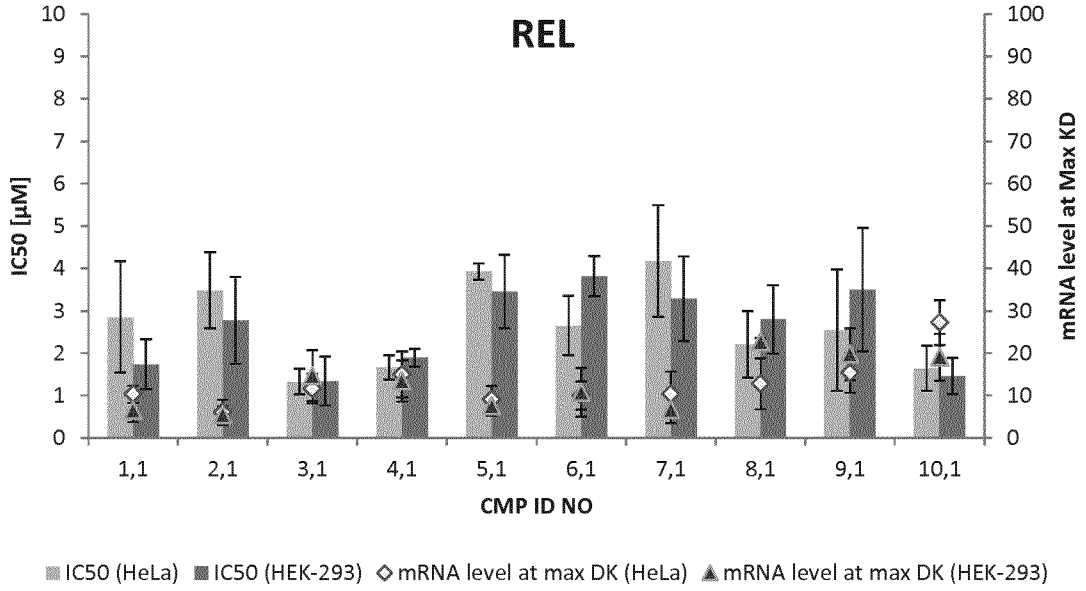

Figure 5B

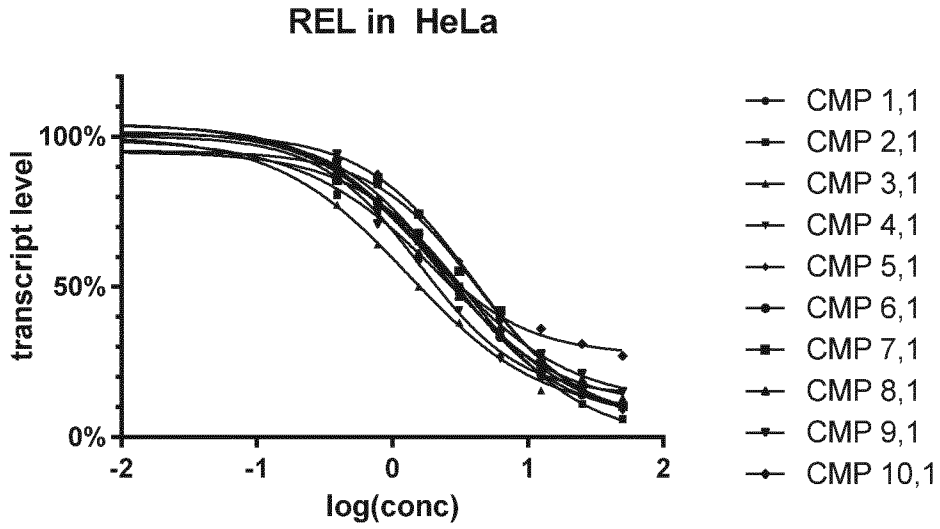

Figure 5C

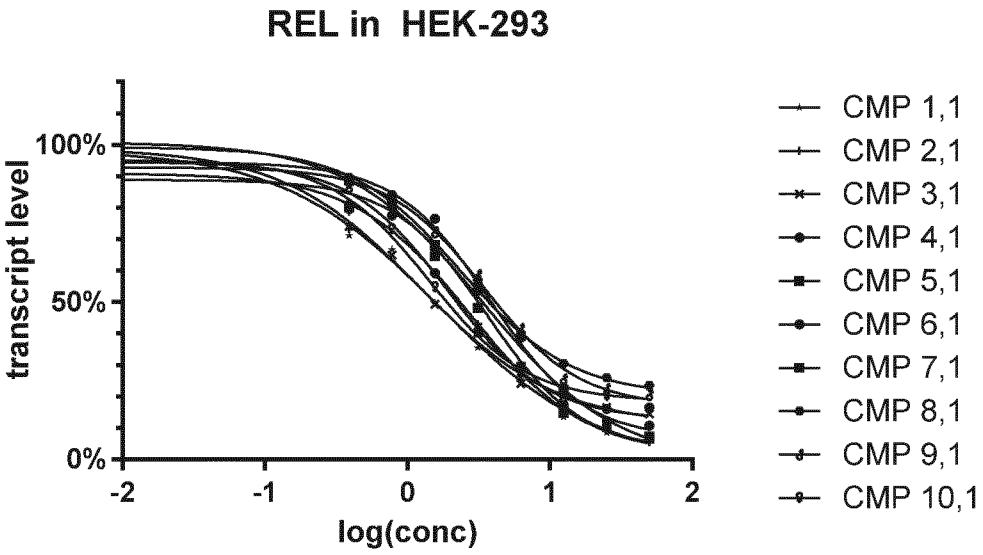

Figure 6

See sequence listing SEQ ID NO 21

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/050582

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113
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 , Sequence Search , CHEM ABS Data, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00/17400 Al (ISIS PHARMACEUTICALS INC [US] ; MONIA BRETT P [US] ; BAKER BRENDA F [US]) 30 March 2000 (2000-03-30) cited in the application the whole document	1-15
A	PI0TR J. KAM0LA ET AL: "In silico and in vitro evaluation of exonic and intronic off-target effects form a critical element of therapeutic AS0 gapmer optimization", NUCLEIC ACIDS RESEARCH , vol . 43, no. 18, 3 September 2015 (2015-09-03) , pages 8638-8650, XP055463091 , ISSN: 0305-1048, D0I: 10. 1093/nar/gkv857 page 8648, right-hand column, paragraph 2 page 8649, left-hand column, paragraph 2	1-15

-/- .

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2018	13/04/2018

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/050582

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/114724 A2 (INTRADIGM CORP [US] ; XIE FRANK Y [US] ; YANG XIAODONG [US] ; LIU YING [U) 17 September 2009 (2009-09-17) abstract; sequences 401 ,402	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/050582

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0017400 A1	30-03-2000	AU 6045499 A	10-04-2000
		US 6001652 A	14-12-1999
		WO 0017400 A1	30-03-2000
W0 2009114724 A2	17-09-2009	US 2011053861 A1	03-03-2011
		W0 2009114724 A2	17-09-2009