OLIGONUCLEOTIDES FOR MODULATING RTEL1 EXPRESSION

FIELD OF INVENTION

The present invention relates to RTEL1 inhibitors, such as oligonucleotides (oligomers) that are complementary to RTEL1, leading to modulation of the expression of RTEL1 or modulation of RTEL1 activity. The invention in particular relates to the use of RTEL1 targeting nucleic acid molecules for use in treating and/or preventing a hepatitis B virus (HBV) infection, in particular a chronic HBV infection. The invention in particular relates to the use of RTEL1 inhibitors for destabilizing cccDNA, such as HBV cccDNA. Also comprised in the present invention is a pharmaceutical composition and its use in the treatment and/or prevention of a HBV infection.

BACKGROUND

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV), a small hepatotropic virus that replicates through reverse transcription. Chronic HBV infection is a key factor for severe liver diseases such as liver cirrhosis and hepatocellular carcinoma. Current treatments for chronic HBV infection are based on administration of pegylated type 1 interferons or nucleos(t)ide analogues, such as lamivudine, adefovir, entecavir, tenofovir disoproxil, and tenofovir alafenamide, which target the viral polymerase, a multifunctional reverse transcriptase. Treatment success is usually measured as loss of hepatitis B surface antigen (HBsAg). However, a complete HBsAg clearance is rarely achieved since Hepatitis B virus DNA persists in the body after infection. HBV persistence is mediated by an episomal form of the HBV genome which is stably maintained in the nucleus. This episomal form is called “covalently closed circular DNA” (cccDNA). The cccDNA serves as a template for all HBV transcripts, including pregenomic RNA (pgRNA), a viral replicative intermediate. The presence of a few copies of cccDNA might be sufficient to reinitiate a full-blown HBV infection. Current treatments for HBV do not target cccDNA. A cure of chronic HBV infection, however, would require the elimination of cccDNA (reviewed by Nassal, Gut. 2015 Dec;64(12):1972-84. doi: 10.1136/gutjnl-2015-309809).

Regulator of telomere elongation helicase 1 (RTEL1) encodes a DNA helicase which functions in the stability, protection and elongation of telomeres and interacts with proteins in the shelterin complex known to protect telomeres during DNA replication. Mutations in this gene have been associated with dyskeratosis congenita and Hoyerall-Hreidarsson syndrome (See for example review by Vannier et al 2014 Trends Cell Biol. Vol 24 p.416).

Located in the nucleus, RTEL1 functions as an ATP-dependent DNA helicase implicated in telomere-length regulation, DNA repair and the maintenance of genomic stability. RTEL1 Acts as an anti-recombinase to counteract toxic recombination and limit crossover during meiosis and regulates meiotic recombination and crossover homeostasis by physically dissociating

strand invasion events and thereby promotes non-crossover repair by meiotic synthesis dependent strand annealing (SDSA) as well as disassembly of D loop recombination intermediates. In additional RTEL1 disassembles T loops and prevents telomere fragility by counteracting telomeric G4-DNA structures, which together ensure the dynamics and stability of the telomere.

RTEL1 has been identified in a siRNA screen as a stabilizer of HPV episomes: (Edwards et al 2013 PLoS One Vol 8, e75406). siRNA targeting RTEL1 has likewise been used to identify interactants with RTEL1 in Hoyeraal-Hreidarsson syndrome (Schertzer et al 2015 Nucleic Acid Res Vol 43 p. 1834). In addition, RTEL1 was identified as a HIV host dependency factor from a siRNA screen for essential host proteins to provide targets for inhibition HIV infection (WO 2007/094818).

To our knowledge RTEL1 has never been identified as a cccDNA dependency factor in the context of cccDNA stability and maintenance, nor have molecules inhibiting RTEL1 ever been suggested as cccDNA destabilizers for the treatment of HBV infection.

OBJECTIVE OF THE INVENTION

The present invention shows that there is a correlation between the inhibition of RTEL1 and reduction of cccDNA in an HBV infected cell, which is relevant in the treatment of HBV infected individuals. An objective of the present invention is to identify RTEL1 inhibitors which reduce cccDNA in an HBV infected cell. Such RTEL1 inhibitors can be used in the treatment of HBV infection.

The present invention further identifies novel nucleic acid molecules, which are capable of inhibiting the expression of RTEL1 *in vitro and in vivo*.

SUMMARY OF INVENTION

The present invention relates to oligonucleotides targeting a nucleic acid capable of modulating the expression of RTEL1 and to treat or prevent diseases related to the functioning of the RTEL1.

Accordingly, in a first aspect the invention provides a RTEL1 inhibitor for use in the treatment and/or prevention of Hepatitis B virus (HBV) infection. In particular, a RTEL1 inhibitor capable of reducing cccDNA and/or pre-genomic RNA (pgRNA) is useful. Such an inhibitor is advantageously selected from a nucleic acid molecule of 12 to 60 nucleotides in length, which is capable of reducing RTEL1 mRNA, such as a single stranded antisense oligonucleotide, a siRNA or a shRNA complementary to mammalian RTEL1

In a further aspect the invention relates to an oligonucleotide of 12-60 nucleotides, such as 12-30 nucleotides, comprising a contiguous nucleotides sequence of at least 10 nucleotides, in particular of 16 to 20 nucleotides, which is complementary to a mammalian RTEL1. Such an

oligonucleotide is capable of inhibiting the expression of RTEL1. The oligonucleotide can be a single stranded antisense oligonucleotide or a shRNA nucleic acid molecule.

The antisense oligonucleotide can have a gapmer design. Preferably, the antisense oligonucleotide is capable of inhibiting the expression of RTEL1 by cleavage of the target nucleic acid. The cleavage is preferably achieved via nuclease recruitment.

In a further aspect, the invention provides pharmaceutical compositions comprising the antisense oligonucleotide of the invention and a pharmaceutically excipient.

In a further aspect, the invention provides methods for *in vivo* or *in vitro* method for modulation of RTEL1 expression in a target cell which is expressing RTEL1, by administering an antisense oligonucleotide 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 RTEL1 comprising administering a therapeutically or prophylactically effective amount of the antisense oligonucleotide of the invention to a subject suffering from or susceptible to the disease, disorder or dysfunction.

Further aspects of the invention are conjugates of nucleic acid molecules of the invention and pharmaceutical compositions comprising the molecules of the invention. In particular conjugates targeting the liver are of interest, such as GalNAc clusters.

BRIEF DESCRIPTION OF FIGURES

Figure 1: Illustrates exemplary antisense oligonucleotide conjugates, where the oligonucleotide either is represented as a wavy line (A-D) or as "oligonucleotide" (E-H) or as T_2 (I) and the asialoglycoprotein receptor targeting conjugate moieties are trivalent N-acetylgalactosamine moieties. Compounds A to D comprise a di-lysine brancher molecule, a PEG3 spacer and three terminal GalNAc carbohydrate moieties. In compound A and B the oligonucleotide is attached directly to the asialoglycoprotein receptor targeting conjugate moiety without a linker. In compound C and D the oligonucleotide is attached to the asialoglycoprotein receptor targeting conjugate moiety via a C6 linker. Compounds E-I comprise a commercially available trebler brancher molecule and spacers of varying length and structure and three terminal GalNAc carbohydrate moieties.

DEFINITIONS

HBV infection

The term "hepatitis B virus infection" or "HBV infection" is commonly known in the art and refers to an infectious disease that is caused by the hepatitis B virus (HBV) and affects the liver. A HBV infection can be an acute or a chronic infection. Chronic hepatitis B virus (CHB) infection is a global disease burden affecting 248 million individuals worldwide. Approximately 686,000

deaths annually are attributed to HBV-related end-stage liver diseases and hepatocellular carcinoma (HCC) (GBD 2013; Schweitzer et al., 2015). WHO projected that without expanded intervention, the number of people living with CHB infection will remain at the current high levels for the next 40-50 years, with a cumulative 20 million deaths occurring between 2015 and 2030 (WHO 2016). CHB infection is not a homogenous disease with singular clinical presentation. Infected individuals have progressed through several phases of CHB-associated liver disease in their life; these phases of disease are also the basis for treatment with standard of care (SOC). Current guidelines recommend treating only selected CHB-infected individuals based on three criteria - serum ALT level, HBV DNA level, and severity of liver disease (EASL, 2017). This recommendation was due to the fact that SOC i.e. nucleos(t)ide analogs (NAs) and pegylated interferon-alpha (PEG-IFN), are not curative and must be administered for long periods of time thereby increasing their safety risks. NAs effectively suppress HBV DNA replication; however, they have very limited/no effect on other viral markers. Two hallmarks of HBV infection, hepatitis B surface antigen (HBsAg) and covalently closed circular DNA (cccDNA), are the main targets of novel drugs aiming for HBV cure. In the plasma of CHB individuals, HBsAg subviral (empty) particles outnumber HBV virions by a factor of 103 to 105 (Ganem & Prince, 2014); its excess is believed to contribute to immunopathogenesis of the disease, including inability of individuals to develop neutralizing anti-HBs antibody, the serological marker observed following resolution of acute HBV infection.

cccDNA (covalently closed circular DNA)

cccDNA is the viral genetic template that resides in the nucleus of infected hepatocytes, where it gives rise to all HBV RNA transcripts needed for productive infection and is responsible for viral persistence during natural course of chronic HBV infection (Locarnini & Zoulim, 2010 Antivir Ther. 15 Suppl 3:3-14. doi: 10.3851/IMP1619). Acting as a viral reservoir, cccDNA is the source of viral rebound after cessation of treatment, necessitating long term, often, lifetime treatment. PEG-IFN can only be administered to a small subset of CHB due to its various side effects.

Consequently, novel therapies that can deliver a complete cure, defined by degradation or elimination of HBV cccDNA, to the majority of CHB patients are highly needed.

Compound

Herein, the term "compound" means any molecule capable of inhibition RTEL1 expression or activity. Particular compounds of the invention are nucleic acid molecules, such as RNAi molecules or antisense oligonucleotides according to the invention or any conjugate comprising such a nucleic acid molecule. For example, herein the compound may be a nucleic acid molecule targeting RTEL1, in particular an antisense oligonucleotide or a siRNA.

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, which may be used interchangeably.

The oligonucleotides referred to in in the description and claims are generally therapeutic oligonucleotides below 70 nucleotides in length. The oligonucleotide may be or comprise a single stranded antisense oligonucleotide, or may be another oligomeric nucleic acid molecule, such as a CRISPR RNA, a siRNA, shRNA, an aptamer, or a ribozyme. Therapeutic oligonucleotide molecules are commonly made in the laboratory by solid-phase chemical synthesis followed by purification and isolation. shRNA's are however often delivered to cells using lentiviral vectors from which they are then transcribed to produce the single stranded RNA that will form a stem loop (hairpin) RNA structure that is capable of interacting with the RNA interference machinery (including the RNA-induced silencing complex (RISC)). In an embodiment of the present invention the shRNA is chemically produced shRNA molecules (not relying on cell based expression from plasmids or viruses).

When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. Generally, the oligonucleotide of the invention is man-made, and is chemically synthesized, and is typically purified or isolated. Although in some embodiments the oligonucleotide of the invention is a shRNA transcribed from a vector upon entry into the target cell. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides.

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

In some embodiments, the oligonucleotide or contiguous nucleotide sequence thereof comprises or consists of 24 or less nucleotides, such as 22, 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 a nucleic acid molecule is said to include from 12 to 25 nucleotides, both 12 and 25 nucleotides are included.

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

The oligonucleotide(s) are for modulating the expression of a target nucleic acid in a mammal. In some embodiments the nucleic acid molecules, such as for siRNAs, shRNAs and antisense oligonucleotides, are typically for inhibiting the expression of a target nucleic acid(s).

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

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

In some embodiments the oligonucleotide comprises phosphorothioate internucleoside linkages.

In some embodiments the oligonucleotide may be conjugated to non-nucleosidic moieties (conjugate moieties).

A library of oligonucleotides is to be understood as a collection of variant oligonucleotidess. The purpose of the library of oligonucleotides can vary. In some embodiments, the library of oligonucleotides is composed of oligonucleotides with overlapping nucleobase sequence targeting one or more mammalian RTEL1 target nucleic acids with the purpose of identifying the most potent sequence within the library of oligonucleotides. In some embodiments, the library of oligonucleotides is a library of oligonucleotide design variants (child nucleic acid molecules) of a parent or ancestral oligonucleotide, wherein the oligonucleotide design variants retaining the core nucleobase sequence of the parent nucleic acid molecule.

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 on a target nucleic acid. The antisense oligonucleotides herein are not essentially double stranded and are therefore not siRNAs or shRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded. It is understood that single stranded oligonucleotides of the present invention can form hairpins or intermolecular duplex structures (duplex between two molecules of the same oligonucleotide), as long as the degree of intra or inter self complementarity is less than 50% across of the full length of the oligonucleotide.

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

Advantageously, the oligonucleotide of the invention comprises one or more modified nucleosides or nucleotides, such as 2' sugar modified nucleosides. Furthermore, it is advantageous that the nucleosides which are not modified are DNA nucleosides.

RNAi molecules

Herein, the term "RNA interference (RNAi) molecule" refers to short double-stranded RNA based oligonucleotide capable of inducing RNA-dependent gene silencing via the RNA-induced silencing complex (RISC) in a cell's cytoplasm, where they interact with the catalytic RISC component argonaute. The RNAi molecule modulates. e g., inhibits, the expression of the target nucleic acid in a cell. e.g. a cell within a subject. such as a mammalian subject. One type of RNAi molecule is a small interfering RNA (siRNA), which is a double-stranded RNA molecule composed of two complementary oligonucleotides, where the binding of one strand to complementary mRNA after transcription, leads to its degradation and loss of translation. A small hairpin RNA (shRNA) is a single stranded RNA-based oligonucleotide that forms a stem loop (hairpin) structure which is able to reduce mRNA via the DICER and RNA reducing silencing complex (RISC). RNAi molecules can be designed based on the sequence of the gene of interest (target nucleic acid). Corresponding RNAi can then be synthesized chemically or by in vitro transcription, or expressed from a vector or PCR product.

siRNA

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

Once inside a cell the antisense strand is incorporated into the RISC complex which mediate target degradation or target inhibition of the target nucleic acid. siRNAs typically comprise modified nucleosides in addition to RNA nucleosides. In one embodiment the siRNA molecule

may be chemically modified using modified internucleotide linkages and 2' sugar modified nucleosides, such as 2'-4' bicyclic ribose modified nucleosides, including LNA and cET or 2' substituted modifications like of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA. In particular 2'fluoro, 2'-O-methyl or 2'-O-methoxyethyl may be incorporated into siRNAs.

In some embodiments all of the nucleotides of an siRNA sense (passenger) strand may be modified with 2' sugar modified nucleosides such as LNA (see WO2004/083430, WO2007/085485 for example). In some embodiments the passenger stand of the siRNA may be discontinuous (see WO2007/107162 for example). The incorporation of thermally destabilizing nucleotides occurring at a seed region of the antisense strand of siRNAs have been reported as useful in reducing off-target activity of siRNAs (see WO2018/098328 for example). Suitably the siRNA comprises a 5' phosphate group or a 5'-phosphate mimic at the 5' end of the antisense strand. In some embodiments the 5' end of the antisense strand is a RNA nucleoside.

In one embodiment, the siRNA molecule further comprises at least one phosphorothioate or methylphosphonate internucleoside linkage. The phosphorothioaie or methylphosphonate internucleoside linkage may be at the 3'- terminus one or both strand (e.g., the antisense strand; or the sense strand); or the phosphorothioate or methylphosphonate internucleoside linkage may be at the 5'-terminus of one or both strands (e.g., the antisense strand; or the sense strand); or the phosphorothioate or methylphosphonate internucleoside linkage may be at the both the 5'- and 3'-terminus of one or both strands (e.g., the antisense strand; or the sense strand). In some embodiments the remaining internucleoside linkages are phosphodiester linkages. In some embodiments siRNA molecules comprise one or more phosphorothioate internucleoside linkages. In siRNA molecules phosphorothioate internucleoside linkages may reduce or the nuclease cleavage in RICS, it is therefore advantageous that not all internucleoside linkages in the antisense strand are modified.

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

For biological distribution, siRNAs may be conjugated to a targeting ligand, and/or be formulated into lipid nanoparticles, for example.

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

shRNA

Short hairpin RNA or shRNA molecules are generally between 40 and 70 nucleotides in length, such as between 45 and 65 nucleotides in length, such as 50 and 60 nucleotides in length, and form a stem loop (hairpin) RNA structure, which interacts with the endonuclease known as Dicer which is believed to processes dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs which are then incorporated into an RNA-induced silencing complex (RISC). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing. RNAi oligonucleotides may be chemically modified using modified internucleotide linkages and 2' sugar modified nucleosides, such as 2'-4' bicyclic ribose modified nucleosides, including LNA and cET or 2' substituted modifications like of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA.

In some embodiments shRNA nucleic acid molecules comprise one or more phosphorothioate internucleoside linkages. In RNAi molecules phosphorothioate internucleoside linkages may reduce or the nuclease cleavage in RICS it is therefore advantageous that not al internucleoside linkages in the stem loop of the shRNA molecule are modified. Phosphorothioate internucleoside linkages can advantageously be place in the 3' and/or 5' end of the stem loop of the shRNA molecule, in particular in the of the part of the molecule that is not complementary to the target nucleic acid (e.g. the sense stand or passenger strand in an siRNA molecule). The region of the shRNA molecule that is complementary to the target nucleic acid may however also be modified in the first 2 to 3 internucleoside linkages in the part that is predicted to become the 3' and/or 5' terminal following cleavage by Dicer.

Contiguous Nucleotide Sequence

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

embodiments, the contiguous nucleotide sequence is 100% complementary to the target nucleic acid.

Nucleotides and nucleosides

Nucleotides and nucleosides are the building blocks of oligonucleotides and polynucleotides, and for the purposes of the present invention include both naturally occurring and non-naturally occurring nucleotides and nucleosides. In nature, nucleotides, such as DNA and RNA nucleotides comprise a ribose sugar moiety, a nucleobase moiety and one or more phosphate groups (which is absent in nucleosides). Nucleosides and nucleotides may also interchangeably be referred to as "units" or "monomers".

Modified nucleoside

The term "modified nucleoside" or "nucleoside modification" as used herein refers to nucleosides modified as compared to the equivalent DNA or RNA nucleoside by the introduction of one or more modifications of the sugar moiety or the (nucleo)base moiety. In a preferred embodiment 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". Nucleosides with an unmodified DNA or RNA sugar moiety are termed DNA or RNA nucleosides herein. Nucleosides with modifications in the base region of the DNA or RNA nucleoside are still generally termed DNA or RNA if they allow Watson Crick base pairing.

Modified internucleoside linkage

The term "modified internucleoside linkage" is defined as generally understood by the skilled person as linkages other than phosphodiester (PO) linkages, that covalently couples two nucleosides together. The oligonucleotides of the invention may therefore comprise one or more modified internucleoside linkages, such as a one or more phosphorothioate internucleoside linkages, or one or more phoshporodithioate internucleoside linkages. 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 G of a gapmer oligonucleotide, as well as in regions of modified nucleosides, such as region F and F'.

In an embodiment, the oligonucleotide comprises one or more internucleoside linkages modified from the natural phosphodiester, such as one or more modified internucleoside linkages 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 75%, 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.

With the oligonucleotide of the invention it is advantageous to use phosphorothioate internucleoside linkages.

Phosphorothioate internucleoside linkages are particularly useful due to nuclease resistance, beneficial pharmacokinetics and ease of manufacture. In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate, such as at least 60%, such as at least 70%, such as at least 75%, 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.

Nuclease resistant linkages, such as phosphorthioate 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. 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. Gapmer oligonucleotides may, in some embodiments comprise one or more phosphodiester linkages in region F or F', or both region F and F', where all the internucleoside linkages in region G may be phosphorothioate.

Advantageously, all the internucleoside linkages of the contiguous nucleotide sequence of the oligonucleotide are phosphorothioate, or all the internucleoside linkages of the oligonucleotide are phosphorothioate linkages.

It is recognized that, as disclosed in EP 2 742 135, antisense oligonucleotides may comprise other internucleoside linkages (other than phosphodiester and phosphorothioate), for example

alkyl phosphonate/methyl phosphonate internucleoside, which according to EP 2 742 135 may for example be tolerated in an otherwise DNA phosphorothioate the gap region.

Nucleobase

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

In some embodiments the nucleobase moiety is modified by changing the purine or pyrimidine into a modified purine or pyrimidine, such as substituted purine or substituted pyrimidine, such as a 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 and DNA nucleosides. The antisense oligonucleotide of the invention is advantageously a chimeric oligonucleotide.

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

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

The following is an example of an oligonucleotide motif (SEQ ID NO: 33) that is fully complementary to the target nucleic acid (SEQ ID NO: 11)

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5'- CTTTGACCAGAGTATGTAAAATTCTC-3' (SEQ ID NO: 11)
  3'-AAACTGGTCTCATACATTTT -5'     (SEQ ID NO: 33)
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Identity

The term "Identity" as used herein, refers to the proportion of nucleotides (expressed in percent) of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which across the contiguous nucleotide sequence, are identical to a reference sequence (e.g. a sequence motif). The percentage of identity is thus calculated by counting the number of aligned nucleobases that are identical (a Match) between two sequences (in the contiguous nucleotide sequence of the compound of the invention and in the reference sequence), dividing that number by the total number of nucleotides in the oligonucleotide and multiplying by 100. Therefore, Percentage of Identity = (Matches x 100)/Length of aligned region (e.g. the contiguous nucleotide sequence). Insertions and deletions are not allowed in the calculation the percentage of identity of a contiguous nucleotide sequence. It will be understood that in determining identity, chemical modifications of the nucleobases are disregarded as long as the functional capacity of the nucleobase to form Watson Crick base pairing is retained (e.g. 5-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

Hybridization

The term "hybridizing" or "hybridizes" as used herein is to be understood as 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 ΔG° is a more accurate representation of binding affinity and is related to the dissociation constant (K_d) of the reaction by $\Delta G° = -RT\ln(K_d)$, where R is the gas constant and T is the absolute temperature. Therefore, a very low ΔG° of the reaction between an oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. ΔG° is the energy associated with a reaction where aqueous concentrations are 1M, the pH is 7, and the temperature is 37°C. The hybridization of oligonucleotides to a target nucleic acid is a spontaneous reaction and for spontaneous reactions ΔG° is less than zero. ΔG° can be measured experimentally, for example, by use of the isothermal titration calorimetry (ITC) method as described in Hansen et al., 1965,*Chem. Comm.* 36–38 and Holdgate et al., 2005, *Drug Discov Today*. The skilled person will know that commercial equipment is available for ΔG° measurements. ΔG° can also be estimated numerically by using the nearest neighbor model as described by SantaLucia, 1998, *Proc Natl Acad Sci USA.* 95: 1460–1465 using appropriately derived thermodynamic parameters described by Sugimoto et al., 1995, *Biochemistry* 34:11211–11216 and McTigue et al., 2004, *Biochemistry* 43:5388–5405. In order to have the possibility of modulating its intended nucleic acid target by hybridization, oligonucleotides of the present invention hybridize to a target nucleic acid with estimated ΔG° 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 ΔG°. The oligonucleotides may hybridize to a target nucleic acid with estimated ΔG° 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 ΔG° 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 RTEL1 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 RTEL1 target nucleic acid.

The oligonucleotide of the invention may for example target exon regions of a mammalian RTEL1 (in particular siRNA and shRNA target exon regions, but also antisense oligonucleotides), or may for example target intron region in the RTEL1 pre-mRNA (in particular antisense oligonucleotides target intron regions). The human RTEL1 gene encodes 15 transcripts of these 7 are protein coding and therefore potential nucleic acid targets. Table 1 lists predicted exon and intron regions of the 7 transcripts, as positioned on the human RTEL1 premRNA of SEQ ID NO: 1. It is understood that the oligonucleotides of the invention can target the mature mRNA sequence of one or more of the listed transcripts in table 1.

Table 1. Transcript-, exonic- and intronic regions in the human RTEL1 premRNA (SEQ ID NO: 1) for the different protein coding RTEL1 mRNA transcripts

Transcript ID	Transcript region		Exonic regions			Intron regions		
	start	end	Exon	start	end	intron	start	end
RTEL1-205 ENST00000370018	1	38444	1	1	657	1	657	1424
			2	1424	1695	2	1695	3489
			3	3489	3687	3	3687	4041
			4	4041	4134	4	4134	4737
			5	4737	4818	5	4818	5020
			6	5020	5080	6	5080	8195
			7	8195	8270	7	8270	9660
			8	9660	9744	8	9744	14747
			9	14747	14812	9	14812	16131
			10	16131	16284	10	16284	20336
			11	20336	20374	11	20374	20459
			12	20459	20537	12	20537	22040
			13	22040	22137	13	22137	22855
			14	22855	22910	14	22910	27714
			15	27714	27788	15	27788	27982
			16	27982	28063	16	28063	29829
			17	29829	29961	17	29961	30128
			18	30128	30241	18	30241	30330
			19	30330	30370	19	30370	30492
			20	30492	30577	20	30577	30719
			21	30719	30796	21	30796	31246
			22	31246	31323	22	31323	31693
			23	31693	31839	23	31839	31941
			24	31941	32056	24	32056	32278
			25	32278	32401	25	32401	32485
			26	32485	32632	26	32632	32996
			27	32996	33138	27	33138	33933
			28	33933	34028	28	34028	34996
			29	34996	35194	29	35194	35334
			30	35334	35474	30	35474	36563
			31	36563	36679	31	36679	36932

Transcript ID	Transcript region		Exonic regions			Intron regions		
	start	end	Exon	start	end	intron	start	end
			32	36932	37165	32	37165	37257
			33	37257	37412	33	37412	37519
			34	37519	37671	34	37671	37969
			35	37969	38444			
RTEL1-203 ENST00000360203	485	38433	1	485	657	1	657	1424
			2	1424	1695	2	1695	3489
			3	3489	3687	3	3687	4041
			4	4041	4134	4	4134	4737
			5	4737	4818	5	4818	5020
			6	5020	5080	6	5080	8195
			7	8195	8270	7	8270	9660
			8	9660	9744	8	9744	14747
			9	14747	14812	9	14812	16131
			10	16131	16284	10	16284	20336
			11	20336	20374	11	20374	20459
			12	20459	20537	12	20537	22040
			13	22040	22137	13	22137	22855
			14	22855	22910	14	22910	27714
			15	27714	27788	15	27788	27982
			16	27982	28063	16	28063	29829
			17	29829	29961	17	29961	30128
			18	30128	30241	18	30241	30330
			19	30330	30370	19	30370	30492
			20	30492	30577	20	30577	30719
			21	30719	30796	21	30796	31246
			22	31246	31323	22	31323	31693
			23	31693	31839	23	31839	31941
			24	31941	32056	24	32056	32278
			25	32278	32401	25	32401	32485
			26	32485	32632	26	32632	32996
			27	32996	33138	27	33138	33933
			28	33933	34028	28	34028	34996
			29	34996	35194	29	35194	35334
			30	35334	35474	30	35474	36563
			31	36563	36679	31	36679	36932
			32	36932	37165	32	37165	37257
			33	37257	37412	33	37412	37519
			34	37519	37841	34	37841	37969
			35	37969	38433			
RTEL1-212 ENST00000508582	482	38171	1	482	657	1	657	1424
			2	1424	1695	2	1695	3489
			3	3489	3687	3	3687	4041
			4	4041	4134	4	4134	4665
			5	4665	4818	5	4818	5020

Transcript ID	Transcript region		Exonic regions			Intron regions		
	start	end	Exon	start	end	intron	start	end
			6	5020	5080	6	5080	8195
			7	8195	8270	7	8270	9660
			8	9660	9744	8	9744	14747
			9	14747	14812	9	14812	16131
			10	16131	16284	10	16284	20336
			11	20336	20374	11	20374	20459
			12	20459	20537	12	20537	22040
			13	22040	22137	13	22137	22855
			14	22855	22910	14	22910	27714
			15	27714	27788	15	27788	27982
			16	27982	28063	16	28063	29829
			17	29829	29961	17	29961	30128
			18	30128	30241	18	30241	30330
			19	30330	30370	19	30370	30492
			20	30492	30577	20	30577	30719
			21	30719	30796	21	30796	31246
			22	31246	31323	22	31323	31693
			23	31693	31839	23	31839	31941
			24	31941	32056	24	32056	32278
			25	32278	32401	25	32401	32485
			26	32485	32632	26	32632	32996
			27	32996	33138	27	33138	33933
			28	33933	34028	28	34028	34996
			29	34996	35194	29	35194	35334
			30	35334	35474	30	35474	36563
			31	36563	36679	31	36679	36932
			32	36932	37165	32	37165	37257
			33	37257	37412	33	37412	37519
			34	37519	37671	34	37671	37969
			35	37969	38171			
RTEL1-201 ENST00000318100	505	38434	1	505	650	1	650	3489
			2	3489	3687	2	3687	4041
			3	4041	4134	3	4134	4737
			4	4737	4818	4	4818	5020
			5	5020	5080	5	5080	8195
			6	8195	8270	6	8270	9660
			7	9660	9744	7	9744	14747
			8	14747	14812	8	14812	16131
			9	16131	16284	9	16284	20336
			10	20336	20374	10	20374	20459
			11	20459	20537	11	20537	22040
			12	22040	22137	12	22137	22855
			13	22855	22910	13	22910	27714
			14	27714	27788	14	27788	27982

Transcript ID	Transcript region		Exonic regions			Intron regions		
	start	end	Exon	start	end	intron	start	end
			15	27982	28063	15	28063	29829
			16	29829	29961	16	29961	30128
			17	30128	30241	17	30241	30330
			18	30330	30370	18	30370	30492
			19	30492	30577	19	30577	30719
			20	30719	30796	20	30796	31246
			21	31246	31323	21	31323	31693
			22	31693	31839	22	31839	31941
			23	31941	32056	23	32056	32278
			24	32278	32401	24	32401	32485
			25	32485	32632	25	32632	32996
			26	32996	33138	26	33138	33933
			27	33933	34028	27	34028	34996
			28	34996	35194	28	35194	35334
			29	35334	35474	29	35474	36563
			30	36563	36679	30	36679	36932
			31	36932	37165	31	37165	37257
			32	37257	37412	32	37412	37519
			33	37519	37671	33	37671	37969
			34	37969	38434			
RTEL1-202 ENST00000356810	551	16284	1	551	650	1	650	1424
			2	1424	1695	2	1695	3489
			3	3489	3687	3	3687	4041
			4	4041	4134	4	4134	4587
			5	4587	4818	5	4818	5020
			6	5020	5080	6	5080	8195
			7	8195	8270	7	8270	9660
			8	9660	9744	8	9744	14747
			9	14747	14812	9	14812	16131
			10	16131	16284			
RTEL1-206 ENST00000425905	30530	33067	1	30530	30577	1	30577	30719
			2	30719	30796	2	30796	31246
			3	31246	31323	3	31323	31941
			4	31941	32056	4	32056	32278
			5	32278	32401	5	32401	32485
			6	32485	32632	6	32632	32996
			7	32996	33067			
RTEL1-214 ENST00000646389	811	3653	1	811	943	1	943	1424
			2	1424	1695	2	1695	3489
			3	3489	3653			

Suitably, the target nucleic acid encodes an RTEL1 protein, in particular mammalian RTEL1, such as human RTEL1 (See for example tables 2 and 3) which provides the pre-mRNA sequences for human and monkey, RTEL1.

In some embodiments, the target nucleic acid is selected from SEQ ID NO: 1 and/or 2 or naturally occurring variants thereof (e.g. sequences encoding a mammalian RTEL1 protein in table 1).

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.

For *in vivo* or *in vitro* application, the oligonucleotide of the invention is typically capable of inhibiting the expression of the RTEL1 target nucleic acid in a cell which is expressing the RTEL1 target nucleic acid. The contiguous sequence of nucleobases of the oligonucleotide of the invention is typically complementary to the RTEL1 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 RNA or DNA, such as a messenger RNA, such as a mature mRNA (e.g. the exonic regions of the transcripts listed in table 1) or a pre-mRNA.

In some embodiments the target nucleic acid is a RNA or DNA which encodes mammalian RTEL1 protein, such as human RTEL1, e.g. the human RTEL1 mRNA sequence, such as that disclosed as SEQ ID NO 1. Further information on exemplary target nucleic acids is provided in tables 2 and 3.

Table 2. Genome and assembly information for RTEL1 across species.

Species	Chr.	Strand	Genomic coordinates		Assembly	ensembl gene_id
			Start	End		
Human	20	fwd	63657810	63696253	GRCh38.p12	ENSG00000258366
Cynomolgus monkey	10	fwd	95853726	95890939	Macaca_fascicularis_5.0	ENSMFAG00000043680

Fwd = forward strand. The genome coordinates provide the pre-mRNA sequence (genomic sequence). The NCBI reference provides the mRNA sequence (cDNA sequence).

Table 3. Sequence details for RTEL1 across species.

Species	RNA type	Length (nt)	SEQ ID NO
Human	premRNA	38444	1
Monkey	premRNA	37214	2

Note SEQ ID NO 2 comprises regions of multiple NNNNs, where the sequencing has been unable to accurately refine the sequence, and a degenerate sequence is therefore included.

For the avoidance of doubt the compounds of the invention are complementary to the actual target sequence and are not therefore degenerate compounds.

In some embodiments, the target nucleic acid is SEQ ID NO 1.
In some embodiments, the target nucleic acid is SEQ ID NO 2.

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 with a nucleobase sequence that is complementary to the contiguous nucleotide sequence of the oligonucleotide of the invention. This region of the target nucleic acid may interchangeably be referred to as the target nucleotide sequence, target sequence or target region. In some embodiments the target sequence is longer than the complementary sequence of a 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.

In some embodiments the target sequence is a sequence selected from the group consisting of a human RTEL1 mRNA exon, such as a RTEL1 human mRNA exon selected from the list in table 1 above.

In some embodiments the target sequence is a sequence selected from the group consisting of a human RTEL1 mRNA intron, such as a RTEL1 human mRNA intron selected from the list in table 1 above.

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

The target sequence to which the oligonucleotide is complementary or hybridizes to generally comprises a contiguous nucleobases sequence of at least 10 nucleotides. The contiguous nucleotide sequence is between 10 to 35 nucleotides, such as 12 to 30, such as 14 to 20, such as 16 to 20 contiguous nucleotides.In one embodiment of the invention the target sequence is selected from the group consisting of

SEQ ID NO: 3-21 as shown in table 4.

Table 4: Target sequences on human RTEL1 premRNA (SEQ ID NO: 1)

SEQ ID NO	Target Sequence	Start on SEQ ID 1	End on SEQ ID 1
3	gaccactgtccttccatg	8294	8311
4	ttcagagattcaagttataataaagctcttcttatattgaggggga	8677	8722
5	aggaatagggttggtttt	9377	9394

SEQ ID NO	Target Sequence	Start on SEQ ID 1	End on SEQ ID 1
6	ccttactacctgtcccg	9667	9683
7	acaattacttgttggatgcc	9722	9741
8	agcttctaacccaaccag	10921	10938
9	tataaacctaaatgtaaaagc	11482	11502
10	ttcaccaaaatttaaagctt	11622	11641
11	ctttgaccagagtatgtaaaattctc	11752	11777
12	aagacgtgttcaaagatt	12868	12885
13	ggacctactgtttttg	13234	13250
14	ggacctactgttttattcc	13550	13568
15	gtcccttctcttcctcctgtag	14725	14746
16	cgtgatctttgacgaagct	14785	14803
17	cgcaaacctttctgga	14874	14889
18	agcctgtgtgtggagtatgagca	33025	33047
19	cgtttccgtgttggtctggg	34571	34590
20	gactacaagggttccgatg	35104	35122
21	agtttgaggaggtctgtatc	35370	35389

In some embodiments, the target sequence is selected from a region shown in Table 5A or 5B.

Target Cell

The term a "target cell" as used herein refers to a cell which is expressing the target nucleic acid. For the therapeutic use of the present invention it is advantageous if the target cell is infected with HBV. In some embodiments the target cell may be *in vivo* or *in vitro*. In some embodiments the target cell is a mammalian cell such as a rodent cell, such as a mouse cell or a rat cell, or a woodchuck cell or a primate cell such as a monkey cell (e.g. a cynomolgus monkey cell) or a human cell.

In preferred embodiments the target cell expresses RTEL1 mRNA, such as the RTEL1 pre-mRNA or RTEL1 mature mRNA. The poly A tail of RTEL1 mRNA is typically disregarded for antisense oligonucleotide targeting.

Further, the target cell may be a hepatocyte. In one embodiment the target cell is HBV infected primary human hepatocytes, either derived from HBV infected individuals or from a HBV infected mouse with a humanized liver (PhoenixBio, PXB-mouse).

In accordance with the present invention, the target cell may be infected with HBV. Further, the target cell may comprise HBV cccDNA. Thus, the target cell preferably comprises RTEL1 mRNA, such as the RTEL1 pre-mRNA or RTEL1 mature mRNA, and HBV cccDNA.

Naturally occurring variant

The term "naturally occurring variant" refers to variants of RTEL1 gene or transcripts which originate from the same genetic loci as the target nucleic acid, but may differ for example, by

virtue of degeneracy of the genetic code causing a multiplicity of codons encoding the same amino acid, or due to alternative splicing of pre-mRNA, or the presence of polymorphisms, such as single nucleotide polymorphisms (SNPs), and allelic variants. Based on the presence of the 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 RTEL1 target nucleic acid, such as a target nucleic acid of SEQ ID NO 1 and/or 2. In some embodiments the naturally occurring variants have at least 99% homology to the human RTEL1 target nucleic acid of SEQ ID NO: 1. In some embodiments the naturally occurring variants are known polymorphisms.

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 RTEL1 when compared to the amount of RTEL1 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).

One type of modulation is the ability of an oligonucleotide to inhibit, down-regulate, reduce, suppress, remove, stop, block, prevent, lessen, lower, avoid or terminate expression of RTEL1, e.g. by degradation of mRNA or blockage of transcription. Another type of modulation is an oligonucleotide's ability to restore, increase or enhance expression of RTEL1, e.g. by repair of splice sites or prevention of splicing or removal or blockage of inhibitory mechanisms such as microRNA repression.

Sugar modifications

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

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

Such modifications include those where the ribose ring structure is modified, *e.g.* by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a 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 (WO2011/017521) or tricyclic

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

Sugar modifications also include modifications made via altering 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.

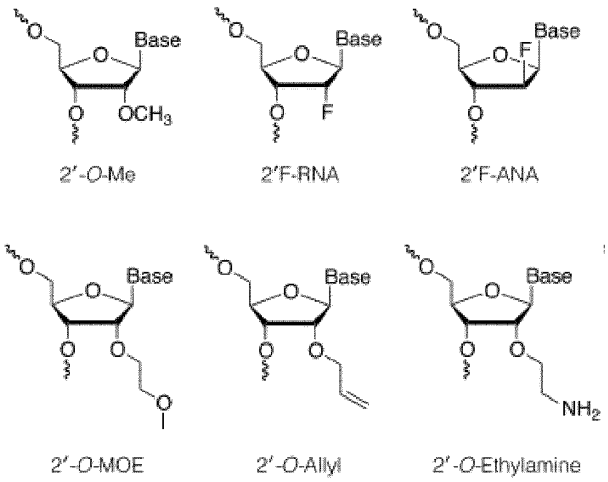
High affinity modified nucleosides

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).

2' sugar modified nucleosides

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

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



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

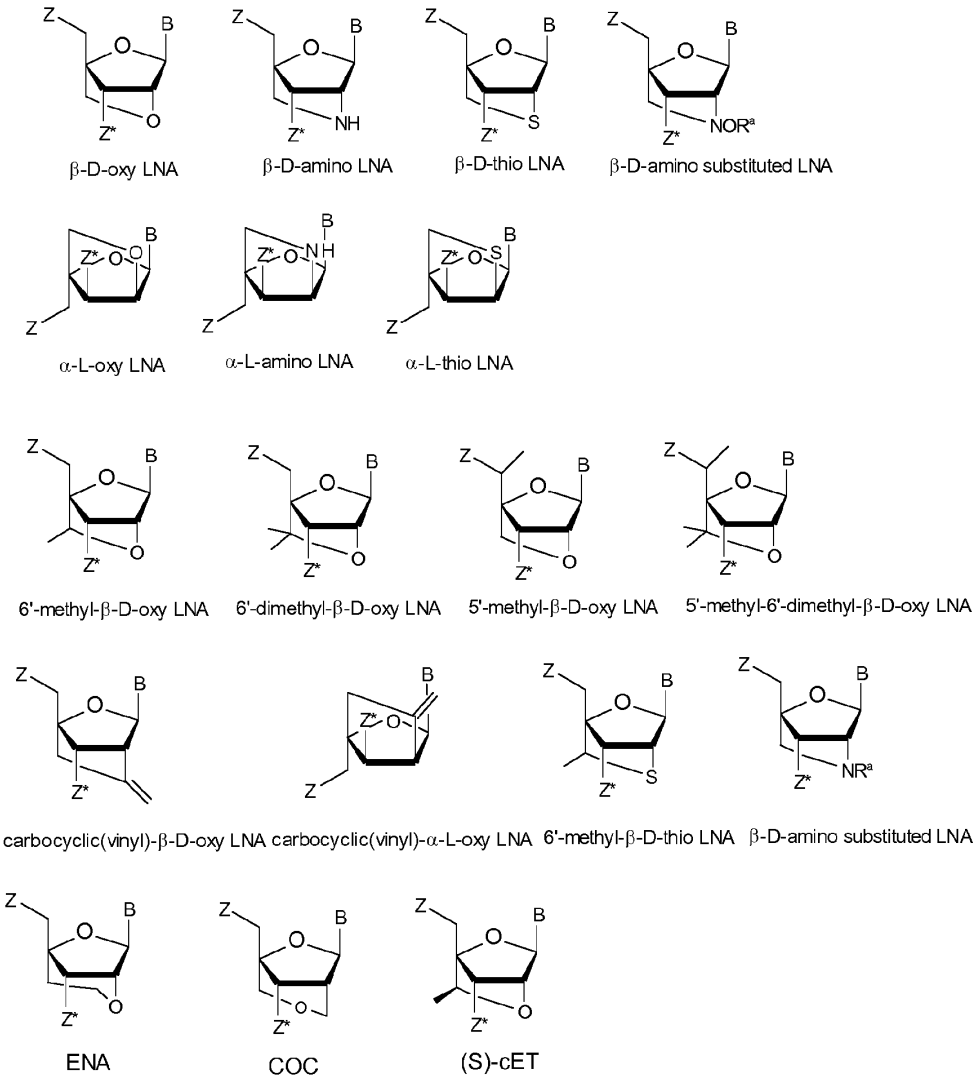
Locked Nucleic Acid Nucleosides (LNA nucleoside)

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

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

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

Scheme 1



Particular LNA nucleosides are beta-D-oxy-LNA, 6'-methyl-beta-D-oxy LNA such as (S)-6'-methyl-beta-D-oxy-LNA (ScET) and ENA.

Pharmaceutically acceptable salts

The term "pharmaceutically acceptable salts" refers to those salts which retain the biological effectiveness and properties of the free bases or free acids, which are not biologically or otherwise undesirable. The salts are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, particularly hydrochloric acid, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic

acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, N-acetylcystein. In addition, these salts may be prepared form addition of an inorganic base or an organic base to the free acid. Salts derived from an inorganic base include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium salts. Salts derived from organic bases include, but are not limited to salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, lysine, arginine, N-ethylpiperidine, piperidine, polyamine resins. The compound of formula (I) can also be present in the form of zwitterions. Particularly preferred pharmaceutically acceptable salts of compounds of formula (I) are the salts of hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid and methanesulfonic acid.

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 WO 01/23613 (hereby incorporated by reference). For use in determining RHase H activity, recombinant human RNase H1 is available from Creative Biomart® (Recombinant Human RNASEH1 fused with His tag expressed in E. coli).

Gapmer

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

the affinity of the oligonucleotide for the target nucleic acid (*i.e.* are affinity enhancing sugar modified nucleosides). In some embodiments, the one or more sugar modified nucleosides in region F and F' are 2' sugar modified nucleosides, such as high affinity 2' sugar modifications, such as independently selected from LNA and 2'-MOE.

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

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

The overall length of the gapmer design F-G-F' may be, for example 12 to 32 nucleosides, such as 13 to 24, such as 14 to 22 nucleosides, Such as from 14 to17, such as 16 to18 nucleosides. By way of example, the gapmer oligonucleotide of the present invention can be represented by the following formulae:

F_{1-8}-G_{5-18}-F'_{1-8}, such as

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

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

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

In an aspect of the invention the antisense oligonucleotide or contiguous nucleotide sequence thereof consists of or comprises a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise or consist of 1- 8 nucleosides, of which 1-4 are 2' sugar modified and defines the 5' and 3' end of the F and F' region, and G is a region between 6 and 18, such as 6 and 16, nucleosides which are capable of recruiting RNaseH. In some embodiments the G region consists of DNA nucleosides.

Regions F, G and F' are further defined below and can be incorporated into the F-G-F' formula.

Gapmer - Region G

Region G (gap region) of the gapmer is a region of nucleosides which enables the oligonucleotide to recruit RNaseH, such as human RNase H1, typically DNA nucleosides. RNaseH is a cellular enzyme which recognizes the duplex between DNA and RNA, and enzymatically cleaves the RNA molecule. Suitably gapmers may have a gap region (G) of at least 5 or 6 contiguous DNA nucleosides, such as 5 – 18 contiguous DNA nucleosides, 5 – 17 contiguous DNA nucleosides, such as 5 – 16 contiguous DNA nucleosides, such as 6 – 15

contiguous DNA nucleosides, such as 7-14 contiguous DNA nucleosides, such as 8 – 12 contiguous DNA nucleotides, such as 8 – 12 contiguous DNA nucleotides in length. The gap region G may, in some embodiments consist of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 contiguous DNA nucleosides. Cytosine (C) DNA in the gap region may in some instances be methylated, such residues are either annotated as 5'-methyl-cytosine (^{me}C or with an e instead of a c). Methylation of cytosine DNA in the gap is advantageous if cg dinucleotides are present in the gap to reduce potential toxicity, the modification does not have significant impact on efficacy of the oligonucleotides. 5' substituted DNA nucleosides, such as 5' methyl DNA nucleoside have been reported for use in DNA gap regions (EP 2 742 136).

In some embodiments the gap region G may consist of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 contiguous phosphorothioate linked DNA nucleosides. In some embodiments, all internucleoside linkages in the gap are phosphorothioate linkages.

Whilst traditional gapmers have a DNA gap region, there are numerous examples of modified nucleosides which allow for RNaseH recruitment when they are used within the gap region. Modified nucleosides which have been reported as being capable of recruiting RNaseH when included within a gap region include, for example, 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. The modified nucleosides used in such gapmers may be nucleosides which adopt a 2' endo (DNA like) structure when introduced into the gap region, *i.e.* modifications which allow for RNaseH recruitment). In some embodiments the DNA Gap region (G) described herein may optionally contain 1 to 3 sugar modified nucleosides which adopt a 2' endo (DNA like) structure when introduced into the gap region.

Gapmer - flanking regions, F and F'

Region F is positioned immediately adjacent to the 5' DNA nucleoside of region G. The 3' most nucleoside of region F is a sugar modified nucleoside, such as a high affinity sugar modified nucleoside, for example a 2' substituted nucleoside, such as a MOE nucleoside, or an LNA nucleoside.

Region F' is positioned immediately adjacent to the 3' DNA nucleoside of region G. The 5' most nucleoside of region F' is a sugar modified nucleoside, such as a high affinity sugar modified nucleoside, for example a 2' substituted nucleoside, such as a MOE nucleoside, or an LNA nucleoside.

Region F is 1 – 8 contiguous nucleotides in length, such as 2-6, such as 3-4 contiguous nucleotides in length. Advantageously the 5' most nucleoside of region F is a sugar modified nucleoside. In some embodiments the two 5' most nucleoside of region F are sugar modified nucleoside. In some embodiments the 5' most nucleoside of region F is an LNA nucleoside. In some embodiments the two 5' most nucleoside of region F are LNA nucleosides. In some embodiments the two 5' most nucleoside of region F are 2' substituted nucleoside nucleosides, such as two 3' MOE nucleosides. In some embodiments the 5' most nucleoside of region F is a 2' substituted nucleoside, such as a MOE nucleoside.

Region F' is 2 – 8 contiguous nucleotides in length, such as 3-6, such as 4-5 contiguous nucleotides in length. Advantageously, embodiments the 3' most nucleoside of region F' is a sugar modified nucleoside. In some embodiments the two 3' most nucleoside of region F' are sugar modified nucleoside. In some embodiments the two 3' most nucleoside of region F' are LNA nucleosides. In some embodiments the 3' most nucleoside of region F' is an LNA nucleoside. In some embodiments the two 3' most nucleoside of region F' are 2' substituted nucleoside nucleosides, such as two 3' MOE nucleosides. In some embodiments the 3' most nucleoside of region F' is a 2' substituted nucleoside, such as a MOE nucleoside.

It should be noted that when the length of region F or F' is one, it is advantageously an LNA nucleoside.

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

In some embodiments, region F and F' independently comprises both LNA and a 2' substituted modified nucleosides (mixed wing design).

In some embodiments, region F and F' consists of only one type of sugar modified nucleosides, such as only MOE or only beta-D-oxy LNA or only ScET. Such designs are also termed uniform flanks or uniform gapmer design.

In some embodiments, all the nucleosides of region F or F', or F and F' are LNA nucleosides, such as independently selected from beta-D-oxy LNA, ENA or ScET nucleosides. In some embodiments region F consists of 1-5, such as 2-4, such as 3-4 such as 1, 2, 3, 4 or 5 contiguous LNA nucleosides. In some embodiments, all the nucleosides of region F and F' are beta-D-oxy LNA nucleosides.

In some embodiments, all the nucleosides of region F or F', or F and F' are 2' substituted nucleosides, such as OMe or MOE nucleosides. In some embodiments region F consists of 1,

2, 3, 4, 5, 6, 7, or 8 contiguous OMe or MOE nucleosides. In some embodiments only one of the flanking regions can consist of 2' substituted nucleosides, such as OMe or MOE nucleosides. In some embodiments it is the 5' (F) flanking region that consists 2' substituted nucleosides, such as OMe or MOE nucleosides whereas the 3' (F') flanking region comprises at least one LNA nucleoside, such as beta-D-oxy LNA nucleosides or cET nucleosides. In some embodiments it is the 3' (F') flanking region that consists 2' substituted nucleosides, such as OMe or MOE nucleosides whereas the 5' (F) flanking region comprises at least one LNA nucleoside, such as beta-D-oxy LNA nucleosides or cET nucleosides.

In some embodiments, all the modified nucleosides of region F and F' are LNA nucleosides, such as independently selected from beta-D-oxy LNA, ENA or ScET nucleosides, wherein region F or F', or F and F' may optionally comprise DNA nucleosides (an alternating flank, see definition of these for more details). In some embodiments, all the modified nucleosides of region F and F' are beta-D-oxy LNA nucleosides, wherein region F or F', or F and F' may optionally comprise DNA nucleosides (an alternating flank, see definition of these for more details).

In some embodiments the 5' most and the 3' most nucleosides of region F and F' are LNA nucleosides, such as beta-D-oxy LNA nucleosides or ScET nucleosides.

In some embodiments, the internucleoside linkage between region F and region G is a phosphorothioate internucleoside linkage. In some embodiments, the internucleoside linkage between region F' and region G is a phosphorothioate internucleoside linkage. In some embodiments, the internucleoside linkages between the nucleosides of region F or F', F and F' are phosphorothioate internucleoside linkages.

LNA Gapmer

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

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

MOE Gapmers

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

Region D' or D'' in an oligonucleotide

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

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

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

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

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

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

F-G-F'; in particular F_{1-8}-G_{5-16}-F'_{2-8}

D'-F-G-F', in particular D'_{1-3}-F_{1-8}-G_{5-16}-F'_{2-8}

F-G-F'-D'', in particular F_{1-8}-G_{5-16}-F'_{2-8}-D''_{1-3}

D'-F-G-F'-D'', in particular D'_{1-3}- F_{1-8}-G_{5-16}-F'_{2-8}-D''_{1-3}

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

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. At 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 WO2013/033230 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 ASGPR, see for example WO 2014/076196, WO 2014/207232 and WO 2014/179620 (hereby incorporated by reference). Such conjugates serve to enhance uptake of the oligonucleotide to the liver while reducing its presence in the kidney, thereby increasing the liver/kidney ratio of a conjugated oligonucleotide compared to the unconjugated version of the same oligonucleotide.

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 a preferred embodiment 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 a preferred embodiment 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. Prophylactic can be understood as preventing an HBV infection from turning into a chronic HBV infection or the prevention of severe liver diseases such as liver cirrhosis and hepatocellular carcinoma caused by a chronic HBV infection.

Prevention

Herein the term "preventing", "prevention" or "prevents" relates to a prophylactic treatment, i.e. to a measure or procedure the purpose of which is to prevent, rather than to cure a disease. Prevention means that a desired pharmacological and/or physiological effect is obtained that is prophylactic in terms of completely or partially preventing a disease or symptom thereof. Accordingly, herein "preventing a HBV infection" includes preventing a HBV infection from occurring in a subject, and preventing the occurrence of symptoms of a HBV infection. In the present invention in particular the prevention of HBV infection in children from HBV infected mothers are contemplated. Also contemplated is the prevention of an acute HBV infection turning into a chronic HBV infection.

Patient

For the purposes of the present invention the "subject" (or "patient") may be a vertebrate. In context of the present invention, the term "subject" includes both humans and other animals, particularly mammals, and other organisms. Thus, the herein provided means and methods are applicable to both human therapy and veterinary applications. Accordingly, herein the subject may be an animal such as a mouse, rat, hamster, rabbit, guinea pig, ferret, cat, dog, chicken, sheep, bovine species, horse, camel, or primate. Preferably, the subject is a mammal. More preferably the subject is human.

DETAILED DESCRIPTION OF THE INVENTION

HBV cccDNA in infected hepatocytes is responsible for persistent chronic infection and reactivation, being the template for all viral subgenomic transcripts and pre-genomic RNA (pgRNA) to ensure both newly synthesized viral progeny and cccDNA pool replenishment via intracellular nucleocapsid recycling. In the context of the present invention it was for the first time shown that RTEL1 is associated with cccDNA stability. This knowledge allows for the opportunity to destabilize cccDNA in HBV infected subjects which in turn opens the opportunity for a complete cure of chronically infected HBV patients.

One aspect of the present invention is a RTEL1 inhibitor for use in the treatment and/or prevention of Hepatitis B virus (HBV) infection, in particular a chronic HBV infection.

The RTEL1 inhibitor can for example be a small molecule that specifically binds to RTEL1 protein, wherein said inhibitor prevents or reduces binding of RTEL1 protein to cccDNA.

An embodiment of the invention is a RTEL1 inhibitor which is capable of reducing cccDNA and/or pgRNA in an infected cell, such as an HBV infected cell.

In a further embodiment, the RTEL1 inhibitor is capable of reducing HBsAg and/or HBeAg *in vivo* in an HBV infected individual.

The Oligonucleotides of the Invention

Therapeutic oligonucleotides are potentially excellent RTEL1 inhibitors since they can target the RTEL1 transcript and promote its degradation either via the RNA interference pathway or via RNaseH cleavage. Alternatively, oligonucleotides such as aptamers can also act as inhibitors of RTEL1 protein interactions.

One aspect of the present invention is a RTEL1 targeting oligonucleotide for use in treatment and/or prevention of Hepatitis B virus (HBV) infection. Such an oligonucleotide can be selected from the group consisting of single stranded antisense oligonucleotide; siRNA molecule; or shRNA molecule.

The present section describes novel oligonucleotides suitable for use in treatment and/or prevention of Hepatitis B virus (HBV) infection.

The oligonucleotides of the present invention are capable of inhibiting expression of RTEL1 *in vitro* and *in vivo*. The inhibition is achieved by hybridizing an oligonucleotide to a target nucleic acid encoding RTEL1 or which is involved in the regulation of RTEL1. The target nucleic acid may be a mammalian RTEL1 sequence, such as the sequence of SEQ ID NO: 1 and/or 2

In some embodiments the 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%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% inhibition compared to the normal expression level of the target. In some embodiments, the oligonucleotide of the invention may be capable of inhibiting expression levels of RTEL1 mRNA by at least 60% or 70% *in vitro* using 10 µM in PXB-PHH cells. In some embodiments, the oligonucleotide of the invention may be capable of inhibiting expression levels of RTEL1 protein by at least 50% *in vitro* using 10µM PXB-PHH cells, this range of target reduction is advantageous in terms of selecting nucleic acid molecules with good correlation to the cccDNA reduction. Suitably, the examples provide assays which may be used to measure RTEL1 RNA or protein inhibition (e.g. example 1). The target inhibition 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 inhibition of RTEL1 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' sugar modified nucleosides, including LNA, present within the oligonucleotide sequence.

An aspect of the present invention relates to an oligonucleotides of 12 to 60 nucleotides in length, which comprises a contiguous nucleotide sequence of at least 10 nucleotides in length, such as at least 12 to 30 nucleotides in length, which is at least 95% complementary, such as fully complementary, to a mammalian RTEL1 target nucleic acid, in particular a human RTEL1 nucleic acid. These oligonucleotides are capable of inhibiting the expression of RTEL1.

An aspect of the invention relates to an oligonucleotide according to the invention which is an antisense oligonucleotide of 12 to 30 nucleotides in length, comprising a contiguous nucleotide sequence of at least 10 nucleotides, such as 10 to 30 nucleotides in length which is at least 90% complementary, such as fully complementary, to a mammalian RTEL1.

A further aspect of the present invention relates to an oligonucleotide according to the invention comprising a contiguous nucleotide sequence of 12 to 20, such as 15 to 22, nucleotides in length with at least 90% complementarity, such as fully complementary, to the target nucleic acid of SEQ ID NO: 1.

In some embodiments, the oligonucleotide comprises a contiguous sequence of 10 to 30 nucleotides in length, which is at least 90% complementary, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or 100% complementary with a region of the target nucleic acid or a target sequence.

It is advantageous if the oligonucleotide 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 antisense oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid of SEQ ID NO: 1.

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

In some embodiments, the oligonucleotide comprises a contiguous nucleotide sequence of 15 to 22 nucleotides in length with at least 90% complementary, such as 100% complementarity, to

a corresponding target sequence present in SEQ ID NO: 1, wherein the target sequence is selected from the group consisting of SEQ ID NO: 3 to 21 (table 4) or region 1A to 959A in Table 5A.

Table 5A: Regions of SEQ ID NO 1 which may be targeted using an oligonucleotide of the invention

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
1A	1594	1623	30	321A	14295	14320	26	641A	24499	24514	16
2A	1636	1685	50	322A	14308	14352	45	642A	24560	24575	16
3A	1687	1708	22	323A	14323	14352	30	643A	24621	24636	16
4A	1773	1794	22	324A	14326	14352	27	644A	24682	24697	16
5A	1810	1824	15	325A	14334	14351	18	645A	24717	24753	37
6A	1824	1870	47	326A	14340	14364	25	646A	24842	24857	16
7A	2890	2907	18	327A	14340	14359	20	647A	24902	24918	17
8A	2931	2952	22	328A	14348	14362	15	648A	24932	24962	31
9A	2984	2999	16	329A	14374	14406	33	649A	25018	25056	39
10A	3002	3021	20	330A	14416	14446	31	650A	25160	25176	17
11A	3026	3081	56	331A	14462	14489	28	651A	25219	25251	33
12A	3083	3100	18	332A	14505	14521	17	652A	25259	25278	20
13A	3175	3202	28	333A	14523	14541	19	653A	25332	25346	15
14A	3205	3228	24	334A	14577	14598	22	654A	25363	25379	17
15A	3253	3270	18	335A	14725	14762	38	655A	25367	25383	17
16A	3289	3320	32	336A	14764	14781	18	656A	25405	25435	31
17A	3353	3368	16	337A	14783	14808	26	657A	25405	25436	32
18A	3374	3388	15	338A	14874	14905	32	658A	25407	25425	19
19A	3443	3472	30	339A	14974	15030	57	659A	25410	25436	27
20A	3525	3547	23	340A	15032	15059	28	660A	25418	25435	18
21A	3549	3581	33	341A	15084	15098	15	661A	25475	25495	21
22A	3584	3612	29	342A	15087	15106	20	662A	25502	25518	17
23A	3648	3675	28	343A	15108	15126	19	663A	25559	25582	24
24A	3681	3708	28	344A	15147	15180	34	664A	25596	25640	45
25A	3716	3731	16	345A	15183	15202	20	665A	25671	25688	18
26A	3733	3755	23	346A	15230	15247	18	666A	25796	25816	21
27A	3757	3775	19	347A	15255	15270	16	667A	25818	25832	15
28A	3797	3811	15	348A	15272	15298	27	668A	25834	25857	24
29A	3823	3844	22	349A	15288	15312	25	669A	25867	25881	15
30A	3846	3881	36	350A	15319	15349	31	670A	25928	25943	16
31A	3922	3955	34	351A	15359	15373	15	671A	25986	26001	16
32A	3957	3975	19	352A	15370	15385	16	672A	26014	26037	24
33A	4016	4036	21	353A	15382	15400	19	673A	26187	26210	24
34A	4038	4053	16	354A	15388	15408	21	674A	26212	26228	17
35A	4067	4098	32	355A	15410	15435	26	675A	26268	26286	19
36A	4100	4150	51	356A	15435	15452	18	676A	26300	26319	20
37A	4271	4290	20	357A	15456	15498	43	677A	26359	26394	36
38A	4312	4330	19	358A	15459	15474	16	678A	26396	26426	31

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
39A	4345	4362	18	359A	15479	15498	20	679A	26465	26482	18
40A	4364	4379	16	360A	15486	15502	17	680A	26505	26529	25
41A	4420	4448	29	361A	15528	15543	16	681A	26547	26565	19
42A	4480	4512	33	362A	15543	15561	19	682A	26576	26600	25
43A	4527	4552	26	363A	15572	15591	20	683A	26588	26603	16
44A	4651	4674	24	364A	15623	15642	20	684A	26588	26606	19
45A	4733	4784	52	365A	15646	15660	15	685A	26609	26624	16
46A	4786	4811	26	366A	15662	15690	29	686A	26615	26638	24
47A	4830	4848	19	367A	15702	15740	39	687A	26615	26642	28
48A	4857	4874	18	368A	15740	15754	15	688A	26632	26669	38
49A	4915	4937	23	369A	15743	15773	31	689A	26649	26669	21
50A	4944	4966	23	370A	15746	15761	16	690A	26658	26672	15
51A	4969	4983	15	371A	15764	15789	26	691A	26695	26716	22
52A	4995	5015	21	372A	15777	15803	27	692A	26706	26725	20
53A	5017	5033	17	373A	15791	15816	26	693A	26713	26735	23
54A	5035	5075	41	374A	15832	15848	17	694A	26715	26733	19
55A	5109	5136	28	375A	15855	15873	19	695A	26737	26768	32
56A	5155	5173	19	376A	15870	15890	21	696A	26755	26770	16
57A	5175	5191	17	377A	15878	15908	31	697A	26756	26789	34
58A	5209	5225	17	378A	15880	15898	19	698A	26759	26789	31
59A	5241	5261	21	379A	15891	15908	18	699A	26787	26813	27
60A	5263	5287	25	380A	15896	15916	21	700A	26795	26812	18
61A	5299	5346	48	381A	15911	15929	19	701A	26815	26829	15
62A	5394	5409	16	382A	15911	15930	20	702A	26861	26880	20
63A	5428	5447	20	383A	15947	15963	17	703A	26862	26882	21
64A	5472	5514	43	384A	16023	16056	34	704A	26865	26883	19
65A	5579	5606	28	385A	16068	16091	24	705A	26868	26883	16
66A	5617	5632	16	386A	16083	16097	15	706A	26870	26885	16
67A	5690	5711	22	387A	16129	16150	22	707A	26871	26892	22
68A	5713	5754	42	388A	16170	16229	60	708A	26880	26898	19
69A	5727	5741	15	389A	16245	16265	21	709A	26889	26910	22
70A	5756	5770	15	390A	16269	16300	32	710A	26908	26924	17
71A	5812	5854	43	391A	16308	16334	27	711A	26917	26939	23
72A	5871	5886	16	392A	16336	16356	21	712A	26948	26962	15
73A	5896	5932	37	393A	16336	16358	23	713A	26955	26973	19
74A	5992	6009	18	394A	16360	16391	32	714A	27097	27113	17
75A	6011	6038	28	395A	16360	16397	38	715A	27101	27128	28
76A	6057	6072	16	396A	16425	16465	41	716A	27112	27127	16
77A	6101	6122	22	397A	16472	16493	22	717A	27116	27183	68
78A	6127	6165	39	398A	16498	16515	18	718A	27133	27165	33
79A	6187	6203	17	399A	16545	16562	18	719A	27188	27209	22
80A	6210	6227	18	400A	16564	16586	23	720A	27218	27232	15
81A	6243	6261	19	401A	16588	16613	26	721A	27218	27234	17
82A	6271	6299	29	402A	16615	16639	25	722A	27235	27253	19
83A	6378	6393	16	403A	16651	16667	17	723A	27237	27253	17

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
84A	6468	6496	29	404A	16669	16695	27	724A	27237	27251	15
85A	6498	6526	29	405A	16696	16716	21	725A	27237	27252	16
86A	6558	6574	17	406A	16704	16718	15	726A	27238	27254	17
87A	6720	6737	18	407A	16732	16760	29	727A	27238	27252	15
88A	6735	6749	15	408A	16737	16760	24	728A	27238	27253	16
89A	6785	6825	41	409A	16849	16865	17	729A	27239	27255	17
90A	6879	6894	16	410A	16853	16868	16	730A	27239	27253	15
91A	6921	6959	39	411A	16853	16867	15	731A	27239	27254	16
92A	6995	7060	66	412A	16882	16897	16	732A	27240	27254	15
93A	7062	7084	23	413A	16885	16902	18	733A	27240	27255	16
94A	7114	7146	33	414A	16914	16938	25	734A	27241	27255	15
95A	7155	7186	32	415A	16942	16956	15	735A	27269	27320	52
96A	7188	7203	16	416A	16990	17004	15	736A	27281	27297	17
97A	7230	7267	38	417A	17016	17042	27	737A	27286	27307	22
98A	7281	7299	19	418A	17097	17115	19	738A	27340	27358	19
99A	7291	7316	26	419A	17105	17119	15	739A	27360	27404	45
100A	7344	7369	26	420A	17105	17126	22	740A	27411	27438	28
101A	7375	7391	17	421A	17114	17128	15	741A	27458	27474	17
102A	7400	7414	15	422A	17133	17158	26	742A	27531	27572	42
103A	7427	7441	15	423A	17160	17174	15	743A	27575	27600	26
104A	7437	7453	17	424A	17162	17178	17	744A	27602	27616	15
105A	7449	7463	15	425A	17166	17183	18	745A	27618	27637	20
106A	7467	7481	15	426A	17178	17199	22	746A	27670	27684	15
107A	7500	7518	19	427A	17187	17201	15	747A	27707	27721	15
108A	7532	7546	15	428A	17203	17223	21	748A	27723	27747	25
109A	7573	7587	15	429A	17213	17230	18	749A	27772	27816	45
110A	7607	7621	15	430A	17213	17235	23	750A	27772	27790	19
111A	7659	7685	27	431A	17237	17259	23	751A	27829	27847	19
112A	7732	7767	36	432A	17249	17279	31	752A	27850	27868	19
113A	7779	7793	15	433A	17267	17285	19	753A	27870	27905	36
114A	7844	7882	39	434A	17273	17288	16	754A	27927	27942	16
115A	7888	7910	23	435A	17297	17315	19	755A	27963	27987	25
116A	7966	7980	15	436A	17300	17315	16	756A	27989	28083	95
117A	8033	8047	15	437A	17302	17317	16	757A	28085	28103	19
118A	8049	8063	15	438A	17303	17324	22	758A	28120	28138	19
119A	8160	8178	19	439A	17312	17330	19	759A	28167	28188	22
120A	8180	8195	16	440A	17346	17375	30	760A	28190	28207	18
121A	8216	8237	22	441A	17349	17375	27	761A	28209	28231	23
122A	8239	8341	103	442A	17357	17374	18	762A	28234	28250	17
123A	8357	8373	17	443A	17363	17382	20	763A	28260	28303	44
124A	8415	8430	16	444A	17371	17385	15	764A	28427	28444	18
125A	8449	8465	17	445A	17420	17447	28	765A	28446	28462	17
126A	8541	8560	20	446A	17524	17551	28	766A	28464	28484	21
127A	8574	8596	23	447A	17562	17580	19	767A	28503	28519	17
128A	8677	8703	27	448A	17622	17636	15	768A	28521	28536	16

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
129A	8705	8722	18	449A	17702	17734	33	769A	28538	28565	28
130A	8748	8763	16	450A	17730	17745	16	770A	28595	28612	18
131A	8792	8807	16	451A	17733	17755	23	771A	28694	28709	16
132A	8796	8811	16	452A	17743	17758	16	772A	28701	28715	15
133A	8799	8818	20	453A	17810	17824	15	773A	28715	28751	37
134A	8807	8821	15	454A	17900	17940	41	774A	28801	28825	25
135A	8814	8828	15	455A	17942	17968	27	775A	28832	28846	15
136A	8837	8853	17	456A	17988	18002	15	776A	28846	28870	25
137A	8837	8851	15	457A	18007	18024	18	777A	28878	28893	16
138A	8841	8858	18	458A	18026	18042	17	778A	28895	28911	17
139A	8884	8911	28	459A	18044	18059	16	779A	28938	28961	24
140A	8918	8937	20	460A	18126	18159	34	780A	29010	29025	16
141A	8918	8933	16	461A	18179	18205	27	781A	29057	29072	16
142A	8969	9005	37	462A	18237	18253	17	782A	29119	29134	16
143A	8969	8999	31	463A	18272	18290	19	783A	29179	29193	15
144A	8969	9000	32	464A	18299	18314	16	784A	29235	29256	22
145A	8971	8989	19	465A	18328	18344	17	785A	29330	29349	20
146A	8974	9000	27	466A	18329	18344	16	786A	29367	29381	15
147A	8982	8999	18	467A	18347	18361	15	787A	29530	29556	27
148A	9024	9042	19	468A	18380	18402	23	788A	29587	29605	19
149A	9026	9042	17	469A	18385	18399	15	789A	29652	29692	41
150A	9026	9040	15	470A	18406	18421	16	790A	29695	29710	16
151A	9026	9041	16	471A	18446	18473	28	791A	29722	29742	21
152A	9027	9043	17	472A	18527	18543	17	792A	29743	29768	26
153A	9027	9041	15	473A	18554	18569	16	793A	29770	29797	28
154A	9027	9042	16	474A	18631	18645	15	794A	29818	29836	19
155A	9028	9044	17	475A	18673	18693	21	795A	29838	29873	36
156A	9028	9042	15	476A	18746	18765	20	796A	29875	29946	72
157A	9028	9043	16	477A	18797	18824	28	797A	29948	29983	36
158A	9029	9045	17	478A	18842	18860	19	798A	30028	30048	21
159A	9029	9043	15	479A	18872	18892	21	799A	30046	30060	15
160A	9029	9044	16	480A	18901	18915	15	800A	30051	30067	17
161A	9030	9046	17	481A	18901	18940	40	801A	30069	30090	22
162A	9030	9044	15	482A	18942	18976	35	802A	30093	30107	15
163A	9030	9045	16	483A	18951	18976	26	803A	30116	30136	21
164A	9031	9047	17	484A	18971	18994	24	804A	30138	30202	65
165A	9031	9045	15	485A	18998	19016	19	805A	30220	30262	43
166A	9031	9046	16	486A	19020	19039	20	806A	30303	30320	18
167A	9032	9048	17	487A	19027	19043	17	807A	30349	30372	24
168A	9032	9046	15	488A	19027	19050	24	808A	30387	30418	32
169A	9032	9047	16	489A	19088	19102	15	809A	30417	30441	25
170A	9033	9047	15	490A	19109	19129	21	810A	30476	30516	41
171A	9033	9048	16	491A	19128	19145	18	811A	30524	30576	53
172A	9034	9048	15	492A	19240	19258	19	812A	30602	30628	27
173A	9038	9052	15	493A	19280	19366	87	813A	30658	30680	23

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
174A	9046	9064	19	494A	19372	19387	16	814A	30682	30747	66
175A	9069	9090	22	495A	19422	19444	23	815A	30749	30799	51
176A	9074	9089	16	496A	19446	19462	17	816A	30801	30821	21
177A	9078	9093	16	497A	19489	19506	18	817A	30823	30844	22
178A	9095	9110	16	498A	19546	19571	26	818A	30908	30922	15
179A	9101	9124	24	499A	19597	19615	19	819A	30924	30980	57
180A	9126	9161	36	500A	19624	19648	25	820A	31027	31045	19
181A	9135	9155	21	501A	19680	19695	16	821A	31047	31080	34
182A	9147	9162	16	502A	19713	19727	15	822A	31086	31113	28
183A	9163	9186	24	503A	19775	19792	18	823A	31128	31146	19
184A	9203	9222	20	504A	19789	19803	15	824A	31150	31164	15
185A	9210	9253	44	505A	19811	19825	15	825A	31166	31193	28
186A	9210	9230	21	506A	19838	19862	25	826A	31229	31271	43
187A	9223	9254	32	507A	20241	20257	17	827A	31276	31310	35
188A	9241	9256	16	508A	20259	20290	32	828A	31312	31333	22
189A	9258	9272	15	509A	20309	20381	73	829A	31400	31417	18
190A	9266	9303	38	510A	20404	20419	16	830A	31419	31433	15
191A	9291	9308	18	511A	20470	20492	23	831A	31456	31470	15
192A	9311	9329	19	512A	20495	20557	63	832A	31517	31569	53
193A	9370	9394	25	513A	20593	20609	17	833A	31578	31599	22
194A	9406	9420	15	514A	20626	20646	21	834A	31661	31689	29
195A	9569	9591	23	515A	20648	20669	22	835A	31706	31739	34
196A	9653	9708	56	516A	20683	20699	17	836A	31741	31763	23
197A	9712	9758	47	517A	20718	20735	18	837A	31765	31805	41
198A	9771	9788	18	518A	20749	20765	17	838A	31807	31855	49
199A	9812	9829	18	519A	20751	20765	15	839A	31819	31834	16
200A	9844	9862	19	520A	20769	20785	17	840A	31851	31866	16
201A	9872	9917	46	521A	20773	20791	19	841A	31857	31872	16
202A	9958	9983	26	522A	20777	20798	22	842A	31938	31984	47
203A	9985	10002	18	523A	20779	20798	20	843A	31986	32032	47
204A	10017	10054	38	524A	20779	20797	19	844A	32034	32071	38
205A	10113	10132	20	525A	20798	20819	22	845A	32082	32097	16
206A	10113	10130	18	526A	20800	20819	20	846A	32124	32151	28
207A	10120	10137	18	527A	20800	20818	19	847A	32197	32216	20
208A	10183	10204	22	528A	20819	20840	22	848A	32233	32262	30
209A	10185	10204	20	529A	20819	20853	35	849A	32264	32289	26
210A	10185	10202	18	530A	20821	20840	20	850A	32306	32325	20
211A	10192	10209	18	531A	20821	20853	33	851A	32357	32408	52
212A	10192	10210	19	532A	20821	20839	19	852A	32410	32459	50
213A	10231	10251	21	533A	20833	20851	19	853A	32474	32492	19
214A	10236	10251	16	534A	20833	20855	23	854A	32494	32508	15
215A	10320	10337	18	535A	20841	20864	24	855A	32527	32543	17
216A	10338	10353	16	536A	20855	20869	15	856A	32545	32560	16
217A	10397	10415	19	537A	20866	20895	30	857A	32570	32636	67
218A	10563	10584	22	538A	20881	20902	22	858A	32697	32713	17

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
219A	10591	10607	17	539A	20881	20915	35	859A	32744	32765	22
220A	10703	10723	21	540A	20883	20902	20	860A	32801	32823	23
221A	10766	10784	19	541A	20883	20915	33	861A	32865	32892	28
222A	10805	10822	18	542A	20883	20901	19	862A	32944	32959	16
223A	10844	10870	27	543A	20895	20913	19	863A	32962	32985	24
224A	10873	10893	21	544A	20895	20917	23	864A	32998	33104	107
225A	10895	10913	19	545A	20903	20926	24	865A	33126	33140	15
226A	10915	10942	28	546A	20917	20931	15	866A	33142	33194	53
227A	10961	10975	15	547A	20928	20946	19	867A	33213	33252	40
228A	10983	10999	17	548A	20937	20951	15	868A	33277	33298	22
229A	11001	11015	15	549A	20955	20973	19	869A	33318	33365	48
230A	11021	11035	15	550A	20975	20993	19	870A	33375	33390	16
231A	11033	11059	27	551A	20975	20997	23	871A	33402	33417	16
232A	11061	11082	22	552A	20983	21004	22	872A	33419	33443	25
233A	11084	11104	21	553A	20983	21017	35	873A	33456	33488	33
234A	11124	11154	31	554A	20985	21004	20	874A	33509	33542	34
235A	11156	11170	15	555A	20985	21017	33	875A	33562	33583	22
236A	11175	11192	18	556A	20985	21003	19	876A	33607	33622	16
237A	11227	11260	34	557A	20997	21015	19	877A	33655	33700	46
238A	11239	11254	16	558A	20997	21019	23	878A	33704	33720	17
239A	11274	11302	29	559A	21005	21028	24	879A	33735	33753	19
240A	11290	11305	16	560A	21019	21033	15	880A	33755	33780	26
241A	11299	11317	19	561A	21030	21048	19	881A	33806	33820	15
242A	11305	11329	25	562A	21030	21052	23	882A	33829	33845	17
243A	11344	11361	18	563A	21057	21075	19	883A	33916	33962	47
244A	11372	11400	29	564A	21057	21079	23	884A	33964	33982	19
245A	11402	11416	15	565A	21067	21085	19	885A	33989	34026	38
246A	11418	11445	28	566A	21088	21118	31	886A	34028	34072	45
247A	11457	11471	15	567A	21127	21153	27	887A	34089	34104	16
248A	11482	11511	30	568A	21155	21169	15	888A	34113	34130	18
249A	11550	11566	17	569A	21155	21180	26	889A	34141	34158	18
250A	11622	11645	24	570A	21205	21220	16	890A	34281	34309	29
251A	11722	11737	16	571A	21222	21283	62	891A	34377	34407	31
252A	11745	11777	33	572A	21347	21370	24	892A	34423	34498	76
253A	11824	11844	21	573A	21431	21445	15	893A	34507	34521	15
254A	11824	11840	17	574A	21463	21487	25	894A	34524	34545	22
255A	12622	12638	17	575A	21489	21518	30	895A	34552	34596	45
256A	12673	12691	19	576A	21520	21535	16	896A	34688	34703	16
257A	12693	12724	32	577A	21551	21573	23	897A	34742	34759	18
258A	12747	12763	17	578A	21574	21591	18	898A	34770	34798	29
259A	12783	12806	24	579A	21595	21618	24	899A	34860	34882	23
260A	12818	12837	20	580A	21622	21641	20	900A	34919	34938	20
261A	12856	12885	30	581A	21664	21678	15	901A	34950	34988	39
262A	12890	12912	23	582A	21758	21789	32	902A	34990	35012	23
263A	12914	12945	32	583A	21799	21816	18	903A	35022	35048	27

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
264A	12984	13016	33	584A	21820	21852	33	904A	35063	35182	120
265A	13001	13016	16	585A	21865	21882	18	905A	35184	35210	27
266A	13004	13022	19	586A	21890	21905	16	906A	35222	35241	20
267A	13004	13021	18	587A	21917	21932	16	907A	35245	35275	31
268A	13014	13034	21	588A	21956	21976	21	908A	35277	35297	21
269A	13166	13191	26	589A	21975	21993	19	909A	35319	35355	37
270A	13228	13251	24	590A	22007	22035	29	910A	35367	35397	31
271A	13283	13319	37	591A	22014	22034	21	911A	35433	35457	25
272A	13295	13310	16	592A	22036	22051	16	912A	35461	35486	26
273A	13317	13332	16	593A	22036	22068	33	913A	35490	35509	20
274A	13354	13381	28	594A	22070	22132	63	914A	35546	35560	15
275A	13383	13430	48	595A	22174	22203	30	915A	35573	35593	21
276A	13446	13468	23	596A	22205	22219	15	916A	35597	35613	17
277A	13449	13468	20	597A	22229	22254	26	917A	35968	35999	32
278A	13471	13487	17	598A	22276	22299	24	918A	35997	36011	15
279A	13500	13518	19	599A	22309	22353	45	919A	36037	36051	15
280A	13547	13568	22	600A	22359	22373	15	920A	36097	36118	22
281A	13631	13650	20	601A	22385	22403	19	921A	36117	36132	16
282A	13663	13679	17	602A	22443	22460	18	922A	36278	36295	18
283A	13680	13694	15	603A	22462	22490	29	923A	36350	36364	15
284A	13744	13764	21	604A	22499	22520	22	924A	36366	36392	27
285A	13766	13803	38	605A	22601	22623	23	925A	36433	36458	26
286A	13768	13803	36	606A	22646	22661	16	926A	36460	36483	24
287A	13777	13797	21	607A	22663	22682	20	927A	36530	36547	18
288A	13789	13804	16	608A	22713	22735	23	928A	36549	36566	18
289A	13804	13827	24	609A	22737	22772	36	929A	36600	36625	26
290A	13823	13844	22	610A	22793	22826	34	930A	36627	36665	39
291A	13840	13854	15	611A	22851	22903	53	931A	36759	36774	16
292A	13840	13855	16	612A	22905	22928	24	932A	36765	36782	18
293A	13841	13855	15	613A	22934	22985	52	933A	36815	36850	36
294A	13851	13874	24	614A	23071	23089	19	934A	36873	36891	19
295A	13851	13873	23	615A	23094	23121	28	935A	36894	36934	41
296A	13853	13871	19	616A	23174	23208	35	936A	36969	36994	26
297A	13855	13874	20	617A	23249	23276	28	937A	36996	37016	21
298A	13862	13882	21	618A	23279	23311	33	938A	37023	37040	18
299A	13890	13905	16	619A	23313	23328	16	939A	37093	37112	20
300A	13897	13927	31	620A	23450	23470	21	940A	37118	37142	25
301A	13926	13940	15	621A	23488	23503	16	941A	37144	37163	20
302A	13957	13971	15	622A	23511	23529	19	942A	37242	37324	83
303A	13966	13980	15	623A	23555	23570	16	943A	37352	37368	17
304A	13995	14025	31	624A	23575	23589	15	944A	37370	37389	20
305A	14027	14048	22	625A	23597	23620	24	945A	37391	37419	29
306A	14048	14067	20	626A	23632	23647	16	946A	37421	37438	18
307A	14084	14098	15	627A	23672	23687	16	947A	37444	37491	48
308A	14118	14133	16	628A	23737	23775	39	948A	37511	37538	28

Reg. A	Position in SEQ ID NO 1		Len-gth	Reg. A	Position in SEQ ID NO 1		Len-gth	Reg A	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
309A	14154	14171	18	629A	23746	23760	15	949A	37567	37614	48
310A	14173	14210	38	630A	23833	23847	15	950A	37636	37680	45
311A	14198	14218	21	631A	23872	23911	40	951A	37723	37765	43
312A	14200	14218	19	632A	23919	23936	18	952A	37773	37801	29
313A	14237	14265	29	633A	24050	24068	19	953A	37803	37822	20
314A	14242	14265	24	634A	24083	24111	29	954A	37824	37853	30
315A	14242	14264	23	635A	24111	24125	15	955A	37855	37887	33
316A	14244	14262	19	636A	24131	24164	34	956A	37889	37908	20
317A	14246	14265	20	637A	24167	24189	23	957A	37920	37939	20
318A	14253	14271	19	638A	24204	24227	24	958A	37988	38020	33
319A	14273	14293	21	639A	24236	24285	50	959A	38022	38049	28
320A	14290	14304	15	640A	24438	24453	16				

In some embodiments, the oligonucleotide comprises a contiguous nucleotide sequence of 16 to 20, such as 15 to 22, nucleotides in length with at least 90% complementary, such as 100% complementarity, to a corresponding target sequence present in SEQ ID NO: 1, wherein the target sequence is selected from the group consisting of SEQ ID NO: 3 to 21 (table 4) or region B1 to B28 in Table 5B.

Table 5B: Regions of SEQ ID NO 1 which may be targeted using an oligonucleotide of the invention

Reg. B	Position in SEQ ID NO 1		Len-gth	Reg. B	Position in SEQ ID NO 1		Len-gth	Reg. B	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
1	8295	8312	17	11	11622	11641	19	21	18085	18101	16
2	8684	8704	20	12	11753	11773	20	22	22425	22441	16
3	9668	9684	16	13	11755	11772	17	23	33030	33048	18
4	9669	9684	15	14	11756	11776	20	24	35103	35123	20
5	9722	9741	19	15	11757	11776	19	25	35371	35390	19
6	9723	9741	18	16	11758	11778	20	26	35636	35655	19
7	9724	9742	18	17	12868	11885	17	27	35638	35654	16
8	10921	10937	16	18	13234	13252	18	28	36915	36931	16
9	11483	11503	20	19	13551	13569	18				
10	11512	11531	19	20	14786	14804	18				

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

In some embodiments, the contiguous nucleotide sequence of the oligonucleotide which is complementary to the target nucleic acids comprises or consists of 12 to 30, such as from 13 to 25, such as from 15 to 23, such as from 16 to 22, contiguous nucleotides in length.

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

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

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting of sequences listed in table 6 (Materials and Method section).

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 22 to 237 (see motif sequences listed in table 6). In a particular embodiment the oligonucleotide or contiguous nucleotide sequence is selected from SEQ ID NO: 22; 23; 24; 25; 26; 27; 28; 29; 32; 35; 36; 37; 38; 39; 40; 41; 42; 42; 42; 43; 43; 46; 49; 83; 109; 130; 203; and 232.

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

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

The oligonucleotide of the invention may be designed with modified nucleosides and RNA nucleosides (in particular for siRNA and shRNA molecules) or DNA nucleosides (in particular for single stranded antisense oligonucleotides). Advantageously, high affinity modified nucleosides are used.

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. Suitable modifications are described in the “Definitions” section under “modified nucleoside”, “high affinity modified nucleosides”, “sugar modifications”, “2’ sugar modifications” and Locked nucleic acids (LNA)”.

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

In a further embodiment the oligonucleotide comprises at least one modified internucleoside linkage. Suitable internucleoside modifications are described in the "Definitions" section under "Modified internucleoside linkage". It is advantageous if at least 2 to 3 internucleoside linkages at the 5' or 3' end of the oligonucleotide are phosphorothioate internucleoside linkages. For single stranded antisense oligonucleotides it is advantageous if at least 75%, such as all, the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages. In some embodiments all the internucleotide linkages in the contiguous sequence of the single stranded antisense oligonucleotide are phosphorothioate linkages.

In 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, ScET 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. It is advantageous for the nuclease stability of the oligonucleotide or contiguous nucleotide sequence to have at least 1 LNA nucleoside at the 5' end and at least 2 LNA nucleosides at the 3' end of the nucleotide sequence.

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

In the current invention an advantageous structural design is a gapmer design as described in the "Definitions" section under for example "Gapmer", "LNA Gapmer" and "MOE gapmer". In the present invention it is advantageous if the antisense oligonucleotide of the invention is a gapmer with an F-G-F' design. In some embodiments the gapmer is an LNA gapmer with uniform flanks.

In some embodiments of the invention the LNA gapmer is selected from the following uniform flank designs: 2-12-3, 4-14-2, 3-10-3, 3-9-3, 2-15-2, 2-12-4, 1-13-2, 3-13-2, 4-13-2, 2-12-2, 3-

12-2, 3-15-2, 3-14-2, 3-13-3, 2-14-4, 3-12-3, 1-14-3, 3-14-3, 2-14-3, 2-15-3, 3-11-3, 1-12-3, 1-11-4, 1-13-2, 2-13-2, 2-16-2, 1-14-2, 1-17-3 and 1-18-2.

Table 6 (Materials and Method section) lists preferred designs of each motif sequence.

In all instances the F-G-F' design may further include region D' and/or D'' as described in the "Definitions" section under "Region D' or D'' in an oligonucleotide". In some embodiments the oligonucleotide of the invention has 1, 2 or 3 phosphodiester linked nucleoside units, such as DNA units, at the 5' or 3' end of the gapmer region. In some embodiments the oligonucleotide of the invention consists of two 5' phosphodiester linked DNA nucleosides followed by a F-G-F' gapmer region as defined in the "Definitions" section. Oligonucleotides that contain phosphodiester linked DNA units at the 5' or 3' end are suitable for conjugation and may further comprise a conjugate moiety as described herein. For delivery to the liver ASGPR targeting moieties are particular advantageous as conjugate moieties.

For some embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 22_1; 23_1; 24_1; 25_1; 26_1; 27_1; 28_1; 29_1; 30_1; 31_1; 32_1; 33_1; 34_1; 35_1; 36_1; 37_1; 38_1; 39_1; 40_1; 41_1; 42_1; 42_2; 42_3; 43_1; 43_2; 44_1; 45_1; 46_1; 47_1; 48_1; 49_1; 130_1; 109_1; 83_1; 203_1 and 232_1 (see Table 6).

Conjugates

Since HBV infection primarily affects the hepatocytes in the liver it is advantageous to conjugate the RTEL1 inhibitor to a conjugate moiety that will increase the delivery of the inhibitor to the liver compared to the unconjugated inhibitor. In one embodiment liver targeting moieties are selected from moieties comprising cholesterol or other lipids or conjugate moieties capable of binding to the asialoglycoprotein receptor (ASGPR).

In some embodiments, the invention provides a conjugate comprising a nucleic acid molecule of the invention covalently attached to a conjugate moiety.

The asialoglycoprotein receptor (ASGPR) conjugate moiety comprises one or more carbohydrate moieties capable of binding to the asialoglycoprotein receptor (ASPGR targeting moieties) with affinity equal to or greater than that of galactose. The affinities of numerous galactose derivatives for the asialoglycoprotein receptor have been studied (see for example: Jobst, S.T. and Drickamer, K. JB.C. 1996, 271, 6686) or are readily determined using methods typical in the art.

In one embodiment the conjugate moiety comprises at least one asialoglycoprotein receptor targeting moiety selected from group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-

galactosamine and N-isobutanoylgalactosamine. Advantageously the asialoglycoprotein receptor targeting moiety is N-acetylgalactosamine (GalNAc).

To generate the ASGPR conjugate moiety the ASPGR targeting moieties (preferably GalNAc) can be attached to a conjugate scaffold. Generally, the ASPGR targeting moieties can be at the same end of the scaffold. In one embodiment, the conjugate moiety consists of two to four terminal GalNAc moieties linked to a spacer which links each GalNAc moiety to a brancher molecule that can be conjugated to the antisense oligonucleotide.

In a further embodiment, the conjugate moiety is mono-valent, di-valent, tri-valent or tetra-valent with respect to asialoglycoprotein receptor targeting moieties. Advantageously the asialoglycoprotein receptor targeting moiety comprises N-acetylgalactosamine (GalNAc) moieties.

GalNAc conjugate moieties can include, for example, those described in WO 2014/179620 and WO 2016/055601 and PCT/EP2017/059080 (hereby incorporated by reference), as well as small peptides with GalNAc moieties attached such as Tyr-Glu-Glu-(aminohexyl GalNAc)3 (YEE(ahGalNAc)3; a glycotripeptide that binds to asialoglycoprotein receptor on hepatocytes, see, e.g., Duff, et al., Methods Enzymol, 2000, 313, 297); lysine-based galactose clusters (e.g., L3G4; Biessen, et al., Cardovasc. Med., 1999, 214); and cholane-based galactose clusters (e.g., carbohydrate recognition motif for asialoglycoprotein receptor).

The ASGPR conjugate moiety, in particular a trivalent GalNAc conjugate moiety, may be attached to the 3'- or 5'-end of the oligonucleotide using methods known in the art. In one embodiment the ASGPR conjugate moiety is linked to the 5'-end of the oligonucleotide.

In one embodiment the conjugate moiety is a tri-valent N-acetylgalactosamine (GalNAc), such as those shown in figure 1, in particular as shown in figure 1D.

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) to covalently attach the conjugate moiety to the oligonucleotide. 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 salt

The compounds according to the present invention may exist in the form of their pharmaceutically acceptable salts. The term "pharmaceutically acceptable salt" refers to conventional acid-addition salts or base-addition salts that retain the biological effectiveness and properties of the compounds of the present invention and are formed from suitable non-toxic organic or inorganic acids or organic or inorganic bases. Acid-addition salts include for example those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, sulfamic acid, phosphoric acid and nitric acid, and those derived from organic acids such as *p*-toluenesulfonic acid, salicylic acid, methanesulfonic acid, oxalic acid, succinic acid, citric acid, malic acid, lactic acid, fumaric acid, and the like. Base-addition salts include those derived from ammonium, potassium, sodium and, quaternary ammonium hydroxides, such as for example, tetramethyl ammonium hydroxide. The chemical modification of a pharmaceutical compound into a salt is a technique well known to pharmaceutical chemists in order to obtain improved physical and chemical stability, hygroscopicity, flowability and solubility of compounds. It is for example described in Bastin, Organic Process Research & Development 2000, 4, 427-435 or in Ansel, In: Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th ed. (1995), pp. 196 and 1456-1457. For example, the pharmaceutically acceptable salt of the compounds provided herein may be a sodium salt.

In a further aspect the invention provides a pharmaceutically acceptable salt of the antisense oligonucleotide or a conjugate thereof. In a preferred embodiment, the pharmaceutically acceptable salt is a sodium or a potassium salt.

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 - 300μM solution.

Suitable formulations for use in the present invention are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (Science 249:1527-1533, 1990). WO 2007/031091 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.

In some embodiments, the oligonucleotide or oligonucleotide conjugates of the invention, or pharmaceutically acceptable salt thereof is in a solid form, such as a powder, such as a lyophilized powder.

Compounds, 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 off the oligonucleotide once the prodrug is delivered to the site of action, e.g. the target cell.

Administration

The compounds, oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions of the present invention may be administered topical (such as, to the skin, inhalation, ophthalmic or otic) or enteral (such as, orally or through the gastrointestinal tract) or parenteral (such as, intravenous, subcutaneous, intra-muscular, intracerebral, intracerebroventricular or intrathecal).

In a preferred embodiment the oligonucleotide or pharmaceutical compositions of the present invention are administered by a parenteral route including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intrathecal or intracranial, e.g. intracerebral or intraventricular, intravitreal administration. In one embodiment 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 inhibitor of the present invention, such as the compound, oligonucleotide, oligonucleotide conjugate or pharmaceutical composition of the invention is for use in a combination treatment with another therapeutic agent. The therapeutic agent can for example be the standard of care for the diseases or disorders described above.

By way of example, the oligomer or the oligomer conjugate of the present invention may be used in combination with other actives, such as oligonucleotide-based antivirals – such as sequence specific oligonucleotide-based antivirals - acting either through antisense (including other LNA oligomers), siRNAs (such as ARC520), aptamers, morpholinos or any other antiviral, nucleotide sequence-dependent mode of action.

By way of further example, the oligomer or the oligomer conjugate of the present invention may be used in combination with other actives, such as immune stimulatory antiviral compounds, such as interferon (e.g. pegylated interferon alpha), TLR7 agonists (e.g. GS-9620), or therapeutic vaccines.

By way of further example, the oligomer or the oligomer conjugate of the present invention may be used in combination with other actives, such as small molecules, with antiviral activity. These other actives could be, for example, nucleoside/nucleotide inhibitors (eg entecavir or tenofovir disoproxil fumarate), encapsidation inhibitors, entry inhibitors (eg Myrcludex B).

In certain embodiments, the additional therapeutic agent may be an HBV agent, a Hepatitis C virus (HCV) agent, a chemotherapeutic agent, an antibiotic, an analgesic, a nonsteroidal anti-inflammatory (NSAID) agent, an antifungal agent, an antiparasitic agent, an anti-nausea agent, an anti-diarrheal agent, or an immunosuppressant agent.

In particular, related embodiments, the additional HBV agent may be interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated), ribavirin; an HBV RNA replication inhibitor; a second antisense oligomer; an HBV therapeutic vaccine; an HBV

prophylactic vaccine; lamivudine (3TC); entecavir (ETV); tenofovir diisoproxil fumarate (TDF); telbivudine (LdT); adefovir; or an HBV antibody therapy (monoclonal or polyclonal).

In other particular related embodiments, the additional HCV agent may be interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated); ribavirin; pegasys; an HCV RNA replication inhibitor (e.g., ViroPharma's VP50406 series); an HCV antisense agent; an HCV therapeutic vaccine; an HCV protease inhibitor; an HCV helicase inhibitor; or an HCV monoclonal or polyclonal antibody therapy.

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 RTEL1 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 oligonucleotides 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.

Also encompassed by the present invention is an *in vivo* or *in vitro* method for modulating RTEL1 expression in a target cell which is expressing RTEL1, said method comprising administering an oligonucleotide, conjugate compound or pharmaceutical composition of the invention in an effective amount to said cell.

In some embodiments, the target cell, is a mammalian cell in particular a human cell. The target cell may be an *in vitro* cell culture or an *in vivo* cell forming part of a tissue in a mammal. In preferred embodiments the target cell is present in in the liver. The target cell may be a hepatocyte.

One aspect of the present invention is related the oligonucleotides, conjugate compounds or pharmaceutical compositions of the invention for use as a medicament.

In an aspect of the invention the oligonucleotides, conjugate compound or pharmaceutical composition of the invention is capable of reducing the cccDNA level in the infected cells and therefore inhibiting HBV infection. In particular, the antisense oligonucleotide is capable of affecting one or more of the following parameters i) reducing cccDNA and/or ii) reducing pgRNA and/or iii) reducing HBV DNA and/or iv) reducing HBV viral antigens in an infected cell.

For example, nucleic acid molecule that inhibits HBV infection may reduce i) the cccDNA levels in an infected cell by at least 40% such as 50%, 60%, 70%, 80%, or 90% reduction compared to controls; or ii) the level of pgRNA by at least 40% such as 50%, 60%, 70%, 80%, or 90% reduction compared to controls. The controls may be untreated cells or animals, or cells or animals treated with an appropriate control.

Inhibition of HBV infection may be measured *in vitro* using HBV infected primary human hepatocytes or *in vivo* using humanized hepatocytes PXB mouse model (available at PhoenixBio, see also Kakuni et al 2014 Int. J. Mol. Sci. 15:58-74). Inhibition of secretion of HBsAg and/or HBeAg may be measured by ELISA, e.g. by using the CLIA ELISA Kit (Autobio Diagnostic) according to the manufacturers' instructions. Reduction of intracellular cccDNA or HBV mRNA and pgRNA may be measured by qPCR, e.g. as described in the Materials and Methods section. Further methods for evaluating whether a test compound inhibits HBV infection are measuring secretion of HBV DNA by qPCR e.g. as described in WO 2015/173208 or using Northern Blot; in-situ hybridization, or immuno-fluorescence.

Due to the reduction of RTEL1 levels the oligonucleotides, conjugate compounds or pharmaceutical compositions of the present invention can be used to inhibit development of or in the treatment of HBV infection. In particular, the destabilization and reduction of the cccDNA, the oligonucleotides, conjugate compounds or pharmaceutical compositions of the present invention more efficiently inhibits development of or treats a chronic HBV infection as compared to a compound that only reduces secretion of HBsAg.

Accordingly, one aspect of the present invention is related to use of the oligonucleotide, conjugate compounds or pharmaceutical compositions of the invention to reduce cccDNA and/or pgRNA in an HBV infected individual.

A further aspect of the invention relates to the use of the oligonucleotides, conjugate compounds or pharmaceutical compositions of the invention to inhibit development of or treat a chronic HBV infection.

A further aspect of the invention relates to the use of the oligonucleotides, conjugate compounds or pharmaceutical compositions of the invention to reduce the infectiousness of a HBV infected person. In a particular aspect of the invention, the oligonucleotides, conjugate compounds or pharmaceutical compositions of the invention inhibits development of a chronic HBV infection.

The subject to be treated with the oligonucleotides, conjugate compounds or pharmaceutical compositions of the invention (or which prophylactically receives antisense oligonucleotides, conjugate compounds or pharmaceutical compositions of the present invention) is preferably a human, more preferably a human patient who is HBsAg positive and/or HBeAg positive, even more preferably a human patient that is HBsAg positive and HBeAg positive.

Accordingly, the present invention relates to a method of treating a HBV infection, wherein the method comprises administering an effective amount of the oligonucleotides, conjugate compounds or pharmaceutical compositions of the invention. The present invention further relates to a method of preventing liver cirrhosis and hepatocellular carcinoma caused by a chronic HBV infection.

The invention also provides for the use of an oligonucleotide, a conjugate compound or a pharmaceutical composition of the invention for the manufacture of a medicament, in particular a medicament for use in the treatment of HBV infection or chronic HBV infection or reduction of the infectiousness of a HBV infected person. In preferred embodiments the medicament is manufactured in a dosage form for subcutaneous administration.

The invention also provides for the use of an oligonucleotide, a conjugate compound, the pharmaceutical composition of the invention for the manufacture of a medicament wherein the medicament is in a dosage form for intravenous administration.

The oligonucleotide, conjugate or the pharmaceutical composition of the invention may be used in a combination therapy. For example, oligonucleotide, conjugate or the pharmaceutical composition of the invention may be combined with other anti-HBV agents such as interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated), ribavirin, lamivudine (3TC), entecavir, tenofovir, telbivudine (LdT), adefovir, or other emerging anti-HBV agents such as a HBV RNA replication inhibitor, a HBsAg secretion inhibitor, a HBV capsid inhibitor, an antisense oligomer (e.g. as described in WO2012/145697, WO 2014/179629 and WO2017/216390), a siRNA (e.g. described in WO 2005/014806, WO 2012/024170, WO 2012/2055362, WO 2013/003520, WO 2013/159109, WO 2017/027350 and WO2017/015175), a HBV therapeutic vaccine, a HBV prophylactic vaccine, a HBV antibody therapy (monoclonal or polyclonal), or TLR 2, 3, 7, 8 or 9 agonists for the treatment and/or prophylaxis of HBV.

Embodiments of the invention

The following embodiments of the present invention may be used in combination with any other embodiments described herein.

1. A RTEL1 inhibitor for use in the in the treatment and/or prevention of Hepatitis B virus (HBV) infection.
2. The RTEL1 inhibitor for the use of embodiment 1, wherein the RTEL1 inhibitor is administered in an effective amount.
3. The RTEL1 inhibitor for the use of embodiment 1 or 2, wherein the HBV infection is a chronic infection.
4. The RTEL1 inhibitor for the use of embodiments 1 to 3, wherein the RTEL1 inhibitor is capable of reducing cccDNA and/or pgRNA in an infected cell.

5. The RTEL1 inhibitor for the use of any one of embodiments 1 to 4, wherein the RTEL1 inhibitor prevents or reduces the binding of RTEL1 to DNA, such as cccDNA.

6. RTEL1 inhibitor for the use of embodiment 5, wherein said inhibitor is a small molecule that specifically binds to RTEL1 protein, wherein said inhibitor prevents or reduces binding of RTEL1 protein to cccDNA.

7. The RTEL1 inhibitor for the use of any one of embodiments 1 to 5, wherein said inhibitor is an oligonucleotide of 12-60 nucleotides in length comprising or consisting of a contiguous nucleotide sequence of at least 10 nucleotides in length which is at least 90% complementary to a mammalian RTEL1 target nucleic acid.

8. The RTEL1 inhibitor for the use of embodiment 7, which is capable of reducing the level of the RTEL1 target nucleic acid.

9. The RTEL1 inhibitor for the use of embodiment 7 or 8, wherein the target nucleic acid is RNA.

10. The RTEL1 inhibitor for the use of embodiment 9, wherein the RNA is pre-mRNA.

11. The RTEL1 inhibitor for the use of any one of embodiments 7 to 10, wherein the oligonucleotide is selected from an antisense oligonucleotide, siRNA or shRNA.

12. The RTEL1 inhibitor for the use of embodiments 11, wherein the oligonucleotide is a single stranded antisense oligonucleotide or a double stranded siRNA.

13. The RTEL1 inhibitor for the use of any one of embodiments 7 to 12, wherein the mammalian RTEL1 target nucleic acid is selected from SEQ ID NO: 1 or 2.

14. The RTEL1 inhibitor for the use of any one of embodiments 7 to 12, wherein the contiguous nucleotide sequence of the oligonucleotide is at least 98% complementarity to the target nucleic acid of SEQ ID NO: 1 and SEQ ID NO: 2.

15. The RTEL1 inhibitor for the use of any one of embodiments 1 to 14, wherein the cccDNA in an HBV infected cell is reduced by at least 50%, such as 60%, such as 70%, such as 80%, such 90%, such as 95%, such as 100%, when compared to a control.

16. The oligonucleotide for the use of any one of embodiments 7 to 15, wherein the RTEL1 mRNA is reduced by at least 50%, such as 60%, such as 70%, such as 80%, such as 90%, such as 95%, such as 100%, when compared to a control.

17. An oligonucleotide of 12 to 60 nucleotides in length which comprises or consists of a contiguous nucleotide sequence of 12 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 90% complementary, such as 95%, such as 98%, such as fully complementarity, to a mammalian RTEL1 target nucleic acid.

18. The oligonucleotide of embodiment 17, wherein the oligonucleotide is chemically produced.

19. The oligonucleotide of embodiment 17 or 18, wherein the mammalian RTEL1 target nucleic acid is selected from SEQ ID NO: 1 or 2.

20. The oligonucleotide embodiment 17 or 18, wherein the contiguous nucleotide sequence is at least 98% complementarity to the target nucleic acid of SEQ ID NO: 1 and SEQ ID NO: 2.

21. The oligonucleotide of any one of embodiments 17 to 20, wherein the oligonucleotide is 12 to 30 nucleotides in length.

22. The oligonucleotide of any one of embodiments 17 to 21, wherein the oligonucleotide is a RNAi molecule, such as a double stranded siRNA or shRNA

23. The oligonucleotide of any one of embodiments 17 to 21, wherein the oligonucleotide is a single stranded antisense oligonucleotide.

24. The oligonucleotide of any one of embodiments 17 to 23, wherein contiguous nucleotide sequence is complementary to a target sequence selected from SEQ ID NO: 3 to 21 (table 4).

25. The oligonucleotide of embodiment 17 to 24, which is capable of hybridizing to a target nucleic acid of SEQ ID NO: 1 and SEQ ID NO: 2 with a ΔG° below -15 kcal.

26. The oligonucleotide of any one of embodiments 17 to 25, wherein the contiguous nucleotide sequence comprises or consists of at least 14 contiguous nucleotides, particularly 15, 16, 17, 18, 19, 20, 21 or 22 contiguous nucleotides.

27. The oligonucleotide of any one of embodiments 17 to 25, wherein the contiguous nucleotide sequence comprises or consists of from 14 to 22 nucleotides.

28. The oligonucleotide of embodiment 27, wherein the contiguous nucleotide sequence comprises or consists of from 16 to 20 nucleotides.

29. The oligonucleotide of any one of embodiments 17 to 28, wherein the oligonucleotide comprises or consists of 14 to 25 nucleotides in length.

30. The oligonucleotide of embodiment 29, wherein the oligonucleotide comprises or consists of 16 to 22 nucleotides in length.

31. The oligonucleotide of any one of embodiment 17 to 30, wherein the oligonucleotide comprises a sequence selected from SEQ ID NO: 22-237.

32. The oligonucleotide of any one of embodiments 17 to 31, wherein the contiguous nucleotide sequence has zero to three mismatches compared to the target nucleic acids it is complementary to.

33. The oligonucleotide of embodiment 32, wherein the contiguous nucleotide sequence has one mismatch compared to the target nucleic acids.

34. The oligonucleotide of embodiment 32, wherein the contiguous nucleotide sequence has two mismatches compared to the target nucleic acids.

35. The oligonucleotide of embodiment 32, wherein the contiguous nucleotide sequence is fully complementary to both target nucleic acid sequences.

36. The oligonucleotide of embodiment 17 to 35, comprising one or more modified nucleosides.

37. The oligonucleotide of embodiment 36, wherein the one or more modified nucleoside is a high-affinity modified nucleosides.

38. The oligonucleotide of embodiment 36 or 37, wherein the one or more modified nucleoside is a 2' sugar modified nucleoside.

39. The oligonucleotide of embodiment 38, wherein the one or more 2' sugar modified nucleoside is 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, 2'-fluoro-ANA and LNA nucleosides.

40. The oligonucleotide of embodiment 36-39, wherein the one or more modified nucleoside is a LNA nucleoside.

41. The oligonucleotide of embodiment 40, wherein the modified LNA nucleoside is selected from oxy-LNA, amino-LNA, thio-LNA, cET, and ENA.

42. The oligonucleotide of embodiment 40 or 41, wherein the modified LNA nucleoside is oxy-LNA with the following 2'-4' bridge –O-CH_2-.

43. The oligonucleotide of embodiment 42, wherein the oxy-LNA is beta-D-oxy-LNA.

44. The oligonucleotide of embodiment 40 or 41, wherein the modified LNA nucleoside is cET with the following 2'-4' bridge –O-CH(CH_3)-.

45. The oligonucleotide of embodiment 44, wherein the cET is (S)cET, i.e. 6'(S)methyl-beta-D-oxy-LNA.

46. The oligonucleotide of embodiment 40 or 41, wherein the LNA is ENA, with the following 2' – 4' bridge –O-CH_2-CH_2-.

47. The oligonucleotide of any one of embodiments 17 to 46, wherein the oligonucleotide comprises at least one modified internucleoside linkage.

48. The oligonucleotide of embodiment 47, wherein the modified internucleoside linkage is nuclease resistant.

49. The oligonucleotide of embodiment 47 or 48, wherein the modified internucleoside linkages is a phosphorothioate internucleoside linkages.

50. The oligonucleotide any one of embodiments 17 to 49, wherein the oligonucleotide is an antisense oligonucleotide capable of recruiting RNase H.

51. The antisense oligonucleotide of embodiment 50, wherein the antisense oligonucleotide or the contiguous nucleotide sequence is a gapmer.

52. The antisense oligonucleotide of embodiment 51, wherein the antisense oligonucleotide or contiguous nucleotide sequence thereof consists of or comprises a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise or consist of 1- 4 2' sugar modified nucleosides and G is a region between 6 and 18 nucleosides which are capable of recruiting RNaseH.

53. The antisense oligonucleotide of embodiment 52, wherein the 2' sugar modified nucleoside independently is 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.

54. The antisense oligonucleotide of embodiment 52 or 53, wherein one or more of the 2' sugar modified nucleosides in region F and F' is a LNA nucleoside

55. The antisense oligonucleotide of embodiment 54, wherein all the 2' sugar modified nucleosides in region F and F' are LNA nucleosides.

56. The oligonucleotide of embodiment 53 to 55, wherein the LNA nucleoside is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA, alpha-L-amino-LNA, beta-D-thio-LNA, alpha-L-thio-LNA, (S)cET, (R)cET beta-D-ENA and alpha-L-ENA.

57. The antisense oligonucleotide of embodiment 53 to 56, wherein region F and F' consist of identical LNA nucleosides.

58. The antisense oligonucleotide of embodiment 53 to 57, wherein all the 2' sugar modified nucleosides in region F and F' are oxy-LNA nucleosides.

59. The antisense oligonucleotide of any one of embodiments 52 to 58, wherein the nucleosides in region G is DNA and/or alpha-L-LNA nucleosides.

60. The antisense oligonucleotide of embodiment 59, wherein region G consists of at least 75% DNA nucleosides.

61. The antisense oligonucleotide of embodiment 60, where all the nucleosides in region G are DNA nucleosides.

62. The oligonucleotide any one of embodiments 17 to 62, wherein the oligonucleotide is selected from CMP ID NO: 22_1; 23_1; 24_1; 25_1; 26_1; 27_1; 28_1; 29_1; 30_1; 31_1; 32_1; 33_1; 34_1; 35_1; 36_1; 37_1; 38_1; 39_1; 40_1; 41_1; 42_1; 42_2; 42_3; 43_1; 43_2; 44_1; 45_1; 46_1; 49_1; 130_1; 109_1; 83_1; 203_1 and 232_1, or pharmaceutically acceptable salts thereof.

63. A conjugate compound comprising an oligonucleotide according to any one of embodiments 17 to 50 or an antisense oligonucleotide according to any one of embodiments 51 to 62, and at least one conjugate moiety covalently attached to said antisense oligonucleotide.

64. The conjugate compound of embodiment 63, wherein the oligonucleotide is a double stranded siRNA and the conjugate moiety is covalently attached to the sense strand of the siRNA.

65. The conjugate compound of embodiment 63 or 64, wherein the conjugate moiety is selected from carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins, vitamins, viral proteins or combinations thereof.

66. The conjugate compound of any one of embodiments 63 to 65, wherein the conjugate moiety is capable of binding to the asialoglycoprotein receptor.

67. The conjugate compound of embodiment 66, wherein the conjugate moiety comprises at least one asialoglycoprotein receptor targeting moiety selected from group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N-isobutanoylgalactosamine.

68. The conjugate compound of embodiment 67, wherein the asialoglycoprotein receptor targeting moiety is N-acetylgalactosamine (GalNAc).

69. The conjugate compound of embodiment 67 or 68, wherein the conjugate moiety is mono-valent, di-valent, tri-valent or tetra-valent with respect to asialoglycoprotein receptor targeting moieties.

70. The conjugate compound of embodiment 69, wherein the conjugate moiety consists of two to four terminal GalNAc moieties and a spacer linking each GalNAc moiety to a brancher molecule that can be conjugated to the antisense compound.

71. The conjugate compound of embodiment 70, wherein the spacer is a PEG spacer.

72. The conjugate compound of embodiment 66 to 71, wherein the conjugate moiety is a tri-valent N-acetylgalactosamine (GalNAc) moiety.

73. The conjugate compound of embodiment 66 to 72, wherein the conjugate moiety is selected from one of the trivalent GalNAc moieties in figure 1.

74. The conjugate compound of embodiment 73, wherein the conjugate moiety is the trivalent GalNAc moiety in figure 1D.

75. The conjugate compound of embodiment 63-74, comprising a linker which is positioned between the oligonucleotide or the antisense oligonucleotide and the conjugate moiety.

76. The conjugate compound of embodiment 75, wherein the linker is a physiologically labile linker.

77. The conjugate compound of embodiment 76, wherein the physiologically labile linker is nuclease susceptible linker.

78. The oligonucleotide conjugate of embodiment 76 or 77, wherein the physiologically labile linker is composed of 2 to 5 consecutive phosphodiester linkages.

79. The conjugate compound of embodiment 66-78, which display improved cellular distribution between liver vs. kidney or improved cellular uptake into the liver of the conjugate compound as compared to an unconjugated oligonucleotide or antisense oligonucleotide.

80. A pharmaceutical composition comprising a oligonucleotide according to any one of embodiments 17 to 50 or an antisense oligonucleotide according to any one of embodiments 51 to 61, a conjugate compound of embodiment 63 to 79 or acceptable salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant.

81. A method for identifying a compound that prevents, ameliorates and/or inhibits a hepatitis B virus (HBV) infection, comprising:
    a. contacting a test compound with
        i. a RTEL1 polypeptide; or
        ii. a cell expressing RTEL1;
    b. measuring the expression and/or activity of RTEL1 in the presence and absence of said test compound; and
    c. identifying a compound that reduces the expression and/or activity RTEL1 and reduces cccDNA.

82. An *in vivo* or *in vitro* method for modulating RTEL1 expression in a target cell which is expressing RTEL1, said method comprising administering the oligonucleotide of any one of embodiments 17 to 50 or an antisense oligonucleotide according to any one of embodiments 51 to 61, a conjugate compound of embodiment 63 to 79 or the pharmaceutical composition of embodiment 80 in an effective amount to said cell.

83. The method of embodiments 82, wherein the RTEL1 expression is reduced by at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95% in the target cell compared to the level without any treatment or treated with a control.

84. The method of embodiments 82, wherein the target cell is infected with HBV and the cccDNA in an HBV infected cell is reduced by at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95% in the HBV infected target cell compared to the level without any treatment or treated with a control.

85. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of the oligonucleotide any one of embodiments 17 to 50 or an antisense oligonucleotide according to any one of embodiments 51 to 61, a conjugate compound of embodiment 63 to 79 or the pharmaceutical composition of embodiment 80 to a subject suffering from or susceptible to the disease.

86. The oligonucleotide of any one of embodiments 17 to 50 or the antisense oligonucleotide according to any one of embodiments 51 to 61, or the conjugate compound of any one of embodiments 63 to 79 or the pharmaceutical composition of embodiment 80, for use as a medicament for treatment or prevention of a disease in a subject.

87. Use of the oligonucleotide any one of embodiments 17 to 50 or the antisense oligonucleotide according to any one of embodiments 51 to 61, or the conjugate compound of any one of embodiments 63 to 79 for the preparation of a medicament for treatment or prevention of a disease in a subject.

88. The method, the oligonucleotide, the antisense oligonucleotide, the conjugate or the use of embodiments 85 - 87 wherein the subject is a mammal.

89. The method, the oligonucleotide, the antisense oligonucleotide, the conjugate, or the use of embodiment 88, wherein the mammal is human.

The invention will now be illustrated by the following examples which have no limiting character.

EXAMPLES

Materials and methods

Oligonucleotide motif sequences and oligonucleotide compounds

Table 6: list of oligonucleotide motif sequences (indicated by SEQ ID NO), designs of these, as well as specific oligonucleotide compounds (indicated by CMP ID NO) designed based on the motif sequence.

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
22	catggaaggacagtggt	2-12-3	CAtggaaggacagtGGT	22_1	8295
23	agctttattataacttgaat	4-14-2	AGCTttattataacttgaAT	23_1	8684
24	cgggacaggtagtaag	3-10-3	CGGgacaggtagtAAG	24_1	9668

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
25	cgggacaggtagtaa	3-9-3	CGGgacaggtagTAA	25_1	9669
26	gcatccaacaagtaattgt	2-15-2	GCatccaacaagtaattGT	26_1	9722
27	gcatccaacaagtaattg	2-12-4	GCatccaacaagtaATTG	27_1	9723
28	ggcatccaacaagtaatt	3-13-2	GGCatccaacaagtaaTT	28_1	9724
29	ggttgggttagaagct	2-12-2	GGttgggttagaagCT	29_1	10921
30	gcttttacatttaggtttat	3-15-2	GCTtttacatttaggtttAT	30_1	11483
31	catgttcctttctataact	3-14-2	CATgttcctttctataaCT	31_1	11512
32	agctttaaattttggtgaa	3-13-3	AGCtttaaattttggtGAA	32_1	11622
33	ttttacatactctggtcaaa	2-14-4	TTttacatactctggtCAAA	33_1	11753
34	ttttacatactctggtca	3-12-3	TTTtacatactctggTCA	34_1	11755
35	gaattttacatactctggtc	3-14-3	GAAttttacatactctgGTC	35_1	11756
36	gaattttacatactctggt	2-14-3	GAattttacatactctGGT	36_1	11757
37	gagaattttacatactctgg	2-15-3	GAgaattttacatactcTGG	37_1	11758
38	atctttgaacacgtctt	3-11-3	ATCtttgaacacgtCTT	38_1	12868
39	acaaaaaacagtaggtcc	2-12-4	ACaaaaaacagtagGTCC	39_1	13234
40	ggaataaaacagtaggtc	2-12-4	GGaataaaacagtaGGTC	40_1	13551
41	agcttcgtcaaagatcac	3-13-2	AGCttcgtcaaagatcAC	41_1	14786
42	ggtgggtggatgtttc	1-12-3	GgtgggtggatgtTTC	42_1	18085
42	ggtgggtggatgtttc	1-11-4	GgtgggtggatgTTTC	42_2	18085
42	ggtgggtggatgtttc	1-13-2	GgtgggtggatgttTC	42_3	18085
43	ggtggtgtggagaagc	1-12-3	GgtggtgtggagaAGC	43_1	22425
43	ggtggtgtggagaagc	1-13-2	GgtggtgtggagaaGC	43_2	22425
44	gctcatactccacacac	2-13-2	GCtcatactccacacAC	44_1	33030
45	catcggaacccttgtagtcc	2-16-2	CAtcggaacccttgtagtCC	45_1	35103
46	gatacagacctcctcaaac	2-15-2	GAtacagacctcctcaaAC	46_1	35371
47	ggtggaggtggtgctgc	1-14-2	GgtggaggtggtgctGC	47_1	35636
48	aggtggaggtggtgct	1-12-3	AggtggaggtggtGCT	48_1	35638
49	tggtgtgggagtagca	2-12-2	TGgtgtgggagtagCA	49_1	36915
50	cgatggcgagaaatta	4-10-2	CGATggegagaaatTA	50_1	3824
51	taattcagcaaaaaagccca	3-15-2	TAAttcagcaaaaaagccCA	51_1	3858
52	aagaatctgacacccca	2-12-3	AAgaatctgacaccCCA	52_1	3924
53	agacagccaagaatctgacac	1-18-2	AgacagccaagaatctgacAC	53_1	3928
54	cagccaagaatctgaca	2-12-3	CAgccaagaatctgACA	54_1	3929
55	acaggaacccgacag	2-10-3	ACaggaaccegaCAG	55_1	4496
56	gttactctcttgtttcttcac	1-18-2	GttactctcttgtttcttcAC	56_1	4789
57	ttactctcttgtttcttca	1-16-2	TtactctcttgtttcttCA	57_1	4790
57	ttactctcttgtttcttca	1-14-4	TtactctcttgtttcTTCA	57_2	4790
58	gttactctcttgtttcttc	2-15-2	GTtactctcttgtttctTC	58_1	4791
59	cgtgggtggagaagca	1-13-2	CgtgggtggagaagCA	59_1	5717
60	acgtgggtggagaagc	2-12-2	ACgtgggtggagaaGC	60_1	5718

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
61	cagaaactgtaagggca	1-13-3	CagaaactgtaaggGCA	61_1	5815
62	agggatagcagggaagg	2-13-2	AGggatagcagggaaGG	62_1	7246
63	gcttaaacacagacaga	2-11-4	GCttaaacacagaCAGA	63_1	7501
64	tgcttaaacacagacag	3-11-3	TGCttaaacacagaCAG	64_1	7502
65	cagggcagggaagaacag	1-14-3	CagggcagggaagaaCAG	65_1	7845
66	catggaaggacagtgg	3-10-3	CATggaaggacagTGG	66_1	8296
66	catggaaggacagtgg	1-12-3	CatggaaggacagTGG	66_2	8296
67	ccccctcaatataagaa	3-12-2	CCCcctcaatataagAA	67_1	8705
68	aaccaaccctattcctgg	2-14-2	AAccaaccctattcctGG	68_1	9375
68	aaccaaccctattcctgg	1-15-2	AaccaaccctattcctGG	68_2	9375
69	accaaccctattcctg	1-12-3	AccaaccctattcCTG	69_1	9376
70	aaccaaccctattcctg	3-12-2	AACcaaccctattccTG	70_1	9376
71	aaaaccaaccctattcct	3-12-3	AAAaccaaccctattCCT	71_1	9377
72	aaaccaaccctattcc	4-10-2	AAACcaaccctattCC	72_1	9378
73	ggtagtaagggcacacc	1-14-2	GgtagtaagggcacaCC	73_1	9660
74	gacaggtagtaagggcacac	1-17-2	GacaggtagtaagggcacAC	74_1	9661
75	gtagtaagggcacac	3-9-3	GTAgtaagggcaCAC	75_1	9661
76	gacaggtagtaagggcaca	1-16-2	GacaggtagtaagggcaCA	76_1	9662
77	acaggtagtaagggcaca	2-14-2	ACaggtagtaagggcaCA	77_1	9662
78	gacaggtagtaagggca	2-13-2	GAcaggtagtaagggCA	78_1	9664
79	cgggacaggtagtaaggg	1-15-2	CgggacaggtagtaagGG	79_1	9666
80	catccaacaagtaattgt	3-12-3	CATccaacaagtaatTGT	80_1	9722
80	catccaacaagtaattgt	2-13-3	CAtccaacaagtaatTGT	80_2	9722
81	ggcatccaacaagtaattgt	1-16-3	GgcatccaacaagtaatTGT	81_1	9722
82	ggcatccaacaagtaattg	3-14-2	GGCatccaacaagtaatTG	82_1	9723
82	ggcatccaacaagtaattg	1-15-3	GgcatccaacaagtaaTTG	82_2	9723
83	ggcatccaacaagtaat	4-11-2	GGCAtccaacaagtaAT	83_1	9725
83	ggcatccaacaagtaat	3-12-2	GGCatccaacaagtaAT	83_2	9725
84	cgtgaaggagagaacct	2-12-3	CGtgaaggagagaaCCT	84_1	10036
85	acgtgaaggagagaacc	3-12-2	ACGtgaaggagagaaCC	85_1	10037
86	gacgtgaaggagagaacc	2-13-3	GAegtgaaggagagaACC	86_1	10037
86	gacgtgaaggagagaacc	2-14-2	GAegtgaaggagagaaCC	86_2	10037
85	acgtgaaggagagaacc	4-11-2	ACGTgaaggagagaaCC	85_2	10037
87	gacgtgaaggagagaac	2-11-4	GAcgtgaaggagaGAAC	87_1	10038
88	cagtcttgctatgcct	2-12-2	CAgtcttgctatgcCT	88_1	10563
89	ctagaatcaaagctcca	2-12-3	CTagaatcaaagctCCA	89_1	10591
90	acatcgcacttgggc	1-12-2	AcategcacttggGC	90_1	10705
91	cacggcaaacctcacc	1-12-3	CaeggcaaacctcACC	91_1	10851
92	aaccacggcaaacctcac	3-13-2	AACcaeggcaaacctcAC	92_1	10852
93	caaagcaccgagtcacc	1-13-3	CaaagcacegagtcACC	93_1	10873

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
94	tcaaagcaccgagtcac	1-13-3	TcaaagcacegagtCAC	94_1	10874
95	ctggttgggttagaag	2-10-4	CTggttgggttaGAAG	95_1	10923
95	ctggttgggttagaag	2-12-2	CTggttgggttagaAG	95_2	10923
96	tataacttttagtttagc	2-12-4	TAtaacttttagttTAGC	96_1	11501
97	ttcctttctataacttttt	4-12-2	TTCCtttctataacttTT	97_1	11509
98	gttcctttctataacttttt	4-13-2	GTTCctttctataacttTT	98_1	11509
99	gttcctttctataacttt	4-12-2	GTTCctttctataactTT	99_1	11510
100	atgttcctttctataacttt	2-15-3	ATgttcctttctataacTTT	100_1	11510
101	atgttcctttctataactt	2-14-3	ATgttcctttctataaCTT	101_1	11511
102	atgttcctttctataact	2-14-2	ATgttcctttctataaCT	102_1	11512
103	gctttaatctgccttc	1-11-4	GctttaatctgcCTTC	103_1	12697
104	ccgtggctttaatctgc	1-14-2	CegtggctttaatctGC	104_1	12701
105	ccgtggctttaatctg	2-12-2	CCgtggctttaatcTG	105_1	12702
105	ccgtggctttaatctg	3-11-2	CCGtggctttaatcTG	105_2	12702
106	caaaaaacagtaggtcc	2-11-4	CAaaaaacagtagGTCC	106_1	13234
106	caaaaaacagtaggtcc	3-11-3	CAAaaaacagtaggTCC	106_2	13234
107	gaataaaacagtaggtcc	2-12-4	GAataaaacagtagGTCC	107_1	13550
108	ggaataaaacagtaggtcc	4-13-2	GGAAtaaaacagtaggtCC	108_1	13550
108	ggaataaaacagtaggtcc	2-15-2	GGaataaaacagtaggtCC	108_2	13550
108	ggaataaaacagtaggtcc	1-14-4	GgaataaaacagtagGTCC	108_3	13550
109	ggaataaaacagtaggt	3-11-3	GGAataaaacagtaGGT	109_1	13552
109	ggaataaaacagtaggt	2-11-4	GGaataaaacagtAGGT	109_2	13552
110	ggaataaaacagtagg	2-10-4	GGaataaaacagTAGG	110_1	13553
111	cacagagtgtcatggg	1-13-2	CacagagtgtcatgGG	111_1	14032
112	acagcatggaaaggcacg	1-13-4	AcagcatggaaaggCACG	112_1	14523
113	cagcatggaaaggcacg	1-12-4	CagcatggaaaggCACG	113_1	14523
114	tacaggaggaagagaagggac	1-18-2	TacaggaggaagagaagggAC	114_1	14725
115	acaggaggaagagaaggg	1-13-4	AcaggaggaagagaAGGG	115_1	14727
116	tctacaggaggaagagaa	4-12-2	TCTAcaggaggaagagAA	116_1	14730
116	tctacaggaggaagagaa	1-13-4	TctacaggaggaagAGAA	116_2	14730
117	tctacaggaggaagaga	4-11-2	TCTAcaggaggaagaGA	117_1	14731
117	tctacaggaggaagaga	2-12-3	TCtacaggaggaagAGA	117_2	14731
117	tctacaggaggaagaga	2-11-4	TCtacaggaggaaGAGA	117_3	14731
118	cttcgtcaaagatcacg	2-11-4	CTtcgtcaaagatCACG	118_1	14785
119	gcttcgtcaaagatcacg	2-13-3	GCttegtcaaagatcACG	119_1	14785
120	gcttcgtcaaagatcac	3-11-3	GCTtegtcaaagatCAC	120_1	14786
120	gcttcgtcaaagatcac	2-13-2	GCttegtcaaagatcAC	120_2	14786
121	ccagaaaggtttgcg	3-10-2	CCAgaaaggtttgCG	121_1	14874
122	tccagaaaggtttgcg	3-11-2	TCCagaaaggtttgCG	122_1	14874
122	tccagaaaggtttgcg	1-12-3	TccagaaaggtttGCG	122_2	14874

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
123	cagaggcatcggatcag	2-13-2	CAgaggcateggatcAG	123_1	14974
124	cagaggcatcggatca	3-11-2	CAGaggcateggatCA	124_1	14975
125	agcagaggcatcggatc	2-13-2	AGcagaggcateggaTC	125_1	14976
126	attcttcacacatcttc	2-11-4	ATtcttcacacatCTTC	126_1	16133
127	ctatgaacgcacctg	3-9-3	CTAtgaaegcacCTG	127_1	16282
128	ggctatgaacgcacctg	1-14-2	GgctatgaaegcaccTG	128_1	16282
129	gctgggagaagacatag	1-12-4	GctgggagaagacATAG	129_1	16593
130	caaaatgcccttacagtga	4-13-2	CAAAatgcccttacagtGA	130_1	16919
131	caaaatgcccttacagt	2-12-3	CAaaatgcccttacAGT	131_1	16921
132	tgtgcgattttaaaggaaaat	3-15-3	TGTgegattttaaaggaaAAT	132_1	17525
133	catgtgcgattttaaaggaaa	3-15-3	CATgtgegattttaaaggAAA	133_1	17527
134	tgtgcgattttaaaggaa	4-12-2	TGTGegattttaaaggAA	134_1	17528
135	catgtgcgattttaaagga	1-15-3	CatgtgegattttaaaGGA	135_1	17529
136	atgtgcgattttaaagga	3-13-2	ATGtgegattttaaagGA	136_1	17529
137	accctgtcacttaaatatatg	1-18-2	AccctgtcacttaaatataTG	137_1	17712
138	gagggaggtggagcgtt	1-14-2	GagggaggtggagegTT	138_1	17924
139	ctgaagagtggagaagg	2-11-4	CTgaagagtggagAAGG	139_1	18130
139	ctgaagagtggagaagg	1-13-3	CtgaagagtggagaAGG	139_2	18130
140	caataaataaagtgtgagga	3-14-3	CAAtaaataaagtgtgaGGA	140_1	18454
141	caacccagtaaccatgac	3-13-2	CAAcccagtaaccatgAC	141_1	19424
142	caacccagtaaccatga	3-12-2	CAAcccagtaaccatGA	142_1	19425
143	accaacccagtaaccatga	1-16-2	AccaacccagtaaccatGA	143_1	19425
144	gagcaggtgttttatc	3-11-2	GAGcaggtgttttaTC	144_1	19825
145	ggtcgaggaggtgtcac	1-14-2	GgtcgaggaggtgtcAC	145_1	20437
145	ggtcgaggaggtgtcac	2-13-2	GGtcgaggaggtgtcAC	145_2	20437
146	gtcgaggaggtgtcac	1-11-4	GtcgaggaggtgTCAC	146_1	20437
147	ggtcgaggaggtgtca	1-13-2	GgtcgaggaggtgtCA	147_1	20438
147	ggtcgaggaggtgtca	1-12-3	GgtcgaggaggtgTCA	147_2	20438
148	ggtcgaggaggtgtc	2-11-2	GGtcgaggaggtgTC	148_1	20439
149	ccaggtctcaaaaaggg	1-13-3	CcaggtctcaaaaaGGG	149_1	20653
150	attacgctgaggaca	1-10-4	AttaegctgagGACA	150_1	21489
151	cattacgctgaggac	4-9-2	CATTaegctgaggAC	151_1	21490
152	cttgagcattacgc	3-8-3	CTTgagcattaCGC	152_1	21497
153	cgaggagaagaaggcag	3-12-2	CGAggagaagaaggcAG	153_1	22019
153	cgaggagaagaaggcag	2-11-4	CGaggagaagaagGCAG	153_2	22019
154	ccttggtctgaaacgtgat	1-15-3	CcttggtctgaaaegtGAT	154_1	22071
155	ctaacgcctccacgc	1-12-2	CtaaegcctccacGC	155_1	22281
156	ggacaggctctacgg	1-11-3	GgacaggctctaCGG	156_1	22312
157	actaatacagcaggagaagg	2-16-2	ACtaatacagcaggagaaGG	157_1	22964
158	aactaatacagcaggagaagg	1-16-4	AactaatacagcaggagAAGG	158_1	22964

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
158	aactaatacagcaggagaagg	1-17-3	AactaatacagcaggagaAGG	158_2	22964
159	taactaatacagcaggagaag	1-16-4	TaactaatacagcaggaGAAG	159_1	22965
160	ttgaagagccaaccac	1-11-4	TtgaagagccaaCCAC	160_1	24131
161	ccattttcactgtcaag	3-12-2	CCAttttcactgtcaAG	161_1	25605
162	gccattttcactgtcaa	2-12-3	GCcattttcactgtCAA	162_1	25606
163	agaaatgcggagaagc	2-10-4	AGaaatgeggagAAGC	163_1	25796
164	aaatggaaaaaatgaccagc	2-14-4	AAatggaaaaaatgacCAGC	164_1	26188
165	aggacttacgacaaaaccac	1-15-4	AggacttaegacaaaaCCAC	165_1	26505
166	ggacttacgacaaaacca	2-13-3	GGacttaegacaaaaCCA	166_1	26506
167	gacttacgacaaaacca	3-11-3	GACttaegacaaaaCCA	167_1	26506
168	acaccaggacttacgaca	1-14-3	AcaccaggacttaegACA	168_1	26512
169	tagaaattcaacatggc	1-12-4	TagaaattcaacaTGGC	169_1	27376
169	tagaaattcaacatggc	4-11-2	TAGAaattcaacatgGC	169_2	27376
169	tagaaattcaacatggc	2-12-3	TAgaaattcaacatGGC	169_3	27376
170	ctagaaattcaacatggc	2-13-3	CTagaaattcaacatGGC	170_1	27376
171	gtcatcggttcacc	1-9-4	GtcateggttCACC	171_1	27602
172	actcgaagacgcca	2-8-4	ACtegaagacGCCA	172_1	28539
173	gactcgaagacgcc	3-9-2	GACtegaagaegCC	173_1	28540
174	ggcacaagcagaacgac	2-13-2	GGcacaagcagaaegAC	174_1	29235
175	agtcagaacaaaggaggc	1-15-2	AgtcagaacaaaggagGC	175_1	29668
176	gaagtcagaacaaaggag	4-12-2	GAAGtcagaacaaaggAG	176_1	29670
177	gcagaagtcagaacaaagg	1-14-4	GcagaagtcagaacaAAGG	177_1	29672
178	gtgcagaagtcagaacaaa	3-13-3	GTGcagaagtcagaacAAA	178_1	29674
178	gtgcagaagtcagaacaaa	3-14-2	GTGcagaagtcagaacaAA	178_2	29674
179	gtgcagaagtcagaacaa	3-13-2	GTGcagaagtcagaacAA	179_1	29675
180	aaggatgagggagcggac	1-14-3	AaggatgagggagegGAC	180_1	29894
181	gtaaggatgagggagc	2-12-2	GTaaggatgagggaGC	181_1	29898
182	tggtaaggatgagggag	1-12-4	TggtaaggatgagGGAG	182_1	29899
183	cgtacatctgcatctc	2-10-4	CGtacatctgcaTCTC	183_1	29951
184	tgtaagataagaggcaacact	1-18-2	TgtaagataagaggcaacaCT	184_1	30947
185	ttgtaagataagaggcaacac	1-17-3	TtgtaagataagaggcaaCAC	185_1	30948
186	ttgtaagataagaggcaaca	2-14-4	TTgtaagataagaggcAACA	186_1	30949
187	tttgtaagataagaggcaaca	2-17-2	TTtgtaagataagaggcaaCA	187_1	30949
188	tgtaagataagaggcaa	2-11-4	TGtaagataagagGCAA	188_1	30951
189	ctggaaggaaagttggt	2-12-3	CTggaaggaaagttGGT	189_1	31229
190	atagtaagcactgatggtc	3-14-2	ATAgtaagcactgatggTC	190_1	31245
190	atagtaagcactgatggtc	1-14-4	Atagtaagcactgat GGTC	190_2	31245
191	tagtaagcactgatgg	2-11-3	TAgtaagcactgaTGG	191_1	31247
192	catagtaagcactgatg	3-12-2	CATagtaagcactgaTG	192_1	31248
192	catagtaagcactgatg	2-11-4	CAtagtaagcactGATG	192_2	31248

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
193	ctgtaactcacctggc	1-13-2	CtgtaactcacctgGC	193_1	31835
193	ctgtaactcacctggc	2-12-2	CTgtaactcacctgGC	193_2	31835
194	cggatcactcgcccg	1-12-2	CggatcactegccCG	194_1	32000
195	acacaggctactctcgg	1-14-2	AcacaggctactcteGG	195_1	33017
196	acacaggctactctcg	3-10-3	ACAcaggctactcTCG	196_1	33018
197	ccacacacaggctactc	1-14-2	CcacacacaggctacTC	197_1	33021
198	atactccacacacaggct	1-15-2	AtactccacacacaggCT	198_1	33025
199	atactccacacacaggc	1-14-2	AtactccacacacagGC	199_1	33026
200	gctcatactccacacacag	1-16-2	GctcatactccacacacAG	200_1	33028
201	tcatactccacacacag	2-11-4	TCatactccacacACAG	201_1	33028
201	tcatactccacacacag	2-13-2	TCatactccacacacAG	201_2	33028
202	gctcatactccacacaca	1-14-3	GctcatactccacacACA	202_1	33029
202	gctcatactccacacaca	1-15-2	GctcatactccacacaCA	202_2	33029
203	tgctcatactccacacac	1-14-3	TgctcatactccacaCAC	203_1	33030
203	tgctcatactccacacac	1-15-2	TgctcatactccacacAC	203_2	33030
203	tgctcatactccacacac	2-14-2	TGctcatactccacacAC	203_3	33030
204	agcaggaagcagggagaaa	2-15-2	AGcaggaagcagggagaAA	204_1	33562
205	tccgaccacagcgag	2-11-2	TCegaccacagegAG	205_1	33681
206	cagaagccaagggacatg	1-14-3	CagaagccaagggacATG	206_1	34432
206	cagaagccaagggacatg	2-14-2	CAgaagccaagggacaTG	206_2	34432
207	cagaagccaagggacat	2-12-3	CAgaagccaagggaCAT	207_1	34433
208	ccagaccaacacggaaacg	1-14-4	CcagaccaacaeggaAACG	208_1	34571
209	ccagaccaacacggaaac	2-12-4	CCagaccaacaeggAAAC	209_1	34572
210	gaatgggcaaagggtaga	4-12-2	GAATgggcaaagggtaGA	210_1	34742
211	aatgggcaaagggtaga	2-12-3	AAtgggcaaagggtAGA	211_1	34742
210	gaatgggcaaagggtaga	2-14-2	GAatgggcaaagggtaGA	210_2	34742
212	gaatgggcaaagggtag	2-12-3	GAatgggcaaagggTAG	212_1	34743
213	gaacccttgtagtcctg	1-14-2	GaacccttgtagtccTG	213_1	35101
214	aacccttgtagtcct	4-9-2	AACCcttgtagtcCT	214_1	35102
215	ggaacccttgtagtc	2-11-2	GGaacccttgtagTC	215_1	35104
216	atcggaacccttgtagtc	2-14-2	ATeggaacccttgtagTC	216_1	35104
216	atcggaacccttgtagtc	1-15-2	AteggaacccttgtagTC	216_2	35104
217	catcggaacccttgtagtc	1-16-2	CateggaacccttgtagTC	217_1	35104
218	catcggaacccttgtagt	2-14-2	CAtcggaacccttgtaGT	218_1	35105
219	gatacagacctcctcaaact	1-17-2	GatacagacctcctcaaaCT	219_1	35370
220	gatacagacctcctcaaac	2-14-3	GAtacagacctcctcaAAC	220_1	35371
221	gatacagacctcctcaaa	2-13-3	GAtacagacctcctcAAA	221_1	35372
221	gatacagacctcctcaaa	2-12-4	GAtacagacctcctCAAA	221_2	35372
221	gatacagacctcctcaaa	1-13-4	GatacagacctcctCAAA	221_3	35372
222	gatacagacctcctcaa	2-11-4	GAtacagacctccTCAA	222_1	35373

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start position on SEQ ID NO: 1
222	gatacagacctcctcaa	1-13-3	GatacagacctcctCAA	222_2	35373
223	gccccatttaccagtg	1-13-2	GccccatttaccagTG	223_1	35470
224	cccaacaagtgatgct	2-12-2	CCcaacaagtgatgCT	224_1	35965
225	cccaacaagtgatgc	2-11-2	CCcaacaagtgatGC	225_1	35966
226	gtaccaagcccagaagg	1-14-2	GtaccaagcccagaaGG	226_1	36279
227	gtaccaagcccagaag	1-11-4	GtaccaagcccaGAAG	227_1	36280
228	ttcctgatgaagagatg	4-11-2	TTCCtgatgaagagaTG	228_1	36549
229	tcctgatgaagagatg	2-10-4	TCctgatgaagaGATG	229_1	36549
229	tcctgatgaagagatg	3-11-2	TCCtgatgaagagaTG	229_2	36549
230	tgggagtagcatggc	2-11-2	TGggagtagcatgGC	230_1	36911
231	tgtgggagtagcatggc	1-14-2	TgtgggagtagcatgGC	231_1	36911
232	gtgggagtagcatggc	1-13-2	GtgggagtagcatgGC	232_1	36911
230	tgggagtagcatggc	1-11-3	TgggagtagcatGGC	230_2	36911
233	aaacatgctgaaccctg	2-11-4	AAacatgctgaacCCTG	233_1	37254
234	acaaacatgctgaaccct	2-13-3	ACaaacatgctgaacCCT	234_1	37255
234	acaaacatgctgaaccct	1-14-3	AcaaacatgctgaacCCT	234_2	37255
234	acaaacatgctgaaccct	3-13-2	ACAaacatgctgaaccCT	234_3	37255
235	cacaaacatgctgaaccc	2-14-2	CAcaaacatgctgaacCC	235_1	37256
236	cacaaacatgctgaacc	2-12-3	CAcaaacatgctgaACC	236_1	37257
237	tggacgcacaaacatgc	1-12-4	TggaegcacaaacATGC	237_1	37263

Motif sequences represent the contiguous sequence of nucleobases present in the oligonucleotide.

Designs refer to the gapmer design, F-G-F', where each number represents the number of consecutive modified nucleosides, e.g 2' modified nucleosides (first number=5' flank), followed by the number of DNA nucleosides (second number= gap region), followed by the number of modified nucleosides, e.g 2' modified nucleosides (third number=3' flank), optionally preceded by or followed by further repeated regions of DNA and LNA, which are not necessarily part of the contiguous sequence that is complementary to the target nucleic acid.

Oligonucleotide compounds represent specific designs of a motif sequence. Capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine and 5-methyl DNA cytosines are presented by "e", and all internucleoside linkages are phosphorothioate internucleoside linkages.

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.

clonal growth medium (dHCGM). dHCGM is a DMEM medium containing 100 U/ml Penicillin, 100 µg/ml Streptomycin, 20 mM Hepes, 44 mM NaHC03, 15 µg/ml L-proline, 0.25 µg/ml insulin, 50 nM Dexamethazone, 5 ng/ml EGF, 0.1 mM Asc-2P, 2% DMSO and 10% FBS (Ishida et al., 2015). Cells were cultured at 37°C incubator in a humidified atmosphere with 5% CO2. Culture medium was replaced 24 h post-plating and every 2 days until harvest.

Primary Human Hepatocytes (PXB-PHH)

Fresh primary human hepatocytes (PXB-PHH) harvested from humanized mice (uPA/SCID mice) – herein called PHH - were obtained from PhoenixBio Co., Ltd (Japan). Cells were seeded on a collagen I-coated plate at the following cell density: 35,000 cells/well (384-well), 70,000 cells/well (96-well), or, 400,000 cells/well (24-well) in modified hepatocyte clonal growth medium (dHCGM). dHCGM is a DMEM medium containing 100 U/ml Penicillin, 100 µg/ml Streptomycin, 20 mM Hepes, 44 mM NaHC03, 15 µg/ml L-proline, 0.25 µg/ml insulin, 50 nM Dexamethazone, 5 ng/ml EGF, 0.1 mM Asc-2P, 2% DMSO and 10% FBS (Ishida et al., 2015). Cells were cultured at 37°C incubator in a humidified atmosphere with 5% CO2. Culture medium was replaced 24 h post-plating and every 2 days until harvest.

HBV infection and oligonucleotide treatment

PHH were incubated with HBV (purified from CHB individuals) at multiplicity of infection (MOI) of 40 together with 4% PEG for 24 hr; virus inoculum was removed the following day. To allow for cccDNA establishment compound treatment in PHH was started at day 3 post HBV infection. Fresh oligonucleotide dissolved in medium was replenished every 2 days (example 1) or fresh oligonucleotide on day 3, 5, 7 and 9 and after that medium was replenished every 2 days (example 2) until cells were harvested at day 19.

HBV antigen measurements

To evaluate the impact on HBV antigen expression and secretion, supernatants were collected on Day 19. The HBV propagation parameters, HBsAg and HBeAg levels, were measured using CLIA ELISA Kits (Autobio Diagnostic #CL0310-2, #CL0312-2), according to the manufacturer's protocol. Briefly, 25µL of supernatant per well were transferred to the respective antibody coated microtiter plate and 25 µL of enzyme conjugate reagent were added. The plate was incubated for 60min on a shaker at room temperature before the wells were washed five times with washing buffer using an automatic washer. 25 µL of substrate A and B were added to each

well. The plates were incubated on a shaker for 10min at room temperature before luminescence was measured using an Envision luminescence reader (Perkin Elmer).

CCK8 cellular toxicity measurements

To evaluate the impact of cellular toxicity upon treatment of oligonucleotide, cells were treated as described in HBV infection and oligonucleotide treatment and at Day 18, PHH were pre-incubated for 24 hours at 37°C incubator in a humidified atmosphere with 5% CO_2. 10ul of CCK-8 solution was added to 100ul in each well of a 96 well plate and incubated for 1-4 hours. Absorbance at 450nM using a microplate reader (Tecan) was measured for each plate and values are calculated as % of control (untreated cells).

Real-time PCR for intracellular HBV pgRNA and RTEL1 RNA

mRNA was extracted from the cells using a Qiagen BioRobot Universal System and the RNeasy 96 well Extraction Plates (RNeasy 96 BioRobot 8000 Kit (12)/ Cat No./ID: 967152) according to the manufacturer's protocol. The relative HBV and cellular mRNA expression levels were analyzed using Real-time PCR on the ABI QuantStudio 12k Flex.

Beta-actin (ACT B) and HBV pgRNA were quantified by qPCR using TaqMan Fast Advanced Master Mix (Life Technologies, cat no. 4444558) in technical triplicates. Results were normalized over the human ACT B endogenous control. The mRNA expression was analyzed using the comparative cycle threshold 2-ΔΔCt method normalized to the reference gene ACT B and to non-transfected cells. Primers used for ACTB RNA and HBV pgRNA quantification are listed in table 7.

Table 7: ACT B and HBV pgRNA qPCR primers

Parameter	Direction	Primer Sequence	Seq ID No
HBV pgRNA	Fwd	5'- GGAGTGTGGATTCGCACTCCT-3`	238
	Rev	5'- AGATTGAGATCTTCTGCGAC -3'	239
	Probe	[6FAM]-AGGCAGGTCCCCTAGAAGAAGAACTCC-[BHQ1]	240
RTEL1	Fwd	5'- CCATCCTGGACATTGAGGACT-3'	241
	Rev	5'- CAGGTTCCGGGACAGGTAGTA-3'	242
Housekeeping gene primers ACT B (VIC): Hs01060665_g1 (Thermo Fisher Scientific)			

HBV cccDNA quantification

DNA was extracted from HBV infected Primary Human Hepatocytes using an SDS Lysis Buffer and purified using the ZymoResearch Genomic DNA Clean & Concentrator kit (ZymoResearch, cat no. D4067) protocol. cccDNA levels were determined after digestion with T5 exonuclease (New England Biolabs, MA, USA) using 10U of T5 for 500ng of DNA, 1 hour at 37°C in 20ul total volume. After digestion, the samples were diluted to 50ul of which 4ul were used for the qPCR reaction. The mRNA expression was analyzed using the comparative cycle threshold 2-

ΔΔCt method normalized to the reference gene mitochondrial DNA and to non-transfected cells. Quantitative real-time polymerase chain reaction measurements were performed on the QuantStudio 12K Flex PCR System (Applied Biosystems). qPCR was performed with the Fast SYBR™ Green Master Mix (Life Technologies, Cat.No 4385612). Primer are shown in table 8.

Table 8: cccDNA qPCR primers.

Parameter	Direction	Primer Sequence	Seq ID No
cccDNA	Fwd	5′- CGTCTGTGCCTTCTCATCTGC-3`	243
	Rev	5′- GCACAGCTTGGAGGCTTGAA -3′	244
mitochondrial DNA	Fwd	5'- CCGTCTGAACTATCCTGCCC -3'	245
	Rev	5'- GCCGTAGTCGGTGTACTCGT-3'	246

Example 1: Effect of antisense oligonucleotides targeting RTEL1 on HBV parameters in HBV infected PHH

In the following experiment, the effect of RTEL1 knock-down on the HBV parameters, HBsAg, HBeAg, HBV pgRNA and cccDNA, were tested using the oligonucleotide compounds in table 6.

PHH were cultured as described in the Materials and Methods section. The cells were dosed at a final oligonucleotide concentration of 10μM dissolved in dHCGM Medium at Day 3 post HBV infection with a final culture volume of 100 μl/well. The experiment was performed in biological triplicate and cells were harvested at Day 19 post HBV infection replenishing oligonucleotide every 2 days. According to Materials and Methods, cellular toxicity was determined using CCK8, supernatant was collected to measure HBsAg and HBeAg and cells were harvested in two fractions; one for RTEL1 mRNA and pgRNA measurements using one lysis buffer and one for cccDNA measurements using another lysis buffers as described in Materials and Methods. All values are shown in Table 9 as % of control (untreated cells) i.e. for RTEL1 mRNA, cccDNA and pgRNA the lower the value the larger the inhibition.

CCK8 cellular toxicity was measured as described in the Materials and Methods section to confirm that any reduction in the viral parameters is not the cause of cell death, the closer the value is to 100% the lower the toxicity. The results are shown in table 9.

Table 9: RTEL1 mediated cccDNA degradation and inhibition of downstream products

Comp ID NO	**% CCK8 of Control**		**% RTEL1 of Control**		**% cccDNA of Control**		**% pgRNA of Control**		**% HBsAg of Control**		**% HBeAg of Control**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
22_1	93	10	68	12	19	4	55	9	60	48	32	28
23_1	98	7	40	1	46	15	57	18	113	5	140	14
24_1	100	2	9	1	37	13	52	12	106	5	110	24
25_1	99	5	14	0	69	15	57	8	99	16	54	10
26_1	95	4	3	1	38	12	44	3	78	2	74	12
27_1	84	2	4	1	45	16	31	15	106	7	79	8
28_1	92	0	31	14	35	37	7	3	4	0	27	25

29_1	89	7	33	4	24	6	80	15	81	3	54	4
32_1	115	9	25	3	22	3	104	14	100	6	109	14
35_1	97	6	4	1	27	0	115	24	80	5	76	7
36_1	96	7	14	1	43	18	129	35	102	15	91	27
37_1	103	14	8	4	27	11	127	41	91	4	90	9
38_1	107	6	14	3	47	11	129	12	123	5	141	10
39_1	107	13	29	1	26	10	107	61	124	4	112	30
40_1	101	2	48	33	19	5	29	4	94	13	88	19
41_1	112	15	31	9	42	16	129	18	134	31	79	37
42_1	100	1	27	4	17	19	72	13	15	13	22	0
42_2	94	6	23	4	6	4	76	8	30	2	26	1
42_3	100	1	29	5	30	13	78	12	22	5	21	4
43_1	97	1	34	12	8	5	66	3	78	6	49	9
43_2	121	17	48	5	26	4	70	10	95	27	98	36
46_1	124	13	21	4	37	17	130	11	102	3	74	9
49_1	96	9	57	14	36	23	30	10	10	2	15	2

*RTEL1 and HBV pgRNA is normalized to housekeeping gene.

All the tested antisense oligonucleotides targeting RTEL1 display target knockdown and HBV antiviral efficacy at a single time point of measurement against at least one of the parameters cccDNA, pgRNA, HBsAg and HBeAg with no observed cellular toxicity measured by CCK8.

Example 2: Additional oligonucleotide library targeting RTEL1 tested for effect on cccDNA in infected PHH

In the following experiment an additional library of 236 oligonucleotides targeting across the RTEL1 transcript was generated, shown in table 6 as CMP ID NO: 50_1 to 237_1. The ability to reduce RTEL1 as well as cccDNA, was tested.

PHH were cultured as described in the Materials and Methods section. The cells were dosed at a final oligonucleotide concentration of 10μM dissolved in dHCGM Medium at Day 3. 5. 7 and 9 post HBV infection with a final culture volume of 100 μl/well. The experiment was performed in biological triplicate and cells were harvested at Day 19 post HBV infection with replenishing medium every 2 days until day 19.

According to Materials and Methods, cells were harvested in two fractions; one for RTEL1 mRNA using one lysis buffer and one for cccDNA measurements using another lysis buffers as described in Materials and Methods. All values are shown in Table 10 as % of control (untreated cells) i.e. for RTEL1 mRNA and cccDNA the lower the value the larger the inhibition/reduction.

Table 10: RTEL1 reduction and cccDNA degradation following oligonucleotide treatment

Comp ID NO	% RTEL1 of Control		% cccDNA of Control		Comp ID NO	% RTEL1 of Control		% cccDNA of Control	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
50_1	7.01	1.47	91.93	53.33	145_2	57.20	14.77	66.82	56.81
51_1	23.34	5.14	91.90	100.79	146_1	74.65	9.52	58.95	24.13
52_1	4.97	3.32	73.18	18.65	147_1	109.87	31.74	71.13	40.86
53_1	74.82	16.01	52.34	6.08	147_2	49.79	11.64	66.49	6.77
54_1	23.96	11.87	84.18	48.67	148_1	99.53	13.20	82.39	6.25
55_1	35.59	7.17	93.69	8.65	149_1	63.54	1.61	58.26	12.29
56_1	78.85	7.76	79.38	6.37	150_1	38.91	3.01	85.40	38.11
57_1	43.89	3.45	56.65	21.45	151_1	75.08	50.61	99.88	1.35
57_2	33.43	20.36	53.42	24.96	152_1	36.80	3.08	53.63	9.51
58_1	23.10	3.28	55.61	24.65	153_1	38.60	2.68	103.42	45.69
59_1	84.74	22.73	83.30	8.28	153_2	28.05	7.82	74.47	62.74
60_1	64.90	34.31	51.76	9.74	154_1	41.59	7.01	77.58	12.13
61_1	40.04	6.27	105.58	36.39	155_1	68.84	9.18	56.76	20.70
62_1	48.58	7.48	69.29	5.28	156_1	58.21	14.18	69.70	8.08
63_1	12.26	1.26	104.61	34.95	157_1	70.14	24.39	58.48	4.65
64_1	9.23	5.14	101.03	20.54	158_1	48.72	1.12	92.78	2.45
65_1	44.85	17.33	101.27	9.05	158_2	96.47	44.09	63.51	2.33
66_1	69.93	1.72	57.89	17.40	159_1	69.81	9.81	63.86	5.72
66_2	68.17	6.74	86.07	8.18	160_1	18.16	0.57	76.72	8.87
67_1	34.99	34.99	56.88	31.24	161_1	4.70	1.81	63.24	27.23
68_1	49.49	15.69	108.68	37.37	162_1	1.23	0.24	96.00	1.23
68_2	68.22	14.89	100.98	74.61	163_1	18.96	8.11	107.22	28.58
69_1	86.75	3.21	96.31	6.98	164_1	20.71	8.98	100.94	10.67
70_1	30.76	11.20	105.62	22.24	165_1	54.95	11.34	79.93	2.08
71_1	41.40	9.03	77.41	55.10	166_1	83.13	18.00	72.36	39.67
72_1	42.31	3.14	84.91	30.20	167_1	105.82	73.31	69.39	13.42
73_1	56.43	2.53	52.43	7.92	168_1	56.30	26.26	61.25	5.61
74_1	62.53	11.46	63.20	10.15	169_1	68.48	18.97	102.59	5.31
75_1	27.73	0.03	54.09	8.64	169_2	40.76	10.27	71.29	13.34
76_1	68.10	16.93	60.01	41.58	169_3	12.35	7.59	103.66	4.18
77_1	25.25	8.17	97.07	11.51	170_1	62.45	13.28	101.40	4.46
78_1	24.30	4.02	57.29	3.26	171_1	48.84	14.22	56.66	4.08
79_1	34.02	5.55	52.04	8.90	172_1	65.73	10.35	86.22	58.44
80_1	64.76	6.15	92.09	8.09	173_1	74.87	8.18	57.84	1.98
80_2	100.95	0.38	91.57	16.17	174_1	32.77	6.46	65.95	17.39
81_1	14.80	0.00	61.33	9.38	175_1	14.82	5.49	77.83	11.93
82_1	7.11	1.75	54.70	15.77	176_1	10.54	4.41	64.33	32.00
82_2	9.81	1.64	58.75	1.67	177_1	9.41	2.42	60.66	6.42
83_1	36.85	0.00	69.70	7.20	178_1	2.79	1.65	76.11	8.17
83_2	8.21	4.88	66.53	7.60	178_2	1.61	1.09	82.07	37.30
84_1	62.48	11.67	93.31	8.13	179_1	2.40	0.97	100.84	2.49
85_1	44.09	9.50	61.00	9.51	180_1	28.81	9.47	64.36	9.58

Comp ID NO	% RTEL1 of Control		% cccDNA of Control		Comp ID NO	% RTEL1 of Control		% cccDNA of Control	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
85_2	46.72	4.15	97.36	24.50	181_1	39.34	6.66	64.81	42.15
86_1	80.90	31.21	51.63	23.28	182_1	40.14	7.85	50.35	5.83
86_2	78.93	4.86	79.87	49.47	183_1	27.21	11.98	96.97	14.85
87_1	26.46	3.80	58.61	6.02	184_1	54.48	12.67	97.69	3.13
88_1	19.44	0.42	80.75	14.07	185_1	97.74	21.37	57.01	9.95
89_1	86.51	25.84	58.06	27.75	186_1	13.48	2.66	76.92	17.59
90_1	58.49	9.86	97.93	30.79	187_1	99.48	9.54	68.99	6.50
91_1	90.34	8.97	70.59	11.83	188_1	4.89	1.53	75.04	5.16
92_1	53.35	13.77	92.14	13.18	189_1	62.35	14.74	72.42	7.24
93_1	86.17	18.80	68.78	12.94	190_1	30.13	3.34	53.95	16.62
94_1	82.51	34.51	74.00	6.73	190_2	70.42	8.41	57.21	7.44
95_1	28.32	13.87	81.77	0.96	191_1	13.78	2.44	59.40	8.50
95_2	31.64	0.23	54.23	0.02	192_1	33.66	5.65	54.13	15.57
96_1	65.92	43.39	90.65	44.78	192_2	52.23	12.88	81.54	6.25
97_1	67.17	19.66	91.10	45.98	193_1	72.41	7.09	79.15	22.21
98_1	20.59	11.95	70.09	7.77	193_2	45.71	16.85	54.47	27.40
99_1	45.17	11.96	81.29	17.73	194_1	27.32	14.47	76.00	7.91
100_1	17.96	8.21	72.67	33.48	195_1	44.50	2.82	51.02	9.90
101_1	17.68	4.56	59.25	15.63	196_1	7.35	2.01	62.45	40.44
102_1	21.78	7.52	56.99	3.77	197_1	60.12	8.74	59.04	23.63
103_1	19.65	2.57	97.02	4.67	198_1	17.64	9.05	72.08	10.88
104_1	46.82	22.92	55.25	3.33	199_1	11.67	2.87	71.46	24.06
105_1	27.66	3.21	72.99	3.87	200_1	22.21	1.07	105.37	3.83
105_2	48.60	13.68	83.37	14.25	201_1	2.79	2.14	67.89	3.06
106_1	29.66	11.05	102.77	23.24	201_2	2.14	0.14	79.46	18.07
106_2	56.28	1.60	50.25	12.84	202_1	8.36	1.66	81.72	3.96
107_1	47.82	18.86	57.44	34.75	202_2	17.71	3.55	91.16	6.78
108_1	26.06	6.57	53.90	14.97	203_1	6.91	1.51	55.95	4.33
108_2	9.75	2.24	59.30	0.53	203_2	10.41	6.90	75.76	2.97
108_3	51.83	0.77	69.13	12.27	203_3	41.89	0.58	61.00	6.57
109_1	5.91	2.61	52.52	12.19	204_1	94.01	4.50	88.29	7.73
109_2	6.37	1.45	68.41	17.92	205_1	13.51	5.81	109.03	20.51
110_1	11.12	1.90	71.58	47.02	206_1	89.89	7.82	85.70	3.32
111_1	84.19	10.48	55.32	2.17	206_2	67.36	7.93	60.55	9.50
112_1	41.08	12.50	52.72	6.13	207_1	23.15	9.64	60.44	29.16
113_1	100.00	0.00	78.91	6.77	208_1	92.51	27.58	75.99	10.80
114_1	99.08	41.68	57.06	6.19	209_1	92.08	9.26	52.56	6.76
115_1	25.63	22.23	72.30	60.35	210_1	12.27	6.71	76.30	31.02
116_1	87.50	1.46	108.23	75.62	210_2	89.62	18.30	77.25	3.97
116_2	83.18	0.17	105.88	5.43	211_1	39.78	3.92	88.22	14.53
117_1	76.07	4.23	67.01	6.43	212_1	36.19	2.39	56.65	14.73
117_2	109.05	3.40	108.67	30.57	213_1	26.52	14.58	62.72	7.94
117_3	93.69	11.88	76.35	2.30	214_1	21.32	6.14	62.15	15.02

Comp ID NO	% RTEL1 of Control		% cccDNA of Control		Comp ID NO	% RTEL1 of Control		% cccDNA of Control	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
118_1	46.05	10.08	72.32	8.58	215_1	22.10	3.45	97.49	19.57
119_1	54.19	20.46	90.03	4.64	216_1	29.74	1.46	53.53	20.26
120_1	29.75	11.69	90.50	26.06	216_2	31.17	7.18	100.84	59.55
120_2	47.34	0.97	101.98	12.10	217_1	36.16	21.08	73.76	5.05
121_1	34.63	9.13	103.04	31.21	218_1	26.08	3.63	62.45	6.34
122_1	23.84	3.45	60.00	1.08	219_1	27.98	0.88	96.57	11.43
122_2	49.03	3.58	57.10	15.69	220_1	35.67	2.59	103.11	28.19
123_1	87.91	14.47	80.61	3.32	221_1	21.87	3.53	80.38	6.57
124_1	45.34	4.29	97.91	15.74	221_2	38.08	4.00	92.15	12.10
125_1	65.93	8.36	82.38	1.51	221_3	40.64	9.61	107.45	5.84
126_1	19.63	1.74	67.12	12.80	222_1	19.69	3.07	59.72	7.41
127_1	28.07	3.10	59.52	3.75	222_2	30.77	10.21	54.26	1.45
128_1	46.11	9.85	89.13	11.64	223_1	78.85	28.60	90.08	12.56
129_1	83.14	23.83	88.56	9.69	224_1	26.92	15.16	71.93	15.87
130_1	3.01	1.73	51.18	0.78	225_1	30.24	6.80	109.61	36.24
131_1	5.14	1.68	106.08	38.37	226_1	35.09	15.62	107.11	10.01
132_1	94.17	18.86	85.17	13.15	227_1	37.63	12.68	70.20	8.73
133_1	37.12	8.11	71.11	12.56	228_1	87.69	10.70	72.25	4.00
134_1	19.28	5.14	68.39	8.54	229_1	44.99	22.32	77.03	46.39
135_1	17.83	0.86	84.76	4.78	229_2	63.89	35.36	51.67	29.57
136_1	5.76	3.37	91.47	13.69	230_1	73.08	17.58	90.57	53.08
137_1	91.76	19.69	89.85	32.57	230_2	36.46	5.34	61.99	30.47
138_1	73.95	8.38	50.33	10.55	231_1	45.85	28.46	67.18	23.44
139_1	54.46	1.17	70.90	15.24	232_1	15.33	10.56	58.98	30.05
139_2	52.71	12.53	52.57	17.88	233_1	92.94	7.62	60.18	12.23
140_1	53.81	13.82	55.02	39.17	234_1	36.40	26.60	60.64	20.11
141_1	24.70	4.90	93.03	59.35	234_2	36.46	9.00	76.75	15.69
142_1	19.89	3.31	54.31	2.48	234_3	55.70	17.41	72.99	21.80
143_1	31.38	2.16	62.23	54.78	235_1	85.79	19.15	69.06	8.99
144_1	18.80	15.78	65.31	22.34	236_1	50.52	7.64	85.61	8.75
145_1	42.42	4.00	65.22	31.76	237_1	94.23	25.38	79.07	31.59

CCK8 cellular toxicity was measured as described in the Materials and Methods section to assess if reduction in the viral parameters could be caused by cell death, the closer the value is to 100% the lower the toxicity. The results are shown in table 11. For CCK8 values above 80 % of control it is not considered likely that cell death has an impact on the RTEL1 and cccDNA reduction shown in table 10.

Table 11: CCK8 cellular toxicity

Comp ID NO	CCK8 % of Control		Comp ID NO	CCK8 % of Control		Comp ID NO	CCK8 % of Control	
	Mean	SD		Mean	SD		Mean	SD
50_1	116.26	8.82	114_1	103.47	7.90	178_2	117.93	3.77
51_1	105.05	7.05	115_1	107.24	8.20	179_1	114.12	5.05

Comp ID NO	CCK8 % of Control		Comp ID NO	CCK8 % of Control		Comp ID NO	CCK8 % of Control	
	Mean	SD		Mean	SD		Mean	SD
52_1	82.96	6.08	116_1	85.06	7.26	180_1	93.24	2.68
53_1	134.27	5.46	116_2	80.29	5.56	181_1	98.66	2.53
54_1	103.38	9.14	117_1	#N/A	#N/A	182_1	89.46	7.87
55_1	71.55	5.78	117_2	95.10	2.33	183_1	83.55	4.22
56_1	95.93	20.93	117_3	96.89	13.60	184_1	70.62	12.67
57_1	105.94	3.64	118_1	#N/A	#N/A	185_1	97.55	10.88
57_2	111.45	11.03	119_1	85.82	4.56	186_1	96.65	8.31
58_1	107.29	9.64	120_1	85.68	11.44	187_1	54.10	8.19
59_1	88.76	7.99	120_2	93.79	5.14	188_1	101.60	8.41
60_1	107.26	3.18	121_1	#N/A	#N/A	189_1	68.00	10.70
61_1	123.93	12.61	122_1	144.58	44.88	190_1	141.71	16.49
62_1	108.72	7.62	122_2	113.11	8.57	190_2	83.85	6.76
63_1	98.97	11.62	123_1	59.62	9.47	191_1	148.53	8.91
64_1	76.87	11.82	124_1	52.57	2.90	192_1	106.29	5.85
65_1	101.36	5.18	125_1	101.47	7.25	192_2	85.20	10.09
66_1	89.28	6.92	126_1	129.31	9.43	193_1	91.71	5.71
66_2	106.42	0.96	127_1	87.40	4.43	193_2	73.38	2.71
67_1	28.82	0.83	128_1	80.60	7.16	194_1	93.19	4.43
68_1	118.08	11.35	129_1	95.26	0.97	195_1	95.70	2.72
68_2	125.36	11.78	130_1	125.56	4.29	196_1	67.40	8.02
69_1	97.66	16.30	131_1	107.17	5.92	197_1	114.76	21.86
70_1	120.71	12.95	132_1	21.86	2.92	198_1	85.13	6.45
71_1	102.41	6.80	133_1	77.50	10.35	199_1	91.59	3.24
72_1	83.17	6.40	134_1	106.91	0.79	200_1	87.64	6.54
73_1	55.40	2.16	135_1	103.33	1.13	201_1	101.76	5.01
74_1	118.35	4.36	136_1	64.06	3.46	201_2	110.01	8.04
75_1	84.55	0.14	137_1	70.57	14.52	202_1	85.95	4.63
76_1	102.91	13.06	138_1	70.14	17.00	202_2	88.94	3.52
77_1	99.31	8.11	139_1	62.23	1.13	203_1	92.92	2.20
78_1	#N/A	#N/A	139_2	59.93	2.52	203_2	104.16	3.17
79_1	55.12	5.20	140_1	90.30	7.69	203_3	129.86	20.13
80_1	90.80	2.66	141_1	63.42	2.15	204_1	112.00	27.06
80_2	98.59	2.30	142_1	123.38	47.71	205_1	87.02	9.91
81_1	67.03	2.60	143_1	69.90	3.87	206_1	90.88	10.42
82_1	59.63	2.52	144_1	#N/A	#N/A	206_2	67.71	14.83
82_2	68.54	1.17	145_1	#N/A	#N/A	207_1	93.70	3.15
83_1	64.43	2.61	145_2	#N/A	#N/A	208_1	90.05	3.01
83_2	64.11	0.89	146_1	#N/A	#N/A	209_1	69.85	4.14
84_1	#N/A	#N/A	147_1	#N/A	#N/A	210_1	86.07	4.68
85_1	96.19	0.06	147_2	#N/A	#N/A	210_2	84.28	15.59
85_2	90.23	8.54	148_1	#N/A	#N/A	211_1	101.07	4.83
86_1	89.93	1.33	149_1	107.30	13.12	212_1	94.44	4.57
86_2	106.93	12.98	150_1	112.39	27.25	213_1	99.59	14.01
87_1	#N/A	#N/A	151_1	72.22	6.20	214_1	#N/A	#N/A

Comp ID NO	CCK8 % of Control		Comp ID NO	CCK8 % of Control		Comp ID NO	CCK8 % of Control	
	Mean	SD		Mean	SD		Mean	SD
88_1	33.65	2.65	152_1	128.68	44.31	215_1	#N/A	#N/A
89_1	95.60	3.51	153_1	148.38	11.62	216_1	96.46	10.44
90_1	97.32	6.73	153_2	136.75	4.21	216_2	103.47	4.95
91_1	61.96	5.80	154_1	99.55	5.83	217_1	104.08	4.88
92_1	64.15	10.92	155_1	110.71	8.09	218_1	#N/A	#N/A
93_1	92.01	6.19	156_1	116.06	0.75	219_1	112.81	6.71
94_1	96.44	10.38	157_1	106.15	16.19	220_1	93.55	8.40
95_1	80.17	2.61	158_1	98.71	16.36	221_1	#N/A	#N/A
95_2	77.56	1.32	158_2	85.55	5.15	221_2	93.70	5.32
96_1	#N/A	#N/A	159_1	118.35	1.48	221_3	106.21	8.49
97_1	#N/A	#N/A	160_1	165.75	9.61	222_1	97.90	13.52
98_1	107.81	13.88	161_1	158.90	0.88	222_2	103.67	13.27
99_1	#N/A	#N/A	162_1	104.97	1.85	223_1	78.11	5.49
100_1	#N/A	#N/A	163_1	133.83	9.44	224_1	#N/A	#N/A
101_1	#N/A	#N/A	164_1	84.46	17.61	225_1	#N/A	#N/A
102_1	#N/A	#N/A	165_1	95.10	11.69	226_1	98.77	6.85
103_1	114.99	24.45	166_1	74.96	8.17	227_1	132.53	18.49
104_1	103.45	5.92	167_1	68.09	11.24	228_1	90.41	3.32
105_1	75.27	1.94	168_1	90.07	16.56	229_1	107.49	3.69
105_2	61.11	2.24	169_1	92.53	8.41	229_2	92.21	3.07
106_1	101.58	1.06	169_2	102.95	9.23	230_1	93.65	2.24
106_2	101.83	5.69	169_3	100.23	4.07	230_2	105.60	5.47
107_1	#N/A	#N/A	170_1	100.71	10.54	231_1	97.51	5.97
108_1	103.24	5.94	171_1	120.58	39.11	232_1	111.24	3.86
108_2	101.74	5.49	172_1	113.47	9.82	233_1	119.06	18.13
108_3	90.13	13.66	173_1	102.28	2.81	234_1	85.73	12.74
109_1	100.11	4.80	174_1	87.69	3.76	234_2	101.48	9.74
109_2	113.58	2.84	175_1	122.11	5.30	234_3	91.68	23.71
110_1	#N/A	#N/A	176_1	69.43	2.44	235_1	93.48	9.09
111_1	92.73	1.91	177_1	108.37	7.16	236_1	95.73	8.67
112_1	105.20	2.71	178_1	107.27	9.14	237_1	87.22	5.14
113_1	103.59	5.78						

#NA means that the CCK8 was not measured for the indicated compound.

The results show that of the 236 oligonucleotides in the library only 5 % were not able to reduce at least either RTEL1 or cccDNA to at least 80% of the control. From this it can be seen that it is possible to produce oligonucleotides targeting across the entire RTEL1 transcript which are capable of reducing RTEL1 and/or cccDNA, generally the reduction in RTEL1 and cccDNA are not due to cell death, although it may be the case for a few of the compounds.

CLAIMS

1. A RTEL1 inhibitor for use in the treatment and/or prevention of Hepatitis B virus (HBV) infection.
2. The RTEL1 inhibitor for use according to claim 1, wherein the HBV infection is a chronic infection.
3. The RTEL1 inhibitor for use according to claim 1 or 2, wherein the RTEL1 inhibitor is capable of reducing cccDNA in an infected cell.
4. The RTEL1 inhibitor for use according to any one of claims 1 to 3, wherein said inhibitor is an oligonucleotide of 12 to 60 nucleotides in length comprising a contiguous nucleotide sequence of at least 10 nucleotides in length which is at least 95% complementary to a mammalian RTEL1 target nucleic acid, in particular a human RTEL1 nucleic acid, and is capable of reducing RTEL1 mRNA.
5. The RTEL 1 inhibitor for use according to any one of claims 1 to 4 selected from a single stranded antisense oligonucleotide, siRNA or a shRNA molecule.
6. The RTEL 1 inhibitor for use according to any one of claim 1 to 5, wherein the mammalian RTEL1 target nucleic acid is selected from SEQ ID NO: 1 or 2.
7. The RTEL 1 inhibitor for use according to any one of claims 4 to 6, wherein the contiguous nucleotide sequence is at least 98% complementarity to the target nucleic acid of SEQ ID NO: 1 and SEQ ID NO: 2.
8. The RTEL 1 inhibitor for use according to any one of claims 3 to 7, wherein the cccDNA in an HBV infected cell is reduced by at least 60% when compared to a control.
9. The RTEL 1 inhibitor for use according to any one of claims 4 to 7, wherein the RTEL1 mRNA is reduced by at least 60% when compared to a control.
10. A single stranded antisense oligonucleotide of 12-30 nucleotides in length comprising a contiguous nucleotides sequence of at least 10 nucleotides which is complementary to a mammalian RTEL1, in particular a human RTEL1, wherein the oligonucleotide is capable of inhibiting the expression of RTEL1.
11. The antisense oligonucleotide according to claim 10, wherein the contiguous nucleotide sequence is at least 90% complementary, such as fully complementary, to SEQ ID NO: 1.
12. The antisense oligonucleotide according to claim 10 or 11 comprising a contiguous nucleotide sequence of 12 to 25, in particular 15 to 21 nucleotides in length.
13. An oligonucleotide according to any one of claims 11 to 12, wherein the contiguous nucleotide sequence is 100% complementary to a target sequence selected from SEQ ID NO: 3-21.

14. The oligonucleotide according to any one of claims 10 to 13, wherein the oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 22-237.

15. The antisense oligonucleotide according to any one of claims 10 to 14, comprising one or more 2' sugar modified nucleoside.

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

17. The antisense oligonucleotide according to any one of claims 15 or 16, wherein the one or more 2' sugar modified nucleoside is a LNA nucleoside.

18. The antisense oligonucleotide according to any one of claims 10 to 17, where the oligonucleotide comprises at least one phosphorothioate internucleoside linkage.

19. The antisense oligonucleotide according to claim 18, wherein all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

20. The antisense oligonucleotide according to any one of claims 10 to 19, wherein the oligonucleotide is capable of recruiting RNase H.

21. The antisense oligonucleotide according to any one of claims 10 to 20, wherein the antisense oligonucleotide, or contiguous nucleotide sequence thereof, consists or comprises a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise 1 - 4 2' sugar modified nucleosides and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH, such as a region comprising between 6 and 18 DNA nucleosides.

22. A conjugate comprising an oligonucleotide according to any one of claims 10 to 21 and at least one conjugate moiety covalently attached to said oligonucleotide.

23. The conjugate compound of claim 22, wherein the conjugate moiety is selected from one of the trivalent GalNAc moieties in figure 1.

24. The conjugate compound of claim 22 or 23 comprising a physiologically labile linker composed of 2 to 5 linked nucleosides comprising at least two consecutive phosphodiester linkages, wherein the physiologically labile linker covalently bound at the 5' or 3' terminal of the oligonucleotide component.

25. A pharmaceutically acceptable salt of an oligonucleotide according to any one of claims 10 to 21, or of a conjugate according to claim 22 to 24.

26. A pharmaceutical composition comprising an oligonucleotide according to any one of claims 10 to 21, or of a conjugate according to claim 22 to 24 or a pharmaceutically acceptable salt according to claim 25 and a pharmaceutically acceptable excipient.

27. An *in vivo* or *in vitro* method for modulating RTEL1 expression in a target cell which is expressing RTEL1, said method comprising administering an oligonucleotide according to any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26 in an effective amount to said cell.

28. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide according any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26, to a subject suffering from or susceptible to the disease.

29. A method according to claim 28, wherein the disease is Hepatitis B Virus (HBV).

30. An antisense oligonucleotide according any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26 for use in medicine.

31. The use of an oligonucleotide according any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26, for the preparation of a medicament for the treatment or prevention of Hepatitis B Virus HBV.

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