OLIGOMERS AND OLIGOMER CONJUGATES

FIELD OF INVENTION

The invention relates to the field of oligomer therapeutics, and in particular to oligomers and oligomer conjugates targeting Hepatitis B Virus (HBV). In particular, the invention relates to the field of oligomer conjugates therapeutics wherein antisense oligonucleotides are attached to a carrier component. In certain aspects, the invention relates to the field of oligomer conjugates therapeutics wherein antisense oligonucleotides are covalently attached to a carrier component by means of physiologically labile linkers.

More specifically, the present invention relates to oligomers, in particular oligomer conjugates therapeutics, that target HBV mRNA in a cell leading to treatment of the viral disorders.

BACKGROUND

Molecular strategies are being developed to modulate unwanted gene expression that either directly causes, participates in, or aggravates a disease state. One such strategy involves inhibiting gene expression with oligonucleotides complementary in sequence to the messenger RNA of a deleterious target gene. The messenger RNA strand is a copy of the coding DNA strand and is therefore, as the DNA strand, called the sense strand. Oligonucleotides that hybridize to the sense strand are called antisense oligonucleotides. Binding of these strands to mRNA interferes with the translation process and consequently with gene expression.

Certain nucleotide-based compounds have been utilized in various therapeutic applications. In particular, various oligonucleotides have been investigated including single-stranded and double-stranded oligonucleotides, and analogues. To be useful in *in vivo* applications, an oligonucleotide must have a plethora of properties including the ability to penetrate a cell membrane, have good resistance to extra- and intracellular nucleases, have high affinity and specificity for the target and preferably have the ability to recruit endogenous enzymes such as RNAseH, RNAaseIII, RNAseL etc.

A fundamental property of oligonucleotides that underlies many of their potential therapeutic applications is their ability to recognize and hybridize specifically to complementary single stranded nucleic acids employing either Watson-Crick hydrogen bonding (A-T and G-C) or other hydrogen bonding schemes such as the Hoogsteen/reverse Hoogsteen mode. Affinity and specificity are properties commonly employed to characterize hybridization characteristics of a particular oligonucleotide. Affinity is a measure of the binding strength of the oligonucleotide to its complementary target (expressed as the thermostability (Tm) of the duplex). Each nucleobase pair in the duplex adds to the thermostability and thus affinity increases with increasing size (number of nucleobases) of the oligonucleotide. Specificity is a measure of the

ability of the oligonucleotide to discriminate between a fully complementary and a mismatched target sequence.

Modified nucleic acids are known to be used to improve for example stabilisation of the oligonucleotides, in particular for the gapmer designs, such as when 1 or more modified nucleotides are present in either or both of the wing regions. Examples of modifications include 2'-O-alkyl-RNA units, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA (intercalating nucleic acid -Christensen, 2002. Nucl. Acids. Res. 2002 30: 4918-4925, hereby incorporated by reference) units, 2'MOE units, ENA (ethylene nucleic acid), UNA (Unlocked Nucleic Acid, Fluiter et al., Mol. Biosyst., 2009, 10, 1039), Tricyclo DNA (R. Steffens & C. J. Leumann, J. Am. Chem. Soc, 1997, 119, 11548-49), cET-LNA (shown herein), and LNA. Figure 4 presents drawings of some of these analogues.

A particular efficacious modified nucleic acid is referred to as a Locked Nucleic Acids (LNA). LNAs have been reported in the art – for example see International Patent Application WO 99/14226; P. Nielsen et al, J. Chem. Soc., Perkin Trans. 1, 1997, 3423; P. Nielsen et al., Chem. Commun., 1997, 9, 825; N. K. Christensen et al., J. Am. Chem. Soc., 1998, 120, 5458; A. A. Koshkin et al., J. Org. Chem., 1998, 63, 2778; A. A Koshkin et al. J. Am. Chem. Soc. 1998, 120, 13252-53; Kumar et al. Bioorg, & Med. Chem. Lett.,1998, 8, 2219-2222; and S. Obika et al., Bioorg. Med. Chem. Lett., 1999, 515. Interestingly, incorporation of LNA units containing a 2'-O,4'-C-methylene bridge into an oligonucleotide sequence leads to an unprecedented improvement in the hybridization stability of the modified oligonucleotide (see above and e.g., S. K. Singh et al., Chem. Commun, 1998, 455). Oligonucleotides comprising the 2'-O,4'-C-methylene bridge (LNA) units and also the corresponding 2'-thio-LNA (thio-LNA), 2'-HN-LNA (amino-LNA), and 2'-N(R)-LNA (amino-R-LNA) analogue, form duplexes with complementary DNA and RNA with thermal stabilities not previously observed for bi- and tricyclic nucleosides modified oligonucleotides. The increase in Tm per modification varies from +3 to +11°C, and furthermore, the selectivity is also improved. No other DNA analogue has reproducibly shown such high affinity for nucleic acids.

In a particular aspect, the present invention relates to hepatitis B virus (HBV) therapies. Hepatitis B is a viral disease caused by the hepatitis B virus (HBV). It is transmitted parenterally by contaminated material such as blood and blood products, contaminated needles, sexually and vertically from infected or carrier mothers to their offspring. In those areas of the world where the disease is common vertical transmission at an early age results in a high proportion of infected individuals becoming chronic carriers of hepatitis B. It is estimated by the World Health Organization that more than 2 billion people have been infected worldwide, with about 4 million acute cases per year, 1 million deaths per year, and 350-400 million chronic carriers. Approximately 25% of carriers die from chronic hepatitis, cirrhosis, or liver cancer and nearly

75% of chronic carriers are Asian. Hepatitis B virus is the second most significant carcinogen behind tobacco, causing from 60% to 80% of all primary liver cancer. HBV is 100 times more contagious than HIV.

The hepatitis B virus (HBV) is an enveloped, partially double-stranded DNA virus. The compact 3.2 kb HBV genome consists of four overlapping open reading frames (ORF), which encode for the core, polymerase (Pol), envelope and X-proteins. The Pol ORF is the longest and the envelope ORF is located within it, while the X and core ORFs overlap with the Pol ORF. The lifecycle of HBV has two main events: 1) generation of closed circular DNA (cccDNA) from relaxed circular (RC DNA), and 2) reverse transcription of pregenomic RNA (pgRNA) to produce RC DNA. Prior to the infection of host cells, the HBV genome exists within the virion as RC DNA. It has been determined that HBV virions are able to gain entry into host cells by non-specifically binding to the negatively charged proteoglycans present on the surface of human hepatocytes and via the specific binding of HBV surface antigens (HBV sAg). Once the virion has entered the cell, the viral cores and the encapsidated RC DNA are transported by host factors, via a nuclear localization signal, into the nucleus through the Impβ/Impα nuclear transport receptors. Inside the nucleus, host DNA repair enzymes convert the RC DNA into cccDNA. cccDNA acts as the template for all viral mRNAs and as such, is responsible for HBV persistence in infected individuals. The transcripts produced from cccDNA are grouped into two categories; pgRNA and subgenomic RNA. Subgenomic transcripts encode for the three envelope (L, M and S) and X proteins, and pgRNA encodes for Pre-Core, Core, and Pol proteins. Inhibition of HBV gene expression or HBV RNA synthesis leads to the inhibition of HBV viral replication and antigens production. For instance, IFN-α was shown to inhibit HBV replication and viral HBsAg production by decreasing the transcription of pregenomic RNA (pgRNA) and subgenomic RNA from the HBV covalently closed circular DNA (cccDNA) minichromosome. All HBV viral mRNAs are capped and polyadenylated, and then exported to the cytoplasm for translation. In the cytoplasm, the assembly of new virons is initiated and nascent pgRNA is packaged with viral Pol so that reverse transcription of pgRNA, via a single stranded DNA intermediate, into RC DNA can commence. The mature nucleocapsids containing RC DNA are enveloped with cellular lipids and viral L, M, and S proteins and then the infectious HBV particles are then released by budding at the intracellular membrane. Interestingly, non-infectious particles are also produced that greatly outnumber the infectious virions. These empty, enveloped particles (L, M and S), are referred to as subviral particles. Importantly, since subviral particles share the same envelope proteins and as infectious particles, it has been surmised that they act as decoys to the host immune system and have been used for HBV vaccines. The S, M, and L envelope proteins are expressed from a single ORF that contains three different start codons. All three proteins share a 226aa sequence, the

S-domain, at their C-termini. M and L have additional pre-S domains, Pre-S2 and Pre-S2 and Pre-S1, respectively. However, it is the S-domain that has the HBsAg epitope.

Hepatitis B viral infections are a continuing medical problem because, like any rapidly-replicating infectious agent, there are continuing mutations that help some sub- populations of HBV become resistant to current treatment regimens. Currently the recommended therapies for chronic HBV infection by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) include interferon alpha (INFa), pegylated interferon alpha-2a (Peg-IFN2a), entecavir, and tenofovir. However, typical interferon therapy is 48-weeks and results in serious and unpleasant side effects, and HBeAg seroconversion, 24 weeks after therapy has ceased, ranges from only 27-36%. Seroconversion of HBsAg is even lower - only 3% observed immediately after treatment ceases, with an increase to upwards of 12% after 5 years.

The secretion of antiviral cytokines in response to HBV infection by the hepatocytes and/or the intra-hepatic immune cells plays a central role in the viral clearance of infected liver. However, chronically infected patients only display a weak immune response due to various escape strategies adopted by the virus to counteract the host cell recognition systems and the subsequent antiviral responses.

Many observations showed that several HBV viral proteins could counteract the initial host cellular response by interfering with the viral recognition signaling system and subsequently the interferon (IFN) antiviral activity. Among these, the excessive secretion of HBV empty subviral particles (SVPs, HBsAg) may participate to the maintenance of the immunological tolerant state observed in chronically infected patients (CHB). In particular, SVPs could contribute to the absence of antigen presentation by dendritic cells together with the lack of HBV-specific T cell immune activation to enable viral persistence. HBsAg quantification is a significant biomarker to predict the infection outcome; however the achievement of HBsAg seroconversion is rarely observed in chronically infected patients. The reduction of the SVP and HBsAg burden is thought as a pathway to recover anti-viral immune function and the seroconversion to HBsAg-negative after antiviral therapy can be seen as an indication as a functional cure and remains the ultimate goal of therapy. Therefore, targeting HBV gene expression leading to reduction of HBsAg together with HBV DNA levels in CHB patients may significantly improve CHB patient immune reactivation and remission.

The nucleoside and nucleotide therapies entecavir and tenofovir are successful at reducing viral load, but the rates of HBeAg seroconversion and HBsAg loss are even lower than those obtained using IFNa therapy. Other similar therapies, including lamivudine (3TC), telbivudine

(LdT), and adefovir are also used, but for nucleoside/nucleotide therapies in general, the emergence of resistance limits therapeutic efficacy.

US 8,598,334 and WO 2012/145697 mention the use of antisense oligonucleotides to target HBV.

The control of viral infection needs a tight surveillance of the host innate immune system which could respond within minutes to hours after infection to impact on the initial growth of the virus and limit the development of a chronic and persistent infection. Despite the available current treatments based on IFN and nucleos(t)ide analogues, the Hepatitis B virus (HBV) infection remains a major health problem worldwide which concerns an estimated 350 million chronic carriers who have a higher risk of liver cirrhosis and hepatocellular carcinoma.

There is a need for new anti-viral therapies, in particular anti-HBV therapies. There is also a need to have a therapeutic strategy that enables one to target different types of HBV genotypes.

SUMMARY

The present disclosure relates to oligomer conjugates, uses thereof, methods using same, that are suitable for use in medicine, such as the treatment of a viral disorder.

In particular, the present disclosure provides an oligomer or an oligomer conjugate – that is suitable for use in the treatment of a viral disorder - wherein said oligomer or said oligomer of the oligomer conjugate is capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV. In certain embodiments a carrier component is conjugated to the oligomer.

In one aspect, the present disclosure provides an oligomer conjugate selected from the group consisting of:

5'- GN2-C6 ca**G$_S$**^{m}**C$_S$G$_S$**t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G$_S$G**-3' (SEQ ID NO: 815)

5'- GN2-C6 ca**G$_S$**^{m}**C$_S$G$_S$**t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G$_S$G$_S$T**-3' (SEQ ID NO: 814)

5'-GN2-C6 ca**G$_S$**^{m}**C$_S$G$_S$**t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$**A$_S$G$_S$G**-3' (SEQ ID NO: 825)

5'- GN2-C6 ca**A$_S$G$_S$**^{m}**C$_S$**g$_S$a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$**A$_S$**^{m}**C$_S$G**-3' (SEQ ID NO: 808)

5'-GN2-C6 ca**A$_S$G$_S$**g$_S$t$_S$g$_S$a$_S$a$_S$g$_S$^{m}c$_S$g$_S$a$_S$**A$_S$G$_S$T$_S$G**-3' (SEQ ID NO: 826); and

5'- GN2-C6 ca**A$_S$G$_S$**^{m}**C$_S$**g$_S$a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$**^{m}C$_S$G$_S$G**-3' (SEQ ID NO: 807)

wherein uppercase letters denote beta-D-oxy-LNA units; lowercase letters denote DNA units; the subscript “s” denotes a phosphorothioate linkage; superscript m denotes a a DNA or beta-D-oxy-

LNA unit containing a 5-methylcytosine base; GN2-C6 denotes a GalNAc2 carrier component with a C6 linker.

The disclosure provides an oligomer or an oligomer conjugate as herein defined wherein the oligomer or oligomer component of the oligomer conjugate comprises at least 6, preferably at least 7, preferably at least 8, preferably at least 9, preferably at least 10 units, preferably at least 11 units, preferably at least 12 units, preferably at least 13 units, preferably at least 14 units, preferably at least 15 units, preferably at least 16 units that are at least 80% identical, preferably at least 85% identical, preferably at least 90% identical, preferably at least 91% identical, preferably at least 92% identical, preferably at least 93% identical, preferably at least 94% identical, preferably at least 95% identical, preferably at least 96% identical, preferably at least 97% identical, preferably at least 98% identical, preferably at least 99% identical, preferably identical to a region corresponding to a HBV HBx gene or HBsAg gene or to the reverse complement of a target region of a nucleic acid which encodes a HBV HBx or HBV HBsAg.

The disclosure provides an oligomer or an oligomer conjugate as herein defined wherein the oligomer or oligomer component of the oligomer conjugate comprises at least 6, preferably at least 7, preferably at least 8, preferably at least 9, preferably at least 10 units that are identical

to a region corresponding to a HBV HBx gene or HBsAg gene or to the reverse complement of a target region of a nucleic acid which encodes a HBV HBx or HBV HBsAg.

For certain embodiments, the disclosure provides an oligomer or an oligomer conjugate as herein defined wherein the oligomer or oligomer component of the oligomer conjugate comprises less than 20 units, such as less than 19 units, such as less than 18 units, such as less than 17 units, such as 16 or less units.

For certain embodiments, the disclosure provides an oligomer or an oligomer conjugate as herein defined wherein the oligomer or oligomer component of the oligomer conjugate comprises 15 units or 16 units.

The disclosure provides a conjugate comprising the oligomer according to the disclosure, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.

The disclosure provides a pharmaceutical composition comprising the oligomer or the conjugate according to the disclosure, and a pharmaceutically acceptable diluent (such as water or saline), carrier, salt or adjuvant. In another aspect, the present disclosure provides for a pharmaceutical composition comprising an oligomer conjugate according to the disclosure; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.

The disclosure provides the oligomer or the conjugate according to the disclosure for use as a medicament, such as for the treatment of a viral disorder. In another aspect, the present disclosure provides for an oligomer conjugate for use in the treatment of a viral disorder in the liver, wherein said oligomer conjugate according to the disclosure.

The disclosure provides the use of an oligomer or the conjugate according to the disclosure for the manufacture of a medicament for the treatment of a viral disorder. In another aspect, the present disclosure provides for use of the oligomer conjugate according to the disclosure in the manufacture of a medicament for the treatment of a viral disorder in the liver.

The disclosure provides a method of treating a viral disorder, said method comprising administering an effective amount of, an oligomer, an oligomer conjugate or a pharmaceutical composition according to the disclosure, to an animal suffering from, or likely to suffer from a viral disorder (such as an animal suffering from or susceptible to the disease or disorder).

The disclosure provides a method of treating a viral disorder, said method comprising administering an effective amount of, an oligomer, an oligomer conjugate or a pharmaceutical

Also disclosed are methods of treating an animal (a non-human animal or a human) suspected of having, or susceptible to, a disease or condition, associated with expression, or over-expression of HBx or HBsAg by administering to the non-human animal or human a therapeutically or prophylactically effective amount of one or more of the oligomers, conjugates or pharmaceutical compositions of the disclosure. Further, methods of using oligomers for the inhibition of expression of HBx or HBsAg, and for treatment of diseases associated with activity of HBx or HBsAg are provided.

The disclosure further provides a pharmaceutical system comprising a pharmaceutical composition according to the disclosure and an additional pharmaceutical entity/therapeutic entity. The additional pharmaceutical entity may be an oligomer or oligomer conjugate according to the present disclosure. The additional pharmaceutical entity may be an oligomer or oligomer conjugate capable of modulating a target sequence in HBV which is not within HBx or HBsAg. For example, at least one oligomer or oligomer conjugate may be capable of modulating a target sequence within the gene or mRNA for HBV HBsAg, HBeAg, or DNA polymerase.

In one embodiment, a plurality of oligomers or conjugates according to the disclosure are administered to a subject in need of treatment. The oligomers or conjugates may administered with additional pharmaceutical/therapeutic agents.

In one embodiment, the disease or disorder or condition is associated with overexpression of HBx or HBsAg.

The disclosure provides for methods for modulating the expression of HBx or HBsAg in a cell or a tissue, the method comprising the step of contacting the cell or tissue, *in vitro* or *in vivo*, with an effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof, to effect modulation of expression of HBx or HBsAg**.**

The disclosure provides for methods of inhibiting (*e.g.,* by down-regulating) the expression of HBx in a cell or a tissue, the method comprising the step of contacting the cell or tissue, *in vitro* or *in vivo*, with an effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof, to effect down-regulation of expression of HBx or HBsAg**.**

The disclosure provides for a method for the inhibition of HBx or HBsAg in a cell which is expressing HBx or HBsAg, said method comprising administering an oligomer, or a conjugate according to the invention to said cell so as to affect the inhibition of HBx or HBsAg in said cell.

Further provided are methods of down-regulating the expression of HBx or HBsAg in cells or tissues comprising contacting said cells or tissues, *in vitro* or *in vivo*, with an effective amount of one or more of the oligomers, oligomer conjugates or compositions of the disclosure.

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Features of the present disclosure are now provided.

The present disclosure provides an oligomer conjugate for use in the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component. Preferably, for delivering said first oligomer to the liver.

The present disclosure provides an oligomer conjugate suitable for the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component. Preferably, for delivering said first oligomer to the liver.

The present disclosure provides a composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate and at least one additional different oligonucleotide; wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component. Preferably, for delivering said first oligomer to the liver.

The present disclosure provides an oligomer or the oligomer component of the oligomer conjugate that hybridize to a target sequence selected from the group consisting of any one or more of positions: 1530 to 1598; 1264-1278 and 670 to 706 of SEQ ID NO: 3.

The present disclosure provides an oligomer based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);

AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 834),

which is capable of modulating a target sequence in HBx or HBsAg of HBV to treat a viral disorder.

The present disclosure provides a composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer and at least one additional different oligonucleotide; wherein said oligomer comprises at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder.

The present disclosure provides a method for treating a viral disorder, said method comprising administering to a subject in need of treatment an effective amount of an oligomer conjugate according to the disclosure.

The present disclosure provides a method for treating a viral disorder, said method comprising administering to a subject in need of treatment an effective amount of a composition according to the disclosure.

The present disclosure provides a method for treating a viral disorder, said method comprising administering to a subject in need of treatment an effective amount of an oligomer according to the disclosure.

The present disclosure provides a pharmaceutical composition comprising an oligomer conjugate according to the disclosure; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.

The present disclosure provides a pharmaceutical composition comprising a composition according to the disclosure; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.

The present disclosure provides a pharmaceutical composition comprising an oligomer according to the disclosure; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.

The present disclosure provides a pharmaceutical system comprising a pharmaceutical composition according to the disclosure and an additional pharmaceutical entity.

The present disclosure provides a motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);

for constructing an oligomer conjugate according to the disclosure.

The present disclosure provides a motif selected from the group consisting of any one or more of:NO: 13) GCGTAAAGAGAGGT (SEQ ID NO: 11) and CGCGTAAAGAGAGGT (SEQ ID NO 12) for constructing a composition according to the disclosure.

The present disclosure provides a motif selected from the group consisting of any one or more of:AGCGAAGTGCACACG (SEQ ID NO: 20); AGGTGAAGCGAAGTG (SEQ ID NO: 26); AGCGAAGTGCACACGG (SEQ ID NO 18); GAAGTGCACACGG (SEQ ID NO 16); GCGAAGTGCACACGG (SEQ ID NO 17); CGAAGTGCACACG (SEQ ID NO 19) and AGGTGAAGCGAAGT (SEQ ID NO 27) for constructing an oligomer according to the disclosure.

The present disclosure provides a method of manufacturing an oligomer conjugate according to the disclosure, comprising conjugating one or more oligomers according to the disclosure with a carrier component according to the disclosure.

The present disclosure provides a method of manufacturing a composition according to the disclosure, comprising admixing an oligomer conjugate according to the disclosure with a pharmaceutically acceptable diluents, carriers, salts or adjuvants.

BRIEF DESCRIPTION OF FIGURES

Figure 1: Presents a scheme for a LNA oligomer GalNAc conjugation step.

Figure 2: Presents examples of a cholesterol or mono-GalNAc carrier components including a C6 linker moiety (region E) which is used to link the carrier component to the oligomer (region A or to a physiologically labile linker region PL, such as a PO linker). The wavy line represents the covalent link to the oligomer or region PL.

Figure 3: Presents examples of tri-GalNAc carrier components. Conjuagtes 1 – 4 illustrate 4 suitable GalNAc carrier components, and conjugates 1a – 4a refer to the same carrier components with an additional C6 linker moiety (region E) which is used to link the carrier

component to the oligomer (region A or to a physiologically labile linker, region PL, such as a PO linker). The wavy line represents the covalent link to the oligomer or region PL.

Figure 4: Presents examples of oligomer conjugates with trivalent GalNAc carrier components.

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Figure 5: Presents structures for a series of nucleoside analogues.

Figure 6: present the structure of SEQ ID NO: 808

Figure 7: present the structure of SEQ ID NO: 814

Figure 8: present the structure of SEQ ID NO: 815

Figure 9: present the structure of SEQ ID NO: 825

Figure 10: present the structure of SEQ ID NO: 826

Figure 11: HBsAG reduction of SEQ ID NO: 807 at dose 0.28 mpk (■), 1.4 mg/kg (▲) and 7.1 mpk (▼); SEQ ID NO: 808 at dose 7.1 mg/kg (■), 1.42 mg/kg (▲) and 0.29 mg/kg (▼); SEQ ID NO: 814 at dose 0.252 mg/kg (▲) and 1.26 mg/kg (▼),6.15 mg/kg (◆); SEQ ID NO: 815 at dose 0.3 mg/kg (■), 1.5 mg/kg (▲) and 7.5 mg/kg (▼);SEQ ID NO: 825 at dose 0.3 mg/kg (▲), 1.5 mg/kg (▼), and 7.5 mg/kg (◆); SEQ ID NO: 826 at dose 7.1 mg/kg (■), 1.42 mg/kg (▲) and 0.29 mg/kg (▼).

Figure 12: HBeAG reduction of SEQ ID NO: 807 at dose 0.28 mg/kg (■), 1.4 mg/kg (▲) and 7.1 mg/kg (▼); SEQ ID NO: 808 at dose 7.1 mg/kg (■), 1.42 mg/kg (▲) and 0.29 mg/kg (▼); SEQ ID NO: 814 at dose 0.252 mg/kg (▲) and 1.26 mg/kg (▼),6.15 mg/kg (◆); SEQ ID NO: 815 at dose 0.3 mg/kg (■), 1.5 mg/kg (▲) and 7.5 mg/kg (▼);SEQ ID NO: 825 at dose 0.3 mg/kg (▲), 1.5 mg/kg (▼), and 7.5 mg/kg (◆); SEQ ID NO: 826 at dose 7.1 mg/kg (◆), 1.42 mg/kg (▲) and 0.29 mg/kg (▼).

Figure 13: HBV DNA reduction of SEQ ID NO: 807 at dose 0.28 mg/kg (■), 1.4 mg/kg (▲) and 7.1 mg/kg (▼); SEQ ID NO: 808 at dose 7.1 mg/kg (■), 1.42 mg/kg (▲) and 0.29 mg/kg (▼); SEQ ID NO: 814 at dose 0.252 mg/kg (▲) and 1.26 mg/kg (▼),6.15 mg/kg (◆); SEQ ID NO: 815 at dose 0.3 mg/kg (■), 1.5 mg/kg (▲) and 7.5 mg/kg (▼);SEQ ID NO: 825 at dose 0.3 mg/kg (▲), 1.5 mg/kg (▼), and 7.5 mg/kg (◆); SEQ ID NO: 826 at dose 7.1 mg/kg (◆), 1.42 mg/kg (▲) and 0.29 mg/kg (▼).

Figure 14: Presents results from subcutaneous (SC) and intravenous (IV) administration routes of SEQ ID NO: 807 at dose o.2 mg/kg, 1.0 mg/kg and 5.0 mg/kg. A) presents HBsAG reduction. B) presents HBeAG reduction. C) presents DNA reductio

Figure 15: Presents results on unconjugated oligomers (■) (SEQ ID NO:308 and 303) and conjugated oligomers (▼) (SEQ ID NO: 807 and 815) tested at equimolar oligomer dosages. A) presents HBsAG reduction. B) presents HBeAG reduction. C) presents DNA reduction.

DEFINITIONS/ELEMENTS OF THE INVENTION

The following presents definitions of terms that apply to all aspects of the present invention. These definitions are not mutually exclusive. These definitions also teach additional embodiments regarding all aspects of the present invention.

Oligomer/Oligonucleotide

In the context of the present invention, references to “oligomer" and “oligonucleotide" as used herein also apply to the first oligomer region and/or the second oligomer region – such as when the first oligomer region is in the oligomer conjugate or when it is not in the free form (i.e. when not in the oligomer conjugate).

The term “oligomer” refers to a molecule formed by covalent linkage of two or more nucleotides (*i.e.* an oligonucleotide). Therefore as used herein, the terms “oligomer” and “oligonucleotide” are interchangeable and have identical meaning. Herein, a single nucleotide (unit) may also be referred to as a monomer or unit. In some embodiments, the terms “nucleoside”, “nucleotide”, “unit” and “monomer” are used interchangeably. It will be recognised that when referring to a sequence of nucleotides or units, what is referred to is the sequence of bases, such as A, T, G, C or U.

As used herein, the terms “oligomer" and "oligonucleotide" include linear or circular oligomers of natural and/or modified units or linkages, including deoxyribonucleosides, ribonucleosides, substituted and alpha-anomeric forms thereof, peptide nucleic acids (PNA), and the like, capable of specifically binding to a target polynucleotide by way of a regular pattern of unit-to-unit interactions, such as Watson-Crick type of base pairing, Hoogsteen or reverse Hoogsteen types of base pairing, or the like.

The oligonucleotide may be composed of a single region or may be composed of several regions. The oligonucleotide may be “chimeric”, that is, composed of different regions. In the context of this invention "chimeric" antisense compounds are antisense compounds, particularly oligonucleotides, which contain two or more chemical regions, for example, DNA region(s), RNA region(s), PNA region(s) etc. Each chemical region is made up of at least one unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified in order to exhibit one or more sought properties. The sought properties of the oligonucleotide might be to increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. Different regions of the oligonucleotide may therefore have different properties. One ore more regions of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. There are several enzymes with such catalytic effect. A method of digesting RNA at a specific location with an antisense

oligonucleotide and an RNase H has been demonstrated by Minshull et al. (Nucleic Acids Research, 14:6433-6451 (1986)). Rnase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Therefore, activation of RNase H results in cleavage of the RNA target. The efficiency of oligonucleotide inhibition of gene expression might therefore be enhanced. Other enzymes capable of cleaving are Rnase L and Rnase P.

Nucleobase

The term "nucleobase" refers to the base moiety of a nucleotide and covers both naturally occuring a well as non-naturally occurring variants.

Thus, "nucleobase" covers not only the known purine and pyrimidine heterocycles but also heterocyclic analogues and tautomeres thereof.

Examples of nucleobases include, but are not limited to adenine, guanine, cytosine, thymidine, uracil, xanthine, hypoxanthine, 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

In some embodiments, at least one of the nucleobases present in the oligomer is a modified nucleobase selected from the group consisting of 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

Units

As used herein, the term "units" or "monomers" typically indicates nucleoside units linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few monomeric units, e.g., from about 3-4, to about several hundreds of monomeric units. Analogs of phosphodiester linkages include: phosphorothioate, phosphorodithioate, methylphosphornates, phosphoroselenoate, phosphoramidate, and the like.

Nucleosides and Nucleoside analogues

In some embodiments, the terms "nucleoside analogue" and "nucleotide analogue" are used interchangeably.

The term "nucleotide" as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group), such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides, such as DNA or RNA, and non-naturally occurring nucleotides comprising modified sugar and/or base moieties, which are also referred to as "nucleotide analogues" herein. Herein, a single nucleotide (unit) may also be referred to as a monomer or nucleic acid unit.

In field of biochemistry, the term “nucleoside” is commonly used to refer to a glycoside comprising a sugar moiety and a base moiety, and may therefore be used when referring to the nucleotide units, which are covalently linked by the internucleotide linkages between the nucleotides of the oligomer.

In the field of biotechnology, the term “nucleotide” is often used to refer to a nucleic acid monomer or unit, and as such in the context of an oligonucleotide may refer to the base – such as the “nucleotide sequence” – and typically may refer to the nucleobase sequence (*i.e.* the presence of the sugar backbone and internucleoside linkages are implicit).

Likewise, particularly in the case of oligonucleotides where one or more of the internucleoside linkage groups are modified, the term “nucleotide” may refer to a “nucleoside” for example the term “nucleotide” may be used, even when specifiying the presence or nature of the linkages between the nucleosides.

As one of ordinary skill in the art would recognise, the 5’ terminal nucleotide of an oligonucleotide does not comprise a 5’ internucleotide linkage group, although may or may not comprise a 5’ terminal group.

Non-naturally occurring nucleotides include nucleotides which have modified sugar moieties, such as bicyclic nucleotides or 2’ modified nucleotides, such as 2’ substituted nucleotides.

“Nucleotide analogues” are variants of natural nucleotides, such as DNA or RNA nucleotides, by virtue of modifications in the sugar and/or base moieties. Analogues could in principle be merely “silent” or “equivalent” to the natural nucleotides in the context of the oligonucleotide, *i.e.* have no functional effect on the way the oligonucleotide works to inhibit target gene expression. Such “equivalent” analogues may nevertheless be useful if, for example, they are easier or cheaper to manufacture, or are more stable to storage or manufacturing conditions, or represent a tag or label. Preferably, however, the analogues will have a functional effect on the way in which the oligomer works to inhibit expression; for example by producing increased binding affinity to the target and/or increased resistance to intracellular nucleases and/or increased ease of transport into the cell. Specific examples of nucleoside analogues are described by *e.g.* Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-4443 and Uhlmann; *Curr. Opinion in Drug Development*, 2000, 3(2), 293-213, and in Scheme 1 presented in Figure 4.

Linker/Linker Group etc.

The terms “linker group”, "linkage group", “linker”, “linker molecule” or “internucleoside linkage” are intended to mean a group capable of covalently coupling together two entities, such as nucleotides. Specific examples include phosphate groups and phosphorothioate groups. Such linkers may contain a spacer molecule covalently attached to one or more activated groups or functional groups. Optionally, the functional group of the linker molecule can be treated with a

coupling agent to form an activated group.Such linkers also include tether molecules as described herein.

The term “brancher region” is intended to mean a group or region capable of covalently coupling together two or more entities, such as nucleotides or for the generation of carrier component clusters such as galctose clusters.

Alternatively, “linker groups” and “brancher regions” as described herein may be used to covalently couple nucleotide regions and non-nucleotide regions, such a linker group is termed L. For example, a linker group may be used to conjugate an oligomer of the invention to a carrier component described herein. For example, a brancher region may be used to conjugate an oligomer of the invention to one or more carrier components described herein. For example, a brancher region may be used to conjugate one or more oligomers of the invention to a carrier component described herein. For example, a brancher region may be used to conjugate one or more oligomers of the invention to one or more carrier components described herein.

Oligomer conjugate

The term "oligomer conjugate" is intended to indicate a heterogenous molecule formed by the attachment (“conjugation”), such as by the covalent attachment, of the oligomer as described herein to a carrier component.

The linkage, such as covalent conjugation, may be chemical in nature, such as via a linker group, or genetic in nature for example by recombinant genetic technology, such as in a fusion protein with for example a reporter molecule (e.g. green fluorescent protein, β-galactosidase, Histag, etc. Alternatively, the oligomer may be conjugated to the carrier component directly without the need for any tether molecule or linker group.

Carrier component

As used herein, the term “carrier component” relates to a molecular vehicle which is intended to carry or convey the oligomers of the invention to their desired location, such as desired anatomical location.

Carrier components according to the present invention may be used to enhance the activity, cellular distribution and/or cellular uptake of the oligomers.

Any suitable carrier component may be used.

The carrier component may be polynucleotide. However, typically, the carrier component is a non-nucleotide moiety or a non-polynucleotide moiety.

Examples of non-nucleotide or non- polynucleotide moieties include macromolecular agents 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, or combinations thereof. . Typical polymers may be polyethylene glycol and/or polypropylene glycol (PPG).

In some embodiments, the carrier component may be an amino acid, a protein, a peptide or polypeptide. Typically proteins may be enzymes, serum proteins (e.g.human serum albumin (HSA), transferrin or glycoproteins), receptors, antibodies or antibody derivatives thereof like single-chain variable fragments, bispecific antibodies, tribodies etc designed to bind a desired target antigen.

Examples of peptide carrier components are poly(L-lysine), that significantly increases cell penetration, and the antennapedia transport peptide. Such conjugates are described by Lemaitre et al, "Specific antiviral activity of a poly(L-lysine)-conjugated oligodeoxyribonucleotide sequence complementary to vesicular stomatitis virus N protein mRNA initiation site," *Proc. Natl. Acad. Sci.* USA, 84:648-652, 1987; US Patent Nos.: 6,166,089 and 6,086,900. The procedure in the above publication requires that the 3'-terminal nucleotide be a ribonucleotide. The resulting aldehyde groups are then randomly coupled to the epsilon-amino groups of lysine residues of poly(L-lysine) by Schiff base formation, and then reduced with sodium cyanoborohydride. This procedure converts the 3'-terminal ribose ring into morpholine structure antisense oligomers. The peptide segment can also be synthesized by strategies which are compatible with DNA/RNA synthesis e.g. Mmt/Fmoc strategies. In that case the peptide can be synthesized directly before or after the oligonucleotide segment. Also methods exist to prepare the peptide oligonucleotide conjugate post synthetically, e.g., by formation of a disulfide bridge.

In some embodiments, the conjugate moiety may be or comprise a lipophilic conjugate moiety. Lipophilic conjugate moieties may be selected from the group consisting of sterols, stanols, steroids, polycyclic aromatic groups, aliphatic groups, lipids, phospholipids, lipophilic alcohols, fatty acids and fatty esters. In some embodiments, the conjugate moiety comprises cholesterol.

The carrier component may be or comprise a pharmacokinetic modulator, such as a lipophilic or hydrophobic moieties. Such moieties are disclosed within the context of siRNA conjugates in WO2012/082046. The hydrophobic moiety may comprise a C8 – C36 fatty acid, which may be saturated or un-saturated. In some embodiments, C10, C12, C14, C16, C18, C20, C22, C24, C26, C28, C30, C32 and C34 fatty acids may be used. The hydrophobic group may have 16 or more carbon atoms. Exemplary suitable hydrophobic groups may be selected from the group comprising:sterol, cholesterol, palmitoyl, hexadec-8-enoyl, oleyl, (9E, 12E)-octadeca-9,12-dienoyl, dioctanoyl, and C16-C20 acyl. According to WO2012/082046, hydrophobic groups having fewer than 16 carbon atoms are less effective in enhancing polynucleotide targeting, but they may be used in multiple copies (e.g. 2x, such as 2x C8 or C10, C12 or C14) to enhance efficacy. Pharmacokinetic modulators useful as polynucleotide targeting moieties may be

selected from the group consisting of: cholesterol, alkyl group, alkenyl group, alkynyl group, aryl group, aralkyl group, aralkenyl group, and aralkynyl group, each of which may be linear, branched, or cyclic. Pharmacokinetic modulators are preferably hydrocarbons, containing only carbon and hydrogen atoms. However, substitutions or heteroatoms which maintain hydrophobicity, for example fluorine, may be permitted.

WO2007/031091 provides examples of other suitable ligands and carrier components, which are hereby incorporated by reference.

In some embodiments, the carrier component is or comprises a carbohydrate moiety. Carbohydrate conjugate moieties include, but is not limited to, galactose, lactose, n-acetylgalactosamine, mannose and mannose-6-phosphate. Carbohydrate conjugates may be used to enhance delivery or activity in a range of tissues, such as liver and/or muscle. See, for example, EP1495769, WO99/65925, Yang et al., Bioconjug Chem (2009) 20(2): 213-21. Zatsepin & Oretskaya Chem Biodivers. (2004) 1(10): 1401-17.

In addition, the oligomer may further comprise one or more additional conjugate moieties, of which lipophilic or hydrophobic moieties are particularly interesting. These may for example, act as pharmacokinetic modulators, and may be covalently linked to either the carbohydrate conjugate, a linker linking the carbohydrate conjugate to the oligomer or a linker linking multiple carbohydrate conjugates (multi-valent) conjugates, or to the oligomer, optionally via a linker, such as a physiologically labile linker.

In some embodiments, the carrier component comprises an asiaglycoprotein receptor (ASGP-R) targeting moiety with affinity equal to or greater than that of galactose. The ASPG-R targeting moiety may be selected from the group consisting ofgalactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, and N-isobutanoylgalactos-amine. In some embodiments the the asialoglycoprotein receptor targeting conjugate moiety is mono-valent. In other embodiments the carrier component comprises a galactose cluster, such as a di-valent, tri-valent or tetra-valent asialoglycoprotein receptor targeting conjugate moiety (i.e. containing 1, 2, 3 or 4 terminal carbohydrate moieties capable of binding to the asialoglycoprotein receptor). In some embodiments, the carrier component comprises a GalNAc (N-acetylgalactosamine), such as a mono-valent, di-valent, tri-valent of tetra-valent GalNAc. GalNAc conjugates may be used to target the compound to the liver. A preferred carrier component is a N-acetylgalactosamine trimer. GalNAc conjugates have been used with methylphosphonate and PNA antisense oligonucleotides (e.g. US 5,994517 and Hangeland *et al.*, Bioconjug Chem. 1995 Nov-Dec; 6(6):695-701) and siRNAs (e.g. WO2009/126933, WO2012/089352 and WO2012/083046) and with LNA and 2'-MOE modified nucleosides WO 2014/076196 WO 2014/207232, WO

2014/179620 and WO 2014/179627. The GalNAc references and the specific conjugates used therein are hereby incorporated by reference. WO2012/083046 discloses siRNAs with GalNAc conjugate moieties which comprise cleavable pharmacokinetic modulators, which are suitable for use in the present invention, the preferred pharmacokinetic modulators are C16 hydrophobic groups such as palmitoyl, hexadec-8-enoyl, oleyl, (9E, 12E)-octadeca-9,12-dienoyl, dioctanoyl, and C16-C20 acyl. The WO2012/083046 cleavable pharmacokinetic modulators may also be cholesterol.

The carrier component may be selected from the group consisting of: galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, Npropionyl- galactosamine, N-n-butanoyl-galactosamine, N-iso-butanoylgalactos-amine, galactose cluster, and N-acetylgalactosamine trimer and may have a pharmacokinetic modulator selected from the group consisting of: hydrophobic group having 16 or more carbon atoms, hydrophobic group having 16-20 carbon atoms, palmitoyl, hexadec-8-enoyl, oleyl, (9E,12E)-octadeca-9,12dienoyl, dioctanoyl, and C16-C20 acyl, and cholesterol. Certain GalNac clusters disclosed in '046 include: (E)-hexadec-8-enoyl (C16), oleyl (C18), (9,E,12E)-octadeca-9,12-dienoyl (C18), octanoyl (C8), dodececanoyl (C12), C-20 acyl, C24 acyl, dioctanoyl (2xC8). The carrier component -pharmacokinetic modulator may be linked to the polynucleotide via a physiologically labile bond or, e.g. a disulfide bond (PO-linker), or a PEG linker. The invention also relates to the use of phosphodiester linkers between the oligomer and the carrier component (suitably are positioned between the oligomer and the carbohydrate conjugate group).

In one embodiments of the present invention, the oligomer is linked, preferably conjugated, to a carrier component, which may be used to deliver oligomers to the liver of a subject e.g. by increasing the cellular uptake of oligomers.

In a particular embodiment, the carrier component may be GalNAc or a GalNAc cluster. Figure 2 and 3 presents some carier components.

For targeting hepatocytes in liver, a preferred targeting ligand is a galactose cluster.

A galactose cluster comprises a molecule having e.g. comprising two to four terminal galactose derivatives. As used herein, the term galactose derivative includes both galactose and derivatives of galactose having affinity for the ASGP-R equal to or greater than that of galactose. A terminal galactose derivative is attached to a molecule through its C-l carbon. The ASGP-R is unique to hepatocytes and binds branched galactose-terminal glycoproteins. A preferred galactose cluster has three terminal galactosamines or galactosamine derivatives each having affinity for the asialoglycoprotein receptor. A more preferred galactose cluster has three terminal N-acetyl-galactosamines. Other terms common in the art include tri-antennary galactose, tri-valent galactose and galactose trimer. It is known that tri-antennary galactose

derivative clusters are bound to the ASGP-R with greater affinity than bi-antennary or mono-antennary galactose derivative structures (Baenziger and Fiete, 1980, Cell, 22, 611-620; Connolly et al., 1982, 1. Biol. Chern., 257,939-945). Multivalency is required to achieve nM affinity.

A preferred galactose derivative is an N-acetyl-galactosamine (GalNAc). Other saccharides having affinity for the asialoglycoprotein receptor may be selected from the list comprising: galactosamine, N-n-butanoylgalactosamine, and N-iso-butanoylgalactosamine. 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.

A galactose cluster may comprise two or preferably three galactose derivatives each linked to a central branch point. The galactose derivatives are attached to the central branch point through the C-l carbons of the saccharides. The galactose derivative is preferably linked to the branch point via linkers or spacers. A preferred spacer is a flexible hydrophilic spacer (U.S. Patent 5885968; Biessen et al. J. Med. Chern. 1995 Vol. 39 p. 1538-1546). A preferred flexible hydrophilic spacer is a PEG spacer. A preferred PEG spacer is a PEG3 spacer (three ethylene units). The branch point can be any small molecule which permits attachment of the three galactose derivatives and further permits attachment of the branch point to the oligomer. Each galactose derivative (carbohydrate moiety) in a GalNAc cluster (e.g. GalNAc) may be joined to the oligomer via a spacer, such as (poly)ethylene glycol linker (PEG), such as a di, tri, tetra, penta, hexa-ethylene glycol linker. The PEG moiety may form a spacer between the galactose derivative sugar moiety and a peptide (di-lysine is shown) linker.An exemplary branch point group is a di-lysine. A di-lysine molecule contains three amine groups through which three galactose derivatives may be attached and a carboxyl reactive group through which the di-lysine may be attached to the oligomer (se for example figure 4).

The carbohydrate conjugate (e.g. GalNAc), or carbohydrate-linker moiety (e.g. carbohydrate-PEG moiety) may be covalently joined (linked) to the oligomer via a branch point group such as, an amino acid, or peptide, which suitably comprises two or more amino groups (such as 3, 4, or 5), such as lysine, di-lysine or tri-lysine or tetra-lysine. A tri-lysine molecule contains four amine groups through which three carbohydrate conjugate groups, such as galactose & derivatives (e.g. GalNAc) and a further conjugate such as a hydrophobic or lipophilic moiety/group may be attached and a carboxyl reactive group through which the tri-lysine may be attached to the oligomer. The further conjugate, such as lipophilic/hyrodphobic moiety may be attached to the lysine residue that is attached to the oligomer.

In some embodiments, the GalNac cluster comprises a peptide linker, e.g. a Tyr-Asp(Asp) tripeptide or Asp(Asp) dipeptide, which is attached to the oligomer via a biradical linker, for example the GalNac cluster may comprise biradical linkers such as those illustrated as Conj 3, 3a , 4 and 4a in figure 3.

Alternative brancher molecules may be selected from the from the group consisting of 1,3-bis-[5-(4,4'-dimethoxytrityloxy)pentylamido]propyl-2-[(2-cyanoethyl)-(N,N-diisopropyl)] phosphoramidite (Glen Research Catalogue Number: 10-1920-xx), tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]ethyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research Catalogue Number: 10-1922-xx), tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]methyleneoxypropyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite and 1-[5-(4,4'-dimethoxy-trityloxy)pentylamido]-3-[5-fluorenomethoxy-carbonyl-oxy-pentylamido]-propyl-2-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research Catalogue Number: 10-1925-xx). WO 2014/179620 and European application No. 14188444.5 describes the generation of various GalNAc conjugate moieties (hereby incorporated by reference). Attachment of the branch point to oligomer may occur through a linker or spacer. A preferred spacer is a flexible hydrophilic spacer. A preferred flexible hydrophilic spacer is a PEG spacer or C6 linker. A preferred PEG spacer is a PEG3 spacer (three ethylene units). In a preferred embodiment the linker is a physiologically labile linker. The galactose cluster may be attached to the 3' or 5' end of the oligomer using methods known in the art. In preferred embodiments the asialoglycoprotein receptor targeting conjugate moiety is linked to the 5'-end of the oligonucleotide.

A preferred galactose cluster comprises three terminal GalNAc moieties linked via a PEG spacer to a di-lysine brancher molecule (a GalNAc cluster). Preferably, the PEG spacer is a 3PEG spacer. Preferred GalNAc clusters are Conj 1, 1a, 2 and 2a. Most preferred is Conj2a (also termed GalNAc2).

Galactose clusters are presented in Figure 3.Carrier component linker

The carrier component may be linked to the first oligomer region, and/or the second oligomer region, by means of a linker (L). Any suitable linker may be used.

A carbohydrate conjugate (e.g. GalNAc) may be linked to the oligomer via a biocleavable linker also termed a physiologically labile linker.

As used herein, a physiologically labile bond is a labile bond that is cleavable under conditions normally encountered or analogous to those encountered within a mammalian body (also referred to as a physiologically labile linker). Physiologically labile linkage groups are selected such that they undergo a chemical transformation (e.g., cleavage) when present in certain physiological conditions. Mammalian intracellular conditions 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 or hydrolytic enzymes

Chemical transformation (cleavage of the labile bond) may be initiated by the addition of a pharmaceutically acceptable agent to the cell or may occur spontaneously when a molecule containing the labile bond reaches an appropriate intra-and/or extra-cellular environment. For example, a pH labile bond may be cleaved when the molecule enters an acidified endosome. Thus, a pH labile bond may be considered to be an endosomal cleavable bond. Enzyme cleavable bonds may be cleaved when exposed to enzymes such as those present in an endosome or lysosome or in the cytoplasm. A disulfide bond may be cleaved when the molecule enters the more reducing environment of the cell cytoplasm. Thus, a disulfide may be considered to be a cytoplasmic cleavable bond. As used herein, a pH-labile bond is a labile bond that is selectively broken under acidic conditions (pH<7). Such bonds may also be termed endosomally labile bonds, since cell endosomes and lysosomes have a pH less than 7. An example of another physiologically labile linker is a di-lysine as used in the GalNAc clusters in figure 3.

In some embodiments, the oligomer conjugate of the invention comprises a physiologically labile linker (region PL, also referred to as a biocleavable linker or nuclease susceptible linker), which joins the oligomer (region A) of the invention to the carrier component (or region C).

For physiologically labile linkers associated with a carrier component for targeted delivery it is preferred that, the cleavage rate seen in the target tissue (for example muscle, liver, kidney or a tumor) is greater than that found in blood serum. Suitable methods for determining the level (%) of cleavage in target tissue versus serum are described in the “Tissue specific In vitro linker cleavage assay” section. In some embodiments, the physiologically labile linker (also referred to as the biocleavable linker, or nuclease susceptible linker) in a compound of the invention, are at least about 20% cleaved, such as at least about 30% cleaved, such as at least about 40% cleaved, such as at least about 50% cleaved, such as at least about 60% cleaved, such as at least about 70% cleaved, such as at least about 75% cleaved in the “Tissue specific *In vitro* linker cleavage assay” in the “Materials and methods” section. In some embodiments, the cleavage (%) in serum, as used in the “Tissue specific *In vitro* linker cleavage assay”, is less than about 20%, such as less than about 10%, such as less than 5%, such as less than about 1%.

In some embodiments, the oligomer conjugate of the invention comprises three regions: i) a first region (region A), which comprises 10 – 18 contiguous nucleotides; ii) a second region (region

PL) which comprises a physiologically labile linker and iii) a third region (C) which comprises a carrier component, wherein the third region is covalent linked to the second region which is covalently linked to the first region.

In some embodiments region A and region PL is covalently linked via a phosphate nucleoside linkage (e.g. phosphodiester, phosphorothioate, phosphodithioate, boranophosphate or methylphosphonate) or a triazol group. In some embodiments region PL and region C is covalently linked via a phosphate nucleoside linkage (e.g. phosphodiester, phosphorothioate, phosphodithioate, boranophosphate or methylphosphonate) or a triazol group. In some embodiments region PL and region C is covalently linked via a second linker such as region E linkers described below.

In some embodiments, the physiologically labile linker may be situated either at the 5' end and/or the 3'-end of the oligomer (region A). In a preferred embodiment the physiologically labile linker is at the 5'-end.

In some embodiments, the physiologically labile linker is attached at its 3'-end to the 5'-end of region A and the carrier component (region C) is attached to the 5'-end or the the physiologically labile linker (e.g. PO-linker), optionally via an additional linker region E.

Nuclease susceptible linker - phosphodiester linker (PO-linker)

In some embodiments, the physiologically labile linker is susceptible to nuclease(s) which may for example, be expressed in the target cell – and as such, as detailed herein, the linker may be a short region (e.g. 1 – 10) phosphodiester linked nucleosides, such as DNA nucleosides.

In some embodiments, which may be the same or different, the physiologically labile linker (region PL) is susceptible to S1 nuclease cleavage. Susceptibility to S1 cleavage may be evaluated using the S1 nuclease assay described in the "S1 nuclease cleavage assay" section. In some embodiments, the physiologically labile linker (also referred to as the physiologically labile linker, or nuclease susceptible linker), such as region PL, in a compound of the invention, are at least about 30% cleaved, such as at least about 40% cleaved, such as at least about 50% cleaved, such as at least about 60% cleaved, such as at least about 70% cleaved, such as at least about 80% cleaved, such as at least about 90% cleaved, such as at least 95% cleaved after 120min incubation with S1 nuclease as described in the "S1 nuclease cleavage assay in the "Materials and methods" section.

In some embodiments, the physiologically labile linker (region PL) is a nuclease susceptible linker, which 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. In some embodiments, the nuclease susceptible linker is a phosphodiester nucleotide linker, such a linker is also termed a PO-linker. In preferred

embodiments the nuclease susceptible linker (PO-linker) comprises at least one phosphodiester linked nucleoside. Preferably, the nuclease susceptible PO-linker comprises at least two consecutive phosphodiester linkages, such as at least 3 or 4 or 5 consecutive phosphodiester linkages.

In some embodiments, the nucleosides in the PO-linker are (optionally independently) selected from the group consisting of DNA and RNA or modifications thereof which do not interfere with nuclease cleavage. Modifications of DNA and RNA nucleosides which do not interfere with nuclease cleavage may be non-naturally occurring nucleobases. Certain sugar-modified nucleosides may also allow nuclease cleavage such as an alpha-L-oxy-LNA. In some embodiments, all the nucleosides of the PO-linker comprise (optionally independently) either a 2'-OH ribose sugar (RNA) or a 2'-H sugar - i.e. RNA or DNA. In some embodiments, the nucleosides of the PO-linker are DNA nucleosides. In some embodiments, at least two consecutive nucleosides of the PO-linker are DNA or RNA nucleosides (such as at least 3 or 4 or 5 consecutive DNA or RNA nucleosides). Preferably the PO-linker consists of between 1 to 5 or 1 to 4, such as 2, 3, 4 consecutive phosphodiester linked DNA nucleosides. In preferred embodiments the PO-linker is so short that it does not recruit RNAseH. In some embodiments, the PO-linker comprises no more than 3 or no more than 4 consecutive phospodiester linked DNA and/or RNA nucleosides (such as DNA nucleosides).

In some embodiments, the PO-linker is not complementary to the target nucleic acid sequence or to the oligomer in region A.

In some embodiments, the PO-linker is complementary with the target nucleic acid sequence. In this respect region A and the PO-linker together may form a single contiguous sequence which is complementary to the target sequence.

In some embodiments, region A and the PO-linker form a single contiguous nucleotide sequence of 10 – 22, such as 12 - 20 nucleotides in length. In this context region A can be differentiated from the PO-linker in that it starts with at least one, preferably at least two, modified nucleosides with increased binding affinity to the target nucleic acid (e.g. LNA or nucleosides with a 2' substituted sugar moiety) and region A on its own is capable of modulation of the expression the target nucleic acid in a relevant cell line. Furthermore, if region A comprises DNA or RNA nucleosides these are linked with nuclease resistant internucleoside linkage, such phosphorothioate or boranophosphate.

In some aspects the internucleoside linkage between the first (region A) and the second region (the PO-linker) may be considered part of the second region.

In some embodiments, the sequence of bases in the PO-linker is selected to provide an optimal endonuclease cleavage site, based upon the predominant endonuclease cleavage enzymes

present in the target tissue or cell or sub-cellular compartment. In this respect, by isolating cell extracts from target tissues and non-target tissues, endonuclease cleavage sequences for use in the PO-linker may be selected based upon a preferential cleavage activity in the desired target cell (e.g. liver/hepatocytes) as compared to a non-target cell (e.g. kidney). In this respect, the potency of the compound for target down-regulation may be optimized for the desired tissue/cell.

In some embodiments the PO-linker comprises a dinucleotide of sequence AA, AT, AC, AG, TA, TT, TC, TG, CA, CT, CC, CG, GA, GT, GC, or GG, wherein C may be 5-methylcytosine, and/or T may be replaced with U. Preferably, the internucleoside linkage is a phosphodiester linkage. In a preferred embodiment the PO-linker is a CA dinucleotide with at least two phosphodiester linkages (one being to Region A). In some embodiments the PO-linker comprises a trinucleotide of sequence AAA, AAT, AAC, AAG, ATA, ATT, ATC, ATG, ACA, ACT, ACC, ACG, AGA, AGT, AGC, AGG, TAA, TAT, TAC, TAG, TTA, TTT, TTC, TAG, TCA, TCT, TCC, TCG, TGA, TGT, TGC, TGG, CAA, CAT, CAC, CAG, CTA, CTG, CTC, CTT, CCA, CCT, CCC, CCG, CGA, CGT, CGC, CGG, GAA, GAT, GAC, CAG, GTA, GTT, GTC, GTG, GCA, GCT, GCC, GCG, GGA, GGT, GGC, and GGG wherein C may be 5-methylcytosine and/or T may be replaced with U. Preferably, the internucleoside linkages are phosphodiester linkages. In some embodiments the PO-linker comprises a trinucleotide of sequence AAAX, AATX, AACX, AAGX, ATAX, ATTX, ATCX, ATGX, ACAX, ACTX, ACCX, ACGX, AGAX, AGTX, AGCX, AGGX, TAAX, TATX, TACX, TAGX, TTAX, TTTX, TTCX, TAGX, TCAX, TCTX, TCCX, TCGX, TGAX, TGTX, TGCX, TGGX, CAAX, CATX, CACX, CAGX, CTAX, CTGX, CTCX, CTTX, CCAX, CCTX, CCCX, CCGX, CGAX, CGTX, CGCX, CGGX, GAAX, GATX, GACX, CAGX, GTAX, GTTX, GTCX, GTGX, GCAX, GCTX, GCCX, GCGX, GGAX, GGTX, GGCX, and GGGX, wherein X may be selected from the group consisting of A, T, U, G, C and analogues thereof, wherein C may be 5-methylcytosine and/or T may be replaced with U. Preferably, the internucleoside linkages are phosphodiester linkages. It will be recognized that when referring to (naturally occurring) nucleobases A, T, U, G, C, these may be substituted with nucleobase analogues which function as the equivalent natural nucleobase (e.g. base pair with the complementary nucleoside).

In some embodiments, region PL is a phosphodiester nucleotide linker (PO-linker) covalently attached to a lipophilic conjugate moiety, such as a lipid, a fatty acid, sterol, such as cholesterol or tocopherol. In some embodiments, region PL is a phosphodiester nucleotide linker (PO-linker) covalently attached to a asialoglycoprotein receptor targeting moiety, such as a GalNAc carrier component.

The concept of inserting a physiologically labile linker between the oligomer and the carrier component is described in detail in WO 2014/076195 (hereby incorporated by reference, in particular figures 3 and 4 are incorporated by reference).

Alternative linkers (Region E)

In some instances linkers are not necessarily physiologically labile but primarily serves to covalently connect a third region, e.g. a carrier component (region C), to an oligomer (region A). Herein these linkers are also termed region E. The oligomer conjugates of the present invention can be constructed of the following regional elements A-C/C-A, A-PL-C/C-PL-A, A-PL-E-C/C-E-PL-A, A-E-PL-C/C-PL-E-A or A-E-C/C-E-A.

In some embodiments, the linker E comprises a chain structure or an oligomer of repeating units such as ethylene glycol, amino acid units or amino alkyl groups. The linker E can have at least two functionalities, one for attaching to the oligomer (optionally with a physiologically labile linker) and the other for attaching to the carrier component. Example linker functionalities can be electrophilic for reacting with nucleophilic groups on the oligomer or carrier component, or nucleophilic for reacting with electrophilic groups. In some embodiments, linker functionalities include amino, hydroxyl, carboxylic acid, thiol, phosphoramidate, phosphorothioate, phosphate, phosphite, unsaturations (e.g., double or triple bonds), and the like. For example, a carbohydrate carrier component (e.g. GalNAc) may be linked to the oligomer via a linker, such as (poly)ethylene glycol linker (PEG), such as a di, tri, tetra, penta, hexa-ethylene glycol linker.

In some embodiments the linker (region E) 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 E) is a C6 amino alkyl group. The amino alkyl group may be added to the oligomer (region A or region A-L/L-A) as part of standard oligomer synthesis, for example using a (e.g. protected) amino alkyl phosphoramidite. The linkage group between the amino alkyl and the oligomer may for example be a phosphorothioate or a phosphodiester, or one of the other nucleoside linkage groups referred to herein. The amino alkyl group is covalently linked to the 5' or 3'-end of the oligomer. Commercially available amino alkyl linkers are for example 3'-Amino-Modifier reagent for linkage at the 3'-end of the oligomer and for linkage at the 5 '-end of an oligomer 5'- Amino-Modifier C6 is available. These reagents are available from Glen Research Corporation (Sterling, Va.). These compounds or similar ones were utilized by Krieg, et al, Antisense Research and Development 1991, 1, 161 to link fluorescein to the 5'- terminus of an oligomer. A wide variety of further linker groups are known in the art and can be useful in the attachment of carrier components to oligomers. A review of many of the useful linker groups can be found in, for example, Antisense Research and Applications, S. T. Crooke and B. Lebleu, Eds., CRC Press, Boca Raton, Fla., 1993, p. 303-350. Other compounds such as acridine have been attached to the 3 '-terminal phosphate group of an oligomer via a polymethylene linkage (Asseline, et al., Proc. Natl. Acad. Sci. USA 1984, 81, 3297). Any of the above groups can be used as a single linker (region E) or in combination with one or more further linkers (region E-E'or region E-L or L-E).

Linkers and their use in preparation of conjugates of oligomers are provided throughout the art such as in WO 96/11205 and WO 98/52614 and U.S. Pat. Nos. 4,948,882; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,580,731; 5,486,603; 5,608,046; 4,587,044; 4,667,025; 5,254,469; 5,245,022; 5,112,963; 5,391,723; 5,510475; 5,512,667; 5,574,142; 5,684,142; 5,770,716; 6,096,875; 6,335,432; and 6,335,437,WO 2012/083046 each of which is incorporated by reference in its entirety.Figure 3 present example GalNAc clusters, Conj 1, 2, 3, 4 and Conj1a, 2a, 3a and 4a having an optional C6 linker which joins the GalNac cluster to the oligomer.

Asiaglycoprotein receptor (ASGP-R) targeting moiety

As used herein, the term "asiaglycoprotein receptor (ASGP-R) targeting moiety" relates to a moiety which interacts with ASGP-R and thereby brings an oligomer into contact with, or into proximity to, a cell expressing surface ASGP-R.

Conjugate moiety

In some embodiments, the carrier component is a conjugate moiety.

In addition, one or more conjugate moieties may be attached to the oligomer or the oligomer conjugate in addition to the carrier component.

In some embodiments, one or more conjugate moieties may be attached to the oligomer of the present invention.

The conjugate moiety may be a non-nucleotide moiety or a non-polynucleotide moiety.

The conjugate moiety may enhance the activity, cellular distribution or cellular uptake of the oligomer of the invention. Such moieties include, but are not limited to, antibodies, polypeptides, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g. Hexyl-s-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipids, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-o-hexadecyl-rac-glycero-3-h-phosphonate, a polyamine or a polyethylene glycol chain, an adamantane acetic acid, a palmityl moiety, an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

The oligomers of the invention may also be conjugated to active drug substances for example, aspirin, ibuprofen, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

In certain embodiments the conjugated moiety is a sterol, such as cholesterol.

In various embodiments, the conjugate moiety comprises or consists of a positively charged polymer, such as a positively charged peptides of, for example from 1 -50, such as 2 – 20 such as 3–10 amino acid residues in length, and/or polyalkylene oxide such as polyethylglycol(PEG) or polypropylene glycol – see WO 2008/034123, hereby incorporated by reference. Suitably

the positively charged polymer, such as a polyalkylene oxide may be attached to the oligomer of the invention via a linker such as the releasable linker described in WO 2008/034123.

The oligomers and oligomer conjugates of the present invention may include – as a conjugate moiety – an appropriate ligand-binding molecule. For example, the oligonucleotides may be conjugated for therapeutic administration to ligand-binding molecules which recognize cell-surface molecules, such as according to International Patent Application WO 91/04753. The ligand-binding molecule may comprise, for example, an antibody against a cell surface antigen, an antibody against a cell surface receptor, a growth factor having a corresponding cell surface receptor, an antibody to such a growth factor, or an antibody which recognizes a complex of a growth factor and its receptor. Methods for conjugating ligand-binding molecules to oligonucleotides are detailed in WO 91/04753. Further, conjugation methods and methods to improve cellular uptake which may be used are described in the following international patent applications WO 9640961, WO9964449, WO9902673, WO9803533, WO0015265 and US patents 5856438 and 5138045.

By way of a further example, the conjugate moiety may be a growth factor such as transferrin or folate. Transferrin-polylysine-oligonucleotide complexes or folate-polylysine-oligonucleotide complexes may be prepared for uptake by cells expressing high levels of transferrin or folate receptor. The preparation of transferrin complexes as carrier components facilitating oligonucleotide uptake into cells is described by Wagner et al., *Proc. Natl. Acad. Sci. USA* 87, 3410-3414 (1990). Cellular delivery of folate-macromolecule conjugates via folate receptor endocytosis, including delivery of an antisense oligonucleotide, is described by Low et al., U.S. Patent 5,108,921. Also see, Leamon et al., *Proc. Natl. Acad. Sci.* 88, 5572 (1991).

Target nucleic acid/target sequence

In preferred aspects, the terms “target nucleic acid” and “target sequence”, as used herein refer to the DNA or RNA encoding a HBx or HBsAg polypeptide, such as a sequence contained within any of SEQ ID No. 1 or SEQ ID No. 2. The terms “target nucleic acid” and “target sequence” therefore include HBx- or HBsAg-encoding nucleic acids or naturally occurring variants thereof, and RNA nucleic acids derived therefrom, preferably mRNA, such as pre-mRNA, although preferably mature mRNA. In some embodiments, for example when used in research or diagnostics the “target nucleic acid” ” or “target sequence” may be a cDNA or a synthetic oligonucleotide derived from the above DNA or RNA nucleic acid targets. The oligomer according to the invention is preferably capable of hybridising to the target nucleic acid.

Identity/homology

As used herein, the terms “homologous” and “homology” are interchangeable with the terms “identity” and “identical”.

Corresponding to/corresponds to

The terms "corresponding to" and "corresponds to" refer to the comparison between the nucleotide sequence of the oligomer (*i.e.* the nucleobase or base sequence) or a contiguous nucleotide sequence thereof and the equivalent contiguous nucleotide sequence of a further sequence selected from either i) a sub-sequence of the reverse complement of the nucleic acid target, such as the mRNA which encodes the target sequence protein, such as any sequence within SEQ ID No. 1 or SEQ ID No. 2 or SEQ ID No. 3 and/or ii) the sequence of any of the specific nucleotide sequences provided herein, or sub-sequence thereof. Nucleotide analogues are compared directly to their equivalent or corresponding nucleotides. A sequence which corresponds to a further sequence under i) or ii) typically is identical to that sequence over the length of the first sequence (such as the contiguous nucleotide sequence) or, as described herein may, in some embodiments, is at least 80% homologous to a corresponding sequence, such as at least 85%, at least 90%, at least 91%, at least 92%at least 93%, at least 94%, at least 95%, at least 96% homologous, such as 100% homologous (identical). The percentage sequence identity may be calculated by counting the number of aligned bases that are identical between the 2 sequences, dividing by the total number of units in the oligomer, and multiplying by 100.

The terms "corresponding nucleotide analogue" and "corresponding nucleotide" are intended to indicate that the nucleotide in the nucleotide analogue and the naturally occurring nucleotide are identical. For example, when the 2-deoxyribose unit of the nucleotide is linked to an adenine, the "corresponding nucleotide analogue" contains a pentose unit (different from 2-deoxyribose) linked to an adenine.

Complementary

The term, "complementary" means that two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3'-end of each sequence binds to the 5'-end of the other sequence and each A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence. Normally, the complementary sequence of the oligonucleotide has at least 90%, preferably 95%, most preferably 100%, complementarity to a defined sequence.

In determining the degree of “complementarity” between oligomers of the invention (or regions thereof) and the target sequence, such as the HBx or HBsAg target sequence, the degree of “complementarity” (also, “homology” or “identity”) is expressed as the percentage identity (or percentage homology) between the sequence of the oligomer (or region thereof) and the

sequence of the target region (or the reverse complement of the target region) that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical between the 2 sequences, dividing by the total number of contiguous units in the oligomer, and multiplying by 100. In such a comparison, if gaps exist, it is preferable that such gaps are merely mismatches rather than areas where the number of units within the gap differs between the oligomer of the invention and the target region.

In particular, the term "complementary" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid. Two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3'-end of each sequence binds to the 5'-end of the other sequence and each nucleobase A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence. In determining the degree of complementarity between oligomers of the invention (or regions thereof) and the target sequence, such as the HBx or HBsAg target sequence, the degree of complementarity is expressed as the percentage complementarity between the sequence of the oligomer (or region thereof) and the sequence of the target region that best aligns therewith. The percentage is calculated by counting the number of aligned bases that form pairs between the 2 sequences, dividing by the total number of contiguous units in the oligomer, and multiplying by 100. In such a comparison a nucleobase/nucleotide which does not align is termed a mismatch. Normally, the complementary sequence of a oligonucleotide of the present invention has at least 80%, preferably 85%, preferably 90%, more preferably 95%, most preferably 100%, complementarity to a defined sequence.

The term "complementary sequence" as it refers to a polynucleotide sequence, relates to the base sequence in another nucleic acid molecule by the base-pairing rules. More particularly, the term or like term refers to the hybridization or base pairing between nucleotides or nucleic acids, such as, for instance, between the two strands of a double stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single stranded nucleic acid to be sequenced or amplified. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 95% of the nucleotides of the other strand, usually at least about 98%, and more preferably from about 99 to about 100%. Complementary polynucleotide sequences can be identified by a variety of approaches including use of well-known computer algorithms and software, for example the BLAST program.

Reverse complement/reverse complementary/reverse complementarity

The terms “reverse complement”, “reverse complementary” and “reverse complementarity” as used herein are interchangeable with the terms “complement”, “complementary” and “complementarity”.

Mismatch

The term “mismatch” – that is sometimes referred to as a non-complementary nucleobase - refers to a nucleobase or nucleotide at a given position in a first nucleic acid that does not make Watson- Crick base paring with the corresponding nucleobase or nucleotide in a second nucleic acid when the first nucleic acid is aligned with thee second nucleic acid. The first nucleic acid can for example be an oligomer or oligomer conjugate according to the invention and the second nucleic acid can for example be a target sequence. In the oligomer or the oligomer conjugate containing multiple mismatches, the mismatches can either be adjacent to each other or interspersed.

Naturally occurring variant thereof

The term “naturally occurring variant thereof” refers to variants of the target sequence which exist naturally within the defined taxonomic group, such as HBV genotypes A-H. Typically, when referring to “naturally occurring variants” of a polynucleotide the term may also encompass any allelic variant of the target sequence encoding genomic DNA which are found by chromosomal translocation or duplication, and the RNA, such as mRNA derived therefrom. “Naturally occurring variants” may also include variants derived from alternative splicing of the target sequence mRNA. When referenced to a specific polypeptide sequence, *e.g.*, the term also includes naturally occurring forms of the protein which may therefore be processed, *e.g.* by co- or post-translational modifications, such as signal peptide cleavage, proteolytic cleavage, glycosylation, etc.

Downstream

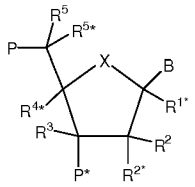
As used herein, the term "downstream" when used in reference to a direction along a nucleotide sequence means in the direction from the 5’ to the 3’ end. Similarly, the term "upstream" means in the direction from the 3' to the 5' end.

LNA

The term “LNA” refers to a bicyclic nucleoside analogue, known as “Locked Nucleic Acid”. It may refer to an LNA unit, or, when used in the context of an “LNA oligonucleotide”, LNA refers to an oligonucleotide containing one or more such bicyclic nucleotide analogues. LNA nucleotides are characterised by the presence of a linker group (such as a bridge) between C2’ and C4’ of the ribose sugar ring – for example as shown as the biradical R^{4*} - R^{2*} as described below.

As used herein, the terms “LNA oligonucleotide” and “LNA-modified oligonucleotide” include any oligonucleotide either fully or partially modified with LNA units. Thus, an LNA-modified oligonucleotide may be composed entirely of LNA units, or a LNA-modified oligonucleotide may comprise one LNA unit.

The LNA used in the oligonucleotide compounds of the invention preferably has the structure of the general formula I



Formula 1

wherein for all chiral centers, asymmetric groups may be found in either R or S orientation;

wherein X is selected from -O-, -S-, -N(R^{N*})-, -C(R^6R^{6*})-, such as, in some embodiments –O-; B is selected from hydrogen, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, nucleobases including naturally occurring and nucleobase analogues, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; preferably, B is a nucleobase or nucleobase analogue;

P designates an internucleotide linkage to an adjacent unit, or a 5'-terminal group, such internucleotide linkage or 5'-terminal group optionally including the substituent R^5 or equally applicable the substituent R^{5*};

P* designates an internucleotide linkage to an adjacent unit, or a 3'-terminal group; R^{4*} and R^{2*} together designate a bivalent linker group consisting of 1 - 4 groups/atoms selected from -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -N(R^a)-, and >C=Z, wherein Z is selected from -O-, -S-, and -N(R^a)-, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, optionally substituted C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together

may designate optionally substituted methylene (=CH_2), wherein for all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, and;

each of the substituents R^{1*}, R^2, R^3, R^5, R^{5*}, R^6 and R^{6*}, which are present is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene; wherein R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl; and basic salts and acid addition salts thereof. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{4*} and R^{2*} together designate a biradical consisting of functional groups selected from the group consisting of $C(R^aR^b)$-$C(R^aR^b)$-, $C(R^aR^b)$-O-, $C(R^aR^b)$-NR^a-, $C(R^aR^b)$-S-, and $C(R^aR^b)$-$C(R^aR^b)$-O-, wherein each R^a and R^b may optionally be independently selected. In some embodiments, R^a and R^b may be, optionally independently selected from the group consisting of hydrogen and $_{C1-6}$alkyl, such as methyl, such as hydrogen.

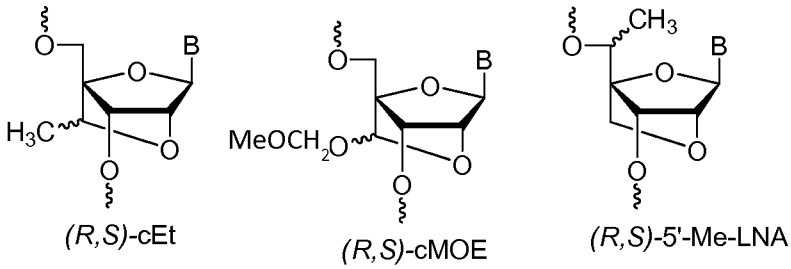
In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-$CH(CH_2OCH_3)$- (2'O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem) – in either the R- or S-configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-$CH(CH_2CH_3)$- (2'O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem). – in either the R- or S- configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-$CH(CH_3)$-. – in either the R- or S- configuration.In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-CH_2-O-CH_2- - (Seth at al., 2010, J. Org. Chem).

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-NR-CH_3- - (Seth at al., 2010, J. Org. Chem) .

In some embodiments, the LNA units have a structure selected from the following group:



(R,S)-cEt *(R,S)*-cMOE *(R,S)*-5'-Me-LNA

In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

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

In some embodiments, R^{1*}, R^2, R^3 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

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

In some embodiments, R^5 and R^{5*} are each independently selected from the group consisting of H, $–CH_3$, $-CH_2-CH_3$,- CH_2-O-CH_3, and $-CH=CH_2$. Suitably in some embodiments, either R^5 or R^{5*} are hydrogen, where as the other group (R^5 or R^{5*} respectively) is selected from the group consisting of C_{1-5} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl or substituted acyl (-C(=O)-); wherein each substituted group is mono or poly substituted with substituent groups independently selected from halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, OJ_1, SJ_1, NJ_1J_2, N_3, $COOJ_1$, CN, $O-C(=O)NJ_1J_2$, $N(H)C(=NH)NJ,J_2$ or $N(H)C(=X)N(H)J_2$ wherein X is O or S; and each J_1 and J_2 is, independently, H, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} aminoalkyl, substituted C_{1-6} aminoalkyl or a protecting group. In some embodiments either R^5 or R^{5*} is substituted C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is substituted methylene wherein preferred substituent groups include one or more groups independently selected from F, NJ_1J_2, N_3, CN, OJ_1, SJ_1, $O-C(=O)NJ_1J_2$, $N(H)C(=NH)NJ$, J_2 or $N(H)C(O)N(H)J_2$. In some embodiments each J_1 and J_2 is, independently H or C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is methyl, ethyl or methoxymethyl. In some embodiments either R^5 or R^{5*} is methyl. In a further embodiment either R^5 or R^{5*} is ethylenyl. In some embodiments either R^5 or R^{5*} is substituted acyl. In some embodiments either R^5 or R^{5*} is $C(=O)NJ_1J_2$. For all chiral centers, asymmetric groups may be

found in either *R* or *S* orientation. Such 5' modified bicyclic nucleotides are disclosed in WO 2007/134181, which is hereby incorporated by reference in its entirety.

In some embodiments B is a nucleobase, including nucleobase analogues and naturally occurring nucleobases, such as a purine or pyrimidine, or a substituted purine or substituted pyrimidine, such as a nucleobase referred to herein, such as a nucleobase selected from the group consisting of adenine, cytosine, thymine, adenine, uracil, and/or a modified or substituted nucleobase, such as 5-thiazolo-uracil, 2-thio-uracil, 5-propynyl-uracil, 2'thio-thymine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, and 2,6-diaminopurine.

In some embodiments, R^{4*} and R^{2*} together designate a biradical selected from -C(R^aR^b)-O-, -C(R^aR^b)-C(R^cR^d)-O-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-O-, -C(R^aR^b)-O-C(R^cR^d)-, -C(R^aR^b)-O-C(R^cR^d)-O-, -C(R^aR^b)-C(R^cR^d)-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-, -C(R^a)=C(R^b)-C(R^cR^d)-, -C(R^aR^b)-N(R^c)-, -C(R^aR^b)-C(R^cR^d)- N(R^e)-, -C(R^aR^b)-N(R^c)-O-, and -C(R^aR^b)-S-, -C(R^aR^b)-C(R^cR^d)-S-, wherein R^a, R^b, R^c, R^d, R^e, and R^f each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2). For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In a further embodiment R^{4*} and R^{2*} together designate a biradical (bivalent group) selected from -CH_2-O-, -CH_2-S-, -CH_2-NH-, -CH_2-N(CH_3)-, -CH_2-CH_2-O-, -CH_2-CH(CH_3)-, -CH_2-CH_2-S-, -CH_2-CH_2-NH-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-O-, -CH_2-CH_2-CH(CH_3)-, -CH=CH-CH_2-, -CH_2-O-CH_2-O-, -CH_2-NH-O-, -CH_2-N(CH_3)-O-, -CH_2-O-CH_2-, -CH(CH_3)-O-, and –CH(CH_2-O-CH_3)-O-, and/or, -CH_2-CH_2-, and -CH=CH- For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{4*} and R^{2*} together designate the biradical C(R^aR^b)-N(R^c)-O-, wherein R^a and R^b are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen, and; wherein R^c is selected from the group consisting of

hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} together designate the biradical $C(R^aR^b)$-O-$C(R^cR^d)$ -O-, wherein R^a, R^b, R^c, and R^d are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} form the biradical –CH(Z)-O-, wherein Z is selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ_1, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ^3C(=X)NJ_1J_2$ and CN, wherein each J_1, J_2 and J_3 is, independently, H or C_{1-6} alkyl, and X is O, S or NJ_1. In some embodiments Z is C_{1-6} alkyl or substituted C_{1-6} alkyl. In some embodiments Z is methyl. In some embodiments Z is substituted C_{1-6} alkyl. In some embodiments said substituent group is C_{1-6} alkoxy. In some embodiments Z is CH_3OCH_2-. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some some embodiments, R^{1*}, R^2, R^{3*} are hydrogen, and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181.

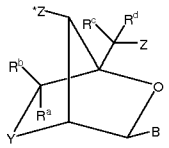
In some embodiments, R^{4*} and R^{2*} together designate a biradical which comprise a substituted amino group in the bridge such as consist or comprise of the biradical $–CH_2$-$N(R^c)$-, wherein R^c is C_{1-12} alkyloxy. In some embodiments R^{4*} and R^{2*} together designate a biradical $–Cq_3q_4$-NOR -, wherein q_3 and q_4 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl; wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ_1, SJ_1, NJ_1J_2, $COOJ_1$, CN, O-$C(=O)NJ_1J_2$, $N(H)C(=NH)N\ J_1J_2$ or $N(H)C(=X=N(H)J_2$ wherein X is O or S; and each of J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/150729 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted

C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181. In some embodiments R^{4*} and R^{2*} together designate a biradical (bivalent group) $C(R^aR^b)$-O-, wherein R^a and R^b are each independently halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_1 SJ_1, SOJ_1, SO_2J_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; or R^a and R^b together are $=C(q3)(q4)$; q_3 and q_4 are each, independently, H, halogen, C_1-C_{12}alkyl or substituted C_1-C_{12} alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2- C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, OJ_1, SJ_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, $O\text{-}C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$. and; each J_1 and J_2 is, independently, H, C1-C_6 alkyl, substituted C1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, C1-C_6 aminoalkyl, substituted C1-C_6 aminoalkyl or a protecting group. Such compounds are disclosed in WO2009006478A, hereby incorporated in its entirety by reference.

In some embodiments, R^{4*} and R^{2*} form the biradical - Q -, wherein Q is $C(q_1)(q_2)C(q_3)(q_4)$, $C(q_1)=C(q_3)$, $C[=C(q_1)(q_2)]\text{-}C(q_3)(q_4)$ or $C(q_1)(q_2)\text{-}C[=C(q_3)(q_4)]$; q_1, q_2, q_3, q_4 are each independently. H, halogen, C_{1-12} alkyl, substituted C_{1-12} alkyl, C_{2-12} alkenyl, substituted C_{1-12} alkoxy, OJ_1, SJ_1, SOJ_1, SO_2J_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)\text{-}NJ_1J_2$, $C(=O)$ J_1, $\text{-}C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; each J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group; and, optionally wherein when Q is $C(q_1)(q_2)(q_3)(q_4)$ and one of q_3 or q_4 is CH_3 then at least one of the other of q_3 or q_4 or one of q_1 and q_2 is other than H. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/154401 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181 or WO2009/067647 (alpha-L-bicyclic nucleic acids analogs).

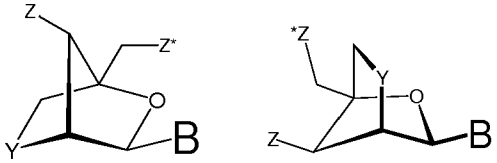
Further bicyclic nucleoside analogues and their use in antisense oligonucleotides are disclosed in WO2011/115818, WO2011/085102, WO2011/017521, WO09/100320, WO10/036698, WO09/124295 & WO09/006478. Such nucleoside analogues may in some aspects be useful in the compounds of present invention.

In some embodiments the LNA used in the oligonucleotide compounds of the invention preferably has the structure of the general formula II:

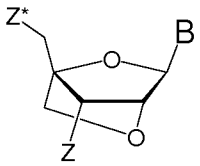


Formula II

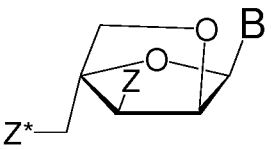
wherein Y is selected from the group consisting of -O-, -CH_2O-, -S-, -NH-, N(R^e) and/or –CH_2-; Z and Z* are independently selected among an internucleotide linkage, R^H, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base moiety (nucleobase), and R^H is selected from hydrogen and C_{1-4}-alkyl; R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkoxyalkyl, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2); and R^H is selected from hydrogen and C_{1-4}-alkyl. In some embodiments R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen and C_{1-6} alkyl, such as methyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, for example, two exemplary stereochemical isomers include the beta-D and alpha-L isoforms, which may be illustrated as follows:



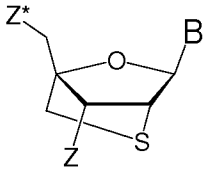
Specific exemplary LNA units are shown below:



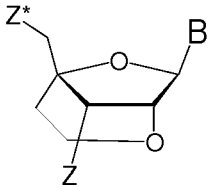
β-D-oxy-LNA



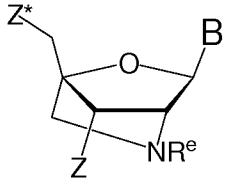
α-L-Oxy-LNA



β-D-thio-LNA



β-D-ENA



β-D-amino-LNA

The term "thio-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from S or -CH_2-S-. Thio-LNA can be in both beta-D and alpha-L-configuration.

The term "amino-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from -N(H)-, N(R)-, CH_2-N(H)-, and -CH_2-N(R)- where R is selected from hydrogen and C_{1-4}-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

The term "oxy-LNA" comprises a locked nucleotide in which Y in the general formula above represents –O-. Oxy-LNA can be in both beta-D and alpha-L-configuration.

The term "ENA" comprises a locked nucleotide in which Y in the general formula above is -CH_2-O- (where the oxygen atom of –CH_2-O- is attached to the 2'-position relative to the base B). R^e is hydrogen or methyl.

In some exemplary embodiments LNA is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA and beta-D-thio-LNA, in particular beta-D-oxy-LNA.

LNA-modified antisense oligonucleotides may be used in combinations. For instance, a cocktail of several different LNA modified oligonucleotides, directed against different regions of the same gene, may be administered simultaneously or separately.

Headmer

A "headmer" is defined as an oligomer that comprises a region X' and a region Y' that is contiguous thereto, with the 5'-most unit of region Y' linked to the 3'-most unit of region X'. Region X' comprises a contiguous stretch of non-RNase recruiting nucleoside analogues and

region Y' comprises a contiguous stretch (such as at least 7 contiguous units) of DNA units or nucleoside analogue units recognizable and cleavable by the RNase.

Tailmer

A "tailmer" is defined as an oligomer that comprises a region X'' and a region Y'' that is contiguous thereto, with the 5'-most unit of region Y'' linked to the 3'-most unit of the region X''. Region X'' comprises a contiguous stretch (such as at least 7 contiguous units) of DNA units or nucleoside analogue units recognizable and cleavable by the RNase, and region X'' comprises a contiguous stretch of non-RNase recruiting nucleoside analogues.

Chimeric Oligomers/Mixmers

"Chimeric" oligomers, called "mixmers", consist of an alternating composition of (i) DNA units or nucleoside analogue units recognizable and cleavable by RNase, and (ii) non-RNase recruiting nucleoside analogue units.

Photochemically active groups

In the present context, the term "photochemically active groups" refers to compounds which are able to undergo chemical reactions upon irradiation with light. Illustrative examples of functional groups herein are quinones, especially 6-methyl-1,4-naphtoquinone, anthraquinone, naphtoquinone, and 1,4-dimethyl-anthraquinone, diazirines, aromatic azides, benzophenones, psoralens, diazo compounds, and diazirino compounds.

Based on

As used herein, the term "based on" means that the oligomer or the oligomer of the oligomer conjugate comprises at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%, preferably all of the nucleotides of the core motif or sequence and optionally wherein one or more of the nucleotides may be a modified nucleotide.

Accordingly, for certain embodiments, the term "based on" means that the oligomer or the oligomer of the oligomer conjugate comprises all of the nucleotides of the core motif or sequence and optionally wherein one or more of the nucleotides may be a modified nucleotide.

For certain embodiments, the term "based on" means that the oligomer or the oligomer of the oligomer conjugate comprises all of the nucleotides of the core motif or sequence and wherein one or more of the nucleotides is a modified nucleotide.

For certain embodiments, the term "based on" means that the oligomer or the oligomer of the oligomer conjugate comprises at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%, preferably all of the nucleotides of the core motif or sequence and wherein one or more of the nucleotides may be a modified nucleotide; and wherein said

oligomer is a gapmer of the motif X-Y-Z wherein each of X and Z is independently a wing comprising at least one modified nucleotide and Y is a central region of nucleotides.

Accordingly, for certain embodiments, the term “based on” means that the oligomer or the oligomer of the oligomer conjugate comprises all of the nucleotides of the core motif or sequence and wherein one or more of the nucleotides may be a modified nucleotide; and wherein said oligomer is a gapmer of the motif X-Y-Z wherein each of X and Z is independently a wing comprising at least one modified nucleotide and Y is a central region of nucleotides.

Stability

The term "stability" in reference to duplex or triplex formation generally designates how tightly an antisense oligonucleotide binds to its intended target sequence; more particularly, “stability” designates the free energy of formation of the duplex or triplex under physiological conditions. Melting temperature under a standard set of conditions, e.g., as described below, is a convenient measure of duplex and/or triplex stability. Preferably, antisense oligonucleotides of the invention are selected that have melting temperatures of at least 45°C when measured in 100mM NaCl, 0.1mM EDTA and10 mM phosphate buffer aqueous solution, pH 7.0 at a strand concentration of both the antisense oligonucleotide and the target nucleic acid of 1.5 µM. Thus, when used under physiological conditions, duplex or triplex formation will be substantially favored over the state in which the antisense oligonucleotide and its target are dissociated. It is understood that a stable duplex or triplex may in some embodiments include mismatches between base pairs and/or among base triplets in the case of triplexes. Preferably, LNA modified antisense oligonucleotides of the invention form perfectly matched duplexes and/or triplexes with their target nucleic acids.

Potent inhibitor

In some embodiments the oligomer or oligomer conjugate is a potent inhibitor, in particular of HBx or HBsAg.

As used herein, the phrase “potent inhibitor” refers to an oligomer with an IC50 of less than 5nM as determined by a lipofectamin transfection assay. In some embodiments, the IC50 is less than 4nM, such as less than 2nM.

A “potent inhibitor” as determined by “gymnosis”, where gymnosis describes the delivery of the oligomer to the cultured cell without the use of a transfection agent, refers to an IC50 of less than 5 µm in a gymnosis assay or in a in vivo AAV/HBV Mouse Model. In some embodiments, the IC50 is less than 2 µm, such as less than 1 µm.

Treat/Treatment

The terms “treat”/”treatment” etc. include one or more of to cure, to alleviate, to prevent or to detect. In certain preferred embodiments, the terms “treat”/”treatment” etc. mean to cure or to

alleviate. The term “alleviate” includes to alleviate the symptoms and/or the conditions attributed with or associated with a viral disorder.

Thus, in some aspects, the present invention relates to oligomers or oligomer conjugates suitable for, and uses thereof and methods using same, to cure, to alleviate, to prevent or to detect a viral disorder.

Accordingly, in certain aspects, the present invention relates to oligomers or oligomer conjugates suitable for, and uses thereof and methods using same, to cure or to alleviate a viral disorder.

In some embodiments, 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 recognised that treatment as referred to herein may, In some embodiments, be prophylactic.

"Treating" a disease or condition in a subject or "treating" a subject having a disease or condition refers to subjecting the individual to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease or condition is decreased or stabilized.

A patient who is in need of treatment is a patient suffering from or likely to suffer from the disease or disorder.

By "treating prophyllactically" a disease or condition in a subject is meant reducing or eliminating the risk of developing (i.e., the incidence) of or reducing the severity of the disease or condition prior to the appearance of at least one symptom of the disease.

Agent

The term "agent" means any compound, for example, an antibody, or a therapeutic agent, a detectable label (e.g., a marker, tracer, or imaging compound).

Therapeutic agent

The term "therapeutic agent" means any compound having a biological activity. Therapeutic agents may be useful for treating conditions or diseases. Specific therapeutic agents according to the invention may be oligomers. Specific therapeutic agents may be oligomers or oligomer conjugates according to the present invention.

Delivering said oligomer to the liver

As used herein, the term “delivering said oligomer to the liver” relates to the process by which an oligomer is brought into contact with, or into proximity to the cells or tissues of the liver. This includes the delivery of the oligomer to the blood supply within or surrounding the liver. The

oligomer may be delivered or carried from the site of entry into the body to the liver or tissues surrounding the liver.

Delivering said oligomer to a hepatocyte

As used herein, the term "delivering said oligomer to a hepatocyte" relates to the process by which an oligomer is brought into contact with, or into proximity to, a hepatocyte of the liver. This includes the delivery of the oligomer to the blood supply within or surrounding a hepatocyte. The oligomer may be delivered or carried from the site of entry into the body to a hepatocyte.

Viral disorder

The term "viral disorder" in the context of the present invention refers to any disorder or disease which is associated with viral infection.

Pharmaceutical carriers/ Pharmaceutically acceptable carriers

"Pharmaceutical carriers" or "pharmaceutically acceptable carriers" are to be distinguished from the "carrier component" of the invention as described above. "Pharmaceutical carrier" and "carrier component" are to be treated as mutually exclusive terms in the context of the present invention.

Administering/Administration

The terms "administering" and "administration" are intended to mean a mode of delivery including, without limitation, intra-arterially, intra-nasally, intra-peritoneally, intravenously, intramuscularly, sub-cutaneously, transdermally or per os. A daily dosage can be divided into one, two or more doses in a suitable form to be administered at one, two or more times throughout a time period.

DETAILED DESCRIPTION OF THE INVENTION

INTRODUCTION

The present invention relates to oligomers (also referred to as oligomeric molecules) and/or oligomer conjugates.

The oligomers and oligomer conjugates are useful for the treatment of a viral disorder.

In particular embodiments, the oligomers or oligomer conjugates of the invention are capable of modulating a target sequence in HBV HBx or HBsAg.

The oligomers of the invention may be conjugated to a carrier component.

Preferably, the carrier component may be capable of delivering the oligomer to the liver of a subject to be treated.

The present invention therefore employs oligomers or oligomer conjugates for use in modulating the function of nucleic acid molecules encoding HBV HBx or HBsAg, such as the HBV nucleic acid molecule presented as SEQ ID no 1 or SEQ ID No 2, and naturally occurring variants of such nucleic acid molecules encoding HBV HBx or HBV HBsAg.

OLIOGOMER FEATURES

The oligomer or the oligomer of the oligomer conjugate may consist or comprise of a contiguous nucleotide sequence of from 8-50, 8-30, 8-25, 8-20, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, or 8-12 nucleotides in length, preferably 8-16 nucleotides in length, more preferably 10 to 20 nucleotides in length.

For some embodiments, the oligomer or the oligomer of the oligomer conjugate may comprise or consist of a contiguous nucleotide sequence of from 10 – 50, such as 10 – 30, 10-20, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, or 10-12 nucleotides in length, preferably 10-20 nucleotides in length, more preferably 10 to 18 nucleotides in length, most preferably 10 to 16 nucleotides in length.

For some embodiments, the oligomer or the oligomer of the oligomer conjugate may comprise or consist of a contiguous nucleotide sequence of from 12 – 50, such as 12 – 30, 12-20, 12-18, 12-17, 12-16, 12-15, 12-14 or 12-13 nucleotides in length, preferably 12-16 nucleotides in length.

For some embodiments, the oligomer or the oligomer of the oligomer conjugate may comprise or consist of a contiguous nucleotide sequence of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides in length.

For some embodiments, the oligomers or the oligomer component of the oligomer conjugates of the present invention may comprise or consist of a contiguous nucleotide sequence of a total of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides in length.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate comprises or consists of a contiguous nucleotide sequence of a total of from 8 – 22, such as 8-20, such as 8 – 18, such as 8 – 17 or 8 – 16, contiguous nucleotides in length.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate comprises or consists of a contiguous nucleotide sequence of a total of 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleotides in length.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate comprises or consists of a contiguous nucleotide sequence of a total of 15 or 16 contiguous nucleotides in length.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate according to the invention consists of no more than 22 nucleotides, such as no more than 20 nucleotides, such as no more than 18 nucleotides - such as 15, or 16 or 17 nucleotides.

In some embodiments, preferably the oligomer or the oligomer of the oligomer conjugate of the invention comprises less than 20 nucleotides.

It should be understood that when a range is given for an oligomer, or contiguous nucleotide sequence length it includes the lower an upper lengths provided in the range, for example from (or between) 10– 30, includes both 10 and 30.

The length of the oligonucleotide moieties is sufficiently large to ensure that specific binding takes place only at the desired target polynucleotide and not at other fortuitous sites, as explained in many references, e.g., Rosenberg et al., International application PCT/US92/05305; or Szostak et al., *Meth. Enzymol*, 68:419-429 (1979). The upper range of the length is determined by several factors, including the inconvenience and expense of synthesizing and purifying oligomers greater than about 30-40 nucleotides in length, the greater tolerance of longer oligonucleotides for mismatches than shorter oligonucleotides, whether modifications to enhance binding or specificity are present, whether duplex or triplex binding is desired, and the like.

The oligomer or the oligomer of the oligomer conjugate may comprise or consist of a simple sequence of natural occurring nucleotides – preferably 2'-deoxynucleotides (referred to here generally as "DNA"), but also possibly ribonucleotides (referred to here generally as "RNA"), or a combination of such naturally occurring nucleotides and one or more non-naturally occurring nucleotides, *i.e.* nucleotide analogues. Such nucleotide analogues may suitably enhance the affinity of the oligomer for the target sequence.

Examples of suitable and preferred nucleotide analogues are provided by WO2007/031091or are referenced therein.

Examples of nucleotide analogues include nucleotides that have been modified. Examples of such modifications include modifying the sugar moiety to provide a 2'-substituent group or to produce a bridged (locked nucleic acid) structure which enhances binding affinity and may also provide increased nuclease resistance. These modified nucleotide analogues can therefore be affinity-enhancing nucleotide analogues.

Incorporation of affinity-enhancing nucleotide analogues in the oligomer or the oligomer of the oligomer conjugate, such as LNA or 2'-substituted sugars, can allow the size of the specifically binding oligomer to be reduced, and may also reduce the upper limit to the size of the oligomer before non-specific or aberrant binding takes place.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate comprises at least 1 nucleotide analogue. In some embodiments the oligomer or the oligomer of the oligomer conjugate comprises at least 2 nucleotide analogues. In some embodiments, the oligomer or the oligomer of the oligomer conjugate comprises from 2-8 nucleotide analogues, *e.g.* 6 or 7 nucleotide analogues. In the by far most preferred embodiments, at least one of said nucleotide analogues is a locked nucleic acid (LNA); for example at least 2, 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8, of the nucleotide analogues may be LNA. In some embodiments all the nucleotides analogues may be LNA.

It will be recognised that when referring to a preferred nucleotide sequence motif or nucleotide sequence, which consists of only nucleotides, the oligomers or the oligomers of the oligomer conjugates of the invention which are defined by that sequence may comprise a corresponding nucleotide analogue in place of one or more of the nucleotides present in said sequence, such as LNA units or other nucleotide analogues, which raise the duplex stability/T_m of the oligomer/target duplex (*i.e.* affinity enhancing nucleotide analogues).

A preferred nucleotide analogue is LNA, such as oxy-LNA (such as beta-D-oxy-LNA, and alpha-L-oxy-LNA), and/or amino-LNA (such as beta-D-amino-LNA and alpha-L-amino-LNA) and/or thio-LNA (such as beta-D-thio-LNA and alpha-L-thio-LNA) and/or ENA (such as beta-D-ENA and alpha-L-ENA). Most preferred is beta-D-oxy-LNA.

In some embodiments the nucleotide analogues present within the oligomer or the oligomer of the oligomer conjugate of the invention (such as in region W and region Y for gapmers – as mentioned herein) are independently selected from, for example: 2'-O-alkyl-RNA units, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA (intercalating nucleic acid -Christensen, 2002. Nucl. Acids. Res. 2002 30: 4918-4925, hereby incorporated by reference) units and 2'MOE units. In some embodiments there is only one of the above types of nucleotide analogues present in the oligomer of the invention, or contiguous nucleotide sequence thereof.

In some embodiments the nucleotide analogues are 2'-O-methoxyethyl-RNA (2'MOE), 2'-fluoro-DNA units or LNA nucleotide analogues, and as such the oligonucleotide of the invention may comprise nucleotide analogues which are independently selected from these three types of analogue, or may comprise only one type of analogue selected from the three types. In some embodiments at least one of said nucleotide analogues is 2'-MOE-RNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-MOE-RNA nucleotide units. In some embodiments at least one of said nucleotide analogues is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-fluoro-DNA nucleotide units.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate according to the invention comprises at least one Locked Nucleic Acid (LNA) unit, such as 1, 2, 3, 4, 5, 6, 7, or 8

LNA units, such as from 3 – 7 or 4 to 8 LNA units, or 3, 4, 5, 6 or 7 LNA units. In some embodiments, all the nucleotide analogues are LNA.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate may comprise both beta-D-oxy-LNA, and one or more of the following LNA units: thio-LNA, amino-LNA, oxy-LNA, and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments all LNA cytosine units are 5'methyl-Cytosine.

In some embodiments of the invention, the oligomer or the oligomer of the oligomer conjugate may comprise both LNA and DNA units. Preferably the combined total of LNA and DNA units is 10-25, such as 10 – 24, preferably 10-20, such as 10 – 18, even more preferably 12-16. In some embodiments of the invention, the nucleotide sequence of the oligomer, such as the contiguous nucleotide sequence consists of at least one LNA and the remaining nucleotide units are DNA units.

In some embodiments the oligomer or the oligomer of the oligomer conjugate comprises only LNA nucleotide analogues and naturally occurring nucleotides (such as RNA or DNA, most preferably DNA nucleotides), optionally with modified internucleotide linkages such as phosphorothioate.

In some embodiments, any mismatches between the nucleotide sequence of the oligomer and the target sequence are preferably found in regions outside the affinity enhancing nucleotide analogues. Examples of such regions outside the affinity enhancing nucleotide analogues in gapmers include region X as referred to herein and/or region V as referred to herein and/or region Z as referred to herein, and/or at the site of non modified nucleotides in the oligonucleotide, and/or in regions which are 5' or 3' to the contiguous nucleotide sequence.

In some embodiments, the oligomer or the oligomer of the oligomer conjugate of the invention does not comprise RNA (units). It is preferred that the oligomer or the oligomer of the oligomer conjugate according to the invention is a linear molecule or is synthesised as a linear molecule. Preferably the oligomer or the oligomer of the oligomer conjugate is a single stranded molecule, and preferably does not comprise short regions of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to equivalent regions within the same oligomer (*i.e.* duplexes). In this regard, the oligomer or the oligomer of the oligomer conjugate is not (essentially) double stranded. In some embodiments, the oligomer or the oligomer of the oligomer conjugate is essentially single stranded. In various embodiments, the oligomer or the oligomer of the oligomer conjugate of the invention may consist entirely of the contiguous nucleotide region. Thus, preferably the oligomer or the oligomer of the oligomer conjugate is not substantially self-complementary.

Hence, in certain aspects, the invention provides an oligomer or an oligomer conjugate having an oligomer component from 5–50, such as 5–30, or such as 5-20, such as 8–30, such as 8-20, such as 8-18, such as 8-16, such as 10-16, such as 10-15, such as 12-16, such as 12-15 nucleotides in length which comprises a contiguous nucleotide sequence (a first region) of a total of at least 5 such as at least 8 nucleotides, wherein said contiguous nucleotide sequence (a first region) is at least 80% (e.g., 85%, 90%, 95%, 98%, or 99%) homologous to a region corresponding to the reverse complement of a HBV HBx or HBsAg gene or mRNA, such any sequence within any of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 or naturally occurring variant thereof. Thus, for example, the oligomer or the oligomer component of the oligomer conjugate hybridizes to a single stranded nucleic acid molecule having the sequence of a portion of any of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, preferably within any of SEQ ID No. 1 and SEQ ID No. 2. The oligomer or oligomer component may hybridize to a single stranded nucleic acid molecule having the sequence of a portion sequence shown as position 200 to 1900 of SEQ ID No. 3). The oligomer or the oligomer component of the oligomer conjugate may hybridize to a single stranded nucleic acid molecule having the sequence shown as position 1264 to 1598 or 691 to 706 of SEQ ID NO: 3. The oligomer or the oligomer component of the oligomer conjugate may hybridize to a single stranded nucleic acid molecule with a sequence selected from the group consisting of the following positions in SEQ ID NO 3: position1 to 1944, position 157 to 1840, position 1196 to 1941, position 1376 to 1840 and position 3158-3182. Preferably, the oligomer conjugate hybridize to a single stranded nucleic acid molecule with a sequence selected within position1530 to 1598 of SEQ ID NO: 3, more preferrable within position 1577 to 1598 of SEQ ID NO: 3 and most preferably within position 1530 to 1543 of SEQ ID NO: 3. An oligomer or the oligomer component of the oligomer conjugate may hybridize to a single stranded nucleic acid molecule selected from the group consisting of positions: 1264-1278; 1265-1277; 1530-1543; 1530-1544; 1531-1543; 1551-1565; 1551-1566; 1577-1589; 1577-1591; 1577-1592; 1578-1590; 1578-1592; 1583-1598; 1584-1598; and 1585-1598; or 670-706, 691-705; 691-706; 692-706; 693-706; and 694-706 of SEQ ID NO: 3. Preferably, an oligomer or the oligomer component of the oligomer conjugate may hybridize to a single stranded nucleic acid molecule within position1530 to 1598 of SEQ ID NO: 3, more preferably withn positions 1530-1543 or positions 1577-1598 of SEQ ID NO: 3.

For certain embodiments, the invention provides an oligomer or an oligomer conjugate having an oligomer based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);

GCGTAAAGAGAGGT (SEQ ID NO: 11);

AGCGAAGTGCACACG (SEQ ID NO: 20);

AGGTGAAGCGAAGTG (SEQ ID NO: 26);

AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852),

which is capable of modulating a target sequence in HBx or HBsAg of HBV to treat a viral disorder.

The oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGgtgaagcgaAGTG (SEQ ID NO: 668);
AGCgaagtgcacaCGG (SEQ ID NO: 308);
CGAaccactgaACA (SEQ ID NO: 297);
CGCagtatggaTC (SEQ ID NO: 300);
AGGtgaagcgaagTGC (SEQ ID NO: 315);
AGGtgaagcgaaGTG (SEQ ID NO: 316);
GAAccactgaacAAA (SEQ ID NO: 294);
CGAaccactgaacAAA (SEQ ID NO: 295);
CGAaccactgaaCAA(SEQ ID NO: 296);
CGAaccactgaAC (SEQ ID NO: 298);
CCGcagtatggaTCG (SEQ ID NO: 299);
CGCgtaaagagaGGT (SEQ ID NO: 302);

AGAaggcacagaCGG (SEQ ID NO: 304);
GAGaaggcacagaCGG (SEQ ID NO: 305);
GAAgtgcacacGG (SEQ ID NO: 306);
GCGaagtgcacaCGG (SEQ ID NO: 307);
CGAagtgcacaCG (SEQ ID NO: 309);
GAAccactgaaCAAA (SEQ ID NO: 585);
CGAAccactgaacAAA (SEQ ID NO: 588)
GAAgtgcacaCGG (SEQ ID NO: 628);
TAGtaaactgagCCA (SEQ ID NO: 678);
CGAaccactgAAC (SEQ ID NO: 600);
AGGtgaagcgaAGT (SEQ ID NO: 317); and
CGAaccactgAACA (SEQ ID NO: 597),

wherein uppercase letters denote LNA units and lower case letters denote DNA units.

In certain embodiments, the oligomer, or additional oligomer, or oligomer component of the oligomer conjugate, or oligomer component of the additional oligomer conjugate may comprise a sequence based on a sequence selected from a list of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15);
AGCGAAGTGCACACGG (SEQ ID NO 18);

GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
AGCGAAGTGCACACG (SEQ ID NO: 20);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGTG (SEQ ID NO: 26); and
AGGTGAAGCGAAGT (SEQ ID NO 27).

In another embodiment the motif sequence is selected from:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26); and
AGCGAAGTGCACACGG (SEQ ID NO 18)

In another embodiment the motif sequence is selected from GCGTAAAGAGAGG (SEQ ID NO: 13) GCGTAAAGAGAGGT (SEQ ID NO: 11) and CGCGTAAAGAGAGGT (SEQ ID NO 12).

In another embodiment the motif sequence is selected from AGCGAAGTGCACACG (SEQ ID NO: 20); AGGTGAAGCGAAGTG (SEQ ID NO: 26); AGCGAAGTGCACACGG (SEQ ID NO 18); GAAGTGCACACGG (SEQ ID NO 16); GCGAAGTGCACACGG (SEQ ID NO 17); CGAAGTGCACACG (SEQ ID NO 19) and AGGTGAAGCGAAGT (SEQ ID NO 27).

In another embodiment the motif sequence is selected from CGAACCACTGAACA (SEQ ID NO: 7); GAACCACTGAACAAA (SEQ ID NO 4); CGAACCACTGAACAAA (SEQ ID NO 5); CGAACCACTGAACAA (SEQ ID NO 6); CGAACCACTGAAC (SEQ ID NO 8) and TAGTAAACTGAGCCA (SEQ ID NO: 852).

In another embodiment the motif sequence is selected from CCGCAGTATGGATCG (SEQ ID NO 9) and CGCAGTATGGATC (SEQ ID NO: 10).

In another embodiment the motif sequence is selected from AGAAGGCACAGACGG (SEQ ID NO 14) and GAGAAGGCACAGACGG (SEQ ID NO 15).

In certain embodiments, the oligomer, or additional oligomer, or oligomer component of the oligomer conjugate, or oligomer component of the additional oligomer conjugate may comprise or consist of a sequence selected from the group presented below:

GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGgtgaagcgaAGTG (SEQ ID NO: 668);
AGCgaagtgcacaCGG (SEQ ID NO: 308);
CGAaccactgaACA (SEQ ID NO: 297);
CGCagtatggaTC (SEQ ID NO: 300);
AGGtgaagcgaagTGC (SEQ ID NO: 315);
AGGtgaagcgaaGTG (SEQ ID NO: 316);
GAAccactgaacAAA (SEQ ID NO: 294);
CGAaccactgaacAAA (SEQ ID NO: 295);
CGAaccactgaaCAA(SEQ ID NO: 296);
CGAaccactgaAC (SEQ ID NO: 298);
CCGcagtatggaTCG (SEQ ID NO: 299);
CGCgtaaagagaGGT (SEQ ID NO: 302);
AGAaggcacagaCGG (SEQ ID NO: 304);
GAGaaggcacagaCGG (SEQ ID NO: 305);

GAAgtgcacacGG (SEQ ID NO: 306);
GCGaagtgcacaCGG (SEQ ID NO: 307);
CGAagtgcacaCG (SEQ ID NO: 309);
GAAccactgaaCAAA (SEQ ID NO: 585);
CGAAccactgaacAAA (SEQ ID NO: 588)
GAAgtgcacaCGG (SEQ ID NO: 628);
TAGtaaactgagCCA (SEQ ID NO: 678);
CGAaccactgAAC (SEQ ID NO: 600);
AGGtgaagcgaAGT (SEQ ID NO: 317); and
CGAaccactgAACA (SEQ ID NO: 597).

wherein uppercase letters denote affinity enhancing nucleotide analogues and lower case letters denote DNA units.

In another embodiment the sequence is selected from:
GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
CGCgtaaagagaGGT (SEQ ID NO: 302);
AGAaggcacagaCGG (SEQ ID NO: 304);
GAGaaggcacagaCGG (SEQ ID NO: 305);
GAAgtgcacacGG (SEQ ID NO: 306);
GCGaagtgcacaCGG (SEQ ID NO: 307);
GAAgtgcacaCGG (SEQ ID NO: 628);
AGCgaagtgcacaCGG (SEQ ID NO: 308);
CGAagtgcacaCG (SEQ ID NO: 309);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGGtgaagcgaagTGC (SEQ ID NO: 315);
AGGtgaagcgaaGTG (SEQ ID NO: 316);
AGGtgaagcgaAGT (SEQ ID NO: 317); and
AGgtgaagcgaAGTG (SEQ ID NO: 668).

In one embodiment the sequence is selected from:
GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGgtgaagcgaAGTG (SEQ ID NO: 668); and
AGCgaagtgcacaCGG (SEQ ID NO: 308).

In one embodiment the sequence is selected from GCGtaaagagaGG (SEQ ID NO: 303) GCGtaaagagaGGT(SEQ ID NO: 301); GCGtaaagagAGG (SEQ ID NO: 618) and CGCgtaaagagaGGT (SEQ ID NO: 302).

In another embodiment the sequence is selected from AGCgaagtgcacACG (SEQ ID NO: 310); AGGtgaagcgaagTGC (SEQ ID NO: 315); AGGtgaagcgaaGTG (SEQ ID NO: 316); GAAgtgcacaCGG (SEQ ID NO: 628); AGgtgaagcgaAGTG (SEQ ID NO: 668); AGCgaagtgcacaCGG (SEQ ID NO: 308); GAAgtgcacacGG (SEQ ID NO: 306); GCGaagtgcacaCGG (SEQ ID NO: 307); CGAagtgcacaCG (SEQ ID NO: 309); and AGGtgaagcgaAGT (SEQ ID NO: 317).

In another embodiment the sequence is selected from CGAaccactgaACA (SEQ ID NO: 297); GAAccactgaacAAA (SEQ ID NO: 294); CGAaccactgaacAAA (SEQ ID NO: 295); CGAaccactgaaCAA (SEQ ID NO: 296); CGAaccactgaAC (SEQ ID NO: 298); CGAaccactgAACA (SEQ ID NO: 597) and TAGtaaactgagCCA (SEQ ID NO: 678).

In another embodiment the sequence is selected from CCGcagtatggaTCG (SEQ ID NO: 299) and CGCagtatggaTC (SEQ ID NO: 300).

In another embodiment the sequence is selected from AGAaggcacagaCGG (SEQ ID NO: 304) and GAGaaggcacagaCGG (SEQ ID NO: 305).

The oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

5'- GN2-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G$-3' (SEQ ID NO: 815)

5'- GN2-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G_S T$-3' (SEQ ID NO: 814)

5'-GN2-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S A_S G_S G$-3' (SEQ ID NO: 825)

5'- GN2-C6 ca$A_S G_S$ $^{m}C_S g_S a_S a_S g_S t_S g_S c_S a_S c_S A_S$ $^{m}C_S G$-3' (SEQ ID NO: 808)

5'-GN2-C6 ca$A_S G_S g_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S A_S G_S T_S G$-3' (SEQ ID NO: 826)

5'- GN2-C6 ca$A_S G_S$ $^{m}C_S g_S a_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 807)

5'- GN2-C6 ca$G_S A_S A_S c_S c_S a_S c_S t_S g_S a_S a_S c_S A_S A_S A$-3' (SEQ ID NO: 799)

5'- GN2-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S a_S c_S A_S A_S A$-3' (SEQ ID NO: 800)

5'- GN2-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S a_S$ $^{m}C_S A_S A$-3' (SEQ ID NO: 801)

5'- GN2-C6 ca$^{m}C_S$ $^{m}C_S G_S c_S a_S g_S t_S a_S t_S g_S g_S a_S T_S$ $^{m}C_S G$-3' (SEQ ID NO: 802)

5'- GN2-C6 ca$^{m}C_S G_S$ $^{m}C_S g_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G_S T$-3' (SEQ ID NO: 803)

5'- GN2-C6 ca$A_S G_S A_S a_S g_S g_S c_S a_S c_S a_S g_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 804)

5'- GN2-C6 ca$G_S A_S G_S a_S a_S g_S g_S c_S a_S c_S a_S g_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 805)

5'- GN2-C6 caG_S $^mC_S$$G_S$$a_S$$a_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 806)

5'- GN2-C6 ca$A_S$$G_S$$G_S$$t_S$$g_S$$a_S$$a_S$$g_S$ ^{m}c$_S$$g_S$$a_S$$a_S$$g_S$$T_S$$G_S$ ^{m}C-3' (SEQ ID NO: 809)

5'- GN2-C6 ca$A_S$$G_S$$G_S$$t_S$$g_S$$a_S$$a_S$$g_S$ ^{m}c$_S$$g_S$$a_S$$a_S$$G_S$$T_S$G-3' (SEQ ID NO: 810)

5'- GN2-C6 ca$^mC_S$$G_S$$A_S$$a_S$$c_S$$c_S$$a_S$$c_S$$t_S$$g_S$$a_S$$A_S$ ^mC_SA-3' (SEQ ID NO: 811)

5'- GN2-C6 ca$^mC_S$$G_S$$A_S$$a_S$$c_S$$c_S$$a_S$$c_S$$t_S$$g_S$$a_S$$A_S$ ^{m}C-3' (SEQ ID NO: 812)

5'- GN2-C6 ca$^mC_S$$G_S$ $^mC_S$$a_S$$g_S$$t_S$$a_S$$t_S$$g_S$$g_S$$a_S$$T_S$ ^{m}C-3' (SEQ ID NO: 813)

5'- GN2-C6 ca$G_S$$A_S$$A_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ ^{m}c$_S$$G_S$G-3' (SEQ ID NO: 816)

5'-GN2-C6 ca$^mC_S$$G_S$$A_S$$a_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ ^mC_SG-3' (SEQ ID NO: 817)

5'-GN2-C6 ca$A_S$$G_S$$G_S$$t_S$$g_S$$a_S$$a_S$$g_S$ ^{m}c$_S$$g_S$$a_S$$A_S$$G_S$T-3' (SEQ ID NO: 818)

5'-GN2-C6 ca$G_S$$A_S$$A_S$$c_S$$c_S$$a_S$$c_S$$t_S$$g_S$$a_S$$a_S$ $^mC_S$$A_S$$A_S$A-3' (SEQ ID NO: 819)

5'-GN2-C6 ca $^mC_S$$G_S$$A_S$$A_S$$c_S$$c_S$$a_S$$c_S$$t_S$$g_S$$a_S$$a_S$$c_S$$A_S$$A_S$A-3' (SEQ ID NO: 820)

5'-GN2-C6 ca$G_S$$A_S$$A_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 821)

5'-GN2-C6 ca$T_S$$A_S$$G_S$$t_S$$a_S$$a_S$$a_S$$c_S$$t_S$$g_S$$a_S$$g_S$ ^mC_S $^mC_S$$A_S$3' (SEQ ID NO: 822)

5'-GN2-C6 ca$^mC_S$$G_S$$A_S$$a_S$$c_S$$c_S$$a_S$$c_S$$t_S$$g_S$$A_S$$A_S$ ^{m}C-3' (SEQ ID NO: 823)

5'-GN2-C6 ca$^mC_S$$G_S$$A_S$$a_S$$c_S$$c_S$$a_S$$c_S$$t_S$$g_S$$A_S$$A_S$ ^mC_SA-3' (SEQ ID NO: 824)

wherein uppercase letters denote beta-D-oxy-LNA units; lowercase letters denote DNA units; the subscript "s" denotes a phosphorothioate linkage; superscript m denotes a a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base;GN2-C6 denotes a GalNAc2 carrier component with a C6 linker.

In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 caG_S $^mC_S$$G_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$a_S$$G_S$G-3' (SEQ ID NO: 815)

5'- GN2-C6 caG_S $^mC_S$$G_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$a_S$$G_S$$G_S$T-3' (SEQ ID NO: 814)

5'-GN2-C6 caG_S $^mC_S$$G_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$A_S$$G_S$G-3' (SEQ ID NO: 825)

5'- GN2-C6 ca$^mC_S$$G_S$ $^mC_S$$g_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$a_S$$G_S$$G_S$T-3' (SEQ ID NO: 803)

5'- GN2-C6 ca$A_S$$G_S$$A_S$$a_S$$g_S$$g_S$$c_S$$a_S$$c_S$$a_S$$g_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 804)

5'- GN2-C6 ca$G_S$$A_S$$G_S$$a_S$$a_S$$g_S$$g_S$$c_S$$a_S$$c_S$$a_S$$g_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 805)

5'- GN2-C6 ca$G_S$$A_S$$A_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ ^{m}c$_S$$G_S$G-3' (SEQ ID NO: 816)

5'- GN2-C6 caG_S $^mC_S$$G_S$$a_S$$a_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 806)

5'-GN2-C6 ca$G_S$$A_S$$A_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 821)

5'- GN2-C6 ca$A_S$$G_S$ $^mC_S$$g_S$$a_S$$a_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ $^mC_S$$G_S$G-3' (SEQ ID NO: 807)

5'-GN2-C6 ca$^{m}C_{S}G_{S}A_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G$-3' (SEQ ID NO: 817)

5'- GN2-C6 ca$A_{S}G_{S}{}^{m}C_{S}g_{S}a_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}A_{S}{}^{m}C_{S}G$-3' (SEQ ID NO: 808)

5'- GN2-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}a_{S}g_{S}T_{S}G_{S}{}^{m}C$-3' (SEQ ID NO: 809)

5'- GN2-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}a_{S}G_{S}T_{S}G$-3' (SEQ ID NO: 810)

5'-GN2-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}A_{S}G_{S}T$-3' (SEQ ID NO: 818)

5'-GN2-C6 ca$A_{S}G_{S}g_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}A_{S}G_{S}T_{S}G$-3' (SEQ ID NO: 826)

In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}a_{S}G_{S}G$-3' (SEQ ID NO: 815)

5'- GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}a_{S}G_{S}G_{S}T$-3' (SEQ ID NO: 814)

5'-GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}A_{S}G_{S}G$-3' (SEQ ID NO: 825)

5'- GN2-C6 ca$A_{S}G_{S}{}^{m}C_{S}g_{S}a_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}A_{S}{}^{m}C_{S}G$-3' (SEQ ID NO: 808)

5'-GN2-C6 ca$A_{S}G_{S}g_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}A_{S}G_{S}T_{S}G$-3' (SEQ ID NO: 826)

5'- GN2-C6 ca$A_{S}G_{S}{}^{m}C_{S}g_{S}a_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G_{S}G$-3' (SEQ ID NO: 807)

In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}a_{S}G_{S}G$-3' (SEQ ID NO: 815)

5'- GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}a_{S}G_{S}G_{S}T$-3' (SEQ ID NO: 814)

5'-GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}A_{S}G_{S}G$-3' (SEQ ID NO: 825)

5'- GN2-C6 ca$^{m}C_{S}G_{S}{}^{m}C_{S}g_{S}t_{S}a_{S}a_{S}a_{S}g_{S}a_{S}g_{S}a_{S}G_{S}G_{S}T$-3' (SEQ ID NO: 803)

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In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 ca$A_{S}G_{S}{}^{m}C_{S}g_{S}a_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}A_{S}{}^{m}C_{S}G$-3' (SEQ ID NO: 808)

5'- GN2-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}a_{S}g_{S}T_{S}G_{S}{}^{m}C$-3' (SEQ ID NO: 809)

5'- GN2-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}a_{S}G_{S}T_{S}G$-3' (SEQ ID NO: 810)

5'-GN2-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}A_{S}G_{S}T$-3' (SEQ ID NO: 818)

5'-GN2-C6 ca$G_{S}A_{S}A_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G_{S}G$-3' (SEQ ID NO: 821)

5'-GN2-C6 ca$A_{S}G_{S}g_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}A_{S}G_{S}T_{S}G$-3' (SEQ ID NO: 826)

5'- GN2-C6 ca$A_{S}G_{S}{}^{m}C_{S}g_{S}a_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G_{S}G$-3' (SEQ ID NO: 807)

5'-GN2-C6 ca$^{m}C_{S}G_{S}A_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G$-3' (SEQ ID NO: 817)

5'- GN2-C6 ca$G_{S}{}^{m}C_{S}G_{S}a_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G_{S}G$-3' (SEQ ID NO: 806)

5'- GN2-C6 ca**G$_S$A$_S$A$_S$**g$_s$t$_s$g$_s$c$_s$a$_s$c$_s$a$_s$^{m}c$_s$**G$_S$G**-3' (SEQ ID NO: 816)

In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 ca**G$_S$A$_S$A$_S$**c$_s$c$_s$a$_s$c$_s$t$_s$g$_s$a$_s$a$_s$c$_s$**A$_S$A$_S$A**-3' (SEQ ID NO: 799)

5'- GN2-C6 ca^m**C$_S$G$_S$A$_S$**a$_s$c$_s$c$_s$a$_s$c$_s$t$_s$g$_s$a$_s$a$_s$c$_s$**A$_S$A$_S$A**-3' (SEQ ID NO: 800)

5'- GN2-C6 ca^m**C$_S$G$_S$A$_S$**a$_s$c$_s$c$_s$a$_s$c$_s$t$_s$g$_s$a$_s$a$_s$^{m}**C$_S$A$_S$A**-3' (SEQ ID NO: 801)

5'- GN2-C6 ca^m**C$_S$G$_S$A$_S$**a$_s$c$_s$c$_s$a$_s$c$_s$t$_s$g$_s$a$_s$**A$_S$**^{m}**C$_S$A**-3' (SEQ ID NO: 811)

5'- GN2-C6 ca^m**C$_S$G$_S$A$_S$**a$_s$c$_s$c$_s$a$_s$c$_s$t$_s$g$_s$a$_s$**A$_S$**^{m}**C**-3' (SEQ ID NO: 812)

5'-GN2-C6 ca^m**C$_S$G$_S$A$_S$**a$_s$c$_s$c$_s$a$_s$c$_s$t$_s$g$_s$**A$_S$A$_S$**^{m}**C$_S$A**-3' (SEQ ID NO: 824)

5'-GN2-C6 ca**T$_S$A$_S$G$_S$**t$_s$a$_s$a$_s$a$_s$c$_s$t$_s$g$_s$a$_s$g$_s$^{m}**C$_S$**^{m}**C$_S$A$_S$**3' (SEQ ID NO: 822)

In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 ca^m**C$_S$**^{m}**C$_S$G$_S$**c$_s$a$_s$g$_s$t$_s$a$_s$t$_s$g$_s$g$_s$a$_s$**T$_S$**^{m}**C$_S$G**-3' (SEQ ID NO: 802)

5'- GN2-C6 ca^m**C$_S$G$_S$**^{m}**C$_S$**a$_s$g$_s$t$_s$a$_s$t$_s$g$_s$g$_s$a$_s$**T$_S$**^{m}**C**-3' (SEQ ID NO: 813)

In another embodiment the oligomer conjugate is selected from:

5'- GN2-C6 ca**A$_S$G$_S$A$_S$**a$_s$g$_s$g$_s$c$_s$a$_s$c$_s$a$_s$g$_s$a$_s$^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 804)

5'- GN2-C6 ca**G$_S$A$_S$G$_S$**a$_s$a$_s$g$_s$g$_s$c$_s$a$_s$c$_s$a$_s$g$_s$a$_s$^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 805)

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif GCGTAAAGAGAGG (SEQ ID NO: 13), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif AGCGAAGTGCACACG (SEQ ID NO: 20), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif GCGTAAAGAGAGGT (SEQ ID NO: 11), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif AGGTGAAGCGAAGTG (SEQ ID NO: 26), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif AGCGAAGTGCACACGG (SEQ ID NO 18), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif CGAACCACTGAACA (SEQ ID NO: 7), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on the sequence motif CCGCAGTATGGATCG (SEQ ID NO 9), or a sub-sequence of thereof.

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on GCGtaaagagaGG (SEQ ID NO: 303).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on GCGtaaagagaGGT(SEQ ID NO: 301).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on GCGtaaagagAGG (SEQ ID NO: 618).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on AGCgaagtgcacACG (SEQ ID NO: 310).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on AGgtgaagcgaAGTG (SEQ ID NO: 668).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on AGCgaagtgcacaCGG (SEQ ID NO: 308).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on CGAaccactgaACA (SEQ ID NO: 297).

In some embodiments the oligomer or oligomer conjugate according to the invention consists of or comprises or is based on CCGcagtatggaTCG (SEQ ID NO: 299)

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'- GN2-C6 ca**G**$_S$^{m}**C**$_S$**G**$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G**$_S$**G**-3' (SEQ ID NO: 815).

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'- GN2-C6 ca**G**$_S$^{m}**C**$_S$**G**$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G**$_S$**G**$_S$**T**-3' (SEQ ID NO: 814).

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'-GN2-C6 ca**G**$_S$^{m}**C**$_S$**G**$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$**A**$_S$**G**$_S$**G**-3' (SEQ ID NO: 825).

In some embodiments the oligomer conjugate according to the invention consists of or comprises5'- GN2-C6 ca**$A_S$$G_S$**^{m}**$C_S$**$g_S a_S a_S g_S t_S g_S c_S a_S c_S$**$A_S$**^{m}**$C_S$G**-3' (SEQ ID NO: 808).

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'-GN2-C6 ca**$A_S$$G_S$**$g_S t_S g_S a_S a_S g_S$$^{m}c_S g_S a_S$**$A_S$$G_S$$T_S$G**-3' (SEQ ID NO: 826).

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'- GN2-C6 ca**$A_S$$G_S$**^{m}**$C_S$**$g_S a_S a_S g_S t_S g_S c_S a_S c_S a_S$^{m}**$C_S$$G_S$G**-3' (SEQ ID NO: 807).

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'- GN2-C6 ca^{m}**$C_S$$G_S$$A_S$**$a_S c_S c_S a_S c_S t_S g_S a_S$**$A_S$**^{m}**$C_S$A**-3' (SEQ ID NO: 811).

In some embodiments the oligomer conjugate according to the invention consists of or comprises 5'- GN2-C6 ca^{m}**C_S**^{m}**$C_S$$G_S$**$c_S a_S g_S t_S a_S t_S g_S g_S a_S$**$T_S$**^{m}**$C_S$G**-3' (SEQ ID NO: 802).

GAPMER

Preferably, the oligomer or the oligomer component of the oligomer conjugate of the invention is a gapmer (sometimes referred to as a gapmer oligomer). Preferably, the oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, wherein each wing independently comprises one or more LNA units.

Typically, a gapmer oligomer of the present invention or the gapmer oligomer component of the oligomer conjugate of the invention can be represented by any one of the following formulae:

W – X – Y;

V - W – X – Y;

W – X – Y – Z;

V - W – X – Y - Z ;

wherein for each formula:

W represents a region of one or more affinity enhancing nucleotide analogues (region W)

X represents a region comprising a stretch of nucleotides capable of recruiting an RNAse (region X)

Y represents a region of one or more affinity enhancing nucleotide analogues (region Y)

V represents a region of one or more nucleotide units (region V)

Z represents a region of one or more nucleotide units (region Z).

Any one of regions V, W, X, Y or Z may contain additional nucleotides or affinity enhancing nucleotide analogues.

Typically, therefore, a gapmer oligomer is an oligomer which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNAse, such as RNAseH, such as a region of at least 6 or 7 DNA nucleotides that is region X; wherein region X is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogues, such as from 1–6 nucleotide analogues 5' and 3' to the contiguous stretch of nucleotides which is capable of recruiting RNAse – these regions are region W and region Y respectively.

In some embodiments, the units which are capable of recruiting RNAse are selected from the group consisting of DNA units, alpha-L-LNA units, C4' alkylayted DNA units (see PCT/EP2009/050349 and Vester *et al.*, Bioorg. Med. Chem. Lett. 18 (2008) 2296 – 2300, hereby incorporated by reference), and UNA (unlinked nucleic acid) nucleotides (see Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference). UNA is unlocked nucleic acid, typically where the C2 – C3 C-C bond of the ribose has been removed, forming an unlocked "sugar" residue. Preferably the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), W-X-Y, or optionally W-X-Y-Z or V-W-X-Y, wherein: region W (W) (5' region) consists or comprises at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region X (X) consists or comprises at least five consecutive nucleotides which are capable of recruiting RNAse (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), such as DNA nucleotides, and; region Y (Y) (3' region) consists or comprises at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region V (V) and/or region Z (Z), when present consists or comprises 1, 2 or 3 nucleotide units, such as DNA nucleotides.

In some embodiments, region W consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 2, 3 or 4 LNA units.

In some embodiments, region Y consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 2, 3 or 4 nucleotide analogues, such as 3 or 4 LNA units.

In some embodiments, region X consists or comprises 5, 6, 7, 8, 9, 10, 11 or 12 consecutive nucleotides which are capable of recruiting RNAse, or from 6-10, or from 7-9, such as 8 consecutive nucleotides which are capable of recruiting RNAse.

In some embodiments region X consists or comprises at least one DNA nucleotide unit, such as 1-12 DNA units, preferably from 4-12 DNA units, more preferably from 6-10 DNA units, such as from 7-10 DNA units, most preferably 8, 9 or 10 DNA units.

In some embodiments, region W consist of 2, 3 or 4 nucleotide analogues, such as LNA, region X consists of 7, 8, 9 or 10 DNA units, and region Y consists of 2, 3 or 4 nucleotide analogues, such as LNA. Such designs include (W-X-Y) 3-10-3, 3-10-4, 4-10-3, 3-9-3, 3-9-4, 4-9-3, 3-8-3, 3-8-4, 4-8-3, 3-7-3, 3-7-4, 4-7-3, 3-10-2, 3-9-2, 3-8-2, 3-7-2, 4-10-2, 4-9-2, 4-8-2, 4-7-2; and may further include region V, which may have 1, 2 or 3 nucleotide units, such as DNA units and/or region Z, which may have 1, 2 or 3 nucleotide units, such as DNA units.

Further gapmer designs are disclosed in WO2004/046160, which is hereby incorporated by reference. WO2008/113832, which claims priority from US provisional application 60/977,409 hereby incorporated by reference, refers to 'shortmer' gapmer oligomers. In some embodiments, oligomers presented here may be such shortmer gapmers.

In some embodiments the oligomer is consisting of a contiguous nucleotide sequence of a total of 10, 11, 12, 13 or 14 nucleotide units, wherein the contiguous nucleotide sequence is of formula (5' – 3'), W-X-Y, or optionally W-X-Y-Z or V-W-X-Y, wherein; W consists of 1, 2 or 3 nucleotide analogue units, such as LNA units; X consists of 7, 8 or 9 contiguous nucleotide units which are capable of recruiting RNAse when formed in a duplex with a complementary RNA molecule (such as a mRNA target); and Y consists of 1, 2 or 3 nucleotide analogue units, such as LNA units. When present, V consists of 1 or 2 DNA units. When present, Z consists of 1 or 2 DNA units.

In some embodiments W consists of 1 LNA unit. In some embodiments W consists of 2 LNA units. In some embodiments W consists of 3 LNA units.

In some embodiments Y consists of 1 LNA unit. In some embodiments Y consists of 2 LNA units. In some embodiments Y consists of 3 LNA units.

In some embodiments X consists of 7 nucleotide units. In some embodiments X consists of 8 nucleotide units. In some embodiments X consists of 9 nucleotide units. In certain embodiments, region X consists of 10 nucleoside units. In certain embodiments, region X comprises 1 – 10 DNA units. In some embodiments X comprises from 1 – 9 DNA units, such as 2, 3, 4, 5, 6, 7, 8 or 9 DNA units. In some embodiments X consists of DNA units. In some embodiments X comprises at least one LNA unit which is in the alpha-L configuration, such as 2, 3, 4, 5, 6, 7, 8 or 9 LNA units in the alpha-L-configuration. In some embodiments X comprises at least one alpha-L-oxy LNA unit or wherein all the LNA units in the alpha-L-configuration are alpha-L-oxy LNA units.

In some embodiments the number of nucleotides present in W-X-Y are selected from the group consisting of (nucleotide analogue units – region X – nucleotide analogue units): 1-8-1, 1-8-2, 2-8-1, 2-8-2, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 1-8-4, 2-8-4, or;1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-

2, 1-9-3, 3-9-1, 4-9-1, 1-9-4, or; 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1, 3-10-2, 2-10-3, 3-10-3.

In some embodiments the number of nucleotides in W-X-Y are selected from the group consisting of: 2-7-1, 1-7-2, 2-7-2, 3-7-3, 2-7-3, 3-7-2, 3-7-4, and 4-7-3; in some embodiments preferred motifs are 3-10-3, 3-9-3, 3-8-3, 3-8-2.

In certain embodiments, each of regions W and Y consists of 2 or 3 LNA units, and region X consists of 8 or 9 or 10 nucleoside units, preferably DNA units.

In some embodiments both W and Y consist of 2 or 3 LNA units each, and X consists of 8 or 9 nucleotide units, preferably DNA units.

In various embodiments, other gapmer designs include those where regions W and/or Y consists of 3, 4, 5 or 6 nucleoside analogues, such as units containing a 2'-O-methoxyethyl-ribose sugar (2'-MOE) or units containing a 2'-fluoro-deoxyribose sugar, and region X consists of 8, 9, 10, 11 or 12 nucleosides, such as DNA units, where regions W-X-Y have 3-9-3, 3-10-3, 5-10-5 or 4-12-4 units.

Further gapmer designs are disclosed in WO 2007/146511A2, hereby incorporated by reference.

Accordingly, the oligomer or the oligomer component of the oligomer conjugate may comprise at least 1, at least 2 or at least 3 modified nucleotides at the 5' end of the above sequences. For example, one or more preferably all of the modified nucleotides may be LNA units.

The oligomer or the oligomer component of the oligomer conjugate may comprise at least 1, at least 2 or at least 3 modified nucleotides at the 3' end of the above sequences. For example, one or more preferably all of the modified nucleotides may be LNA units.

The oligomer or the oligomer component of the oligomer conjugate may comprise at least 1, at least 2 or at least 3 modified nucleotides at the 5' and/or 3' end of the sequences disclosed herein. For example, one or more preferably all of the modified nucleotides may be LNA units.

For certain embodiments, the oligomer or the oligomer component of the oligomer conjugate may comprise at least 2 or at least 3 modified nucleotides at the 5' and/or 3' end of the sequences disclosed herein. For example, two or more preferably all of the nucleotides may be LNA units.

For certain embodiments, the oligomer or the oligomer component of the oligomer conjugate may comprise at least 3 modified nucleotides at the 5' and/or 3' end of the sequences disclosed herein. For example, three or more preferably all of the modified nucleotides may be LNA units.

For certain embodiments, the oligomer or the oligomer component of the oligomer conjugate may comprise 3 modified nucleotides at the 5' and/or 3' end of the sequences disclosed herein. For example the modified nucleotides may be LNA units.

For certain embodiments, the oligomer or the oligomer component of the oligomer conjugate may comprise 3 modified nucleotides at the 5' and at the 3' end of the sequences disclosed herein. For example one or more preferably all of the modified nucleotides may be LNA units.

For some embodiments, the oligomer may comprise an additional CA dinucleotide motif.

In certain aspects, preferably the oligomer is not GAGGCATAGCAGCAGG (SEQ ID NO: 102).

For certain embodiments of the invention, the oligomer or oligomer component of the oligomer conjugate comprises any one of the motifs: 2-8-2, 3-8-3, 2-8-3, 3-8-2, 2-9-2, 3-9-3, 2-9-3, 3-9-2, 2-10-2, 3-10-3, 3-10-2, 2-10-3 wherein the first number is the number of LNA units in an LNA wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in an LNA wing region.

For certain embodiments of the invention, the oligomer or oligomer component of the oligomer conjugate comprises any one of the motifs 3-8-3, 3-8-2, 3-9-3, 3-9-2, 3-10-3, 3-10-2 wherein the first number is the number of LNA units in an LNA wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in an LNA wing region.

For certain embodiments, the invention provides an oligomer or an oligomer conjugate as herein defined wherein the oligomer or oligomer component of the oligomer conjugate is a gapmer, and wherein the overall sequence comprises at least 6, preferably at least 7, preferably at least 8, preferably at least 9, preferably at least 10 units, preferably at least 11 units, preferably at least 12 units that are at least 80%, at least 85%, at least 90%, at least 95%, at least 98% identical to a region corresponding to part of the HBV HBx gene, such as part of SEQ ID No. 1, or part of the HBsAg gene, such as SEQ ID No. 2, or to the reverse complement of a target region of a nucleic acid which encodes a HBV HBx or HBV HBsAg.

For certain embodiments, the invention provides an oligomer or an oligomer conjugate as herein defined wherein the oligomer or oligomer component of the oligomer conjugate is a gapmer, and wherein the sequence of the central region comprises at least 6, preferably at least 7, preferably at least 8, preferably at least 9, preferably at least 10 units, that are at least 80%, at least 85%, at least 90%, at least 95%, at least 98% identical to a region corresponding to part of the HBV HBx gene, such as part of SEQ ID No. 1, or part of the HBsAg gene, such as SEQ ID No. 2, or to the reverse complement of a target region of a nucleic acid which encodes a HBV HBx or HBV HBsAg.

For certain embodiments of the invention, the oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852),

wherein1, 2, 3 or 4 of the three to four 5' terminal nucleotides are modified nucleotides, for example LNA units; and 1, 2, 3 or 4 of the three to four 3' terminal nucleotides are modified nucleotides, for example LNA units.

For certain embodiments of the invention, the oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
AGCGAAGTGCACACG (SEQ ID NO: 20);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);

CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852),
wherein

2 or 3 of the three 5' terminal nucleotides are modified nucleotides, for example LNA units;

2 or 3 of the three 3' terminal nucleotides are modified nucleotides, for example LNA units.

For certain embodiments of the invention, the oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)

GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852)

,

wherein

the three 5' terminal nucleotides are modified nucleotides, for example LNA units; 2 or 3 of the three 3' terminal nucleotides are modified nucleotides, for example LNA units.

For certain embodiments of the invention, the oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGgtgaagcgaAGTG (SEQ ID NO: 668);
AGCgaagtgcacaCGG (SEQ ID NO: 308);
CGAaccactgaACA (SEQ ID NO: 297);
CGCagtatggaTC (SEQ ID NO: 300);
AGGtgaagcgaagTGC (SEQ ID NO: 315)
AGGtgaagcgaaGTG (SEQ ID NO: 316);
GAAccactgaacAAA (SEQ ID NO: 294);
CGAaccactgaacAAA (SEQ ID NO: 295);
CGAaccactgaaCAA(SEQ ID NO: 296);
CGAaccactgaAC (SEQ ID NO: 298);
CCGcagtatggaTCG (SEQ ID NO: 299);
CGCgtaaagagaGGT (SEQ ID NO: 302);
AGAaggcacagaCGG (SEQ ID NO: 304);
GAGaaggcacagaCGG (SEQ ID NO: 305);
GAAgtgcacacGG (SEQ ID NO: 306);
GCGaagtgcacaCGG (SEQ ID NO: 307);
CGAagtgcacaCG (SEQ ID NO: 309);
GAAccactgaaCAAA (SEQ ID NO: 585);
CGAAccactgaacAAA (SEQ ID NO: 588)

GAAgtgcacaCGG (SEQ ID NO: 628);
TAGtaaactgagCCA (SEQ ID NO: 678);
CGAaccactgAAC (SEQ ID NO: 600);
AGGtgaagcgaAGT (SEQ ID NO: 317); and
CGAaccactgAACA (SEQ ID NO: 597),

wherein upper case letters denote nucleotides which may be modified nucleotides, for example LNA units.

For certain embodiments of the invention, the oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

5'- AM-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G$-3' (SEQ ID NO: 303)

5'- AM-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G_S T$-3' (SEQ ID NO: 301)

5'-AM-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S A_S G_S G$-3' (SEQ ID NO: 618)

5'- AM-C6 ca$A_S G_S$ $^{m}C_S g_S a_S a_S g_S t_S g_S c_S a_S c_S A_S$ $^{m}C_S G$-3' (SEQ ID NO: 310)

5'-AM-C6 ca$A_S G_S g_S t_S g_S a_S a_S a_S g_S$ $^{m}c_S g_S a_S A_S G_S T_S G$-3' (SEQ ID NO: 668)

5'- AM-C6 ca$A_S G_S$ $^{m}C_S g_S a_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 308)

5'- AM-C6 ca$G_S A_S A_S c_S c_S a_S c_S t_S g_S a_S a_S c_S A_S A_S A$-3' (SEQ ID NO: 294)

5'- AM-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S a_S c_S A_S A_S A$-3' (SEQ ID NO: 295)

5'- AM-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S a_S$ $^{m}C_S A_S A$-3' (SEQ ID NO: 296)

5'- AM-C6 ca$^{m}C_S$ $^{m}C_S G_S c_S a_S g_S t_S a_S t_S g_S g_S a_S T_S$ $^{m}C_S G$-3' (SEQ ID NO: 299)

5'- AM-C6 ca$^{m}C_S G_S$ $^{m}C_S g_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G_S T$-3' (SEQ ID NO: 302)

5'- AM-C6 ca$A_S G_S A_S a_S g_S g_S c_S a_S c_S a_S g_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 304)

5'- AM-C6 ca$G_S A_S G_S a_S a_S g_S g_S c_S a_S c_S a_S g_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 305)

5'- AM-C6 caG_S $^{m}C_S G_S a_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 307)

5'- AM-C6 ca$A_S G_S G_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S a_S g_S T_S G_S$ ^{m}C-3' (SEQ ID NO:315)

5'- AM-C6 ca$A_S G_S G_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S a_S G_S T_S G$-3' (SEQ ID NO: 316)

5'- AM-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S A_S$ $^{m}C_S A$-3' (SEQ ID NO: 297)

5'- AM-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S A_S$ ^{m}C-3' (SEQ ID NO: 298)

5'- AM-C6 ca$^{m}C_S G_S$ $^{m}C_S a_S g_S t_S a_S t_S g_S g_S a_S T_S$ ^{m}C-3' (SEQ ID NO: 300)

5'- AM-C6 ca$G_S A_S A_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}c_S G_S G$-3' (SEQ ID NO: 306)

5'-AM-C6 ca$^{m}C_S G_S A_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G$-3' (SEQ ID NO: 309)

5'-AM-C6 ca**A$_S$G$_S$G$_S$**t$_S$g$_S$a$_S$a$_S$g$_S$ ^{m}c$_S$g$_S$a$_S$**A$_S$G$_S$T**-3' (SEQ ID NO: 317)

5'-AM-C6 ca**G$_S$A$_S$A$_S$**c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$ ^{m}**C$_S$A$_S$A$_S$A**-3' (SEQ ID NO: 585)

5'-AM-C6 ca^m**C$_S$G$_S$A$_S$A$_S$**c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$c$_S$**A$_S$A$_S$A**-3' (SEQ ID NO: 588)

5'-AM-C6 ca**G$_S$A$_S$A$_S$**g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$ ^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 628)

5'-AM-C6 ca**T$_S$A$_S$G$_S$**t$_S$a$_S$a$_S$a$_S$c$_S$t$_S$g$_S$a$_S$g$_S$ ^{m}**C$_S$** ^{m}**C$_S$A$_S$**3' (SEQ ID NO: 678)

5'-AM-C6 ca^m**C$_S$G$_S$A$_S$**a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$**A$_S$A$_S$** ^{m}**C**-3' (SEQ ID NO: 600)

5'-AM-C6 ca^{m}C$_S$G$_S$A$_S$a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$A$_S$A$_S$ ^{m}C$_S$A-3' (SEQ ID NO: 597)

wherein

uppercase letters denote beta-D-oxy-LNA units;

lowercase letters denote DNA units;

the subscript “s” denotes a phosphorothioate linkage;

superscript m denotes a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base;

AM-C6 is an amino-C6 linker;wherein the 5’ terminal group “AM-C6 c a” is optional.

AM-C6 is an amino-C6 linker: 6-aminohexanol in the 5’-end of the oligonucleotide linked via a phosphodiester or phorphothioate

Accrodingly, for certain embodiments of the invention, the oligomer or the oligomer component of the oligomer conjugate may be based on a sequence selected from the group consisting of any one or more of:

G^{m}CGtaaagagaGG(SEQ ID NO: 303);

G^{m}CGtaaagagaGGT(SEQ ID NO: 301);

G^{m}CGtaaagagAGG (SEQ ID NO: 618);

AG^{m}CgaagtgcacA^{m}CG (SEQ ID NO: 310)

AGgtgaag^{m}cgaAGTG (SEQ ID NO: 668);

AG^{m}Cgaagtgcaca^{m}CGG (SEQ ID NO: 308);

^{m}CGAaccactgaA^{m}CA (SEQ ID NO: 297);

^{m}CG^{m}CagtatggaT^{m}C (SEQ ID NO: 300);

AGGtgaagcgaagTGC (SEQ ID NO: 315);

AGGtgaag^{m}cgaaGTG (SEQ ID NO: 316);

GAAccactgaacAAA (SEQ ID NO: 294);

^{m}CGAaccactgaacAAA (SEQ ID NO: 295);

^{m}CGAaccactgaa^{m}CAA(SEQ ID NO: 296);

^{m}CGAaccactgaA^{m}C (SEQ ID NO: 298);

^{m}C^{m}CGcagtatggaT^{m}CG (SEQ ID NO: 299);

^{m}CG^{m}CgtaaagagaGGT (SEQ ID NO: 302);
AGAaggcacaga^{m}CGG (SEQ ID NO: 304);
GAGaaggcacaga^{m}CGG (SEQ ID NO: 305);
GAAgtgcaca^{m}cGG (SEQ ID NO: 306);
G^{m}CGaagtgcaca^{m}CGG (SEQ ID NO: 307);
^{m}CGAagtgcaca^{m}CG (SEQ ID NO: 309);
GAAccactgaa^{m}CAAA (SEQ ID NO: 585);
^{m}CGAAccactgaacAAA (SEQ ID NO: 588)
GAAgtgcaca^{m}CGG (SEQ ID NO: 628);
TAGtaaactgag^{m}C^{m}CA (SEQ ID NO: 678);
^{m}CGAaccactgAA^{m}C (SEQ ID NO: 600);
AGGtgaag^{m}cgaAGT (SEQ ID NO: 317); and
^{m}CGAaccactgAA^{m}CA (SEQ ID NO: 597),

wherein

uppercase letters denote beta-D-oxy-LNA units;

lowercase letters denote DNA units;

all internucleoside linkages are phosphorothioate linkages;

superscript m denotes a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base.

In certain preferred aspects, the oligomer or oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of modified nucleotides in the wing region, preferably at least one being an LNA unit, preferably all being an LNA unit, the second number is the number of nucleotides in the gap region, and the third number is the number of modified nucleotides in the wing region, preferably at least one being an LNA unit, preferably all being an LNA unit.

As indicated, the oligomer of the invention may comprise or may be a gapmer. Alternatively expressed, a gapmer oligomer is an oligomer which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNAse, such as RNAseH, such as a region of at least 6 or 7 DNA nucleotides, referred to herein in as region GH (GH), wherein region GH is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogues, such as from 1 – 6 nucleotide analogues 5' and 3' to the contiguous stretch of nucleotides which is capable of recruiting RNAse – these regions are referred to as regions GX' (GX') and GZ' (GZ'). Regions GX, GH and GZ correspond to regions W, X and Y, respectively.

In some embodiments, the units which are capable of recruiting RNAse are selected from the group consisting of DNA units, alpha-L-LNA units, C4' alkylayted DNA units (see PCT/EP2009/050349 and Vester *et al.*, Bioorg. Med. Chem. Lett. 18 (2008) 2296 – 2300,

hereby incorporated by reference), and UNA (unlinked nucleic acid) nucleotides (see Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference). UNA is unlocked nucleic acid, typically where the C2 – C3 C-C bond of the ribose has been removed, forming an unlocked "sugar" residue. Preferably the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), GX'-GH-GZ', wherein; region GX' (GX') (5' region) consists or comprises of at least one nucleotide analogue, such as at least one BNA (e.g. LNA) unit, such as from 1-6 nucleotide analogues, such as BNA (e.g. LNA) units, and; region GH (H) consists or comprises of at least five consecutive nucleotides which are capable of recruiting RNAse (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), such as DNA nucleotides, and; region GZ' (GZ') (3'region) consists or comprises of at least one nucleotide analogue, such as at least one BNA (e.g LNA unit), such as from 1-6 nucleotide analogues, such as BNA (e.g. LNA) units.

In some embodiments, region GX' consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as BNA (e.g. LNA) units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units; and/or region GZ' consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as BNA (e.g. LNA) units, such as from 2-5 nucleotide analogues, such as 2-5 BNA (e.g. LNA units), such as 3 or 4 nucleotide analogues, such as 3 or 4 BNA (e.g. LNA) units.

In some embodiments GH consists or comprises of 5, 6, 7, 8, 9, 10, 11 or 12 consecutive nucleotides which are capable of recruiting RNAse, or from 6-10, or from 7-9, such as 8 consecutive nucleotides which are capable of recruiting RNAse. In some embodiments region GH consists or comprises at least one DNA nucleotide unit, such as 1-12 DNA units, preferably from 4-12 DNA units, more preferably from 6-10 DNA units, such as from 7-10 DNA units, most preferably 8, 9 or 10 DNA units.

In some embodiments region GX' consist of 3 or 4 nucleotide analogues, such as BNA (e.g. LNA), region X' consists of 7, 8, 9 or 10 DNA units, and region Z' consists of 3 or 4 nucleotide analogues, such as BNA (e.g. LNA). Such designs include (GX'-GH-GZ') 3-10-3, 3-10-4, 4-10-3, 3-9-3, 3-9-4, 4-9-3, 3-8-3, 3-8-4, 4-8-3, 3-7-3, 3-7-4, 4-7-3.

In some embodiments the oligomer, e.g. region GX', is consisting of a contiguous nucleotide sequence of a total of 10, 11, 12, 13 or 14 nucleotide units, wherein the contiguous nucleotide sequence comprises or is of formula (5' – 3'), GX'-GH-GZ' wherein; GX' consists of 1, 2 or 3 nucleotide analogue units, such as BNA (e.g. LNA) units; GH consists of 7, 8 or 9 contiguous nucleotide units which are capable of recruiting RNAse when formed in a duplex with a complementary RNA molecule (such as a mRNA target); and GZ' consists of 1, 2 or 3 nucleotide analogue units, such as BNA (e.g. LNA) units.

In some embodiments GX' consists of 1 BNA (e.g. LNA) unit. In some embodiments GX' consists of 2 BNA (e.g. LNA) units. In some embodiments GX' consists of 3 BNA (e.g. LNA) units. In some embodiments GZ' consists of 1 BNA (e.g. LNA) units. In some embodiments GZ' consists of 2 BNA (e.g. LNA) units. In some embodiments GZ' consists of 3 BNA (e.g. LNA) units. In some embodiments GH consists of 7 nucleotide units. In some embodiments GH consists of 8 nucleotide units. In some embodiments GH consists of 9 nucleotide units. . In certain embodiments, region GH consists of 10 nucleoside units. In certain embodiments, region GH consists or comprises 1 – 10 DNA units. In some embodiments GH comprises of from 1 – 9 DNA units, such as 2, 3, 4, 5, 6, 7 , 8 or 9 DNA units. In some embodiments GH consists of DNA units. In some embodiments GH comprises of at least one BNA unit which is in the alpha-L configuration, such as 2, 3, 4, 5, 6, 7, 8 or 9 LNA units in the alpha-L-configuration. In some embodiments GH comprises of at least one alpha-L-oxy BNA/LNA unit or wherein all the LNA units in the alpha-L- configuration are alpha-L-oxy LNA units. In some embodiments the number of nucleotides present in GX'-GH-GZ' are selected from the group consisting of (nucleotide analogue units – region GH – nucleotide analogue units): 1-8-1, 1-8-2, 2-8-1, 2-8-2, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 1-8-4, 2-8-4, or;1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-2, 1-9-3, 3-9-1, 4-9-1, 1-9-4, or; 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1. In some embodiments the number of nucleotides in GX'-GH-GZ' are selected from the group consisting of: 2-7-1, 1-7-2, 2-7-2, 3-7-3, 2-7-3, 3-7-2, 3-7-4, and 4-7-3. In certain embodiments, each of regions GX' and GH consists of three BNA (e.g. LNA) units, and region GH consists of 8 or 9 or 10 nucleoside units, preferably DNA units. In some embodiments both GX' and GZ' consists of two BNA (e.g. LNA) units each, and GH consists of 8 or 9 nucleotide units, preferably DNA units. In various embodiments, other gapmer designs include those where regions GX' and/or GZ' consists of 3, 4, 5 or 6 nucleoside analogues, such as units containing a 2'-O-methoxyethyl-ribose sugar (2'-MOE) or units containing a 2'-fluoro-deoxyribose sugar, and region H consists of 8, 9, 10, 11 or 12 nucleosides, such as DNA units, where regions GX'-GH-GZ' have 3-9-3, 3-10-3, 5-10-5 or 4-12-4 units.

BNA and LNA Gapmers: The terms BNA and LNA are used interchangeably. A BNA gapmer is a gapmer oligomer (region GA) which comprises at least one BNA nucleotide. A LNA gapmer is a gapmer oligomer (region GA) which comprises at least one LNA nucleotide.

INTERNUCLEOTIDE LINKAGES

The units of the oligomers and oligomer conjugates described herein are coupled together via linkage groups. Suitably, each unit is linked to the 3' adjacent unit via a linkage group.

The person having ordinary skill in the art would understand that, in the context of the present invention, the 5' unit at the end of an oligomer does not comprise a 5' linkage group, although it may or may not comprise a 5' terminal group.

The nucleotides of the oligomer of the invention or contiguous nucleotides sequence thereof are coupled together via linkage groups. Suitably each nucleotide is linked to the 3' adjacent nucleotide via a linkage group.

Optionally, the oligonucleotide of the invention or the oligonucleotide conjugate of the invention may comprise one or more linker groups and/or one or more brancher regions. In various embodiments, the linker groups are internucleoside or internucleotide linkages.

Suitable internucleotide linkages include those listed within WO2007/031091, for example the internucleotide linkages listed on the first paragraph of page 34 of WO2007/031091 (hereby incorporated by reference).

For some embodiments, it is preferred to modify the internucleotide linkage from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate or boranophosphate – these two, being cleavable by RNase H, also allow that route of antisense inhibition in reducing the expression of the target gene.

Suitable sulphur (S) containing internucleotide linkages as provided herein may be preferred. Phosphorothioate internucleotide linkages are also preferred, particularly for the gap region (X) of gapmers. Phosphorothioate linkages may also be used for the flanking regions (W and Y, and for linking W or Y to V or Z, and within region V or region Z, as appropriate).

Regions W, X and Y, may however comprise internucleotide linkages other than phosphorothioate, such as phosphodiester linkages, particularly, for instance when the use of nucleotide analogues protects the internucleotide linkages within regions W and Y from endo-nuclease degradation – such as when regions W and Y comprise LNA nucleotides.

The internucleotide linkages in the oligomer may be phosphodiester, phosphorothioate or boranophosphate so as to allow RNase H cleavage of targeted RNA. Phosphorothioate is preferred for improved nuclease resistance and other reasons, such as ease of manufacture.

In one aspect of the oligomer of the invention, the nucleotides and/or nucleotide analogues are linked to each other by means of phosphorothioate groups.

It is recognised that the inclusion of phosphodiester linkages, such as one or two linkages, into an otherwise phosphorothioate oligomer, particularly between or adjacent to nucleotide analogue units (typically in region W and or Y) can modify the bioavailability and/or bio-distribution of an oligomer – see WO2008/113832, hereby incorporated by reference.

In some embodiments, such as the embodiments referred to above, where suitable and not specifically indicated, all remaining linkage groups are either phosphodiester or phosphorothioate, or a mixture thereof.

In some embodiments all the internucleotide linkage groups are phosphorothioate.

When referring to specific gapmer oligonucleotide sequences, such as any of those specific sequences provided herein it will be understood that, in various embodiments, when the linkages are phosphorothioate linkages, alternative linkages, such as those disclosed herein may be used, for example phosphate (phosphodiester) linkages may be used, particularly for linkages between nucleotide analogues, such as LNA units. Likewise, when referring to specific gapmer oligonucleotide sequences, such as any of those specific sequences provided herein, when the C residues are annotated as 5'methyl modified cytosine, in various embodiments, one or more of the Cs present in the oligomer may be unmodified C residues.

WO09124238 refers to oligomeric compounds having at least one bicyclic nucleoside attached to the 3' or 5' termini by a neutral internucleoside linkage. The oligomers of the invention may therefore have at least one bicyclic nucleoside attached to the 3' or 5' termini by a neutral internucleoside linkage, such as one or more phosphotriester, methylphosphonate, MMI, amide-3, formacetal or thioformacetal. The remaining linkages may be phosphorothioate.

CARRIER

In some embodiments, the oligomer of the present invention is linked to one or more carrier components, which may be the same or different.

In some embodiments, the oligomer conjugate has or comprises the structure:

Carrier component – L - First Oligomer Region

wherein L is an optional linker or brancher region or tether molecule or bridging moiety.

Preferably L is selected from a physiologically labile linker (region PL) or an alternative linker (Region E), or a combination of both.

The first oligomer region can be linked to the linker or carrier via the 5'-end illustrated as follows:

Carrier component – L1 - First Oligomer Region

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety; or

Alternatively the first oligomer region can be linked to the linker or carrier via the 3'-end illustrated as follows:First Oligomer Region – L2 - Carrier component

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety.

For certain embodiments, preferably the oligomer conjugate has or comprises the structure:

Carrier component – L1 - First Oligomer Region

wherein L1 is an optional linker.

For certain embodiments, preferably Linker 1 is present.

For certain embodiments, preferably said carrier component is linked, preferably conjugated, to said first oligomer region.

For certain embodiments, preferably said carrier component is linked, preferably conjugated, to the 5' end of said oligomer.

For certain embodiments, preferably said carrier component is linked, preferably conjugated, to the 5' end of said oligomer by means of a linker group or a brancher region or tether molecule or bridging moiety.

For certain embodiments, preferably the linker group or the brancher region is a physiologically labile linker group or a physiologically labile brancher region or physiologically labile tether molecule or physiologically labile bridging moiety.

For certain embodiments, preferably the physiologically labile linker group is a nuclease susceptible linker, preferably a phosphodiester linker.

For certain empodiments the preferred L1 linker is composed of a physiologically labile linker and a C2 – C36 amino alkyl group, including, for example C6 to C12 amino alkyl groups. In a preferred embodiment the L1 linker is composed a PO linker and a C6 amino linker.

In some embodiments, the carrier component is selected from a carbohydrate conjugate or a lipophilic conjugate, or the carrier component comprises both a carbohydrate and a lipophilic conjugate.

The carbohydrate conjugate moiety may for example be selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, Nacetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine, and N-isobutanoylgalactose-amine. Preferably the carbohydrate conjugate moiety is an asialoglycoprotein receptor targeting conjugate moiety. The lipophilic conjugate may be a hydrophobic group, such as a C16-20 hydrophobic group, a sterol, cholesterol. Other carbohydrate and lipophilic groups which may be used are, for example, disclosed herein.

For some embodiments, the oligomer may comprise an additional CA dinucleotide motif. Preferably, in the context of the oligomer conjugate, the CA motif is positioned between the carrier component and the oligomer. The CA motif preferably comprise a phosphidiester linkage and serves as a PO linker between the oligomer and the carrier component.

HEPATITIS B VIRUS (HBV)

It is intended that all oligomers and oligomer conjugates as described herein are capable of being used to treat a viral disorder, in particular a disorder associated with HBV. Such uses form part of the present invention.

It is intended that all oligomers and oligomer conjugates as described herein are capable of being used in the manufacture of a medicament to treat a viral disorder, in particular a disorder associated with HBV. Such uses form part of the present invention.

It is intended that all oligomers and oligomer conjugates as described herein are capable of being administered to a subject as part of a method for alleviating, curing or treating a viral disorder, in particular a disorder associated with HBV. Such methods form part of the present invention.

It is intended that all oligomers and oligomer conjugates as described herein are capable of comprising part of a pharmaceutical composition to treat a viral disorder, in particular a disorder associated with HBV. Such pharmaceutical compositions form part of the present invention.

Hepatitis B virus (HBV) is a species of the genus *Orthohepadnavirus*, which is a part of the *Hepadnaviridae* family. The virus causes the disease hepatitis B. In addition to causing hepatitis, infection with HBV can lead to cirrhosis and hepatocellular carcinoma. It has also been suggested that it may increase the risk of pancreatic cancer.

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins, and into eight genotypes (A–H) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination. The genotypes differ by at least 8%. Type F which diverges from the other genomes by 14% is the most divergent type known. Type A is prevalent in Europe, Africa and South-east Asia, including the Philippines. Type B and C are predominant in Asia; type D is common in the Mediterranean area, the Middle East and India; type E is localized in sub-Saharan Africa; type F (or H) is restricted to Central and South America. Type G has been found in France and Germany. Genotypes A, D and F are predominant in Brazil and all genotypes occur in the United States with frequencies dependent on ethnicity.

HBV has a circular DNA genome, however, the DNA is not fully double-stranded as one end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full length strand) and 1700–2800 nucleotides long (for the short length strand).

There are four known genes encoded by the HBV genome (C, P, S, and X). The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P.

HBx

The HBx polypeptide is a 154 residue protein which interferes with transcription, signal transduction, cell cycle progress, protein degradation, apoptosis and chromosomal stability in the host. It forms a heterodimeric complex with its cellular target protein (HBX interacting protein: HBXIP), and this interaction dysregulates centrosome dynamics and mitotic spindle formation. It interacts with DDB1 (Damaged DNA Binding Protein 1) redirecting the ubiquitin ligase activity of the CUL4-DDB1 E3 complexes, which are intimately involved in the intracellular regulation of DNA replication and repair, transcription and signal transduction.

Although it lacks significant sequence identity with any known vertebrate proteins, it is likely to have evolved from a DNA glycosylase. Transgenic mice expressing the X protein in liver are more likely than the wild type to develop hepatocellular carcinoma. This is because the X protein promotes cell cycle progression while binding to and inhibiting tumour suppressor protein p53 from performing their role. Experimental observations also suggest that HBx protein increases TERT and telomerase activity, prolonging the lifespan of hepatocytes and contributing to malignant transformation.

In a study purifying cancerous liver cells infected with HBV, the level of expression of protein arginine methyltransferase 1 (PRMT1) was found to be associated with changes in transcription due to the methyltransferase function of PRMT1. Overexpression causes a reduction in the number of HBV genes transcribed, while conversely, reduced expression causes an increase. PRMT1 was also found to be recruited by HBV DNA during the replication process to regulate the transcription process. Increased HBx expression in turn leads to an inhibition of PRMT1-mediated protein methylation, benefiting viral replication.

The HBx target sequence comprises sequences starting at the first reported transcription start site at position 1196 to the polyadenylation site at position 1941. The sequence from position 1196 to 1941 of the U95551 sequence is presented as SEQ ID No. 1. The U95551 sequence is presented as SEQ ID No. 3.

HBsAg

HBsAg (also known as Major surface antigen, HBV major surface antigen, HBV surface antigen and ‘S’) is the surface antigen of the HBV.

Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced.

The HBsAg is made up of three glycoproteins that are encoded by the same gene. The proteins are translated in the same reading frame but start at a different AUG start codon; thus, all have the same C-terminus. The largest protein is the L protein (42kd) and contained within this is the M glycoprotein. The S glycoprotein (27kD) is contained within the M protein. The HBsAg protein is also secreted into the patient's serum where it can be seen as spherical (mostly self-associated S protein) or filamentous particles (also mostly S protein but with some L and M). The former are smaller than the true virus but the filaments can be quite large (several hundred nanometers).

S-HBsAg is 226 amino acid residues in length. It is an integral membrane glycoprotein which is anchored in the ER lipid bilayer through an amino-terminal transmembrane domain (TMD-I) between residues 4 and 24. It comprises a downstream cytosolic loop (CYL-I) between residues 24 and 80, a second transmembrane domain (TMD-II) between residues 80 and 100, and an antigenic loop (AGL) encompassing residues 101 to 164, facing the ER lumen (or the surface of extracellular particles). The carboxyl terminus (residues 165 to 226) is predicted to contain two TMDs (TMD-III and -IV), located at positions 173 to 193 and 202 to 222, respectively, separated by a short sequence (residues 194 to 201) referred to here as cytosolic loop II (CYL-II) because it is predicted to reside at the cytosolic side of the ER membrane. The M-HBsAg protein sequence is longer than that of S-HBsAg by 55 residues (the pre-S2 domain) at its amino terminus; it is coassembled with the latter in the viral envelope but is dispensable for both morphogenesis and in vitro infectivity. L-HBsAg comprises the entire M polypeptide with an additional amino-terminal extension (pre-S1) of 108 to 119 residues depending on the HBV genotype. It has been described with two topologies, with the amino-terminal pre-S domain (pre-S1 plus pre-S2) being either cytosolic at the ER membrane (internal on secreted virions) or luminal (exposed at the virion surface). The internal conformation is involved in recruiting the nucleocapsid for virion assembly, whereas the external position corresponds to a receptor-binding function at viral entry.

All three envelope proteins are synthesized at the endoplasmic reticulum (ER) membrane, where they aggregate through protein-protein interactions leading primarily to the secretion of empty S-HBsAg-coated subviral particles (SVPs) It is only when L-HBsAg is present in the envelope protein aggregates at the ER membrane that the HBV nucleocapsid can be recruited in the budding complex and released as a mature virion. Owing to the overwhelming activity of S-HBsAg for self-assembly, in comparison to that of L-HBsAg, HBV virion formation occurs only on rare occasions.

The HBsAg target sequence comprises sequences starting of the circularized U95551 sequence from the transcription start site at position 3158 to 3182 and from position 1 to the polyadenylation site at position 1941. Thus, the HBsAg target sequence comprises sequences

of the combined sequences from position 3158 to 3182 and position 1 to 1944 of the circularized U95551 sequence. This target sequence is presented as SEQ ID no. 2. The U95551 sequence is presented as SEQ ID No. 3.

TARGET

In a preferred aspect, suitably the oligomer or the oligomer conjugate of the invention is capable of modulating a target sequence in HBx or HBsAg of Hepatitis B Virus (HBV). In this regard, the oligomer of the invention can affect the inhibition of a target sequence, typically in a mammalian cell such as a human cell, such as a liver cell.

In some embodiments, the oligomer or the oligomer conjugate of the invention binds to the target nucleic acid and modulates expression by at least 10% or 20% compared to the normal expression level, more preferably at least a 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% compared to the normal expression level (such as the expression level in the absence of the oligomer(s) or oligomer conjugate(s)).

In one embodiment, the oligomer or the oligomer conjugate of the invention is capable of down-regulating (e.g. inhibiting, reducing or removing) expression of the HBx or HBsAg gene. In this regard, the oligomer of the invention can affect the inhibition of HBx or HBsAg. Such inhibition may typically occur in a mammalian cell such as a human cell, such as a liver cell. In some embodiments, the oligomers of the invention bind to the target nucleic acid and affect inhibition of expression of at least 10% or 20% compared to the normal expression level, more preferably at least a 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% inhibition compared to the normal expression level (such as the expression level in the absence of the oligomer(s) or oligomer conjugate(s)).

In some embodiments, such modulation is seen when using from 0.04 and 25nM, such as from 0.8 and 20nM concentration of the compound of the invention. In the same or a different embodiment, the modulation of expression is less than 100%, such as less than 98%, less than 95%, less than 90%, less than 80%, such as less than 70%. Modulation of expression level may be determined by measuring protein levels, *e.g.* by the methods such as SDS-PAGE followed by western blotting using suitable antibodies raised against the target protein. Alternatively, modulation of expression levels can be determined by measuring levels of mRNA, *e.g.* by northern blotting or quantitative RT-PCR. When measuring via mRNA levels, the level of down-regulation when using an appropriate dosage, such as from 0.04 and 25nM, such as from 0.8 and 20nM concentration, is, in some embodiments, typically to a level of from 10-20% the normal levels in the absence of the compound, conjugate or composition of the invention.

As illustrated herein the cells may be *in vitro* transfected cells. The concentration of the oligomer or the oligomer conjugate used may, in some embodiments, be 5nM. The oligomer

concentration used may, in some embodiments be 25nM. The concentration of the oligomer or the oligomer conjugate used may, in some embodiments be 1nM. It should be noted that the concentration of oligomer used to treat the cell is typically performed in an *in vitro* cell assay, using transfection (Lipofecton), as illustrated in the examples. In the absence of a transfection agent, the concentration of the oligomer or the oligomer conjugate required to obtain the down-regulation of the target is typically between 1 and 25μM, such as 5μM.

The invention therefore provides a method of down-regulating or inhibiting the expression of HBx or HBsAg protein and/or mRNA in a cell which is expressing HBx or HBsAg protein and/or mRNA, said method comprising administering the oligomer or conjugate according to the invention to said cell to down-regulate or inhibit the expression of HBx or HBsAg protein and/or mRNA in said cell. Suitably the cell is a mammalian cell such as a human cell. The administration may occur, in some embodiments, *in vitro*. The administration may occur, in some embodiments, *in vivo*.

For certain embodiments, the oligomer or the oligomer component of the oligomer conjugate that targets HBx also binds to HBsAg.

TARGET SEQUENCE

The oligomers or oligomer conjugates comprise or consist of a contiguous nucleotide sequence which corresponds to the reverse complement of a nucleotide sequence present in the target sequence.

The target sequence may be a gene or a mRNA, such as a coding or a non-coding region of a gene or mRNA. For example, the target sequence may be a coding or non-coding exon. The target sequence may comprise at least part of an exon. The target sequence may be part of an exon, such as within an exon.

In the practice of the present invention, the target sequence may be single-stranded or double-stranded DNA or RNA; however, single-stranded DNA or RNA targets are preferred. It is understood that the target sequence to which the antisense oligonucleotides of the invention are directed include allelic forms of the targeted gene and the corresponding mRNAs including splice variants. There is substantial guidance in the literature for selecting particular sequences for antisense oligonucleotides given a knowledge of the sequence of the target polynucleotide, e.g., Cook S.T. *Antisense Drug Technology, Principles, Strategies, and Applications,* Marcel Dekker, Inc, 2001; Peyman and Ulmann, *Chemical Reviews*, 90:543-584, 1990; and Crooke, *Ann. Rev. Pharmacol. Toxicol.*, 32:329-376 (1992). mRNA targets may include the 5' cap site, tRNA primer binding site, the initiation codon site, the mRNA donor splice site, and the mRNA acceptor splice site.

Where the target polynucleotide sequence comprises a mRNA transcript, sequence complementary oligonucleotides can hybridize to any desired portion of the transcript. Such oligonucleotides are, in principle, effective for inhibiting translation, and capable of inducing the effects described herein. It is hypothesized that translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Thus, oligonucleotides may be complementary to the 5'-region of mRNA transcript. Oligonucleotides may be complementary to the mRNA, including the initiation codon (the first codon at the 5' end of the translated portion of the transcript), or codons adjacent to the initiation codon.

In one embodiment, the target sequence may have identity between HBV genotypes A-H (described in detail below).

In one embodiment, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with any one or more of the HBV genotypes A-H. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype A. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype B. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype C. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype D. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype E. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype F. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype G. For example, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with HBV genotype H.

In one embodiment, the target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% identity between two or more of the HBV genotypes A-H.

In one embodiment, the target sequence may have identity between two or more of HBV genotypes A, B, C and D. The target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% between HBV genotypes A and B.

In one embodiment, the target sequence may have identity between two or more of HBV genotypes A, B, C and D. The target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% between HBV genotypes A and C.

In one embodiment, the target sequence may have identity between three or more of HBV genotypes A, B, C and D. The target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% identity between HBV genotypes A, B, C and D.

In one embodiment, the target sequence may have identity between three or more of HBV genotypes A, B, C and D. The target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% identity between HBV genotypes A, B and C.

In one embodiment, the target sequence may have identity between three or more of HBV genotypes A, B, C and D. The target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% identity between HBV genotypes A, B, and D.

In one embodiment, the target sequence may have identity between all of HBV genotypes A, B, C and D. The target sequence may have at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity between HBV genotypes A, B, C and D.

In various embodiments, the target sequence is within the sequence shown as SEQ ID No. 3.

In one embodiment, the target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof.

In various embodiments, the target sequence is HBx or HBsAg or a naturally-occurring variant thereof.

In various embodiments, the target sequence is within the sequence shown as SEQ ID No. 1.

In various embodiments, the target sequence is within the sequence shown as SEQ ID No. 2.

In various embodiments, the target sequence is selected from one or more of the following positions in SEQ ID NO 3: position1 to 1944, position 157 to 1840, position 1196 to 1941, position 1376 to 1840 and position 3158-3182. Preferably, the target sequence is selected from position1530 to 1598 of SEQ ID NO: 3, more preferrable from position 1577 to 1598 of SEQ ID NO: 3 and most preferably from position 1530 to 1543 of SEQ ID NO: 3.

In various embodiments, the target sequence may be selected from the group consisting of any one or more of positions:

1264-1278;
1265-1277;
1530-1543;
1530-1544;
1531-1543;
1551-1565;
1551-1566;

1577-1589;
1577-1591;
1577-1592;
1577 to 1598;
1578-1590;
1578-1592;
1583-1598;
1584-1598;
1585-1598;
670-706
670-684
691-705;
691-706;
692-706;
693-706;
694-706;
of SEQ ID No. 3.

In an aspect, the oligomer or oligomer conjugate of the invention is capable of targeting from 8-30, 8-20, 8-18, 8-16, 8-14, 8-12 or 8-10 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID No. 3; preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect, the oligomer or oligomer conjugate of the invention is capable of targeting at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID No. 3; preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 8 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3; preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 9 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to

1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 10 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3; preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 11 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 12 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 13 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 14 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 15 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In an aspect the oligomer or oligomer conjugate of the invention is capable of targeting at least 16 contiguous nucleotides within the sequence shown as position 1200 to 1900, preferably

position 1530 to 1598, more preferrable position 1577 to 1598, most preferably position 1530 to 1543 of SEQ ID NO: 3 of SEQ ID NO: 3 preferably wherein said oligomer or oligomer conjugate is complementary to said contiguous nucleotides.

In one embodiment, the target sequence comprises a sequence within the sequence set forth in SEQ ID NO: 1 or SEQ ID No 2 or SEQ ID No 3 or a sequence having at least 80% identity thereto. Thus, the oligomer or oligomer conjugate can comprise or consist of a core motif selected from the group presented herein, wherein said oligomer or oligomer conjugate (or contiguous nucleotide portion thereof) may optionally have one, two, or three mismatches against said selected motif sequence:

GCGTAAAGAGAGG (SEQ ID NO: 13);
AGCGAAGTGCACACG (SEQ ID NO: 20);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852)

In one embodiment, the target sequence comprises the sequence set forth below or a sequence having at least 80% identity to any thereto. Thus, the oligomer or oligomer conjugate can comprise or consist of a sequence hybridizing to a target sequence selected from the group presented below, wherein said oligomer or oligomer conjugate (or contiguous nucleotide portion thereof) may optionally have one, two, or three mismatches against said selected target sequence.

Target Sequence

acggggcgcacctctctttacgcg (SEQ ID NO: 827)
cgtgtgcacttcgcttcacctc (SEQ ID NO: 828)
ccgtctgtgccttctc (SEQ ID NO: 829)
cgatccatactgcgg (SEQ ID NO: 830)
tggctcagtttacta (SEQ ID NO: 831)
ctagtgccatttgtt(SEQ ID NO: 833)

In some embodiments, the oligomer or oligomer conjugate may tolerate 1, 2, 3, or 4 (or more) mismatches, when hybridising to the target sequence and still sufficiently bind to the target to show the desired effect, *i.e.* down-regulation of the target. Mismatches may, for example, be compensated by increased length of the oligomer nucleotide sequence and/or an increased number of nucleotide analogues, such as LNA, present within the nucleotide sequence.

In some embodiments, the contiguous nucleotide sequence comprises no more than 3, such as no more than 2 mismatches when hybridizing to the target sequence, such as to the corresponding region of a nucleic acid which encodes HBx or HBsAg.

In some embodiments, the contiguous nucleotide sequence comprises no more than a single mismatch when hybridizing to the target sequence, such as the corresponding region of a nucleic acid which encodes HBx or HBsAg.

If the target is HBx, the nucleotide sequence of the oligomer or oligomer conjugate of the invention or the contiguous nucleotide sequence preferably has at least 80% identity (sometimes referred to as homology or homologous) to a corresponding sequence selected from the nucleotide sequences presented herein, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous, at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

If the target is HBx, the nucleotide sequence of the oligomer or oligomer conjugate of the invention or the contiguous nucleotide sequence preferably has at least 80% identity (sometimes referred to as homology or homologous) to the reverse complement of a corresponding sequence within the sequence presented as SEQ ID No. 1, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous, at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

If the target is HBx, the nucleotide sequence of the oligomer or oligomer conjugate of the invention or the contiguous nucleotide sequence is preferably at least 80% complementary to a sub-sequence present within the sequence presented as SEQ ID No. 1,, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%

complementary, at least 97% complementary, at least 98% complementary, at least 99% complementary, such as 100% complementary (perfectly complementary).

In some embodiments if the target is HBx, the oligomer or oligomer conjugate (or contiguous nucleotide portion thereof) is selected from, or comprises, one of the sequences presented herein, or a sub-sequence of at least 10 contiguous nucleotides thereof, wherein said oligomer or oligomer conjugate (or contiguous nucleotide portion thereof) may optionally comprise one, two, or three mismatches when compared to the sequence.

If the target is HBsAg, the nucleotide sequence of the oligomer or oligomer conjugate of the invention or the contiguous nucleotide sequence preferably has at least 80% identity (sometimes referred to as homology or homologous) to a corresponding sequence selected from the group presented herein, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous, at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

If the target is HBsAg, the nucleotide sequence of the oligomer or oligomer conjugate of the invention or the contiguous nucleotide sequence preferably has at least 80% identity (sometimes referred to as homology or homologous) to the reverse complement of a corresponding sequence present within the sequence presented as SEQ ID No. 2, such as at least 85%, at least 90%, at least 91%, at least 92%at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous, at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

If the target is HBsAg, the nucleotide sequence of the oligomer or oligomer conjugate of the invention or the contiguous nucleotide sequence is preferably at least 80% complementary to a sub-sequence present within the sequence presented as SEQ ID No. 2, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% complementary, at least 97% complementary, at least 98% complementary, at least 99% complementary, such as 100% complementary (perfectly complementary).

In some embodiments if the target is HBsAg, the oligomer or oligomer conjugate (or contiguous nucleotide portion thereof) is selected from, or comprises, one of the sequences selected from the group presented herein, or a sub-sequence of at least 10 contiguous nucleotides thereof, wherein said oligomer or oligomer conjugate (or contiguous nucleotide portion thereof) may optionally comprise one, two, or three mismatches when compared to the sequence.

In some embodiments the sub-sequence may consist of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 contiguous nucleotides, such as from 12 -22, such as from

12-18, such as from 12 -16 nucleotides. Suitably, in some embodiments, the sub-sequence is of the same length as the contiguous nucleotide sequence of the oligomer of the invention.

However, it is recognised that, in some embodiments the nucleotide sequence of the oligomer or oligomer conjugate may comprise additional 5' or 3' nucleotides, such as, independently, 1, 2, 3, 4 or 5 additional nucleotides 5' and/or 3', which are non-complementary to the target sequence. In this respect the oligomer of the invention, may, in some embodiments, comprise a contiguous nucleotide sequence which is flanked 5' and or 3' by additional nucleotides. In some embodiments the additional 5' or 3' nucleotides are naturally occurring nucleotides, such as DNA or RNA. In some embodiments, the additional 5' or 3' nucleotides may represent region D as referred to in the context of gapmer oligomer or oligomer conjugate herein.

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RNAse RECRUITMENT

It is recognised that an oligomeric molecule may function via non RNase mediated degradation of target mRNA, such as by steric hindrance of translation, or other methods.

For some embodiments, the oligomer or oligomer conjugate of the invention is capable of recruiting an endoribonuclease (RNase), such as RNase H.

It is preferable that the oligomer or oligomer conjugate of the invention comprises a region of at least 6, such as at least 7 consecutive nucleotide units, such as at least 8 or at least 9 consecutive nucleotide units (residues), including 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 consecutive nucleotides, which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase. The contiguous sequence which is capable of recruiting RNAse may be region X as referred to in the context of a gapmer as described herein. In some embodiments the size of the contiguous sequence which is capable of recruiting RNAse, such as region X, may be higher, such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotide units.

EP 1 222 309 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. An oligomer is deemed capable of recruiting RNase H if, when provided with the complementary RNA target, it has an initial rate, as measured in pmol/l/min, of at least 1 %, such as at least 5%, such as at least 10% or ,more than 20% of the of the initial rate determined using DNA only oligonucleotide, having the same base sequence but containing only DNA units, with no 2' substitutions, with phosphorothioate linkage groups between all units in the oligonucleotide,, using the methodology provided by Examples 91 - 95 of EP 1 222 309.

In some embodiments, an oligomer is deemed essentially incapable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as

measured in pmol/l/min, is less than 1%, such as less than 5%,such as less than 10% or less than 20% of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Examples 91 - 95 of EP 1 222 309.

In other embodiments, an oligomer is deemed capable of recruiting RNaseH if, when provided with the complementary RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is at least 20%, such as at least 40 %, such as at least 60 %, such as at least 80 % of the initial rate determined using the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Examples 91 - 95 of EP 1 222 309.

Typically the region of the oligomer or oligomer conjugate of the invention which forms the consecutive nucleotide units which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase consists of nucleotide units which form a DNA/RNA like duplex with the RNA target – and include both DNA units and LNA units which are in the alpha-L configuration, particularly preferred being alpha-L-oxy LNA.

The oligomer or oligomer conjugate of the invention may comprise a nucleotide sequence which comprises both nucleotides and nucleotide analogues, and may be in the form of a gapmer, a headmer or a mixmer.

In some embodiments, in addition to enhancing affinity of the oligomer for the target region, some nucleoside analogues also mediate RNase (*e.g.,* RNaseH) binding and cleavage. Since α-L-LNA units recruit RNaseH activity to a certain extent, in some embodiments, gap regions (*e.g.,* region X as referred to herein) of oligomers containing α-L-LNA units consist of fewer units recognizable and cleavable by the RNaseH, and more flexibility in the mixmer construction is introduced.

SYNTHESIS

The present invention provides a method of manufacturing an oligomer conjugate, comprising conjugating at least one oligomer to a carrier component, wherein said oligomer conjugate is suitable for treating a viral disorder.

The present invention also provides a method of manufacturing a polyoligomer conjugate as described herein, comprising attaching one or more oligomers to a linker group (E or L) or a symmetrical brancher region F which is then attached to a carrier component as described herein, wherein said oligomer conjugate is suitable for treating a viral disorder.

In some embodiments the symmetrical brancher region is either 1,3-pentylamidopropyl (from 1,3-bis-[5-(4,4'-dimethoxytrityloxy)pentylamido]propyl-2-[(2-cyanoethyl)-(N,N-

diisopropyl)]-phosphoramidite), tris-2,2,2-(propyloxymethyl)ethyl (from tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]ethyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite) or tris-2,2,2-(propyloxymethyl)methyleneoxypropyl (from tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]methyleneoxypropyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite). In some embodiments the asymmetrical brancher region is 1,3-pentylamidopropyl (from 1-[5-(4,4'-dimethoxytrityloxy)pentylamido]-3-[5-fluorenomethoxycarbonyloxypentylamido]-propyl-2-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite),Glen Reseach, USA provides such suitable branchers (e.g. catalog number 0-1920, 10-1922 and 10-1925)..

Linker groups and brancher regions as described herein may be cleavable or non-cleavable.

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

ACTIVATED OLIGOMERS

The term “activated oligomer,” as used herein, refers to an oligomer of the invention that is covalently linked (i.e., functionalized) to at least one functional moiety that permits covalent linkage of the oligomer to one or more conjugated moieties, i.e., moieties that are not themselves nucleic acids or units, to form the conjugates herein described. Typically, a functional moiety will comprise a chemical group that is capable of covalently bonding to the oligomer via, e.g., a 3’-hydroxyl group or the exocyclic NH_2 group of the adenine base, a spacer that is preferably hydrophilic and a terminal group that is capable of binding to a conjugated moiety (e.g., an amino, sulfhydryl or hydroxyl group). In some embodiments, this terminal group is not protected, e.g., is an NH_2 group. In other embodiments, the terminal group is protected, for example, by any suitable protecting group such as those described in “Protective Groups in Organic Synthesis" by Theodora W Greene and Peter G M Wuts, 3rd edition (John Wiley & Sons, 1999). Examples of suitable hydroxyl protecting groups include esters such as acetate ester, aralkyl groups such as benzyl, diphenylmethyl, or triphenylmethyl, and tetrahydropyranyl. Examples of suitable amino protecting groups include benzyl, alpha-methylbenzyl, diphenylmethyl, triphenylmethyl, benzyloxycarbonyl, tert-butoxycarbonyl, and acyl groups such as trichloroacetyl or trifluoroacetyl. In some embodiments, the functional moiety is self-cleaving. In other embodiments, the functional moiety is biodegradable. See e.g., U.S. Patent No. 7,087,229, which is incorporated by reference herein in its entirety.

In some embodiments, oligomers of the invention are functionalized at the 5’ end in order to allow covalent attachment of the conjugated moiety to the 5’ end of the oligomer. In other embodiments, oligomers of the invention can be functionalized at the 3’ end. In still other

embodiments, oligomers of the invention can be functionalized along the backbone or on the heterocyclic base moiety. In yet other embodiments, oligomers of the invention can be functionalized at more than one position independently selected from the 5' end, the 3' end, the backbone and the base.

In some embodiments, activated oligomers of the invention are synthesized by incorporating during the synthesis one or more units that is covalently attached to a functional moiety. In other embodiments, activated oligomers of the invention are synthesized with units that have not been functionalized, and the oligomer is functionalized upon completion of synthesis. In some embodiments, the oligomers are functionalized with a hindered ester containing an aminoalkyl linker, wherein the alkyl portion has the formula $(CH_2)_w$, wherein w is an integer ranging from 1 to 10, preferably about 6, wherein the alkyl portion of the alkylamino group can be straight chain or branched chain, and wherein the functional group is attached to the oligomer via an ester group (-O-C(O)-$(CH_2)_w$NH).

In other embodiments, the oligomers are functionalized with a hindered ester containing a $(CH_2)_w$-sulfhydryl (SH) linker, wherein w is an integer ranging from 1 to 10, preferably about 6, wherein the alkyl portion of the alkylamino group can be straight chain or branched chain, and wherein the functional group attached to the oligomer via an ester group (-O-C(O)-$(CH_2)_w$SH). In some embodiments, sulfhydryl-activated oligonucleotides are conjugated with polymer moieties such as polyethylene glycol or peptides (via formation of a disulfide bond).

Activated oligomers containing hindered esters as described above can be synthesized by any method known in the art, and in particular by methods disclosed in PCT Publication No. WO 2008/034122 and the examples therein, which is incorporated herein by reference in its entirety.

In still other embodiments, the oligomers of the invention are functionalized by introducing sulfhydryl, amino or hydroxyl groups into the oligomer by means of a functionalizing reagent substantially as described in U.S. Patent Nos. 4,962,029 and 4,914,210, i.e., a substantially linear reagent having a phosphoramidite at one end linked through a hydrophilic spacer chain to the opposing end which comprises a protected or unprotected sulfhydryl, amino or hydroxyl group. Such reagents primarily react with hydroxyl groups of the oligomer. In some embodiments, such activated oligomers have a functionalizing reagent coupled to a 5'-hydroxyl group of the oligomer. In other embodiments, the activated oligomers have a functionalizing reagent coupled to a 3'-hydroxyl group. In still other embodiments, the activated oligomers of the invention have a functionalizing reagent coupled to a hydroxyl group on the backbone of the oligomer. In yet further embodiments, the oligomer of the invention is functionalized with more than one of the functionalizing reagents as described in U.S. Patent Nos. 4,962,029 and 4,914,210, incorporated herein by reference in their entirety. Methods of synthesizing such

functionalizing reagents and incorporating them into units or oligomers are disclosed in U.S. Patent Nos. 4,962,029 and 4,914,210.

In some embodiments, the 5'-terminus of a solid-phase bound oligomer is functionalized with a dienyl phosphoramidite derivative, followed by conjugation of the deprotected oligomer with, e.g., an amino acid or peptide via a Diels-Alder cycloaddition reaction.

In various embodiments, the incorporation of units containing 2'-sugar modifications, such as a 2'-carbamate substituted sugar or a 2'-(O-pentyl-N-phthalimido)-deoxyribose sugar into the oligomer facilitates covalent attachment of conjugated moieties to the sugars of the oligomer. In other embodiments, an oligomer with an amino-containing linker at the 2'-position of one or more units is prepared using a reagent such as, for example, 5'-dimethoxytrityl-2'-O-(e-phthalimidylaminopentyl)-2'-deoxyadenosine-3'-N,N-diisopropyl-cyanoethoxy phosphoramidite (see, e.g., Manoharan, et al., Tetrahedron Letters, 1991, 34, 7171.)

In still further embodiments, the oligomers of the invention may have amine-containing functional moieties on the nucleobase, including on the N6 purine amino groups, on the exocyclic N2 of guanine, or on the N4 or 5 positions of cytosine. In various embodiments, such functionalization may be achieved by using a commercial reagent that is already functionalized in the oligomer synthesis.

Some functional moieties are commercially available, for example, heterobifunctional and homobifunctional linking moieties are available from the Pierce Co. (Rockford, Ill.). Other commercially available linking groups are 5'-Amino-Modifier C6 and 3'-Amino-Modifier reagents, both available from Glen Research Corporation (Sterling, Va.). 5'-Amino-Modifier C6 is also available from ABI (Applied Biosystems Inc., Foster City, Calif.) as Aminolink-2, and 3'-Amino-Modifier is also available from Clontech Laboratories Inc. (Palo Alto, Calif.).

POLYOLIGOMERS

As indicated above, in some embodiments of the present invention, the oligomer or the oligomer component of the oligomer conjugate may comprise or be part of a molecule that has two targeting sequences. In some instances, these molecules are called polyoligomers.

In some embodiments, the oligomer conjugate has or comprises the structure:

Carrier component – L1 - First Oligomer Region – L2 – Second Oligomer Region

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 can be the same or different; or

wherein said oligomer conjugate has or comprises the structure:

First Oligomer Region – L2 – Second Oligomer Region - L3 – Carrier component

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 and L3 can be the same or different different; or

wherein said oligomer conjugate has or comprises the structure:

Carrier component 1 – L1 - First Oligomer Region – L2 – Second Oligomer Region - L3 – Carrier component 2

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1, L2 and L3 can be the same or different

wherein Carrier component 1 and Carrier component 2 can be the same or different; or

wherein said oligomer conjugate has or comprises the structure:

First Oligomer Region – L1 - Carrier component 1 – L2 – Second Oligomer Region

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 and L3 can be the same or different; or

wherein said oligomer conjugate has or comprises the structure:

First Oligomer Region – L1 - Carrier component 1 – L2 – Second Oligomer Region - L3 – Carrier component 2

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 can be the same or different

wherein Carrier component 1 and Carrier component 2 can be the same or different; or

wherein said oligomer conjugate has or comprises the structure:

Carrier component 1 – L1 - First Oligomer Region – L2 - Carrier component 2 – L3 – Second Oligomer Region - L4 – Carrier component 3

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1, L2 and L3 can be the same or different

wherein Carrier component 1, Carrier Component 2 and Carrier component 3 can be the same or different.

In some embodiments, preferably the oligomer conjugate for the use according to the present invention wherein said oligomer conjugate has or comprises the structure:

Carrier component – L1 - First Oligomer Region – L2 – Second Oligomer Region

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 can be the same or different.

In some embodiments, the Linker 1 is present.

In some embodiments, the Linker 2 is present.

In some embodiments, the Linker 3 is present.

In some embodiments, the carrier component is linked, preferably conjugated, to said first oligomer region.

In some embodiments, the carrier component is linked, preferably conjugated, to the 5' end of said oligomer.

In some embodiments, each of the first oligomer region and the second oligomer regions is linked, preferably conjugated, by means of a linker or brancher region.

In some embodiments, each of the first oligomer region and the second oligomer regions is linked, preferably conjugated, by means of a physiologically labile linker group or a physiologically labile brancher region.

In some embodiments, the invention provides for a poly-oligomeric compound which may comprise a first region (region PA), a second region (region PB) and a third region (region PC), wherein the first region is covalently linked to at least one further oligomeric compound (region PA'), wherein the first region (region PA) and region PA' are covalently linked via a biocleavable linker (region PB'), which may be, by way of example, as according to the second region as disclosed here, for example a region of at least one phosphodiester linked DNA or RNA (such as DNA), such as two, three, four or five phosphodiester linked DNA or RNA nucleosides (such as DNA nucleosides). Regions PB and PB' may, in some embodiments have the same structure, e.g. the same number of DNA/RNA nucleosides and phosphodiester linkages and/or the same nucleobase sequence. In other embodiments Regions PB and PB' may be different. By way of example such poly oligomeric compounds may have a structure such as: (5' – 3' or 3' – 5') Conjugate/Carrier Compound-PO-ON-PO'-ON', wherein conjugate/carrier compound is region PC, PO is region PB, PO' is region PB', and ON is region PA, and ON' is region PA'.

It should be understood that region PA' may, in some embodiments, comprise multiple further oligomeric compounds (such as a further 2 or 3 oligomeric compounds) linked in series (or in parallel) via biocleavable linkers, for example: Conjugate/Carrier Compound-PO-ON-PO-ON'-PO''-ON'', or Conjugate/Carrier Compound-PO-ON-[PO-ON']n, wherein n may, for example be 1, 2 or 3, and each ON' may be the same or different, and if different may have the same or different targets.

In an aspect, the present invention employs poly-oligomeric compounds (also referred herein as oligomer compounds) for use in modulating, such as inhibiting a target nucleic acid in a cell, for example HBV HBx or HBsAg. The oligomer compound comprises at least two oligomer regions, e.g. (PA and PA') and may comprise further oligomer regions (e.g. PA''). At least one oligomer region is an oligomer which is capable of modulating a target sequence in HBx or HBsAg of HBV, for example an oligomer as provided by the present invention. In certain embodiments, each of PA, PA' (and PA'' if present) may be an oligomer which is capable of modulating a target sequence in HBx or HBsAg of HBV, for example an oligomer as provided by the present invention. PA and PA' may be complements to different positions in the target sequence.

In some embodiments, PA may be an oligomer which is capable of modulating a target sequence in HBx and PA' (and/or PA'' if present) may be oligomers which are capable of modulating a different target sequence. In certain embodiments, PA' (and/or PA'' if present) may be capable of modulating a target sequence in HBsAg of HBV. For example PA' (and/or PA'' if present) may be an oligomer capable of modulating a target sequence in HBsAg of HBV, as described herein.

In some embodiments, PA' may be an oligomer which is capable of modulating a target sequence in HBx and PA (and/or PA'' if present) may be oligomers which are capable of modulating a different target sequence from HBV. In certain embodiments, PA (and/or PA'' if present) may be capable of modulating a target sequence in HBsAg of HBV. For example PA (and/or PA'' if present) may be an oligomer capable of modulating a target sequence in HBsAg of HBV, as described herein.

Each oligomer region may be flanked by a bio-cleavabe region (region PB), which may, for example, be a further region of 1 – 10 contiguous nucleotides (region PB), which comprise at least one phosphodiester linkage. Other physiological labile nucleoside regions may be used.

In some embodiments, the oligomer compounds of the invention are covalently linked to a conjugate group, a targeting group, a reactive group, an activation group, or a blocking group, optionally, via a short region comprising (e.g. 1 – 10) of phosphodiester linked DNA or RNA nucleoside(s). Examples of such groups are the carrier components and conjugate components mentioned herein.

In some embodiments, the compound of the invention does not comprise RNA (units). In some embodiments, the compound according to the invention forms a single contiguous sequence), optionally linked to a function group, such as a conjugate group, and is such a linear molecule or is synthesized as a linear molecule. The oligomeric compound may therefore be single stranded molecule. In some embodiments, the oligomer does not comprise short regions of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to equivalent regions within the same oligomeric compound (i.e. duplexes). The oligomer, in some embodiments, may be not (essentially) double stranded. In some embodiments, the oligomer is essentially not double stranded, such as is not a siRNA.

Oligomer regions PA, PA' and if present PA'' are phosphorothioate oligomers, i.e. at least 70% of the internucleoside linkages within each oligomer region PA, PA' and if present PA'', are phosphorothioate linkages, such as at least 80% or at least 90% or all of the internucleoside linkages if present oligomer regions PA, PA' and PA'' (if present), are phosphorothioate.

In some embodiments, oligomer regions PA, PA' and if present PA'' may form a single contiguous oligonucleotide sequence. Regions PA, PA' and PA'' are interspaced by regions PB, for example regions of 1, 2, 3, 4, or 5 phosphodiester linked DNA nucleosides.

When region PB comprises only 1 nucleoside, at least one, or both of the internucleoside linkages between the region PB nucleoside (e.g. a DNA nucleoside) may be phosphodiester linkages. When region PB comprises only 2 or more nucleosides, the internucleoside linkages between the region PB nucleoside (e.g. the DNA nucleosides) may be phosphodiester linkages and/or may be another internucleoside linkage, such as phosphorothioate linkages.

The oligomers of the invention, such as PA, PA' and if present PA'', do not form part of a siRNA complex. The oligomers of the invention, such as PA, PA' and if present PA'', are non-complementary, e.g. they do not hybridize to one another to form a region of more than 8 or in some embodiments more than 6 contiguous base pairs. In some embodiments, regions PA and PA'' do not hybridize to one another to form a region of more than 4 contiguous base pairs. Exemplary base pairs may be between A-T, G-C or A-U. In the case there are three oligomer regions, PA, PA' and PA'', the non-complementarity is between PA and PA', and PA' and PA'', as well as PA and PA''.

The oligomer regions PA, PA' and if present PA'' are not in the form of a duplex with a (substantially) complementary oligonucleotide – e.g. is not an siRNA.

In some embodiments, oligomer regions PA, PA' and PA'' share the same contiguous nucleotide sequence. In some embodiments, oligomer regions PA and PA' share the same contiguous nucleotide sequence. In this respect the invention provides for a single compound which can be used to deliver multiple copies of an oligomer (i.e. with the same contiguous nucleobase sequence and optionally the same chemical modifications) to the target tissue.

The oligomer regions (PA, PA' and if present PA'') are linked via at least one biocleavable region, referred to as region PB herein (and where there is more than one region PB, region PB' and region PB''). In some embodiments, region PB comprises 1 – 10 nucleosides which form a physiologically labile region between oligomer regions, or between an (or each) oligomer region and a linking group. Regions of DNA phosphodiester nucleosides may be used, but other nucleotide regions may be used if they are suitably physiologically labile.

In some embodiments, the internucleoside linkage between the oligomer region (PA, PA' or if present PA'') and (each) second region PB, is a phosphodiester linked to the first (or only) DNA or RNA nucleoside of region PB comprises at least one phosphodiester linked DNA or RNA nucleoside.

The region PB may, in some embodiments, comprise further DNA or RNA nucleosides which may be phosphodiester linked.

As explained herein, region PB may also be used to join a functional group to the oligomeric region(s), optionally via a further linkage group (PY). The use of region PB as a cleavable linker to join functional groups to oligomer is described in detail in PCT/EP2013/073858, which is hereby incorporated by reference.

In some embodiments a region PB is further covalently linked to a third region which may, for example, be a conjugate, a targeting group a reactive group, and/or a blocking group (PC). Group (PC) may be a carrier component as described herein.

In some aspects, the present invention is based upon the provision of a physiologically labile region, the second region, linking the first region, e.g. an antisense oligonucleotide, and a conjugate or functional group, e.g. carrier component. The physiologically labile region may comprises at least one phosphodiester linked nucleoside, such as a DNA or RNA nucleoside, such as 1, 2, 3, 4, 5, 6, 7, 8,9 or 10 phosphodiester linked nucleosides, such as DNA or RNA. In some embodiments, the oligomeric compound comprises a cleavable (physiologically labile) linker. In this respect the cleavable linker is preferably present in region PB (or in some embodiments, between region PA and PB).

In some embodiments, one (or more or all) region PB may comprise or consists of at least one DNA or RNA nucleosides linked to the first region via a phosphodiester linkage. In some aspects, the internucleoside linkage between an oligomer region and second region is considered as part of region PB.

In some embodiments, a (or more or each) region PB comprises or consists of at least between 1 and 10 linked nucleosides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 linked DNA or RNA nucleotides. Whilst a region of DNA/RNA phosphodiester is considered important in the provision of a cleavable linker, it is possible that region PB also comprises sugar-modified nucleoside analogues, such as those referred to under the first region above. However in some embodiments, the nucleosides of region PB are (optionally independently) selected from the group consisting of DNA and RNA. In some embodiments, the nucleosides of region PB are (optionally independently) DNA. It will be recognized that the nucleosides of region PB may comprise naturally occurring or non-naturally occurring nucleobases. Typically, region PB comprises at least one phosphodiester linked DNA or RNA nucleoside (which may, in some embodiments. be the first nucleoside adjacent to an oligomer). If region PB comprises other nucleosides, region PB may also comprise of other nucleoside linkages other than phosphodiester, such as (optionally independently) phosphorothioate, phosphodithioate, boranophosphate or methyl phosphonate. However, in other exemplified embodiments, all the internucleoside linkages in region PB are phosphorothioate. In some embodiments, all the nucleosides of region PB comprise (optionally independently) either a 2'-OH ribose sugar (RNA) or a 2'-H sugar - i.e. RNA or DNA. Between 1 – 5, or 1 -4, such as 2, 3, 4 phosphate (phosphodiester) linked DNA nucleosides have been shown to be particularly useful in the compounds of the invention.

In some embodiments, the second region comprises or consists of at least between 1 and 10 (e.g. phosphodiester) linked DNA or RNA nucleosides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 (e.g. phosphodiester) linked DNA or RNA nucleotides.

In some embodiments, region PB comprises no more than 3 or no more than 4 consecutive DNA or RNA nucleosides (such as DNA nucleosides). As such region PB may be so short as it does not recruit RNAseH, an aspect which may be important in embodiments when region PB does not form a part of a single contiguous nucleobase sequence which is complementary to the target. Shorter region PBs, e.g. of 1 – 4nts in length may also be preferable in some embodiments, as they are unlikely to be the target of sequence specific restriction enzymes. As such it is possible to vary the susceptibility of the region PB to endonuclease cleavage, and thereby fine-tune the rate of activation of the active oligomer in vivo, or even intra-cellular. Suitably, if very rapid activation is required, longer region PBs may be employed and/or region Bs which comprise the recognition sites of (e.g. cell or tissue specific or differentially expressed) restriction enzymes.

In some embodiments, a region PB may be conjugated to a functional group (PC), such as a conjugate, targeting reactive group, an activation group, or blocking group, optinally via a linker group (PY such as those provided herein). Functional groups may also be joined to an oligomer region, or the compound of the invention via other means, e.g. via phosphate nucleoside linkage (e.g. phosphodiester, phosphorothioate, phosphodithioate, boranophosphate or methylphosphonate) or a triazol group. In some aspects, the linkage group is the same as the region PB between at least two of the oligomer regions, and as such may be a phosphodiester linkage.

In some embodiments the DNA or RNA nucleotides of an (or more or each) region PB are independently selected from DNA and RNA nucleotides. In some embodiments the DNA or RNA nucleotides of an (or more or each) region PB are DNA nucleotides. In some embodiments the DNA or RNA nucleotides of an (or more or each) region PB are RNA nucleotides.

In the context of the second region, the term DNA and RNA nucleoside may comprise a naturally occurring or non-naturally occurring base (also referred to as a base analogue or modified base).

It will be recognized that, in some embodiments, an (or more or each) region PB may further comprise other nucleotides or nucleotide analogues. In some embodiments, (or more or each) region PB comprises only DNA or RNA nucleosides. In some embodiments, an (or more or each) region PB comprises more than one nucleoside, the internucleoside linkages in an or each region PB comprise phosphodiester linkages. In some embodiments, when an (or more or each) region PB comprises more than one nucleoside, all the internucleoside linkages in the second region comprise phosphodiester linkages.

In some embodiments, at least two consecutive nucleosides of an (or more or each) region PB are DNA nucleosides (such as at least 3 or 4 or 5 consecutive DNA nucleotides). In some embodiments the at least two consecutive nucleosides an (or more or each) region PB are RNA nucleosides (such as at least 3 or 4 or 5 consecutive RNA nucleotides). In some embodiments the at least two consecutive nucleosides of the (or more or each) region PB are at least one DNA and at least one RNA nucleoside. The internucleoside linkage between a region PA and region PB may be a phosphodiester linkage. In some embodiments, when region PB comprises more than one nucleoside, at least one further internucleoside linkage is phosphodiester – such as the linkage group(s) between the 2 (or 3 or 4 or 5) nucleosides adjacent to a region PA.

A region PB may be flanked on at least one side (either 5' or 3') by the first region, e.g. an antisense oligonucleotide, and on the other side (either 3' or 5' respectfully, via a further oligomer region (PA'), or a conjugate moiety or similar group (e.g. a blocking moiety/group, a targeting moiety/group or therapeutic small molecule moiety), optionally via a linker group (i.e. between the second region and the conjugate/blocking group etc. moiety).

In some embodiments, region PB does not form a complementary sequence when the oligomer region (e.g. PA, PA' and/or PA'') and PB is aligned to the complementary target sequence.

In some embodiments, region PB does form a complementary sequence when the oligomer region (e.g. PA, PA' and/or PA'') and PB is aligned to the complementary target sequence. In this respect region PA and PB together may form a single contiguous sequence which is complementary to the target sequence.

In some embodiments, the sequence of bases in region PB is selected to provide an optimal endonuclease cleavage site, based upon the predominant endonuclease cleavage enzymes present in the target tissue or cell or sub-cellular compartment. In this respect, by isolating cell extracts from target tissues and non-target tissues, endonuclease cleavage sequences for use in region PB may be selected based upon a preferential cleavage activity in the desired target cell (e.g. liver/hepatocytes) as compared to a non-target cell (e.g. kidney). In this respect, the potency of the compound for target down-regulation may be optimized for the desired tissue/cell.

In some embodiments region PB comprises a dinucleotide of sequence AA, AT, AC, AG, TA, TT, TC, TG, CA, CT, CC, CG, GA, GT, GC, or GG, wherein C may be 5-mthylcytosine, and/or T may be replaced with U.

In some embodiments region PB comprises a trinucleotide of sequence AAA, AAT, AAC, AAG, ATA, ATT, ATC, ATG, ACA, ACT, ACC, ACG, AGA, AGT, AGC, AGG, TAA, TAT, TAC, TAG, TTA, TTT, TTC, TAG, TCA, TCT, TCC, TCG, TGA, TGT, TGC, TGG, CAA, CAT, CAC, CAG, CTA, CTG, CTC, CTT, CCA, CCT, CCC, CCG, CGA, CGT, CGC, CGG, GAA, GAT, GAC,

CAG, GTA, GTT, GTC, GTG, GCA, GCT, GCC, GCG, GGA, GGT, GGC, and GGG wherein C may be 5-mthylcytosine and/or T may be replaced with U.

In some embodiments region PB comprises a trinucleotide of sequence AAAX, AATX, AACX, AAGX, ATAX, ATTX, ATCX, ATGX, ACAX, ACTX, ACCX, ACGX, AGAX, AGTX, AGCX, AGGX, TAAX, TATX, TACX, TAGX, TTAX, TTTX, TTCX, TAGX, TCAX, TCTX, TCCX, TCGX, TGAX, TGTX, TGCX, TGGX, CAAX, CATX, CACX, CAGX, CTAX, CTGX, CTCX, CTTX, CCAX, CCTX, CCCX, CCGX, CGAX, CGTX, CGCX, CGGX, GAAX, GATX, GACX, CAGX, GTAX, GTTX, GTCX, GTGX, GCAX, GCTX, GCCX, GCGX, GGAX, GGTX, GGCX, and GGGX, wherein X may be selected from the group consisting of A, T, U, G, C and analogues thereof, wherein C may be 5-mthylcytosine and/or T may be replaced with U. It will be recognized that when referring to (naturally occurring) nucleobases A, T, U, G, C, these may be substituted with nucleobase analogues which function as the equivalent natural nucleobase (e.g. base pair with the complementary nucleoside).

In some embodiments, the compound of the invention may comprise more than one conjugate group (or more than one functional group PX – such as a conjugate, targeting, blocking or activated group or a reactive or activation group), such as 2 or 3 such groups. In some embodiments, region PB is covalently linked, optionally via a [e.g. non-nucleotide] linker group), to at least one functional group, such as two or three functional groups. In some embodiments, the first region (PA) may be covalently linked (e.g. via internucleoside linkages, such as phosphodiester linkages), to two region PBs, for example, one 5' and one 3' to the first region PA, wherein each region PB may be (optionally independently) selected from the region PB described herein.

COMPOSITION

Oligomers of the invention and oligomer conjugates of the invention may be used in pharmaceutical formulations and compositions. Suitably, such compositions comprise a pharmaceutically acceptable diluent, carrier, salt or adjuvant. WO 2007/03109 provides suitable and preferred pharmaceutically acceptable diluent, carrier and adjuvants - which are hereby incorporated by reference. Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations are also provided in WO 2007/03109 - which are also hereby incorporated by reference.

Pharmaceutical compositions of the invention may include a pharmaceutically acceptable carrier that may contain a variety of components that provide a variety of functions, including regulation of drug concentration, regulation of solubility, chemical stabilization, regulation of viscosity, absorption enhancement, regulation of pH, and the like.

The pharmaceutical carrier may comprise a suitable liquid vehicle or excipient and an optional auxiliary additive or additives. The liquid vehicles and excipients are conventional and commercially available. Illustrative thereof are distilled water, physiological saline, aqueous solutions of dextrose, and the like. For water soluble formulations, the pharmaceutical composition preferably includes a buffer such as a phosphate buffer, or other organic acid salt, preferably at a pH in the range of 6.5 to 8. For formulations containing weakly soluble antisense compounds, micro-emulsions may be employed, for example by using a nonionic surfactant such as polysorbate 80 in an amount of 0.04-0.05% (w/v), to increase solubility. Other components may include antioxidants, such as ascorbic acid, hydrophilic polymers, such as, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, dextrins, chelating agents, such as EDTA, and like components well known to those in the pharmaceutical sciences, e.g., *Remington's Pharmaceutical Science*, latest edition (Mack Publishing Company, Easton, Pa.).

Oligonucleotides of the invention include the pharmaceutically acceptable salts thereof, including those of alkaline earth salts, e.g., sodium or magnesium, ammonium or NX_4^+, wherein X is C_1-C_4 alkyl. Other pharmaceutically acceptable salts include organic carboxylic acids such as formic, acetic, lactic, tartaric, malic, isethionic, lactobionic, and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, toluenesulfonic acid and benzenesulfonic; and inorganic acids such as hydrochloric, sulfuric, phosphoric, and sulfamic acids. Pharmaceutically acceptable salts of a compound having a hydroxyl group include the anion of such compound with a suitable cation such as Na^+, NH_4^+, or the like.

The patient should receive a sufficient daily dosage of oligonucleotide to achieve an effective yet safe intercellular concentrations of combined oligonucleotides. Those skilled in the art should be readily able to derive appropriate dosages and schedules of administration to suit the specific circumstance and needs of the patient.

The effectiveness of the treatment may be assessed by routine methods, which are used for determining whether or not remission has occurred. Such methods generally depend upon morphological, cytochemical, cytogenetic, immunologic and molecular analyses. In addition, remission can be assessed genetically by probing the level of expression of one or more relevant genes. The reverse transcriptase polymerase chain reaction (RT-PCR) methodology can be used to detect even very low numbers of mRNA transcript.

OLIGONUCLEOTIDE DELIVERY TECHNIQUES

Oligonucleotides and conjugates of the invention may be preferably administered to a subject orally or topically but may also be administered intravenously by injection. The vehicle is designed accordingly. Alternatively, the oligonucleotide may be administered subcutaneously

via controlled release dosage forms or conventional formulation for intravenous injection.A preferred method of administration of oligonucleotides comprises either topical, systemic or regional perfusion, as is appropriate. According to a method of regional perfusion, the afferent and efferent vessels supplying the extremity containing the lesion are isolated and connected to a low-flow perfusion pump in continuity with an oxygenator and a heat exchanger. The iliac vessels may be used for perfusion of the lower extremity. The axillary vessels are cannulated high in the axilla for upper extremity lesions. Oligonucleotide is added to the perfusion circuit, and the perfusion is continued for an appropriate time period, e.g., one hour. Perfusion rates of from about 100 to about 150 ml/minute may be employed for lower extremity lesions, while half that rate should be employed for upper extremity lesions. Systemic heparinization may be used throughout the perfusion, and reversed after the perfusion is complete. This isolation perfusion technique permits administration of higher doses of chemotherapeutic agent than would otherwise be tolerated upon infusion into the arterial or venous systemic circulation.

In a particular embodiment, oligomers and conjugates of the invention are administered systemically or formulated for systemic administration.

For systemic infusion, the oligonucleotides are preferably delivered via a central venous catheter, which is connected to an appropriate continuous infusion device. Indwelling catheters provide long term access to the intravenous circulation for frequent administration of drugs over extended time periods. They are generally surgically inserted into the external cephalic or internal jugular vein under general or local anesthesia. The subclavian vein is another common site of catheterization. The infuser pump may be external, or may form part of an entirely implantable central venous system such as the INFUSAPORT system available from Infusaid Corp., Norwood, Mass. and the PORT-A-CATH system available from Pharmacia Laboratories, Piscataway, N.J. These devices are implanted into a subcutaneous pocket under local anesthesia. A catheter, connected to the pump injection port, is threaded through the subclavian vein to the superior vena cava. The implant contains a supply of oligonucleotide in a reservoir which may be replenished as needed by injection of additional drug from a hypodermic needle through a self-sealing diaphragm in the reservoir. Completely implantable infusers are preferred, as they are generally well accepted by patients because of the convenience, ease of maintenance and cosmetic advantage of such devices.

Oligonucleotides and conjugates of the invention may be introduced by any of the methods described in U.S. Patent 4,740,463, incorporated herein by reference. One technique is *in vitro* transfection, which can be done by several different methods. One method of transfection involves the addition of DEAE-dextran to increase the uptake of the naked DNA molecules by a recipient cell. See McCutchin, J. H. and Pagano, J. S., *J. Natl. Cancer Inst.* 41, 351-7 (1968). Another method of transfection is the calcium phosphate precipitation technique which depends

upon the addition of Ca^{2+} to a phosphate-containing DNA solution. The resulting precipitate apparently includes DNA in association with calcium phosphate crystals. These crystals settle onto a cell monolayer; the resulting apposition of crystals and cell surface appears to lead to uptake of the DNA. A small proportion of the DNA taken up becomes expressed in a transfectant, as well as in its clonal descendants. See Graham, F. L. and van der Eb, A. J., *Virology* 52, 456-467 (1973) and *Virology* 54, 536-539 (1973).

Transfection may also be carried out by cationic phospholipid-mediated delivery. In particular, polycationic liposomes can be formed from N-[1-(2,3-di-oleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOT-MA). See Felgner et al., *Proc. Natl. Acad. Sci.*, 84, 7413-7417 (1987) (DNA-transfection); Malone et al., *Proc. Natl. Acad. Sci.*, 86, 6077-6081 (1989) (RNA-transfection).

For systemic or regional *in vivo* administration, the amount of oligonucleotides may vary depending on the nature and extent of the disease, the particular oligonucleotides utilized, and other factors. The actual dosage administered may take into account the size and weight of the patient, whether the nature of the treatment is prophylactic or therapeutic in nature, the age, health and sex of the patient, the route of administration, whether the treatment is regional or systemic, and other factors.

In addition to administration with conventional pharmaceutical carriers, the antisense oligonucleotides may be administered by a variety of specialized oligonucleotide delivery techniques. Sustained release systems suitable for use with the pharmaceutical compositions of the invention include semi-permeable polymer matrices in the form of films, microcapsules, or the like, which may comprise polylactides; copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, poly(2-hydroxyethyl methacrylate), and like materials, e.g., Rosenberg et al., International application PCT/US92/05305.

The oligonucleotides and conjugates may be encapsulated in liposomes for therapeutic delivery, as described for example in Liposome Technology, Vol. II, Incorporation of Drugs, Proteins, and Genetic Material, CRC Press. The oligonucleotide, depending upon its solubility, may be present both in the aqueous layer and in the lipidic layer, or in what is generally termed a liposomic suspension. The hydrophobic layer, generally but not exclusively, comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, ionic surfactants such as diacetylphosphate, stearylamine, or phosphatidic acid, and/or other materials of a hydrophobic nature. Also comprised are the novel cationic amphiphiles, termed “molecular umbrellas", that are described in (DeLong et al, *Nucl. Acid. Res.*, 1999, 27(16), 3334-3341).

The embodiments of the present invention may be delivered by means of particulate systems and/or polymers. Particulate systems and polymers for *in vitro* and *in vivo* delivery of polynucleotides have been extensively reviewed by Felgner in *Advanced Drug Delivery Reviews* 5, 163-187 (1990). Techniques for direct delivery are also described in Cook S.T. *Antisense Drug Technology, Principles, Strategies, and Applications,* Marcel Dekker, Inc, 2001.

PRO-DRUGS

The oligonucleotides may be synthesized as pro-drugs carrying lipophilic groups, such as for example methyl-SATE (S-acetylthioethyl) or t-Bu-SATE (S-pivaloylthioethyl) protecting groups, that confers nuclease resistance to the oligo, improve cellular uptake and selectively deprotects after entry into the cell as described in Vives et al. *Nucl. Acids Res*. 1999, Vol. 27, 4071-4076.

CIRCULAR MOLECULES

The oligonucleotides may be synthesized as circular molecules in which the 5´and 3´ ends of the oligonucleotides are covalently linked or held together by an affinity pair one member of which is attached covalently to the 5´ end and the other attached covalently to the 3´end. Such circularization protects the oligonucleotide against degradation by exonucleases and may also improve cellular uptake and distribution. In one aspect of the invention the moiety linking the 5´ and 3´ end of a circular oligonucleotide is cleaved automatically upon entry into any type of human or vertebrate cell thereby linearising the oligonucleotide and enabling it to efficiently hybridize to its target sequence. In another aspect, the moiety linking the 5´ and 3´ends of the oligonucleotide is so designed that cleavage preferably occurs only in the particular type of cells that expresses the mRNA that is the target for the antisense oligonucleotide. For instance, a circular antisense oligonucleotide directed against a gene involved in a viral disorder may be brought into action by linearisation only in the subset of cells expressing the gene in question, for example HBV HBx or HBsAg.

ADDITIONAL PHARMACEUTICAL ENTITY

Oligomers and oligomer conjugates of the invention may be used as the primary therapeutic for the treatment of the disease state, or may be used in combination with non-oligonucleotide drugs.

Accordingly, the present invention provides a pharmaceutical system comprising a pharmaceutical composition as described herein and an additional pharmaceutical entity. The additional pharmaceutical entity may be any therapeutic agent known in the art. For example, the additional pharmaceutical entity may be an antibody, a small molecule therapeutic, a polynucleotide or gene therapy vector (e.g. a vector capable of expressing therapeutic polypeptides or RNAi agents).

Hence, the oligomer or the oligomer conjugate of the present invention may be used in combination with other actives, such as other anti-viral actives.

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, an 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; 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.

The additional pharmaceutical entity may be an oligomer or oligomer conjugate as defined herein.

In certain embodiments the pharmaceutical system may comprise at least one, at least two, at least three, up to a plurality of oligomers or oligomer conjugates provided by the present invention.

In certain embodiments the additional pharmaceutical entity may an oligomer or oligomer conjugate capable of modulating a target sequence in HBV. The oligomers or conjugates may each be capable of modulating a target sequence in HBV HBx or HBsAg. The oligomers or conjugates may be capable of modulating a target sequence in HBV which is not within HBx or HBsAg. For example, at least one oligomer or conjugate may be capable of modulating a target sequence within the HBV gene or mRNA for HBcAg, HBeAg, or DNA polymerase.

In certain embodiments, the oligomer, or additional oligomer, or conjugate, or additional conjugate, may be capable of modulating a target sequence in the HBV gene or mRNA for HBsAg.

When a combination of oligonucleotides targeting different target sequences are employed, the ratio of the amounts of the different types of oligonucleotide may vary over a broad range. According to one preferred embodiment of the invention, the oligonucleotides of all types are present in approximately equal amounts, by molarity.

ADMINISTRATION AND DOSAGE

The present invention also relates pharmaceutical compositions that contain a therapeutically effective amount of a conjugate of the invention. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. 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).

The pharmaceutical compositions of the present invention are intended for parenteral, intranasal, topical, oral, or local administration, such as by a transdermal means, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered parenterally (e.g., by intravenous, intramuscular, or subcutaneous injection), or by oral ingestion, or by topical application or intraarticular injection. Additional routes of administration include intravascular, intra-arterial, intratumor, intraperitoneal, intraventricular, intraepidural, as well as nasal, ophthalmic, intrascleral, intraorbital, rectal, topical, or aerosol inhalation administration. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants or components. Thus, the invention provides compositions for parenteral administration that comprise the above mention agents dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, PBS, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents,

detergents and the like. The invention also provides compositions for oral delivery, which may contain inert ingredients such as binders or fillers for the formulation of a tablet, a capsule, and the like. Furthermore, this invention provides compositions for local administration, which may contain inert ingredients such as solvents or emulsifiers for the formulation of a cream, an ointment, and the like.

In a particular embodiment, oligomers and conjugates of the invention are administered subcutaneously or formulated for subcutaneous administration.

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.

The compositions containing an effective amount can be administered for prophylactic or therapeutic treatments. In prophylactic applications, compositions can be administered to a subject with a clinically determined predisposition or increased susceptibility to development of a tumor or cancer, neurodegenerative disease, or lysosomal disorder. Compositions of the invention can be administered to the patient (e.g., a human) in an amount sufficient to delay, reduce, or preferably prevent the onset of clinical disease or tumorigenesis. In therapeutic applications, compositions are administered to a subject (e.g., a human) already suffering from disease (e.g., a cancer, neurodegenerative disease, or lysosomal storage disorder) in an amount sufficient to cure or at least partially arrest the symptoms of the condition and its complications. An amount adequate to accomplish this purpose is defined as a "therapeutically effective dose," an amount of a compound sufficient to substantially improve some symptom associated with a disease or a medical condition. For example, in the treatment of cancer, neurodegenerative disease, or lysosomal storage disease, an agent or compound which decreases, prevents, delays, suppresses, or arrests any symptom of the disease or condition would be therapeutically effective. A therapeutically effective amount of an agent or compound is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, or the disease or condition symptoms are ameliorated, or the term of the disease or condition is changed or, for example, is less severe or recovery is accelerated in an individual. Amounts effective for this use may depend on the severity of the disease or condition and the weight and

general state of the patient, but generally range from about 0.5 mg to about 3000 mg of the agent or agents per dose per patient. Suitable regimes for initial administration and booster administrations are typified by an initial administration followed by repeated doses at one or more hourly, daily, weekly, or monthly intervals by a subsequent administration. The total effective amount of an agent present in the compositions of the invention can be administered to a mammal as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which multiple doses are administered over a more prolonged period of time (e.g., a dose every 4-6, 8-12, 14-16, or 18-24 hours, or every 2-4 days, 1-2 weeks, once a month). Alternatively, continuous intravenous infusion sufficient to maintain therapeutically effective concentrations in the blood are contemplated.

The therapeutically effective amount of one or more agents present within the compositions of the invention and used in the methods of this invention applied to mammals (e.g., humans) can be determined by the ordinarily-skilled artisan with consideration of individual differences in age, weight, and the condition of the mammal. The agents of the invention are administered to a subject (e.g. a mammal, such as a human) in an effective amount, which is an amount that produces a desirable result in a treated subject (e.g. the slowing or remission of a cancer or neurodegenerative disorder). Therapeutically effective amounts can be determined empirically by those of skill in the art.

The patient may also receive an agent in the range of about 0.1 to 3,000 mg per dose one or more times per week (e.g., 2, 3, 4, 5, 6, or 7 or more times per week), 0.1 to 2,500 (e.g., 2,000, 1,500, 1,000, 500, 100, 10, 1, 0.5, or 0.1) mg dose per week. A patient may also receive an agent of the composition in the range of 0.1 to 3,000 mg per dose once every two or three weeks.Single or multiple administrations of the compositions of the invention comprising an effective amount can be carried out with dose levels and pattern being selected by the treating physician. The dose and administration schedule can be determined and adjusted based on the severity of the disease or condition in the patient, which may be monitored throughout the course of treatment according to the methods commonly practiced by clinicians or those described herein.

The carrier and conjugates of the present invention may be used in combination with either conventional methods of treatment or therapy or may be used separately from conventional methods of treatment or therapy.

When the conjugates of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to an individual. Alternatively, pharmaceutical compositions according to the present invention may be comprised of a

combination of a carrier-agent conjugate of the present invention in association with a pharmaceutically acceptable excipient, as described herein, and another therapeutic or prophylactic agent known in the art.

FURTHER APPICATIONS

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

In research, such oligomers may be used to specifically inhibit the synthesis of the expression product of a target sequence (typically by degrading or inhibiting the mRNA and thereby prevent protein formation) in cells and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention.

In diagnostics the oligomers may be used to detect and quantitate expression of the expression product of a target sequence in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics, an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the expression of a target sequence is treated by administering oligomeric compounds in accordance with this invention. Further provided are methods of treating a mammal, such as treating a human, suspected of having or being prone to a disease or condition, associated with expression of a target sequence by administering a therapeutically or prophylactically effective amount of one or more of the oligomers or compositions of the invention. The oligomer, a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

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

The invention also provides for a method for treating a disorder as referred to herein said method comprising administering a compound according to the invention as herein described, and/or a conjugate according to the invention, and/or a pharmaceutical composition according to the invention to a patient in need thereof.

VIRAL DISORDERS

The oligomers, oligomer conjugates and other compositions according to the invention can be used for the treatment of conditions associated with the target sequence, such as over-expression or expression of a mutated version of the target sequence.

The invention further provides use of a compound of the invention in the manufacture of a medicament for the treatment of a disease, disorder or condition as referred to herein.

In the context of the present invention, said disease, disorder or condition may be a viral disorder.

In one embodiment, the viral disorder is associated with expression or over-expression of HBx or HBsAg. The viral disorder may be a disorder associated with HBV. Examples of such viral disorders include, but are not limited to, hepatitis B, cirrhosis, liver cancer (e.g. hepatocellular carcinoma), cholangiocarcinoma.

In one embodiment, the viral disorder is hepatitis B. As one of ordinary skill would recognise, the term "hepatitis B" in the context of the present invention refers to an infectious disorder of the liver caused by HBV. Hepatitis B may be acute or chronic. Acute disease causes liver inflammation, vomiting, jaundice and occasionally death. Chronic hepatitis B may cause cirrhosis and liver cancer.

In one embodiment, the viral disorder is cirrhosis. As one of ordinary skill would recognise, the term "cirrhosis" in the context of the present invention refers to an advanced liver disease characterised by the presence of fibrosis and regenerative nodules in the liver. These changes can lead to a loss a liver function.

In one embodiment, the viral disorder in liver cancer. As one of ordinary skill would recognise, "liver cancer" in the context of the present invention refers to a malignancy which originates in the liver.

In one embodiment, the viral disorder is hepatocellular carcinoma (HCC). As one of ordinary skill would recognise, "HCC" in the context of the present invention refers to a type of liver cancer which commonly occurs secondary to a viral hepatitis infection, for example hepatitis B. Macroscopically, HCC appears as a nodular or infiltrative tumour. The nodular type may be solitary (large mass) or multiple (when developed as a complication of cirrhosis). Tumour nodules are round to oval, grey or green (if the tumour produces bile), well circumscribed but not encapsulated. The diffuse type is poorly circumscribed and infiltrates the portal veins, or the hepatic veins (rarely). Microscopically, there are four architectural and cytological types (patterns) of HCC: fibrolamellar, pseudoglandular (adenoid), pleomorphic (giant cell) and clear cell. In well differentiated forms, tumour cells resemble hepatocytes, form trabeculae, cords and nests, and may contain bile pigment in cytoplasm. In poorly differentiated forms, malignant epithelial cells are discohesive, pleomorphic, anaplastic, giant. The tumour has a scant stroma and central necrosis because of the poor vascularization.

In one embodiment, the viral disorder is cholangiocarcinoma. As one of ordinary skill would recognise, "cholangiocarcinoma" in the context of the present invention refers to a form of cancer that is composed of mutated epithelial cells (or cells showing characteristics of epithelial

differentiation) that originate in the bile ducts which drain bile from the liver into the small intestine.

Whilst various embodiments disclosed herein are related to a viral disorder associated with HBV infection, the present invention is not limited by these exemplary embodiments. Rather the present invention is applicable to any disorder associated with a viral infection.

Generally stated, one aspect of the invention is directed to a method of treating a mammal suffering from or susceptible to conditions associated with abnormal levels of the expression product of the target sequence, comprising administering to the mammal a therapeutically effective amount of an oligomer targeted to the target sequence. The oligomer of the invention may comprise one or more LNA units. The oligomer, a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

An interesting aspect of the invention is directed to the use of an oligomer (compound) as defined herein or a oligomer conjugate as defined herein for the preparation of a medicament for the treatment of a disease, disorder or condition as referred to herein.

The methods of the invention are preferably employed for treatment or prophylaxis against diseases caused by abnormal levels of HBx or HBsAg.

Alternatively stated, in some embodiments, the invention is furthermore directed to a method for treating abnormal levels of HBx or HBsAg, said method comprising administering a oligomer of the invention, or a conjugate of the invention or a pharmaceutical composition of the invention to a patient in need thereof.

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

The invention further relates to use of a compound, composition, or a conjugate as defined herein for the manufacture of a medicament for the treatment of abnormal levels of HBx or HBsAg or expression of mutant forms of HBx or HBsAg (such as allelic variants, such as those associated with one of the diseases referred to herein).

Moreover, the invention relates to a method of treating a subject suffering from a disease or condition such as those referred to herein.

A patient who is in need of treatment is a patient suffering from or likely to suffer from the disease or disorder.

EXAMPLE OLIGOMERS

Examples of oligomers for use in the present invention are presented in the following Tables.

Table 1 oligonucleotide sequence motifs used to design LNA modified oligomers.

The table indicates the fraction of conservation within all published full length genotype sequences of genotype A, B, C and D (GenBank), meaning that a given oligomer motif will be 100% complementary to the given fraction of target sequences within a genotype. All oligomer motifs were selected such that they will essentially target almost all sequences within genotypes A, B, C and D, thereby allowing for treatment of individual infected with any of these four genotypes.

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 29		X	201	AAAACCCCGCCTGT	0.97	0.98	0.95	0.99
SeqID 30		X	202	AAAACCCCGCCTG	0.96	0.97	0.97	0.97
SeqID 31		X	245	ACGAGTCTAGACTCT	0.96	0.97	0.97	0.97
SeqID 32		X	245	CACGAGTCTAGACTCT	0.96	0.97	0.97	0.97
SeqID 33		X	246	ACGAGTCTAGACTC	0.96	0.97	0.97	0.97
SeqID 34		X	246	CACGAGTCTAGACTC	0.96	0.97	0.97	0.97
SeqID 35		X	246	CCACGAGTCTAGACTC	0.96	0.97	0.97	0.97
SeqID 36		X	247	ACGAGTCTAGACT	0.96	0.97	0.97	0.97
SeqID 37		X	247	CACGAGTCTAGACT	0.96	0.97	0.97	0.97
SeqID 38		X	247	CCACGAGTCTAGACT	0.96	0.97	0.97	0.97
SeqID 39		X	247	ACCACGAGTCTAGACT	0.96	0.98	0.97	0.98
SeqID 40		X	248	ACGAGTCTAGAC	0.96	0.97	0.97	0.98
SeqID 41		X	248	CACGAGTCTAGAC	0.96	0.97	0.97	0.98
SeqID 42		X	248	CCACGAGTCTAGAC	0.96	0.97	0.97	0.97
SeqID 43		X	248	ACCACGAGTCTAGAC	0.96	0.97	0.96	0.97
SeqID 44		X	248	CACCACGAGTCTAGAC	0.99	0.97	0.97	0.98
SeqID 45		X	249	ACCACGAGTCTAGA	0.99	0.97	0.97	0.98
SeqID 46		X	249	CACCACGAGTCTAGA	0.99	0.97	0.97	0.98
SeqID 47		X	249	CCACCACGAGTCTAGA	0.99	0.98	0.97	0.98
SeqID 48		X	250	CCACCACGAGTCTAG	0.99	0.98	0.97	0.98
SeqID 49		X	250	TCCACCACGAGTCTAG	0.99	0.98	0.97	0.98
SeqID 50		X	251	CCACCACGAGTCTA	0.99	0.98	0.97	0.98
SeqID 51		X	251	TCCACCACGAGTCTA	0.98	0.97	0.97	0.98
SeqID 52		X	251	GTCCACCACGAGTCTA	0.99	0.98	0.97	0.98
SeqID 53		X	252	TCCACCACGAGTCT	0.98	0.97	0.97	0.98
SeqID 54		X	252	GTCCACCACGAGTCT	0.98	0.97	0.97	0.98
SeqID 55		X	252	AGTCCACCACGAGTCT	0.98	0.97	0.97	0.98
SeqID 56		X	253	GTCCACCACGAGTC	0.98	0.97	0.97	0.98
SeqID 57		X	253	AGTCCACCACGAGTC	0.98	0.97	0.97	0.98
SeqID 58		X	253	AAGTCCACCACGAGTC	0.98	0.97	0.98	0.99
SeqID 59		X	254	AGTCCACCACGAGT	0.98	0.97	0.98	0.99
SeqID 60		X	254	AAGTCCACCACGAGT	0.98	0.97	0.97	0.99

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 61		X	254	GAAGTCCACCACGAGT	0.98	0.97	0.98	0.99
SeqID 62		X	255	AAGTCCACCACGAG	0.98	0.97	0.98	0.99
SeqID 63		X	255	GAAGTCCACCACGAG	0.97	0.97	0.98	0.99
SeqID 64		X	255	AGAAGTCCACCACGAG	0.97	0.97	0.98	0.99
SeqID 65		X	256	AGAAGTCCACCACGA	0.97	0.97	0.98	0.99
SeqID 66		X	256	GAGAAGTCCACCACGA	0.97	0.97	0.98	0.99
SeqID 67		X	257	GAGAAGTCCACCACG	0.97	0.97	0.97	0.98
SeqID 68		X	257	AGAGAAGTCCACCACG	0.98	0.99	0.98	0.99
SeqID 69		X	258	GAGAGAAGTCCACCAC	0.98	0.99	0.98	0.99
SeqID 70		X	259	GAGAGAAGTCCACCA	0.98	0.99	0.98	0.99
SeqID 71		X	259	TGAGAGAAGTCCACCA	0.98	0.99	0.98	0.99
SeqID 72		X	260	GAGAGAAGTCCACC	0.98	0.99	0.98	0.99
SeqID 73		X	260	TGAGAGAAGTCCACC	0.98	0.99	0.98	0.99
SeqID 74		X	261	TGAGAGAAGTCCAC	0.99	0.96	0.95	0.98
SeqID 75		X	384	AAAACGCCGCAGA	0.98	0.96	0.95	0.98
SeqID 76		X	384	TAAAACGCCGCAGA	0.98	0.96	0.95	0.97
SeqID 77		X	384	ATAAAACGCCGCAGA	0.98	0.96	0.95	0.97
SeqID 78		X	384	GATAAAACGCCGCAGA	0.98	0.97	0.96	0.97
SeqID 79		X	385	ATAAAACGCCGCAG	0.98	0.97	0.96	0.97
SeqID 80		X	385	GATAAAACGCCGCAG	0.98	0.97	0.96	0.97
SeqID 81		X	385	TGATAAAACGCCGCAG	0.98	0.97	0.96	0.98
SeqID 82		X	386	ATAAAACGCCGCA	0.98	0.97	0.96	0.98
SeqID 83		X	386	GATAAAACGCCGCA	0.98	0.97	0.96	0.98
SeqID 84		X	386	TGATAAAACGCCGCA	0.98	0.97	0.96	0.98
SeqID 85		X	386	ATGATAAAACGCCGCA	0.98	0.98	0.97	0.98
SeqID 86		X	387	ATAAAACGCCGC	0.98	0.98	0.97	0.98
SeqID 87		X	387	GATAAAACGCCGC	0.98	0.97	0.97	0.98
SeqID 88		X	387	TGATAAAACGCCGC	0.98	0.97	0.97	0.98
SeqID 89		X	387	ATGATAAAACGCCGC	0.98	0.98	0.97	0.98
SeqID 90		X	388	GATAAAACGCCG	0.98	0.97	0.97	0.98
SeqID 91		X	388	TGATAAAACGCCG	0.98	0.97	0.97	0.98
SeqID 92		X	388	ATGATAAAACGCCG	0.98	0.97	0.97	0.98
SeqID 93		X	389	TGATAAAACGCC	0.98	0.97	0.97	0.98
SeqID 94		X	389	ATGATAAAACGCC	0.98	0.97	0.97	0.98
SeqID 95		X	390	ATGATAAAACGC	1.00	0.99	0.98	0.97
SeqID 96		X	411	TAGCAGCAGGATG	1.00	0.99	0.98	0.97
SeqID 97		X	411	ATAGCAGCAGGATG	1.00	0.99	0.98	0.97
SeqID 98		X	411	CATAGCAGCAGGATG	1.00	0.98	0.98	0.97
SeqID 99		X	411	GCATAGCAGCAGGATG	1.00	0.99	0.98	0.97
SeqID 100		X	412	GCATAGCAGCAGGAT	1.00	0.99	0.98	0.97

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 101		X	412	GGCATAGCAGCAGGAT	0.99	0.98	0.98	0.97
SeqID 102		X	414	GAGGCATAGCAGCAGG	0.99	0.98	0.98	0.97
SeqID 103		X	415	TGAGGCATAGCAGCAG	0.99	0.98	0.98	0.97
SeqID 104		X	416	TGAGGCATAGCAGCA	0.99	0.97	0.96	0.96
SeqID 105		X	416	ATGAGGCATAGCAGCA	0.99	0.98	0.98	0.97
SeqID 106		X	417	TGAGGCATAGCAGC	0.99	0.97	0.96	0.96
SeqID 107		X	417	ATGAGGCATAGCAGC	0.99	0.97	0.96	0.96
SeqID 108		X	417	GATGAGGCATAGCAGC	1.00	0.97	0.96	0.96
SeqID 109		X	418	GATGAGGCATAGCAG	1.00	0.97	0.96	0.96
SeqID 110		X	418	AGATGAGGCATAGCAG	1.00	0.97	0.97	0.96
SeqID 111		X	419	GATGAGGCATAGCA	1.00	0.97	0.97	0.96
SeqID 112		X	419	AGATGAGGCATAGCA	1.00	0.97	0.97	0.96
SeqID 113		X	419	AAGATGAGGCATAGCA	1.00	0.96	0.97	0.98
SeqID 114		X	422	AAGAAGATGAGGCATA	1.00	0.96	0.97	0.98
SeqID 115		X	423	AAGAAGATGAGGCAT	0.98	0.99	1.00	0.99
SeqID 116		X	601	TGGGATGGGAATACA	0.98	0.99	0.99	0.99
SeqID 117		X	601	ATGGGATGGGAATACA	0.98	0.99	1.00	0.99
SeqID 118		X	602	TGGGATGGGAATAC	0.98	0.99	1.00	0.99
SeqID 119		X	602	ATGGGATGGGAATAC	0.97	0.99	0.99	0.99
SeqID 120		X	602	GATGGGATGGGAATAC	0.98	0.99	1.00	0.99
SeqID 121		X	603	ATGGGATGGGAATA	0.97	0.99	0.99	0.99
SeqID 122		X	603	GATGGGATGGGAATA	0.97	0.99	0.99	0.99
SeqID 123		X	604	GATGGGATGGGAAT	0.99	0.97	0.96	0.98
SeqID834			670	TAGTAAACTGAGCCA				
SeqID835			670	CTAGTAAACTGAGCCA				
SeqID836			671	CTAGTAAACTGAGCC				
SeqID837			674	GCACTAGTAAACTGA				
SeqID838			674	GGCACTAGTAAACTGA				
SeqID 124		X	691	AACCACTGAACAAA	0.99	0.97	0.96	0.98
SeqID 4		X	691	GAACCACTGAACAAA	0.99	0.97	0.96	0.98
SeqID 5		X	691	CGAACCACTGAACAAA	0.99	0.97	0.96	0.98
SeqID 6		X	692	CGAACCACTGAACAA	0.99	0.97	0.96	0.98
SeqID 7		X	693	CGAACCACTGAACA	0.99	0.98	0.96	0.98
SeqID 8		X	694	CGAACCACTGAAC	0.99	0.98	0.96	0.98
SeqID 125		X	695	CGAACCACTGAA	0.98	0.97	0.98	0.98
SeqID 126		X	708	GGGGGAAAGCCCT	0.97	0.97	0.98	0.98
SeqID 127		X	708	TGGGGGAAAGCCCT	0.99	0.97	0.97	0.99
SeqID839			1141	CAACGGGGTAAAGGT				
SeqID 128		X	1142	GCAACGGGGTAAAGG	0.99	0.97	0.97	0.99
SeqID 129		X	1143	GCAACGGGGTAAAG	0.99	0.97	0.97	0.99

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 130		X	1144	GCAACGGGGTAAA	0.99	0.98	0.97	0.99
SeqID 131		X	1176	AGCAAACACTTGGCA	0.99	0.98	0.97	0.99
SeqID 132		X	1176	CAGCAAACACTTGGCA	0.99	0.98	0.97	0.99
SeqID 133		X	1177	CAGCAAACACTTGGC	0.99	0.98	0.97	0.99
SeqID 134		X	1177	TCAGCAAACACTTGGC	0.99	0.98	0.97	0.99
SeqID 135		X	1178	TCAGCAAACACTTGG	0.98	0.96	0.96	0.97
SeqID840			1261	CAGTATGGATCGGCA				
SeqID 136	x		1264	GCAGTATGGATCG	0.98	0.95	0.96	0.97
SeqID 137	x		1264	CGCAGTATGGATCG	0.98	0.95	0.96	0.97
SeqID 9	x		1264	CCGCAGTATGGATCG	0.98	0.95	0.96	0.97
SeqID 138	x		1264	TCCGCAGTATGGATCG	0.98	0.95	0.96	0.97
SeqID832			1264	CCGCAGTATGGATCG				
SeqID 10	x		1265	CGCAGTATGGATC	0.98	0.95	0.96	0.97
SeqID 139	x		1265	CCGCAGTATGGATC	0.98	0.95	0.96	0.97
SeqID 140	x		1265	TCCGCAGTATGGATC	0.99	0.96	0.97	0.97
SeqID841			1265	TTCCGCAGTATGGATC				
SeqID842			1266	TTCCGCAGTATGGAT				
SeqID843			1266	GTTCCGCAGTATGGAT				
SeqID 141	x		1266	CGCAGTATGGAT	0.99	0.96	0.97	0.97
SeqID 142	x		1266	CCGCAGTATGGAT	0.99	0.96	0.97	0.97
SeqID 143	x		1266	TCCGCAGTATGGAT	0.99	0.96	0.97	0.97
SeqID 144	x		1267	TCCGCAGTATGGA	0.99	0.95	0.97	0.97
SeqID844			1267	GTTCCGCAGTATGGA				
SeqID845			1267	AGTTCCGCAGTATGGA				
SeqID846			1268	AGTTCCGCAGTATGG				
SeqID847			1268	GAGTTCCGCAGTATGG				
SeqID848			1269	GAGTTCCGCAGTATG				
SeqID849			1269	GGAGTTCCGCAGTATG				
SeqID 145	x		1269	TTCCGCAGTATG	0.99	0.99	0.99	0.99
SeqID850			1525	TAAAGAGAGGTGCGCC				
SeqID851			1526	TAAAGAGAGGTGCGC				
SeqID852			1526	GTAAAGAGAGGTGCGC				
SeqID853			1527	GTAAAGAGAGGTGCG				
SeqID854			1527	CGTAAAGAGAGGTGCG				
SeqID855			1528	CGTAAAGAGAGGTGC				
SeqID856			1528	GCGTAAAGAGAGGTGC				
SeqID857			1529	GCGTAAAGAGAGGTG				
SeqID858			1529	CGCGTAAAGAGAGGTG				
SeqID 146	x		1530	CGTAAAGAGAGGT	0.99	0.98	0.99	0.99
SeqID 11	x		1530	GCGTAAAGAGAGGT	0.99	0.98	0.99	0.99

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 12	x		1530	CGCGTAAAGAGAGGT	0.99	0.98	0.99	0.99
SeqID 147	x		1530	CCGCGTAAAGAGAGGT	0.99	0.98	0.99	0.99
SeqID 148	x		1531	CGTAAAGAGAGG	0.99	0.98	0.99	0.99
SeqID 13	x		1531	GCGTAAAGAGAGG	0.99	0.98	0.99	0.99
SeqID 149	x		1531	CGCGTAAAGAGAGG	0.99	0.98	0.99	0.99
SeqID 150	x		1531	CCGCGTAAAGAGAGG	0.99	0.98	0.99	1.00
SeqID 151	x		1532	CGCGTAAAGAGAG	0.99	0.98	0.99	1.00
SeqID 152	x		1532	CCGCGTAAAGAGAG	0.99	0.98	0.99	1.00
SeqID 153	x		1533	CGCGTAAAGAGA	0.99	0.98	0.99	1.00
SeqID 154	x		1533	CCGCGTAAAGAGA	0.99	0.99	0.99	1.00
SeqID 155	x		1534	CCGCGTAAAGAG	0.98	0.98	0.99	1.00
SeqID 156	x		1547	GGCACAGACGGGGAG	0.98	0.98	0.99	1.00
SeqID 157	x		1547	AGGCACAGACGGGGAG	0.98	0.99	0.99	1.00
SeqID 158	x		1548	GGCACAGACGGGGA	0.98	0.98	0.99	1.00
SeqID 159	x		1548	AGGCACAGACGGGGA	0.98	0.98	0.99	1.00
SeqID 160	x		1548	AAGGCACAGACGGGGA	0.98	0.98	0.99	1.00
SeqID 161	x		1549	AGGCACAGACGGGG	0.98	0.98	0.99	1.00
SeqID 162	x		1549	AAGGCACAGACGGGG	0.98	0.97	0.99	0.99
SeqID 163	x		1549	GAAGGCACAGACGGGG	0.97	0.98	0.98	0.98
SeqID 164	x		1550	AGAAGGCACAGACGGG	0.97	0.98	0.98	0.98
SeqID 14	x		1551	AGAAGGCACAGACGG	0.97	0.98	0.98	0.98
SeqID 15	x		1551	GAGAAGGCACAGACGG	0.97	0.98	0.98	0.98
SeqID 165	x		1552	GAGAAGGCACAGACG	0.99	0.99	0.99	0.98
SeqID859			1552	TGAGAAGGCACAGACG				
SeqID 16	x		1577	GAAGTGCACACGG	0.99	0.99	0.99	0.98
SeqID 166	x		1577	CGAAGTGCACACGG	0.98	0.99	0.99	0.98
SeqID 17	x		1577	GCGAAGTGCACACGG	0.98	0.99	0.99	0.96
SeqID 18	x		1577	AGCGAAGTGCACACGG	0.99	0.99	0.99	0.98
SeqID 19	x		1578	CGAAGTGCACACG	0.98	0.99	0.99	0.98
SeqID 167	x		1578	GCGAAGTGCACACG	0.98	0.99	0.99	0.96
SeqID 20	x		1578	AGCGAAGTGCACACG	0.98	0.99	0.99	0.96
SeqID 21	x		1578	AAGCGAAGTGCACACG	0.98	0.99	0.99	0.98
SeqID 168	x		1579	GCGAAGTGCACAC	0.98	0.99	0.99	0.96
SeqID 169	x		1579	AGCGAAGTGCACAC	0.98	0.99	0.99	0.96
SeqID 170	x		1579	AAGCGAAGTGCACAC	0.98	0.99	0.99	0.96
SeqID 171	x		1579	GAAGCGAAGTGCACAC	0.98	0.99	0.99	0.96
SeqID 172	x		1580	AGCGAAGTGCACA	0.98	0.99	0.99	0.96
SeqID 173	x		1580	AAGCGAAGTGCACA	0.98	0.99	0.99	0.96
SeqID 22	x		1580	GAAGCGAAGTGCACA	0.98	0.99	0.99	0.96

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 174	x		1580	TGAAGCGAAGTGCACA	0.98	0.99	0.99	0.96
SeqID 175	x		1581	AAGCGAAGTGCAC	0.98	0.99	0.99	0.96
SeqID 176	x		1581	GAAGCGAAGTGCAC	0.98	0.99	0.99	0.96
SeqID 177	x		1581	TGAAGCGAAGTGCAC	0.98	0.99	0.99	0.96
SeqID 178	x		1581	GTGAAGCGAAGTGCAC	0.98	0.99	0.99	0.96
SeqID 179	x		1582	AAGCGAAGTGCA	0.98	0.99	0.99	0.96
SeqID 180	x		1582	GAAGCGAAGTGCA	0.98	0.99	0.99	0.96
SeqID 181	x		1582	TGAAGCGAAGTGCA	0.98	0.99	0.99	0.96
SeqID 182	x		1582	GTGAAGCGAAGTGCA	0.98	0.99	0.99	0.96
SeqID 23	x		1582	GGTGAAGCGAAGTGCA	0.98	0.99	0.99	0.96
SeqID 183	x		1583	TGAAGCGAAGTGC	0.98	0.99	0.99	0.96
SeqID 184	x		1583	GTGAAGCGAAGTGC	0.98	0.99	0.99	0.96
SeqID 24	x		1583	GGTGAAGCGAAGTGC	0.98	0.99	0.99	0.96
SeqID 25	x		1583	AGGTGAAGCGAAGTGC	0.98	0.99	0.99	0.96
SeqID 185	x		1584	GTGAAGCGAAGTG	0.98	0.99	0.99	0.96
SeqID 186	x		1584	GGTGAAGCGAAGTG	0.98	0.99	0.99	0.96
SeqID 26	x		1584	AGGTGAAGCGAAGTG	0.98	0.99	0.99	0.96
SeqID 187	x		1584	GAGGTGAAGCGAAGTG	0.98	0.99	0.99	0.96
SeqID 188	x		1585	GTGAAGCGAAGT	0.98	0.99	0.99	0.96
SeqID 189	x		1585	GGTGAAGCGAAGT	0.98	0.99	0.99	0.96
SeqID 27	x		1585	AGGTGAAGCGAAGT	0.98	0.99	0.99	0.96
SeqID 190	x		1585	GAGGTGAAGCGAAGT	0.98	0.99	0.99	0.96
SeqID 191	x		1585	AGAGGTGAAGCGAAGT	0.98	0.99	0.99	0.96
SeqID 192	x		1586	AGAGGTGAAGCGAAG	0.98	0.99	0.99	0.96
SeqID 193	x		1586	CAGAGGTGAAGCGAAG	0.99	0.99	0.99	0.96
SeqID 194	x		1587	AGAGGTGAAGCGAA	0.99	0.99	0.99	0.96
SeqID 195	x		1587	CAGAGGTGAAGCGAA	0.99	0.99	0.98	0.96
SeqID 196	x		1587	GCAGAGGTGAAGCGAA	0.99	0.99	0.99	0.97
SeqID 28	x		1588	CAGAGGTGAAGCGA	0.99	0.99	0.98	0.96
SeqID 197	x		1588	GCAGAGGTGAAGCGA	0.99	0.99	0.98	0.96
SeqID 198	x		1588	TGCAGAGGTGAAGCGA	0.99	0.99	0.98	0.96
SeqID 199	x		1589	TGCAGAGGTGAAGCG	0.99	0.99	0.98	0.96
SeqID 200	x		1589	GTGCAGAGGTGAAGCG	0.99	0.99	0.98	0.96
SeqID 201	x		1590	CGTGCAGAGGTGAAGC	0.99	0.99	0.98	0.96
SeqID 202	x		1591	CGTGCAGAGGTGAAG	0.99	0.99	0.98	0.96
SeqID 203	x		1591	ACGTGCAGAGGTGAAG	1.00	0.99	0.98	0.96
SeqID 204	x		1592	CGTGCAGAGGTGAA	1.00	0.99	0.98	0.96
SeqID 205	x		1592	ACGTGCAGAGGTGAA	1.00	0.99	0.98	0.99
SeqID 206	x		1593	CGTGCAGAGGTGA	1.00	0.99	0.98	0.99
SeqID 207	x		1593	ACGTGCAGAGGTGA	0.98	0.97	0.96	0.97

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 208	x		1616	CGTTCACGGTGGT	0.98	0.96	0.96	0.95
SeqID 209	x		1690	CTCAAGGTCGGTC	0.99	0.97	0.98	0.96
SeqID860			1690	GCCTCAAGGTCGGTC				
SeqID 210	x		1691	CCTCAAGGTCGGT	0.99	0.97	0.98	0.95
SeqID 211	x		1691	GCCTCAAGGTCGGT	0.98	0.97	0.98	0.99
SeqID 212	x		1706	ACAGTCTTTGAAGTA	0.99	0.95	0.95	0.99
SeqID861			1778	ATGCCTACAGCCTCC				
SeqID 213	x		1783	TTTATGCCTACAG	0.99	0.96	0.95	0.99
SeqID 214	x		1784	AATTTATGCCTACA	0.99	0.96	0.95	0.99
SeqID 215	x		1785	AATTTATGCCTAC	0.99	0.95	0.96	0.99
SeqID862			1785	ACCAATTTATGCCTAC				
SeqID 216	x		1787	CCAATTTATGCCT	0.97	0.99	0.99	0.98
SeqID 217	x		1865	GCTTGGAGGCTTGAA	0.97	0.99	0.98	0.98
SeqID 218	x		1865	AGCTTGGAGGCTTGAA	0.97	0.99	0.99	0.98
SeqID 219	x		1866	GCTTGGAGGCTTGA	0.97	0.99	0.99	0.98
SeqID 220	x		1866	AGCTTGGAGGCTTGA	0.97	0.98	0.98	0.98
SeqID 221	x		1866	CAGCTTGGAGGCTTGA	0.97	0.99	0.99	0.98
SeqID 222	x		1867	GCTTGGAGGCTTG	0.97	0.99	0.99	0.98
SeqID 223	x		1867	AGCTTGGAGGCTTG	0.97	0.98	0.98	0.98
SeqID 224	x		1867	CAGCTTGGAGGCTTG	0.97	0.98	0.98	0.98
SeqID 225	x		1867	ACAGCTTGGAGGCTTG	0.97	0.98	0.98	0.98
SeqID 226	x		1868	CACAGCTTGGAGGCTT	0.97	0.98	0.98	0.98
SeqID 227	x		1869	CACAGCTTGGAGGCT	0.97	0.98	0.98	0.98
SeqID 228	x		1869	GCACAGCTTGGAGGCT	0.97	0.98	0.98	0.98
SeqID 229	x		1870	GCACAGCTTGGAGGC	0.97	0.98	0.98	0.98
SeqID 230	x		1870	GGCACAGCTTGGAGGC	0.96	0.98	0.98	0.98
SeqID 231	x		1871	AGGCACAGCTTGGAGG	0.96	0.98	0.98	0.99
SeqID 232	x		1872	AGGCACAGCTTGGAG	0.96	0.97	0.98	0.99
SeqID 233	x		1872	AAGGCACAGCTTGGAG	0.96	0.97	0.98	0.99
SeqID 234	x		1873	AAGGCACAGCTTGGA	0.96	0.97	0.98	0.98
SeqID 235	x		1873	CAAGGCACAGCTTGGA	0.96	0.97	0.98	0.99
SeqID 236	x		1874	AAGGCACAGCTTGG	0.96	0.97	0.98	0.99
SeqID 237	x		1874	CAAGGCACAGCTTGG	0.96	0.97	0.97	0.98
SeqID 238	x		1874	CCAAGGCACAGCTTGG	0.96	0.97	0.98	0.99
SeqID 239	x		1875	CAAGGCACAGCTTG	0.96	0.97	0.97	0.98
SeqID 240	x		1875	CCAAGGCACAGCTTG	0.96	0.97	0.97	0.99
SeqID 241	x		1876	CCAAGGCACAGCTT	0.96	0.97	0.96	0.97
SeqID 242			2272	TGCGAATCCACAC	0.96	0.97	0.96	0.97
SeqID 243			2272	GTGCGAATCCACAC	0.96	0.96	0.98	0.97
SeqID 244			2370	GGAGTTCTTCTTCTA	0.96	0.96	0.98	0.97

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 245			2370	GGGAGTTCTTCTTCTA	0.96	0.96	0.98	0.97
SeqID 246			2371	GGGAGTTCTTCTTCT	0.96	0.96	0.98	0.97
SeqID 247			2371	AGGGAGTTCTTCTTCT	0.96	0.98	0.98	0.97
SeqID 248			2372	AGGGAGTTCTTCTTC	0.96	0.98	0.97	0.97
SeqID 249			2372	GAGGGAGTTCTTCTTC	0.97	0.98	0.98	0.97
SeqID 250			2373	AGGGAGTTCTTCTT	0.97	0.98	0.98	0.97
SeqID 251			2373	GAGGGAGTTCTTCTT	0.97	0.95	0.97	0.95
SeqID 252			2373	CGAGGGAGTTCTTCTT	0.97	0.95	0.97	0.96
SeqID 253			2374	CGAGGGAGTTCTTCT	0.97	0.95	0.97	0.96
SeqID 254			2374	GCGAGGGAGTTCTTCT	0.97	0.96	0.97	0.96
SeqID 255			2375	GCGAGGGAGTTCTTC	0.97	0.96	0.97	0.96
SeqID 256			2375	GGCGAGGGAGTTCTTC	0.97	0.96	0.97	0.96
SeqID 257			2376	GCGAGGGAGTTCTT	0.97	0.96	0.97	0.96
SeqID 258			2376	GGCGAGGGAGTTCTT	0.97	0.96	0.97	0.96
SeqID 259			2376	AGGCGAGGGAGTTCTT	0.98	0.96	0.97	0.96
SeqID 260			2377	GCGAGGGAGTTCT	0.98	0.96	0.97	0.96
SeqID 261			2377	GGCGAGGGAGTTCT	0.98	0.96	0.97	0.96
SeqID 262			2377	AGGCGAGGGAGTTCT	0.98	0.96	0.97	0.96
SeqID 263			2377	GAGGCGAGGGAGTTCT	0.99	0.96	0.98	0.97
SeqID 264			2378	GGCGAGGGAGTTC	0.99	0.96	0.98	0.97
SeqID 265			2378	AGGCGAGGGAGTTC	0.99	0.96	0.98	0.97
SeqID 266			2378	GAGGCGAGGGAGTTC	0.99	0.96	0.97	0.97
SeqID 267			2378	CGAGGCGAGGGAGTTC	0.99	0.96	0.98	0.97
SeqID 268			2379	AGGCGAGGGAGTT	0.99	0.96	0.98	0.97
SeqID 269			2379	GAGGCGAGGGAGTT	0.99	0.96	0.98	0.97
SeqID 270			2379	CGAGGCGAGGGAGTT	0.99	0.96	0.98	0.97
SeqID 271			2379	GCGAGGCGAGGGAGTT	0.99	0.96	0.98	0.97
SeqID 272			2380	GAGGCGAGGGAGT	0.99	0.96	0.98	0.97
SeqID 273			2380	CGAGGCGAGGGAGT	0.99	0.96	0.98	0.97
SeqID 274			2380	GCGAGGCGAGGGAGT	0.99	0.96	0.97	0.96
SeqID 275			2380	TGCGAGGCGAGGGAGT	0.99	0.96	0.98	0.97
SeqID 276			2381	CGAGGCGAGGGAG	0.99	0.96	0.98	0.97
SeqID 277			2381	GCGAGGCGAGGGAG	0.99	0.96	0.97	0.97
SeqID 278			2381	TGCGAGGCGAGGGAG	0.97	0.96	0.96	0.96
SeqID 279			2381	CTGCGAGGCGAGGGAG	0.99	0.96	0.98	0.97
SeqID 280			2382	CGAGGCGAGGGA	0.99	0.96	0.98	0.97
SeqID 281			2382	GCGAGGCGAGGGA	0.99	0.96	0.97	0.97
SeqID 282			2382	TGCGAGGCGAGGGA	0.97	0.96	0.96	0.96
SeqID 283			2382	CTGCGAGGCGAGGGA	0.97	0.96	0.96	0.96
SeqID 284			2382	TCTGCGAGGCGAGGGA	0.97	0.96	0.96	0.96
SeqID 285			2383	TCTGCGAGGCGAGGG	0.97	0.96	0.96	0.96

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligosequence motif	Fraction of conservation within all sequences of genotype			
					A	B	C	D
SeqID 286			2383	GTCTGCGAGGCGAGGG	0.98	0.97	0.96	0.95
SeqID 287			2824	GTTCCCAAGAATAT	0.98	0.97	0.96	0.95
SeqID 288			2824	TGTTCCCAAGAATAT	0.98	0.98	0.97	0.96
SeqID 289			2825	GTTCCCAAGAATA	0.98	0.98	0.97	0.96
SeqID 290			2825	TGTTCCCAAGAATA	0.97	0.97	0.96	0.96
SeqID 291			2825	TTGTTCCCAAGAATA	0.98	0.98	0.97	0.96
SeqID 292			2826	TGTTCCCAAGAAT	0.97	0.97	0.96	0.96
SeqID 293			2826	TTGTTCCCAAGAAT				

Table 2 A subset of oligomer motifs from table 1

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 4		X	691	GAACCACTGAACAAA
SeqID 5		X	691	CGAACCACTGAACAAA
SeqID 6		X	692	CGAACCACTGAACAA
SeqID 7		X	693	CGAACCACTGAACA
SeqID 8		X	694	CGAACCACTGAAC
SeqID 9	X		1264	CCGCAGTATGGATCG
SeqID 10	X		1265	CGCAGTATGGATC
SeqID 11	X		1530	GCGTAAAGAGAGGT
SeqID 12	X		1530	CGCGTAAAGAGAGGT
SeqID 13	X		1531	GCGTAAAGAGAGG
SeqID 14	X		1551	AGAAGGCACAGACGG
SeqID 15	X		1551	GAGAAGGCACAGACGG
SeqID 16	X		1577	GAAGTGCACACGG
SeqID 17	X		1577	GCGAAGTGCACACGG
SeqID 18	X		1577	AGCGAAGTGCACACGG
SeqID 19	X		1578	CGAAGTGCACACG
SeqID 20	X		1578	AGCGAAGTGCACACG
SeqID 21	X		1578	AAGCGAAGTGCACACG
SeqID 22	X		1580	GAAGCGAAGTGCACA
SeqID 23	X		1582	GGTGAAGCGAAGTGCA
SeqID 24	X		1583	GGTGAAGCGAAGTGC
SeqID 25	X		1583	AGGTGAAGCGAAGTGC
SeqID 26	X		1584	AGGTGAAGCGAAGTG
SeqID 27	X		1585	AGGTGAAGCGAAGT
SeqID 28	X		1588	CAGAGGTGAAGCGA

Table 3 LNA oligomers

Upper case letters denote beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters denote DNA, ^{m}c denotes a 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages.

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 294		x	691	GAAccactgaacAAA
SeqID 295		x	691	CGAaccactgaacAAA
SeqID 296		x	692	CGAaccactgaaCAA
SeqID 297		x	693	CGAaccactgaACA
SeqID 298		x	694	CGAaccactgaAC
SeqID 299	x		1264	CCGcagtatggaTCG
SeqID 300	x		1265	CGCagtatggaTC
SeqID 301	x		1530	GCGtaaagagaGGT
SeqID 302	x		1530	CGCgtaaagagaGGT
SeqID 303	x		1531	GCGtaaagagaGG
SeqID 304	x		1551	AGAaggcacagaCGG
SeqID 305	x		1551	GAGaaggcacagaCGG
SeqID 306	x		1577	GAAgtgcaca^{m}cGG
SeqID 307	x		1577	GCGaagtgcacaCGG
SeqID 308	x		1577	AGCgaagtgcacaCGG
SeqID 309	x		1578	CGAagtgcacaCG
SeqID 310	x		1578	AGCgaagtgcacACG
SeqID 311	x		1578	AAG^{m}cgaagtgcacACG
SeqID 312	x		1580	GAAg^{m}cgaagtgcACA
SeqID 313	x		1582	GGTgaag^{m}cgaagtGCA
SeqID 314	x		1583	GGTgaag^{m}cgaagTGC
SeqID 315	x		1583	AGGtgaag^{m}cgaagTGC
SeqID 316	x		1584	AGGtgaag^{m}cgaaGTG
SeqID 317	x		1585	AGGtgaag^{m}cgaAGT
SeqID 318	x		1588	CAGaggtgaagCGA
SeqID 319		x	201	AAAaccc^{m}cgccTGT
SeqID 320		x	202	AAAaccc^{m}cgccTG
SeqID 321		x	245	ACGagtctagacTCT
SeqID 322		x	245	CACgagtctagacTCT
SeqID 323		x	246	ACGagtctagaCTC
SeqID 324		x	246	CACgagtctagaCTC
SeqID 325		x	246	CCA^{m}cgagtctagaCTC
SeqID 326		x	247	ACGagtctagaCT
SeqID 327		x	247	CACgagtctagACT
SeqID 328		x	247	CCA^{m}cgagtctagACT
SeqID 329		x	247	ACCa^{m}cgagtctagACT
SeqID 330		x	248	ACgagtctagAC
SeqID 331		x	248	CACgagtctagAC
SeqID 332		x	248	CCA^{m}cgagtctaGAC
SeqID 333		x	248	ACCa^{m}cgagtctaGAC
SeqID 334		x	248	CACca^{m}cgagtctaGAC

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 335		x	249	ACCa^{m}cgagtctAGA
SeqID 336		x	249	CACca^{m}cgagtctAGA
SeqID 337		x	249	CCAcca^{m}cgagtctAGA
SeqID 338		x	250	CCAcca^{m}cgagtcTAG
SeqID 339		x	250	TCCacca^{m}cgagtcTAG
SeqID 340		x	251	CCAcca^{m}cgagtCTA
SeqID 341		x	251	TCCacca^{m}cgagtCTA
SeqID 342		x	251	GTCcacca^{m}cgagtCTA
SeqID 343		x	252	TCCacca^{m}cgagTCT
SeqID 344		x	252	GTCcacca^{m}cgagTCT
SeqID 345		x	252	AGTccacca^{m}cgagTCT
SeqID 346		x	253	GTCcacca^{m}cgaGTC
SeqID 347		x	253	AGTccacca^{m}cgaGTC
SeqID 348		x	253	AAGtccacca^{m}cgaGTC
SeqID 349		x	254	AGTccacca^{m}cgAGT
SeqID 350		x	254	AAGtccacca^{m}cgAGT
SeqID 351		x	254	GAAgtccacca^{m}cgAGT
SeqID 352		x	255	AAGtccacca^{m}cgAG
SeqID 353		x	255	GAAgtccacca^{m}cgAG
SeqID 354		x	255	AGAagtccacca^{m}cgAG
SeqID 355		x	256	AGAagtccaccaCGA
SeqID 356		x	256	GAGaagtccaccaCGA
SeqID 357		x	257	GAGaagtccaccACG
SeqID 358		x	257	AGAgaagtccaccACG
SeqID 359		x	258	GAGagaagtccacCAC
SeqID 360		x	259	GAGagaagtccaCCA
SeqID 361		x	259	TGAgagaagtccaCCA
SeqID 362		x	260	GAGagaagtccACC
SeqID 363		x	260	TGAgagaagtccACC
SeqID 364		x	261	TGAgagaagtcCAC
SeqID 365		x	384	AAAa^{m}cgc^{m}cgcaGA
SeqID 366		x	384	TAAaa^{m}cgc^{m}cgcAGA
SeqID 367		x	384	ATAaaa^{m}cgc^{m}cgcAGA
SeqID 368		x	384	GATaaaa^{m}cgc^{m}cgcAGA
SeqID 369		x	385	ATAaaa^{m}cgc^{m}cgCAG
SeqID 370		x	385	GATaaaa^{m}cgc^{m}cgCAG
SeqID 371		x	385	TGAtaaaa^{m}cgc^{m}cgCAG
SeqID 372		x	386	ATAaaa^{m}cgc^{m}cgCA
SeqID 373		x	386	GATaaaa^{m}cgc^{m}cGCA
SeqID 374		x	386	TGAtaaaa^{m}cgc^{m}cGCA
SeqID 375		x	386	ATGataaaa^{m}cgc^{m}cGCA
SeqID 376		x	387	ATaaaa^{m}cgc^{m}cGC
SeqID 377		x	387	GATaaaa^{m}cgc^{m}cGC
SeqID 378		x	387	TGAtaaaa^{m}cgcCGC
SeqID 379		x	387	ATGataaaa^{m}cgcCGC

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 380		x	388	GAtaaaa^{m}cgcCG
SeqID 381		x	388	TGAtaaaa^{m}cgcCG
SeqID 382		x	388	ATGataaaa^{m}cgCCG
SeqID 383		x	389	TGataaaa^{m}cgCC
SeqID 384		x	389	ATGataaaa^{m}cgCC
SeqID 385		x	390	ATgataaaa^{m}cGC
SeqID 386		x	411	TAGcagcaggaTG
SeqID 387		x	411	ATAgcagcaggATG
SeqID 388		x	411	CATagcagcaggATG
SeqID 389		x	411	GCAtagcagcaggATG
SeqID 390		x	412	GCAtagcagcagGAT
SeqID 391		x	412	GGCatagcagcagGAT
SeqID 392		x	414	GAGgcatagcagcAGG
SeqID 393		x	415	TGAggcatagcagCAG
SeqID 394		x	416	TGAggcatagcaGCA
SeqID 395		x	416	ATGaggcatagcaGCA
SeqID 396		x	417	TGAggcatagcAGC
SeqID 397		x	417	ATGaggcatagcAGC
SeqID 398		x	417	GATgaggcatagcAGC
SeqID 399		x	418	GATgaggcatagCAG
SeqID 400		x	418	AGAtgaggcatagCAG
SeqID 401		x	419	GATgaggcataGCA
SeqID 402		x	419	AGAtgaggcataGCA
SeqID 403		x	419	AAGatgaggcataGCA
SeqID 404		x	422	AAGaagatgaggcATA
SeqID 405		x	423	AAGaagatgaggCAT
SeqID 406		x	601	TGGgatgggaatACA
SeqID 407		x	601	ATGggatgggaatACA
SeqID 408		x	602	TGGgatgggaaTAC
SeqID 409		x	602	ATGggatgggaaTAC
SeqID 410		x	602	GATgggatgggaaTAC
SeqID 411		x	603	ATGggatgggaATA
SeqID 412		x	603	GATgggatgggaATA
SeqID 413		x	604	GATgggatgggAAT
SeqID 414		x	691	AACcactgaacAAA
SeqID 415		x	695	CGaaccactgAA
SeqID 416		x	708	GGGggaaagccCT
SeqID 417		x	708	TGGgggaaagcCCT
SeqID 418		x	1142	GCAa^{m}cggggtaaAGG
SeqID 419		x	1143	GCAa^{m}cggggtaAAG
SeqID 420		x	1144	GCAa^{m}cggggtaAA
SeqID 421		x	1176	AGCaaacacttgGCA
SeqID 422		x	1176	CAGcaaacacttgGCA
SeqID 423		x	1177	CAGcaaacacttGGC
SeqID 424		x	1177	TCAgcaaacacttGGC

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 425		x	1178	TCAgcaaacactTGG
SeqID 426	x		1264	GCAgtatggatCG
SeqID 427	x		1264	CGCagtatggaTCG
SeqID 428	x		1264	TCCgcagtatggaTCG
SeqID 429	x		1265	CCGcagtatggATC
SeqID 430	x		1265	TCCgcagtatggATC
SeqID 431	x		1266	CGcagtatggAT
SeqID 432	x		1266	CCGcagtatggAT
SeqID 433	x		1266	TCCgcagtatgGAT
SeqID 434	x		1267	TCCgcagtatgGA
SeqID 435	x		1269	TTc^{m}cgcagtaTG
SeqID 436	x		1530	CGTaaagagagGT
SeqID 437	x		1530	CCG^{m}cgtaaagagaGGT
SeqID 438	x		1531	CGtaaagagaGG
SeqID 439	x		1531	CGCgtaaagagAGG
SeqID 440	x		1531	CCG^{m}cgtaaagagAGG
SeqID 441	x		1532	CGCgtaaagagAG
SeqID 442	x		1532	CCG^{m}cgtaaagaGAG
SeqID 443	x		1533	CG^{m}cgtaaagaGA
SeqID 444	x		1533	CCG^{m}cgtaaagaGA
SeqID 445	x		1534	CCg^{m}cgtaaagAG
SeqID 446	x		1547	GGCacaga^{m}cgggGAG
SeqID 447	x		1547	AGGcacaga^{m}cgggGAG
SeqID 448	x		1548	GGCacaga^{m}cggGGA
SeqID 449	x		1548	AGGcacaga^{m}cggGGA
SeqID 450	x		1548	AAGgcacaga^{m}cggGGA
SeqID 451	x		1549	AGGcacaga^{m}cgGGG
SeqID 452	x		1549	AAGgcacaga^{m}cgGGG
SeqID 453	x		1549	GAAggcacaga^{m}cgGGG
SeqID 454	x		1550	AGAaggcacaga^{m}cGGG
SeqID 455	x		1552	GAGaaggcacagACG
SeqID 456	x		1577	CGAagtgcacaCGG
SeqID 457	x		1578	GCGaagtgcacACG
SeqID 458	x		1579	GCGaagtgcacAC
SeqID 459	x		1579	AGCgaagtgcaCAC
SeqID 460	x		1579	AAG^{m}cgaagtgcaCAC
SeqID 461	x		1579	GAAg^{m}cgaagtgcaCAC
SeqID 462	x		1580	AGCgaagtgcaCA
SeqID 463	x		1580	AAG^{m}cgaagtgcACA
SeqID 464	x		1580	TGAag^{m}cgaagtgcACA
SeqID 465	x		1581	AAG^{m}cgaagtgcAC
SeqID 466	x		1581	GAAg^{m}cgaagtgCAC
SeqID 467	x		1581	TGAag^{m}cgaagtgCAC
SeqID 468	x		1581	GTGaag^{m}cgaagtgCAC
SeqID 469	x		1582	AAg^{m}cgaagtgCA

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 470	x		1582	GAAg^{m}cgaagtgCA
SeqID 471	x		1582	TGAag^{m}cgaagtGCA
SeqID 472	x		1582	GTGaag^{m}cgaagtGCA
SeqID 473	x		1583	TGAag^{m}cgaagtGC
SeqID 474	x		1583	GTGaag^{m}cgaagTGC
SeqID 475	x		1584	GTGaag^{m}cgaagTG
SeqID 476	x		1584	GGTgaag^{m}cgaaGTG
SeqID 477	x		1584	GAGgtgaag^{m}cgaaGTG
SeqID 478	x		1585	GTgaag^{m}cgaaGT
SeqID 479	x		1585	GGTgaag^{m}cgaaGT
SeqID 480	x		1585	GAGgtgaag^{m}cgaAGT
SeqID 481	x		1585	AGAggtgaag^{m}cgaAGT
SeqID 482	x		1586	AGAggtgaag^{m}cgAAG
SeqID 483	x		1586	CAGaggtgaag^{m}cgAAG
SeqID 484	x		1587	AGAggtgaag^{m}cGAA
SeqID 485	x		1587	CAGaggtgaag^{m}cGAA
SeqID 486	x		1587	GCAgaggtgaag^{m}cGAA
SeqID 487	x		1588	GCAgaggtgaagCGA
SeqID 488	x		1588	TGCagaggtgaagCGA
SeqID 489	x		1589	TGCagaggtgaaGCG
SeqID 490	x		1589	GTGcagaggtgaaGCG
SeqID 491	x		1590	CGTgcagaggtgaAGC
SeqID 492	x		1591	CGTgcagaggtgAAG
SeqID 493	x		1591	ACGtgcagaggtgAAG
SeqID 494	x		1592	CGTgcagaggtGAA
SeqID 495	x		1592	ACGtgcagaggtGAA
SeqID 496	X		1593	CGTgcagaggtGA
SeqID 497	x		1593	ACGtgcagaggTGA
SeqID 498	x		1616	CGTtca^{m}cggtgGT
SeqID 499	x		1690	CTCaaggt^{m}cggTC
SeqID 500	x		1691	CCTcaaggt^{m}cgGT
SeqID 501	x		1691	GCCtcaaggt^{m}cGGT
SeqID 502	x		1706	ACAgtctttgaaGTA
SeqID 503	x		1783	TTTatgcctacAG
SeqID 504	x		1784	AATttatgcctACA
SeqID 505	x		1785	AATttatgcctAC
SeqID 506	x		1787	CCAatttatgcCT
SeqID 507	x		1865	GCTtggaggcttGAA
SeqID 508	x		1865	AGCttggaggcttGAA
SeqID 509	x		1866	GCTtggaggctTGA
SeqID 510	x		1866	AGCttggaggctTGA
SeqID 511	x		1866	CAGcttggaggctTGA
SeqID 512	x		1867	GCTtggaggctTG
SeqID 513	x		1867	AGCttggaggcTTG
SeqID 514	x		1867	CAGcttggaggcTTG

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 515	x		1867	ACAgcttggaggcTTG
SeqID 516	x		1868	CACagcttggaggCTT
SeqID 517	x		1869	CACagcttggagGCT
SeqID 518	x		1869	GCAcagcttggagGCT
SeqID 519	x		1870	GCAcagcttggaGGC
SeqID 520	x		1870	GGCacagcttggaGGC
SeqID 521	x		1871	AGGcacagcttggAGG
SeqID 522	x		1872	AGGcacagcttgGAG
SeqID 523	x		1872	AAGgcacagcttgGAG
SeqID 524	x		1873	AAGgcacagcttGGA
SeqID 525	x		1873	CAAggcacagcttGGA
SeqID 526	x		1874	AAGgcacagctTGG
SeqID 527	x		1874	CAAggcacagctTGG
SeqID 528	x		1874	CCAaggcacagctTGG
SeqID 529	x		1875	CAAggcacagcTTG
SeqID 530	x		1875	CCAaggcacagcTTG
SeqID 531	x		1876	CCAaggcacagCTT
SeqID 532			2272	TGCgaatccacAC
SeqID 533			2272	GTG^{m}cgaatccaCAC
SeqID 534			2370	GGAgttcttcttCTA
SeqID 535			2370	GGGagttcttcttCTA
SeqID 536			2371	GGGagttcttctTCT
SeqID 537			2371	AGGgagttcttctTCT
SeqID 538			2372	AGGgagttcttcTTC
SeqID 539			2372	GAGggagttcttcTTC
SeqID 540			2373	AGGgagttcttCTT
SeqID 541			2373	GAGggagttcttCTT
SeqID 542			2373	CGAgggagttcttCTT
SeqID 543			2374	CGAgggagttctTCT
SeqID 544			2374	GCGagggagttctTCT
SeqID 545			2375	GCGagggagttcTTC
SeqID 546			2375	GGCgagggagttcTTC
SeqID 547			2376	GCGagggagttCTT
SeqID 548			2376	GGCgagggagttCTT
SeqID 549			2376	AGG^{m}cgagggagttCTT
SeqID 550			2377	GCGagggagttCT
SeqID 551			2377	GGCgagggagtTCT
SeqID 552			2377	AGG^{m}cgagggagtTCT
SeqID 553			2377	GAGg^{m}cgagggagtTCT
SeqID 554			2378	GGCgagggagtTC
SeqID 555			2378	AGG^{m}cgagggagTTC
SeqID 556			2378	GAGg^{m}cgagggagTTC
SeqID 557			2378	CGAgg^{m}cgagggagTTC
SeqID 558			2379	AGG^{m}cgagggagTT
SeqID 559			2379	GAGg^{m}cgagggaGTT

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 560			2379	CGAgg^{m}cgagggaGTT
SeqID 561			2379	GCGagg^{m}cgagggaGTT
SeqID 562			2380	GAGg^{m}cgagggaGT
SeqID 563			2380	CGAgg^{m}cgagggAGT
SeqID 564			2380	GCGagg^{m}cgagggAGT
SeqID 565			2380	TGCgagg^{m}cgagggAGT
SeqID 566			2381	CGAgg^{m}cgagggAG
SeqID 567			2381	GCGagg^{m}cgaggGAG
SeqID 568			2381	TGCgagg^{m}cgaggGAG
SeqID 569			2381	CTG^{m}cgagg^{m}cgaggGAG
SeqID 570			2382	CGagg^{m}cgaggGA
SeqID 571			2382	GCGagg^{m}cgaggGA
SeqID 572			2382	TGCgagg^{m}cgagGGA
SeqID 573			2382	CTG^{m}cgagg^{m}cgagGGA
SeqID 574			2382	TCTg^{m}cgagg^{m}cgagGGA
SeqID 575			2383	TCTg^{m}cgagg^{m}cgaGGG
SeqID 576			2383	GTCtg^{m}cgagg^{m}cgaGGG
SeqID 577			2824	GTTcccaagaaTAT
SeqID 578			2824	TGTtcccaagaaTAT
SeqID 579			2825	GTTcccaagaaTA
SeqID 580			2825	TGTtcccaagaATA
SeqID 581			2825	TTGttcccaagaATA
SeqID 582			2826	TGTtcccaagaAT
SeqID 583			2826	TTGttcccaagAAT
SeqID 584	x		414	GAGGcatagcagCAGG
SeqID 585			691	GAAccactgaaCAAA
SeqID 586			691	GAACcactgaacAAA
SeqID 587			691	CGaaccactgaaCAAA
SeqID 588			691	CGAAccactgaacAAA
SeqID 589			691	CGAaccactgaaCAAA
SeqID 590			691	CGAAccactgaacaAA
SeqID 591			691	CGAAccactgaaCAAA
SeqID 592			692	CGAAccactgaacAA
SeqID 593			692	CGAAccactgaaCAA
SeqID 594			692	CGAaccactgaACAA
SeqID 595			693	CGaaccactgAACA
SeqID 596			693	CGAAccactgaaCA
SeqID 597			693	CGAaccactgAACA
SeqID 598			693	CGAAccactgaACA
SeqID 599			694	CGaaccactgAAC
SeqID 600			694	CGAaccactgAAC
SeqID 601			1264	CCgcagtatggATCG
SeqID 602			1264	CCGCagtatggatCG
SeqID 603			1264	CCGCagtatggaTCG
SeqID 604			1264	CCGcagtatggATCG

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 605			1265	CGCAgtatggaTC
SeqID 606			1265	CGcagtatggATC
SeqID 607			1265	CGCagtatggATC
SeqID 608			1265	CGcagtatgGATC
SeqID 609			1530	GCGTaaagagagGT
SeqID 610			1530	GCgtaaagagAGGT
SeqID 611			1530	GCGtaaagagAGGT
SeqID 612			1530	GCGTaaagagaGGT
SeqID 613			1530	CGCGtaaagagagGT
SeqID 614			1530	CGcgtaaagagAGGT
SeqID 615			1530	CGCGtaaagagaGGT
SeqID 616			1530	CGCgtaaagagAGGT
SeqID 617			1531	GCgtaaagagAGG
SeqID 618			1531	GCGtaaagagAGG
SeqID 619			1531	GCgtaaagaGAGG
SeqID 620			1531	GCGTaaagagaGG
SeqID 621			1551	AGaaggcacagACGG
SeqID 622			1551	AGAaggcacagACGG
SeqID 623			1551	AGAAggcacagaCGG
SeqID 624			1551	GAGAaggcacagaCGG
SeqID 625			1551	GAGaaggcacagACGG
SeqID 626			1551	GAGAaggcacagACGG
SeqID 627			1577	GAagtgcacaCGG
SeqID 628			1577	GAAgtgcacaCGG
SeqID 629			1577	GAAGtgcacaCGG
SeqID 630			1577	GAAgtgcacACGG
SeqID 631			1577	GCgaagtgcacaCGG
SeqID 632			1577	GCGaagtgcacacGG
SeqID 633			1577	GCGAagtgcacacGG
SeqID 634			1577	GCgaagtgcacACGG
SeqID 635			1577	AGCGaagtgcacacGG
SeqID 636			1577	AG^{m}cgaagtgcacACGG
SeqID 637			1577	AG^{m}cgaagtgcacaCGG
SeqID 638			1577	AGCgaagtgcacacGG
SeqID 639			1578	CGaagtgcaCACG
SeqID 640			1578	CGAagtgcacACG
SeqID 641			1578	CGaagtgcacACG
SeqID 642			1578	AGCgaagtgcaCACG
SeqID 643			1578	AGCGaagtgcacACG
SeqID 644			1578	AGCGaagtgcacaCG
SeqID 645			1578	AG^{m}cgaagtgcaCACG
SeqID 646			1578	AAg^{m}cgaagtgcaCACG
SeqID 647			1578	AAGCgaagtgcacaCG
SeqID 648			1578	AAG^{m}cgaagtgcaCACG
SeqID 649			1578	AAGCgaagtgcacACG

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 650			1578	AAGCgaagtgcaCACG
SeqID 651			1580	GAag^{m}cgaagtgCACA
SeqID 652			1580	GAAG^{m}cgaagtgcaCA
SeqID 653			1580	GAAg^{m}cgaagtgCACA
SeqID 654			1580	GAAG^{m}cgaagtgCACA
SeqID 655			1582	GGtgaag^{m}cgaagtGCA
SeqID 656			1582	GGTgaag^{m}cgaagtgCA
SeqID 657			1582	GGTGaag^{m}cgaagtgCA
SeqID 658			1582	GGtgaag^{m}cgaagTGCA
SeqID 659			1583	GGtgaag^{m}cgaagTGC
SeqID 660			1583	GGTgaag^{m}cgaagtGC
SeqID 661			1583	GGTGaag^{m}cgaagtGC
SeqID 662			1583	GGtgaag^{m}cgaaGTGC
SeqID 663			1583	AGgtgaag^{m}cgaagTGC
SeqID 664			1583	AGGtgaag^{m}cgaagtGC
SeqID 665			1583	AGGTgaag^{m}cgaagtGC
SeqID 666			1583	AGgtgaag^{m}cgaaGTGC
SeqID 667			1584	AGGTgaag^{m}cgaagTG
SeqID 668			1584	AGgtgaag^{m}cgaAGTG
SeqID 669			1584	AGGtgaag^{m}cgaAGTG
SeqID 670			1584	AGGTgaag^{m}cgaaGTG
SeqID 671			1585	AGGTgaag^{m}cgaaGT
SeqID 672			1585	AGgtgaag^{m}cgAAGT
SeqID 673			1585	AGGtgaag^{m}cgAAGT
SeqID 674			1585	AGGTgaag^{m}cgaAGT
SeqID 675			1588	CAGAggtgaagcGA
SeqID 676			1588	CAgaggtgaaGCGA
SeqID 677			1588	CAGaggtgaaGCGA
SeqID 678			670	TAGtaaactgagCCA
SeqID 679			670	TAgtaaactgaGCCA
SeqID 680			670	TAGTaaactgagcCA
SeqID 681			670	TAGtaaactgaGCCA
SeqID 682			670	TAGTaaactgagCCA
SeqID 683			670	CTAgtaaactgagCCA
SeqID 684			670	CTagtaaactgaGCCA
SeqID 685			670	CTAGtaaactgagcCA
SeqID 686			671	CTAgtaaactgaGCC
SeqID 687			671	CTagtaaactgAGCC
SeqID 688			671	CTAGtaaactgagCC
SeqID 689			671	CTagtaaactgaGCC
SeqID 690			671	CTAgtaaactgagCC
SeqID 691			674	GCActagtaaacTGA
SeqID 692			674	GCactagtaaaCTGA
SeqID 693			674	GCACtagtaaactGA
SeqID 694			674	GCActagtaaaCTGA

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 695			674	GCACtagtaaacTGA
SeqID 696			674	GGCactagtaaacTGA
SeqID 697			674	GGcactagtaaaCTGA
SeqID 698			674	GGCActagtaaactGA
SeqID 699			1141	CAA^{m}cggggtaaaGGT
SeqID 700			1141	CAa^{m}cggggtaaAGGT
SeqID 701			1141	CAACggggtaaagGT
SeqID 702			1141	CAA^{m}cggggtaaAGGT
SeqID 703			1141	CAACggggtaaaGGT
SeqID 704			1261	CAGtatggat^{m}cgGCA
SeqID 705			1261	CAgtatggat^{m}cGGCA
SeqID 706			1261	CAgtatggat^{m}cgGCA
SeqID 707			1261	CAGtatggat^{m}cggCA
SeqID 708			1265	TTC^{m}cgcagtatggATC
SeqID 709			1265	TTc^{m}cgcagtatgGATC
SeqID 710			1265	TTCCgcagtatggaTC
SeqID 711			1265	TTC^{m}cgcagtatgGATC
SeqID 712			1265	TTCCgcagtatggATC
SeqID 713			1266	TTC^{m}cgcagtatgGAT
SeqID 714			1266	TTc^{m}cgcagtatGGAT
SeqID 715			1266	TTCCgcagtatggAT
SeqID 716			1266	TTC^{m}cgcagtatGGAT
SeqID 717			1266	TTCCgcagtatgGAT
SeqID 718			1266	GTTc^{m}cgcagtatgGAT
SeqID 719			1266	GTtc^{m}cgcagtatGGAT
SeqID 720			1266	GTTC^{m}cgcagtatggAT
SeqID 721			1267	GTtc^{m}cgcagtaTGGA
SeqID 722			1267	GTTC^{m}cgcagtatgGA
SeqID 723			1267	GTtc^{m}cgcagtatGGA
SeqID 724			1267	GTTc^{m}cgcagtatgGA
SeqID 725			1267	AGTtc^{m}cgcagtatGGA
SeqID 726			1267	AGTTc^{m}cgcagtatgGA
SeqID 727			1267	AGttc^{m}cgcagtatGGA
SeqID 728			1267	AGTtc^{m}cgcagtatgGA
SeqID 729			1267	AGttc^{m}cgcagtatgGA
SeqID 730			1268	AGTtc^{m}cgcagtaTGG
SeqID 731			1268	AGttc^{m}cgcagtaTGG
SeqID 732			1268	AGTtc^{m}cgcagtatGG
SeqID 733			1268	AGttc^{m}cgcagtatGG
SeqID 734			1268	GAgttc^{m}cgcagtaTGG
SeqID 735			1268	GAGttc^{m}cgcagtatGG
SeqID 736			1268	GAgttc^{m}cgcagtatGG
SeqID 737			1269	GAGTtc^{m}cgcagtaTG
SeqID 738			1269	GAgttc^{m}cgcagtATG
SeqID 739			1269	GAGttc^{m}cgcagtaTG

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 740			1269	GAgttc^{m}cgcagtaTG
SeqID 741			1269	GGAGttc^{m}cgcagtaTG
SeqID 742			1269	GGagttc^{m}cgcagtATG
SeqID 743			1269	GGAgttc^{m}cgcagtaTG
SeqID 744			1269	GGagttc^{m}cgcagtaTG
SeqID 745			1525	TAAagagaggtg^{m}cGCC
SeqID 746			1525	TAaagagaggtgCGCC
SeqID 747			1525	TAAAgagaggtg^{m}cgCC
SeqID 748			1525	TAAagagaggtgCGCC
SeqID 749			1525	TAAAgagaggtg^{m}cGCC
SeqID 750			1526	TAAagagaggtgCGC
SeqID 751			1526	TAaagagaggtGCGC
SeqID 752			1526	TAAagagaggtGCGC
SeqID 753			1526	TAAAgagaggtgCGC
SeqID 754			1526	GTAaagagaggtgCGC
SeqID 755			1526	GTaaagagaggtGCGC
SeqID 756			1527	GTAaagagaggtGCG
SeqID 757			1527	GTaaagagaggTGCG
SeqID 758			1527	GTAaagagaggTGCG
SeqID 759			1527	GTAAagagaggtGCG
SeqID 760			1527	CGtaaagagaggTGCG
SeqID 761			1527	CGTAaagagaggtgCG
SeqID 762			1527	CGTaaagagaggTGCG
SeqID 763			1527	CGTAaagagaggtGCG
SeqID 764			1528	CGTaaagagaggTGC
SeqID 765			1528	CGtaaagagagGTGC
SeqID 766			1528	CGTAaagagaggtGC
SeqID 767			1528	CGTaaagagagGTGC
SeqID 768			1528	CGTAaagagaggTGC
SeqID 769			1528	GCGtaaagagaggTGC
SeqID 770			1528	GCgtaaagagagGTGC
SeqID 771			1528	GCgtaaagagaggTGC
SeqID 772			1528	GCGtaaagagaggtGC
SeqID 773			1529	GCGtaaagagagGTG
SeqID 774			1529	GCgtaaagagaGGTG
SeqID 775			1529	GCGTaaagagaggTG
SeqID 776			1529	GCGtaaagagaGGTG
SeqID 777			1529	GCGTaaagagagGTG
SeqID 778			1529	CGCgtaaagagagGTG
SeqID 779			1529	^{m}cg^{m}cgtaaagagaGGTG
SeqID 780			1529	CGCGtaaagagaggTG
SeqID 781			1529	CGCgtaaagagaGGTG
SeqID 782			1529	CGCGtaaagagagGTG
SeqID 783			1552	TGAgaaggcacagACG
SeqID 784			1552	TGagaaggcacaGACG

SEQ ID No.	Target HBx	Target HBsAg	Start position on U95551	Oligo_seq
SeqID 785			1552	TGAGaaggcacagaCG
SeqID 786			1552	TGAgaaggcacaGACG
SeqID 787			1552	TGAGaaggcacagACG
SeqID 788			1690	GCctcaaggt^{m}cgGTC
SeqID 789			1690	GCCtcaaggt^{m}cggTC
SeqID 790			1690	GCctcaaggt^{m}cggTC
SeqID 791			1778	ATgcctacagccTCC
SeqID 792			1778	ATGcctacagcctCC
SeqID 793			1778	ATgcctacagcctCC
SeqID 794			1785	ACCAatttatgcCTAC
SeqID 795			1785	ACCaatttatgcCTAC
SeqID 796			1785	ACCAatttatgccTAC
SeqID 797			1785	ACCaatttatgccTAC
SeqID 798			1785	ACcaatttatgcCTAC

Table 4 LNA oligomers with a GalNAc2 conjugate moiety linked via a C6 amino linker and a cleavable ca phosphodiester linkage to the oligomer. The GalNAc2 conjugate moiety can also be substituted with other GalNAc conjugate moieties or sterol moieties. The oligomer sequence motif which these oligomers are based on is a subset from Table 2 and 3.

Upper case letters denote beta-D-oxy LNA, lower case letters denote DNA, ^{m}c/ ^{m}C denotes a 5-methylcytosine DNA/LNA, s denotes phosphorothiate internucleoside linkages. Where nothing is specified the linkage is a phosphodiester internucleoside linkage

SEQ ID No	Design
SeqID 799	5'- GN2-C6 ca**G$_S$A$_S$A$_S$**c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$c$_S$**A$_S$A$_S$A**-3'
SeqID 800	5'- GN2-C6 ca**^{m}C$_S$G$_S$A$_S$**a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$c$_S$**A$_S$A$_S$A**-3'
SeqID 801	5'- GN2-C6 ca**^{m}C$_S$G$_S$A$_S$**a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$**^{m}C$_S$A$_S$A**-3'
SeqID 802	5'- GN2-C6 ca**^{m}C$_S$ ^{m}C$_S$G$_S$**c$_S$a$_S$g$_S$t$_S$a$_S$t$_S$g$_S$g$_S$a$_S$**T$_S$ ^{m}C$_S$G**-3'
SeqID 803	5'- GN2-C6 ca**^{m}C$_S$G$_S$ ^{m}C$_S$**g$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G$_S$G$_S$T**-3'
SeqID 804	5'- GN2-C6 ca**A$_S$G$_S$A$_S$**a$_S$g$_S$g$_S$c$_S$a$_S$c$_S$a$_S$g$_S$a$_S$**^{m}C$_S$G$_S$G**-3'
SeqID 805	5'- GN2-C6 ca**G$_S$A$_S$G$_S$**a$_S$a$_S$g$_S$g$_S$c$_S$a$_S$c$_S$a$_S$g$_S$a$_S$**^{m}C$_S$G$_S$G**-3'
SeqID 806	5'- GN2-C6 ca**G$_S$ ^{m}C$_S$G$_S$**a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$**^{m}C$_S$G$_S$G**-3'
SeqID 807	5'- GN2-C6 ca**A$_S$G$_S$ ^{m}C$_S$**g$_S$a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$**^{m}C$_S$G$_S$G**-3'
SeqID 808	5'- GN2-C6 ca**A$_S$G$_S$ ^{m}C$_S$**g$_S$a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$**A$_S$ ^{m}C$_S$G**-3'
SeqID 809	5'- GN2-C6 ca**A$_S$G$_S$G$_S$**t$_S$g$_S$a$_S$a$_S$g$_S$**^{m}**c$_S$g$_S$a$_S$a$_S$g$_S$**T$_S$G$_S$ ^{m}C**-3'
SeqID 810	5'- GN2-C6 ca**A$_S$G$_S$G$_S$**t$_S$g$_S$a$_S$a$_S$g$_S$**^{m}**c$_S$g$_S$a$_S$a$_S$**G$_S$T$_S$G**-3'

SEQ ID No	Design
SeqID 811	5'- GN2-C6 ca^{m}C$_S$G$_S$A$_S$a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$A$_S$ ^{m}C$_S$A-3'
SeqID 812	5'- GN2-C6 ca^{m}C$_S$G$_S$A$_S$a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$A$_S$ ^{m}C-3'
SeqID 813	5'- GN2-C6 ca^{m}C$_S$G$_S$ ^{m}C$_S$a$_S$g$_S$t$_S$a$_S$t$_S$g$_S$g$_S$a$_S$T$_S$ ^{m}C-3'
SeqID 814	5'- GN2-C6 caG$_S$ ^{m}C$_S$G$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$G$_S$G$_S$T-3'
SeqID 815	5'- GN2-C6 caG$_S$ ^{m}C$_S$G$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$G$_S$G-3'
SeqID 816	5'- GN2-C6 caG$_S$A$_S$A$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$ ^{m}c$_S$G$_S$G-3'
SeqID 817	5'-GN2-C6 ca^{m}C$_S$G$_S$A$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$ ^{m}C$_S$G-3'
SeqID 818	5'-GN2-C6 caA$_S$G$_S$G$_S$t$_S$g$_S$a$_S$a$_S$g$_S$ ^{m}c$_S$g$_S$a$_S$A$_S$G$_S$T-3'
SeqID 819	5'-GN2-C6 caG$_S$A$_S$A$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$ ^{m}C$_S$A$_S$A$_S$A-3'
SeqID 820	5'-GN2-C6 ca^{m}C$_S$G$_S$A$_S$A$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$c$_S$A$_S$A$_S$A-3'
SeqID 821	5'-GN2-C6 caG$_S$A$_S$A$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$ ^{m}C$_S$G$_S$G-3'
SeqID 822	5'-GN2-C6 caT$_S$A$_S$G$_S$t$_S$a$_S$a$_S$a$_S$c$_S$t$_S$g$_S$a$_S$g$_S$ ^{m}C$_S$ ^{m}C$_S$A$_S$3'
SeqID 823	5'-GN2-C6 ca^{m}C$_S$G$_S$A$_S$a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$A$_S$A$_S$ ^{m}C-3'
SeqID 824	5'-GN2-C6 ca^{m}C$_S$G$_S$A$_S$a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$A$_S$A$_S$ ^{m}C$_S$A-3'
SeqID 825	5'-GN2-C6 caG$_S$ ^{m}C$_S$G$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$A$_S$G$_S$G-3'
SeqID 826	5'-GN2-C6 caA$_S$G$_S$g$_S$t$_S$g$_S$a$_S$a$_S$g$_S$ ^{m}c$_S$g$_S$a$_S$A$_S$G$_S$T$_S$G-3'

Prior to conjugation with GalNAc, the LNA oligomers of table 4 are represented as AM-C6 ca – oligomer, where AM-C6 represents an amino linker ready for conjugation and ca is a cleavable phosphodiester linkage. These oligomers are incorporated by reference from table 4 in the priority application GB1408623.5.

EMBODIMENTS

The following embodiments of the present invention, presented as numbered paragraphs, may be used in combination with the other embodiments described herein:

1. An oligomer conjugate for use in the treatment of a viral disorder, wherein said oligomer conjugate comprises:
   a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
   b) a carrier component for delivering said first oligomer to the liver.
2. The oligomer conjugate for the use according to paragraph 1 wherein said carrier component is capable of delivering said oligomer to the liver of a subject to be treated by administration of said oligomer conjugate.
3. The oligomer conjugate for the use according to paragraph 1 or paragraph 2 wherein said carrier component is capable of delivering said oligomer to a hepatocyte of a subject to be treated by administration of said oligomer conjugate.
4. The oligomer conjugate for the use according to any of paragraphs 1 to 3 wherein said carrier component is a carbohydrate conjugate moiety.
5. The oligomer conjugate for the use according to any of paragraphs 1 to 4 wherein said carrier component is an asialoglycoprotein receptor (ASGP-R) targeting moiety.
6. The oligomer conjugate for the use according to paragraphs 4 or 5 wherein said carbohydrate conjugate moiety or ASGP-R targeting moiety is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof.
7. The oligomer conjugate for the use according to any of paragraphs 1 to 6 wherein said carrier component is a GalNAc cluster comprising two to four terminal galactose derivatives, a hydrophilic spacer linking each galactose derivative to a branch point group.
8. The oligomer conjugate for the use according to paragraph 7, wherein the galactose derivatives are GalNAc, the spacer is a PEG spacer and the branch point group is a comprising a peptide, with two or more amino groups, such as a di-lysine or tri-lysine.
9. The oligomer conjugate for the use according to any of paragraphs 1 to 8 wherein said carrier component is GalNAc2.
10. The oligomer conjugate for the use according to any of paragraphs 1 to 9 wherein said first oligomer region has at least 80% complementarity to the target sequence.
11. The oligomer conjugate for the use according to any of paragraphs 1 to 10 wherein said first oligomer region comprises one or more LNA units.

12. The oligomer conjugate for the use according to any of paragraphs 1 to 11 wherein said first oligomer region is a gapmer.
13. The oligomer conjugate for the use according to any of paragraphs 1 to 12 wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, wherein each wing independently comprises one or more LNA units.
14. The oligomer conjugate for the use according to any of paragraphs 1 to 13 wherein said first oligomer region comprises any one of the motifs: 2-8-2, 3-8-3, 2-8-3, 3-8-2, 2-9-2, 3-9-3, 2-9-3, 3-9-2, 2-10-2, 3-10-3, 3-10-2, 2-10-3 wherein the first number is the number of LNA units in the 5' LNA wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the 3' LNA wing region.
15. The oligomer conjugate for the use according to any of paragraph 1 to 14 wherein said first oligomer region is 8-30 nucleotides in length.
16. The oligomer conjugate for the use according to any of paragraphs 1 to 15 wherein said first oligomer region is 10-20 nucleotides in length.
17. The oligomer conjugate for the use according to any of paragraphs 1 to 16 wherein said first oligomer region is 10 to 16 nucleotides in length.
18. The oligomer conjugate for the use according to any of paragraphs 1 to 17 wherein said first oligomer region is 10 to 14 nucleotides in length.
19. The oligomer conjugate for the use according to any of paragraphs 1 to 18 wherein said first oligomer region binds to the target sequence.
20. The oligomer conjugate for the use according to any of paragraphs 1 to 19 wherein said first oligomer region is capable of inhibiting any one or more of the expression, replication or translation of the target sequence.
21. The oligomer conjugate for the use according to any of paragraphs 1 to 20 wherein said first oligomer region is capable of inhibiting the expression of the target sequence.
22. The oligomer conjugate for the use according to any of paragraphs 1 to 21 wherein said target sequence is a gene or a mRNA.
23. The oligomer conjugate for the use according to any of paragraphs 1 to 22 wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof.
24. The oligomer conjugate for the use according to any of paragraphs 1 to 23 wherein said target sequence is a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof.
25. The oligomer conjugate for the use according to any of paragraphs 1 to 24 wherein said target sequence is within the sequence shown as SEQ ID No. 1 and/or SEQ ID No. 2, or a

sequence that has at least 80% identity thereto, preferably at least 85% identity thereto, preferably at least 90% identity thereto, preferably at least 95% identity thereto.

26. The oligomer conjugate for the use according to any of paragraphs 1 to 24 wherein said target sequence is within the sequence shown as SEQ ID No. 1 and SEQ ID No. 2.

27. The oligomer conjugate for the use according to any of paragraphs 1 to 26 wherein said target sequence has at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identity with any one or more of the HBV genotypes A-H.

28. The oligomer conjugate for the use according to any of paragraphs 1 to 27 wherein said target sequence is selected from the group consisting of any one or more of positions:
1264-1278;
1530-1544;
1551-1566;
1577 to 1598;
691-706;
670-684
of SEQ ID NO: 3.

29. The oligomer conjugate for the use according to any of paragraphs 1 to 28 wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:
GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);

CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852).

30. The oligomer conjugate for the use according any of paragraphs 1 to 29 wherein said first oligomer region is based on a sequence selected from the group consisting of any one or more of:
GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGgtgaagcgaAGTG (SEQ ID NO: 668);
AGCgaagtgcacaCGG (SEQ ID NO: 308);
CGAaccactgaACA (SEQ ID NO: 297);
CGCagtatggaTC (SEQ ID NO: 300);
AGGtgaagcgaagTGC (SEQ ID NO: 315);
AGGtgaagcgaaGTG (SEQ ID NO: 316);
GAAccactgaacAAA (SEQ ID NO: 294);
CGAaccactgaacAAA (SEQ ID NO: 295);
CGAaccactgaaCAA(SEQ ID NO: 296);
CGAaccactgaAC (SEQ ID NO: 298);
CCGcagtatggaTCG (SEQ ID NO: 299);
CGCgtaaagagaGGT (SEQ ID NO: 302);
AGAaggcacagaCGG (SEQ ID NO: 304);
GAGaaggcacagaCGG (SEQ ID NO: 305);
GAAgtgcacacGG (SEQ ID NO: 306);
GCGaagtgcacaCGG (SEQ ID NO: 307);
CGAagtgcacaCG (SEQ ID NO: 309);
GAAccactgaaCAAA (SEQ ID NO: 585);
CGAAccactgaacAAA (SEQ ID NO: 588)
GAAgtgcacaCGG (SEQ ID NO: 628);
TAGtaaactgagCCA (SEQ ID NO: 678);
CGAaccactgAAC (SEQ ID NO: 600);
AGGtgaagcgaAGT (SEQ ID NO: 317); and
CGAaccactgAACA (SEQ ID NO: 597).
wherein uppercase letters denote LNA units and lower case letters denote DNA units.

31. The oligomer conjugate for the use according to any of paragraphs 1 to 30 wherein said oligomer conjugate is selected from the group consisting of any one or more of:

5'- GN2-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G$-3' (SEQ ID NO: 815)

5'- GN2-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G_S T$-3' (SEQ ID NO: 814)

5'-GN2-C6 caG_S $^{m}C_S G_S t_S a_S a_S a_S g_S a_S g_S A_S G_S G$-3' (SEQ ID NO: 825)

5'- GN2-C6 ca$A_S G_S$ $^{m}C_S g_S a_S a_S g_S t_S g_S c_S a_S c_S A_S$ $^{m}C_S G$-3' (SEQ ID NO: 808)

5'-GN2-C6 ca$A_S G_S g_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S A_S G_S T_S G$-3' (SEQ ID NO: 826)

5'- GN2-C6 ca$A_S G_S$ $^{m}C_S g_S a_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 807)

5'- GN2-C6 ca$G_S A_S A_S c_S c_S a_S c_S t_S g_S a_S a_S c_S A_S A_S A$-3' (SEQ ID NO: 799)

5'- GN2-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S a_S c_S A_S A_S A$-3' (SEQ ID NO: 800)

5'- GN2-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S a_S$ $^{m}C_S A_S A$-3' (SEQ ID NO: 801)

5'- GN2-C6 ca$^{m}C_S$ $^{m}C_S G_S c_S a_S g_S t_S a_S t_S g_S g_S a_S T_S$ $^{m}C_S G$-3' (SEQ ID NO: 802)

5'- GN2-C6 ca$^{m}C_S G_S$ $^{m}C_S g_S t_S a_S a_S a_S g_S a_S g_S a_S G_S G_S T$-3' (SEQ ID NO: 803)

5'- GN2-C6 ca$A_S G_S A_S a_S g_S g_S c_S a_S c_S a_S g_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 804)

5'- GN2-C6 ca$G_S A_S G_S a_S a_S g_S g_S c_S a_S c_S a_S g_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 805)

5'- GN2-C6 caG_S $^{m}C_S G_S a_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G_S G$-3' (SEQ ID NO: 806)

5'- GN2-C6 ca$A_S G_S G_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S a_S g_S T_S G_S$ ^{m}C-3' (SEQ ID NO: 809)

5'- GN2-C6 ca$A_S G_S G_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S a_S G_S T_S G$-3' (SEQ ID NO: 810)

5'- GN2-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S A_S$ $^{m}C_S A$-3' (SEQ ID NO: 811)

5'- GN2-C6 ca$^{m}C_S G_S A_S a_S c_S c_S a_S c_S t_S g_S a_S A_S$ ^{m}C-3' (SEQ ID NO: 812)

5'- GN2-C6 ca$^{m}C_S G_S$ $^{m}C_S a_S g_S t_S a_S t_S g_S g_S a_S T_S$ ^{m}C-3' (SEQ ID NO: 813)

5'- GN2-C6 ca$G_S A_S A_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}c_S G_S G$-3' (SEQ ID NO: 816)

5'-GN2-C6 ca$^{m}C_S G_S A_S a_S g_S t_S g_S c_S a_S c_S a_S$ $^{m}C_S G$-3' (SEQ ID NO: 817)

5'-GN2-C6 ca$A_S G_S G_S t_S g_S a_S a_S g_S$ $^{m}c_S g_S a_S A_S G_S T$-3' (SEQ ID NO: 818)

5′-GN2-C6 ca$G_sA_sA_sc_sc_sa_sc_st_sg_sa_sa_s{}^{m}C_sA_sA_sA$-3′ (SEQ ID NO: 819)

5′-GN2-C6 ca$^{m}C_sG_sA_sA_sc_sc_sa_sc_st_sg_sa_sa_sc_sA_sA_sA$-3′ (SEQ ID NO: 820)

5′-GN2-C6 ca$G_sA_sA_sg_st_sg_sc_sa_sc_sa_s{}^{m}C_sG_sG$-3′ (SEQ ID NO: 821)

5′-GN2-C6 ca$T_sA_sG_st_sa_sa_sa_sc_st_sg_sa_sg_s{}^{m}C_s{}^{m}C_sA_s$3′ (SEQ ID NO: 822)

5′-GN2-C6 ca$^{m}C_sG_sA_sa_sc_sc_sa_sc_st_sg_sA_sA_s{}^{m}C$-3′ (SEQ ID NO: 823)

5'-GN2-C6 ca$^{m}C_sG_sA_sa_sc_sc_sa_sc_st_sg_sA_sA_s{}^{m}C_sA$-3' (SEQ ID NO: 824)

wherein uppercase letters denote beta-D-oxy-LNA units; lowercase letters denote DNA units; the subscript "s" denotes a phosphorothioate linkage; superscript m denotes a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base; GN2-C6 denotes a GalNAc2 carrier component with a C6 linker.

32. The oligomer conjugate for the use according to any of paragraphs 1 to 29 wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:
GCGTAAAGAGAGG (SEQ ID NO: 13)
GCGTAAAGAGAGGT (SEQ ID NO: 11) and
CGCGTAAAGAGAGGT (SEQ ID NO 12).

33. The oligomer conjugate for the use according to any of paragraphs 1 to 29 wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19) and
AGGTGAAGCGAAGT (SEQ ID NO 27).

34. The oligomer conjugate for the use according to any one of paragraphs 1 to 33 wherein said oligomer conjugate has or comprises the structure:
Carrier component – L1 - First Oligomer Region
wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety; or
wherein said oligomer conjugate has or comprises the structure:
First Oligomer Region – L2 - Carrier component
wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety.

35. The oligomer conjugate for the use according to any one of paragraphs 1 to 34 wherein said oligomer conjugate has or comprises the structure:
Carrier component – L1 - First Oligomer Region
wherein L1 is an optional linker.
36. The oligomer conjugate for the use according to paragraph 34 or paragraph 35 wherein said Linker 1 is present.
37. The oligomer conjugate for the use according to any of paragraphs 1 to 36 wherein said carrier component is linked, preferably conjugated, to the 5' end of said oligomer.
38. The oligomer conjugate for the use according to paragraph 34 to 37 wherein the linker group or the brancher region is a physiologically labile linker group or a physiologically labile brancher region or physiologically labile tether molecule or physiologically labile bridging moiety.
39. The oligomer conjugate for the use according to paragraph 38 wherein the physiologically labile linker group is a nuclease susceptible linker .
40. The oligomer conjugate for the use according to paragraph 38 or 39 wherein the physiologically labile linker further is conjugated with a C6 to C12 amino alkyl group.
41. The oligomer conjugate for the use according to any of paragraphs 1 to 40 which further comprises a second oligomer region which is capable of modulating a target sequence.
42. The oligomer conjugate for the use according to paragraph 41 wherein each of the first oligomer region and the second oligomer regions is capable of modulating a target sequence in HBx or HBsAg of HBV.
43. The oligomer conjugate for the use according to any one of paragraphs 31 or 42 wherein each of the first oligomer region and the second oligomer regions is capable of modulating a target sequence in HBx or HBsAg of HBV; wherein said target regions are different.
44. The oligomer conjugate for the use according to any one of paragraphs 41 to 43 wherein said oligomer conjugate has or comprises the structure:
Carrier component – L1 - First Oligomer Region – L2 – Second Oligomer Region
wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety
wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety
wherein L1 and L2 can be the same or different; or
wherein said oligomer conjugate has or comprises the structure:
First Oligomer Region – L2 – Second Oligomer Region - L3 – Carrier component
wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety
wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety
wherein L2 and L3 can be the same or different ; or
wherein said oligomer conjugate has or comprises the structure:

Carrier component 1 – L1 - First Oligomer Region – L2 – Second Oligomer Region - L3 – Carrier component 2

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1, L2 and L3 can be the same or different

wherein Carrier component 1 and Carrier component 2 can be the same or different; or

wherein said oligomer conjugate has or comprises the structure:

First Oligomer Region – L1 - Carrier component 1 – L2 – Second Oligomer Region

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 and L3 can be the same or different; or

wherein said oligomer conjugate has or comprises the structure:

First Oligomer Region – L1 - Carrier component 1 – L2 – Second Oligomer Region - L3 – Carrier component 2

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 can be the same or different

wherein Carrier component 1 and Carrier component 2 can be the same or different; or.

wherein said oligomer conjugate has or comprises the structure:

Carrier component 1 – L1 - First Oligomer Region – L2 - Carrier component 2 – L3 – Second Oligomer Region - L4 – Carrier component 3

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L3 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1, L2 and L3 can be the same or different

wherein Carrier component 1, Carrier Component 2 and Carrier component 3 can be the same or different.

45. The oligomer conjugate for the use according to any one of paragraphs 39 to 43 wherein said oligomer conjugate has or comprises the structure:

Carrier component – L1 - First Oligomer Region – L2 – Second Oligomer Region

wherein L1 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L2 is an optional linker or brancher region or tether molecule or bridging moiety

wherein L1 and L2 can be the same or different.

46. The oligomer conjugate for the use according to paragraph 45 wherein said Linker 1 is present.
47. The oligomer conjugate for the use according to paragraph 45 or paragraph 46 wherein said Linker 2 is present.
48. The oligomer conjugate for the use according to any of paragraphs 41 to 46 wherein said carrier component is linked, preferably conjugated, to the 5' end of said oligomer.
49. The oligomer conjugate for the use according to any of paragraphs 41 to 48 wherein each of the first oligomer region and the second oligomer regions is linked, preferably conjugated, by means of a linker or brancher region.
50. The oligomer conjugate for the use according to any of paragraphs 41 to 49 wherein each of the first oligomer region and the second oligomer regions is linked, preferably conjugated, by means of a physiologically labile linker group or a physiologically labile brancher region.
51. The oligomer conjugate for the use according to any of paragraphs 1 to 50 wherein said viral disorder is hepatitis B or a disorder associated with HBV
52. The oligomer conjugate for the use according to any of paragraphs 1 to 51 wherein said viral disorder is associated with expression or overexpression of HBx or HBsAg.
53. The oligomer conjugate for the use according to any of paragraphs 1 to 52, wherein said oligomer conjugate is administered subcutaneously.
54. A composition for use in the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate as defined in any one of paragraphs 1 to 53 and at least one additional different oligonucleotide.
55. The composition for the use according to paragraph 54 wherein at least one of said additional different oligonucleotide is an oligomer conjugate.
56. The composition for the use according to paragraph 54 or paragraph 55 wherein each of said additional different oligonucleotide is an oligomer conjugate.
57. The composition for the use according to any one of paragraphs 54 to 56 wherein each of said additional different oligonucleotide is capable of modulating a target sequence in HBV.
58. The composition for the use according to any one of paragraphs 54 to 57 wherein at least one of said additional different oligonucleotide is capable of modulating a target sequence in HBx or HBsAg of HBV.
59. The composition for the use according to any one of paragraphs 54 to 58 wherein at least one of said additional different oligonucleotide is capable of modulating a target sequence in HBx or HBsAg of HBV; and wherein said at least one of said additional different oligonucleotide is capable of modulating a target sequence in HBx or HBsAg of HBV

different to that targeted by an oligomer conjugate as defined in any one of paragraphs 1 to 53.

60. The composition for the use according to any one of paragraphs 54 to 59 wherein each of said additional different oligonucleotide is capable of modulating a target sequence in HBx or HBsAg of HBV.
61. The composition for the use according to any one of paragraphs 54 to 60 wherein each of said additional different oligonucleotide is capable of modulating a target sequence in HBx or HBsAg of HBV; and wherein each of said additional different oligonucleotide is capable of modulating a target sequence in HBx or HBsAg of HBV different to that targeted by an oligomer conjugate as defined in any one of paragraphs 1 to 53.
62. An oligomer conjugate suitable for the treatment of a viral disorder, wherein said oligomer conjugate comprises:
    a)at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
    b)a carrier component for delivering said first oligomer to the liver.
63. An oligomer conjugate according to paragraph 62 wherein said oligomer is as defined in any one of paragraphs 1 to 53.
64. A composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate and at least one additional different oligonucleotide; wherein said oligomer conjugate comprises:
    a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
    b) a carrier component for delivering said first oligomer to the liver.
65. A composition according to paragraph 64 wherein said oligomer conjugate is an oligomer conjugate as defined in any one of paragraphs 1 to 53 or any one of paragraphs 62 to 63.
66. A composition according to paragraph 64 or paragraph 65 wherein said additional different oligonucleotide is an additional different oligonucleotide as defined in any one of paragraphs 62 to 63.
67. An oligomer based on a core motif selected from the group consisting of any one or more of:
    GCGTAAAGAGAGG (SEQ ID NO: 13);
    GCGTAAAGAGAGGT (SEQ ID NO: 11);
    AGCGAAGTGCACACG (SEQ ID NO: 20);
    AGGTGAAGCGAAGTG (SEQ ID NO: 26);
    CGAACCACTGAACA (SEQ ID NO: 7);
    GAACCACTGAACAAA (SEQ ID NO 4);

CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852)

which is capable of modulating a target sequence in HBx or HBsAg of HBV to treat a viral disorder.

68. The oligomer of paragraph 67 wherein said oligomer region is based on a core motif selected from the group consisting of any one or more of:
GCGTAAAGAGAGG (SEQ ID NO: 13)
GCGTAAAGAGAGGT (SEQ ID NO: 11) and
CGCGTAAAGAGAGGT (SEQ ID NO 12).

69. The oligomer of paragraph 67 wherein said oligomer region is based on a core motif selected from the group consisting of any one or more of:
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19) and
AGGTGAAGCGAAGT (SEQ ID NO 27).

70. The oligomer according to any one of paragraphs 67 to 69, wherein said oligomer comprises one or more LNA units.

71. The oligomer according to any of paragraphs 67 to 70 wherein said oligomer is a gapmer.

72. The oligomer according to any of paragraphs 67 to 71 wherein said oligomer comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, wherein each wing independently comprises one or more LNA units.

73. The oligomer according to any of paragraphs 67 to 72 wherein said oligomer any one of the motifs: 2-8-2, 3-8-3, 2-8-3, 3-8-2, 2-9-2, 3-9-3, 2-9-3, 3-9-2, 2-10-2, 3-10-3, 3-10-2, 2-10-3 wherein the first number is the number of LNA units in an LNA wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in an LNA wing region.
74. The oligomer according to any of paragraphs 67 to 73 wherein said first oligomer region is 10-18 nucleotides in length.
75. The oligomer according to any of paragraphs 67 to 74 wherein said first oligomer region is 10 to 16 nucleotides in length.
76. The oligomer according to any of paragraphs 67 to 75 wherein said first oligomer region is 10 to 14 nucleotides in length.
77. The oligomer according to any one of paragraphs 67 to 76 which is based on a sequence selected from the group consisting of any one or more of:
GCGtaaagagaGG(SEQ ID NO: 303);
GCGtaaagagaGGT(SEQ ID NO: 301);
GCGtaaagagAGG (SEQ ID NO: 618);
AGCgaagtgcacACG (SEQ ID NO: 310)
AGgtgaagcgaAGTG (SEQ ID NO: 668);
AGCgaagtgcacaCGG (SEQ ID NO: 308);
CGAaccactgaACA (SEQ ID NO: 297);
CGCagtatggaTC (SEQ ID NO: 300);
AGGtgaagcgaagTGC (SEQ ID NO: 315);
AGGtgaagcgaaGTG (SEQ ID NO: 316);
GAAccactgaacAAA (SEQ ID NO: 294);
CGAaccactgaacAAA (SEQ ID NO: 295);
CGAaccactgaaCAA(SEQ ID NO: 296);
CGAaccactgaAC (SEQ ID NO: 298);
CCGcagtatggaTCG (SEQ ID NO: 299);
CGCgtaaagagaGGT (SEQ ID NO: 302);
AGAaggcacagaCGG (SEQ ID NO: 304);
GAGaaggcacagaCGG (SEQ ID NO: 305);
GAAgtgcacacGG (SEQ ID NO: 306);
GCGaagtgcacaCGG (SEQ ID NO: 307);
CGAagtgcacaCG (SEQ ID NO: 309);
GAAccactgaaCAAA (SEQ ID NO: 585);
CGAAccactgaacAAA (SEQ ID NO: 588)

GAAgtgcacaCGG (SEQ ID NO: 628);

TAGtaaactgagCCA (SEQ ID NO: 678);

CGAaccactgAAC (SEQ ID NO: 600);

AGGtgaagcgaAGT (SEQ ID NO: 317); and

CGAaccactgAACA (SEQ ID NO: 597),

wherein uppercase letters denote affinity enhancing nucleotide analogues and lower case letters denote DNA units.

78. The oligomer according to any of paragraphs 67 to 77 which is based on a selected from a sequence selected from the group consisting of any one or more of:

5'- AM-C6 ca**G$_S$** ^{m}**C$_S$G$_S$**t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G$_S$G**-3' (SEQ ID NO: 303)

5'- AM-C6 ca**G$_S$** ^{m}**C$_S$G$_S$**t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G$_S$G$_S$T**-3' (SEQ ID NO: 301)

5'-AM-C6 caG$_S$ ^{m}C$_S$G$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$**A$_S$G$_S$G**-3' (SEQ ID NO: 618)

5'- AM-C6 ca**A$_S$G$_S$** ^{m}**C$_S$**g$_S$a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$**A$_S$** ^{m}**C$_S$G**-3' (SEQ ID NO: 310)

5'-AM-C6 ca**A$_S$G$_S$**g$_S$t$_S$g$_S$a$_S$a$_S$g$_S$ ^{m}c$_S$g$_S$a$_S$**A$_S$G$_S$T$_S$G**-3' (SEQ ID NO: 668)

5'- AM-C6 ca**A$_S$G$_S$** ^{m}**C$_S$**g$_S$a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$ ^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 308)

5'- AM-C6 ca**G$_S$A$_S$A$_S$**c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$c$_S$**A$_S$A$_S$A**-3' (SEQ ID NO: 294)

5'- AM-C6 ca ^{m}**C$_S$G$_S$A$_S$**a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$c$_S$**A$_S$A$_S$A**-3' (SEQ ID NO: 295)

5'- AM-C6 ca ^{m}**C$_S$G$_S$A$_S$**a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$a$_S$ ^{m}**C$_S$A$_S$A**-3' (SEQ ID NO: 296)

5'- AM-C6 ca ^{m}**C$_S$** ^{m}**C$_S$G$_S$**c$_S$a$_S$g$_S$t$_S$a$_S$t$_S$g$_S$g$_S$a$_S$**T$_S$** ^{m}**C$_S$G**-3' (SEQ ID NO: 299)

5'- AM-C6 ca ^{m}**C$_S$G$_S$** ^{m}**C$_S$**g$_S$t$_S$a$_S$a$_S$a$_S$g$_S$a$_S$g$_S$a$_S$**G$_S$G$_S$T**-3' (SEQ ID NO: 302)

5'- AM-C6 ca**A$_S$G$_S$A$_S$**a$_S$g$_S$g$_S$c$_S$a$_S$c$_S$a$_S$g$_S$a$_S$ ^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 304)

5'- AM-C6 ca**G$_S$A$_S$G$_S$**a$_S$a$_S$g$_S$g$_S$c$_S$a$_S$c$_S$a$_S$g$_S$a$_S$ ^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 305)

5'- AM-C6 ca**G$_S$** ^{m}**C$_S$G$_S$**a$_S$a$_S$g$_S$t$_S$g$_S$c$_S$a$_S$c$_S$a$_S$ ^{m}**C$_S$G$_S$G**-3' (SEQ ID NO: 307)

5'- AM-C6 ca**A$_S$G$_S$G$_S$**t$_S$g$_S$a$_S$a$_S$g$_S$ ^{m}c$_S$g$_S$a$_S$a$_S$g$_S$**T$_S$G$_S$** ^{m}**C**-3' (SEQ ID NO:315)

5'- AM-C6 ca**A$_S$G$_S$G$_S$**t$_S$g$_S$a$_S$a$_S$g$_S$ ^{m}c$_S$g$_S$a$_S$a$_S$**G$_S$T$_S$G**-3' (SEQ ID NO: 316)

5'- AM-C6 ca ^{m}**C$_S$G$_S$A$_S$**a$_S$c$_S$c$_S$a$_S$c$_S$t$_S$g$_S$a$_S$**A$_S$** ^{m}**C$_S$A**-3' (SEQ ID NO: 297)

5'- AM-C6 ca$^{m}C_{S}G_{S}A_{S}a_{S}c_{S}c_{S}a_{S}c_{S}t_{S}g_{S}a_{S}A_{S}{}^{m}C$-3' (SEQ ID NO: 298)

5'- AM-C6 ca$^{m}C_{S}G_{S}{}^{m}C_{S}a_{S}g_{S}t_{S}a_{S}t_{S}g_{S}g_{S}a_{S}T_{S}{}^{m}C$-3' (SEQ ID NO: 300)

5'- AM-C6 ca$G_{S}A_{S}A_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}c_{S}G_{S}G$-3' (SEQ ID NO: 306)

5'-AM-C6 ca$^{m}C_{S}G_{S}A_{S}a_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G$-3' (SEQ ID NO: 309)

5'-AM-C6 ca$A_{S}G_{S}G_{S}t_{S}g_{S}a_{S}a_{S}g_{S}{}^{m}c_{S}g_{S}a_{S}A_{S}G_{S}T$-3' (SEQ ID NO: 317)

5'-AM-C6 ca$G_{S}A_{S}A_{S}c_{S}c_{S}a_{S}c_{S}t_{S}g_{S}a_{S}a_{S}{}^{m}C_{S}A_{S}A_{S}A$-3' (SEQ ID NO: 585)

5'-AM-C6 ca$^{m}C_{S}G_{S}A_{S}A_{S}c_{S}c_{S}a_{S}c_{S}t_{S}g_{S}a_{S}a_{S}c_{S}A_{S}A_{S}A$-3' (SEQ ID NO: 588)

5'-AM-C6 ca$G_{S}A_{S}A_{S}g_{S}t_{S}g_{S}c_{S}a_{S}c_{S}a_{S}{}^{m}C_{S}G_{S}G$-3' (SEQ ID NO: 628)

5'-AM-C6 ca$T_{S}A_{S}G_{S}t_{S}a_{S}a_{S}a_{S}c_{S}t_{S}g_{S}a_{S}g_{S}{}^{m}C_{S}{}^{m}C_{S}A_{S}$3' (SEQ ID NO: 678)

5'-AM-C6 ca$^{m}C_{S}G_{S}A_{S}a_{S}c_{S}c_{S}a_{S}c_{S}t_{S}g_{S}A_{S}A_{S}{}^{m}C$-3' (SEQ ID NO: 600)

5'-AM-C6 ca$^{m}C_{S}G_{S}A_{S}a_{S}c_{S}c_{S}a_{S}c_{S}t_{S}g_{S}A_{S}A_{S}{}^{m}C_{S}A$-3' (SEQ ID NO: 597)

wherein uppercase letters denote beta-D-oxy-LNA units; lowercase letters denote DNA units; the subscript "s" denotes a phosphorothioate linkage; superscript m denotes a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base; AM-C6 is an amino-C6 linker;wherein the 5' terminal group "AM-C6 c a" is optional.

79. The oligomer as defined in any of paragraphs 67 to 78 for use in a medical treatment.
80. The oligomer as defined in any of paragraphs 67 to 79 for use in the treatment of a viral disorder.
81. The oligomer as defined in paragraph 79 or paragraph 80 wherein said oligomer is as defined in any one of paragraphs 1 to 53.
82. The oligomer as defined in paragraph 79 or paragraph 81 wherein said disorder is as defined in any one of paragraphs 1 to 53.
83. A composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer and at least one additional different oligonucleotide; wherein said oligomer comprises at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder.
84. A composition according to paragraph 83 wherein said oligomer is an oligomer as defined in any one of paragraphs 67 to 82.

85. A composition according to paragraph 83 or paragraph 84 wherein said at least one additional different oligonucleotide is an additional different oligonucleotide as defined in any one of paragraphs 54 to 61.
86. A method for treating a viral disorder, said method comprising administering to a subject in need of treatment an effective amount of an oligomer conjugate according to paragraph 62 or paragraph 63 or an oligomer conjugate as defined in any one of paragraphs 1 to 52.
87. A method for treating a viral disorder, said method comprising administering to a subject in need of treatment an effective amount of a composition according to any one of paragraphs 64 to 66 or a composition as defined in any one of paragraphs 54 to 61 or a composition as defined in any one of paragraphs 83 to 85.
88. A method for treating a viral disorder, said method comprising administering to a subject in need of treatment an effective amount of an oligomer according to any one of paragraphs 67 to 82.
89. A pharmaceutical composition comprising an oligomer conjugate according to paragraph 62 or paragraph 63 or an oligomer conjugate as defined in any one of paragraphs 1 to 53; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.
90. A pharmaceutical composition comprising a composition according to any one of paragraphs 64 to 66 or a composition as defined in any one of paragraphs 54 to 61 or a composition as defined in any one of paragraphs 83 to 85; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.
91. A pharmaceutical composition comprising an oligomer according to any one of paragraphs 67 to 82; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.

A pharmaceutical system comprising a pharmaceutical composition according to any one of paragraphs 89 to 91 and an additional pharmaceutical entity.

92. A method of manufacturing an oligomer conjugate according to any one of paragraphs 1 to 52, comprising conjugating one or more oligomers as defined in any one of paragraphs 1 to 53 with a carrier component as defined in any one of paragraphs 1 to 53.
93. A method of manufacturing a composition according to any one of paragraphs 64 to 66, comprising admixing an oligomer conjugate as defined in any one of paragraphs 1 to 53 with a pharmaceutically acceptable diluent, carrier, salt or adjuvant.
94. A method of manufacturing a composition according to paragraph 91, comprising admixing an oligomer as defined in any one of paragraphs 67 to 82 with a pharmaceutically acceptable diluent, carrier, salt or adjuvant.
95. The invention according to any one of the preceding paragraphs wherein the oligomer or oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-

10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of modified nucleotides in the wing region, preferably at least one being an LNA unit, preferably all being an LNA unit, the second number is the number of nucleotides in the gap region, and the third number is the number of modified nucleotides in the wing region, preferably at least one being an LNA unit, preferably all being an LNA unit.

96. An oligomer conjugate substantially as described herein and with reference to the Examples.
97. A composition substantially as described herein and with reference to the Examples.
98. An oligomer substantially as described herein and with reference to the Examples.
99. A method substantially as described herein and with reference to the Examples.

PARTICULAR EMBODIMENTS

The present invention relates to an oligomer conjugate for use in the treatment of a viral disorder. The oligomer conjugate comprises: a) an oligomer capable of modulating a target sequence in HBx or HBsAg of Hepatitis B Virus (HBV) to treat said viral disorder; and b) a carrier component conjugated to said oligomer. Preferably the carrier component is for delivering said first oligomer to the liver.

Preferred aspects for certain embodiments of the present invention are now provided.

In one aspect, the present invention provides an oligomer conjugate for use in the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component;

wherein said first oligomer region is 8-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;

wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof.

In one aspect, the present invention provides an oligomer conjugate suitable for the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component

wherein said first oligomer region is 8-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;

wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof.

In one aspect, the present invention provides a composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate and at least one additional different oligonucleotide; wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component

wherein said first oligomer region is 8-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;

wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof.

In one aspect, the present invention provides an oligomer conjugate for use in the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component;

wherein said first oligomer region is 8-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;

wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;

wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852).

In one aspect, the present invention provides an oligomer conjugate suitable for the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component

wherein said first oligomer region is 8-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;

wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;

wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
AGCGAAGTGCACACG (SEQ ID NO: 20);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);

CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852.

In one aspect, the present invention provides a composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate and at least one additional different oligonucleotide; wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
b) a carrier component

wherein said first oligomer region is 8-16 nucleotides in length;
wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;
wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;
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CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
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CGCAGTATGGATC (SEQ ID NO: 10);
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AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852.

In one aspect, the present invention provides an oligomer conjugate for use in the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
b) a carrier component;

wherein said first oligomer region is 12-16 nucleotides in length;
wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof;
preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;
wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;
wherein the oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides an oligomer conjugate suitable for the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
b) a carrier component

wherein said first oligomer region is 12-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;
wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;
wherein the oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides a composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate and at least one additional different oligonucleotide; wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
b) a carrier component

wherein said first oligomer region is 12-16 nucleotides in length;
wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof;
preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;
wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;
wherein the oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in

the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides an oligomer conjugate for use in the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and

b) a carrier component;

wherein said first oligomer region is 12-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof; preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;

wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;

wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)

GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852;

wherein the oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides an oligomer conjugate suitable for the treatment of a viral disorder, wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
b) a carrier component

wherein said first oligomer region is 12-16 nucleotides in length;
wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof;
preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;
wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;
wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);

CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852);

wherein the oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides a composition suitable for the treatment of a viral disorder, wherein said composition comprises an oligomer conjugate and at least one additional different oligonucleotide; wherein said oligomer conjugate comprises:

a) at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and
b) a carrier component,

wherein said first oligomer region is 12-16 nucleotides in length;
wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
wherein said carrier component is a carbohydrate conjugate moiety, preferably said carrier component is selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine (GalNAc), N-propionyl-galactosamine, N-n-butanoyl-galactosamine, N-isobutanoylgalactose-amine or a cluster of any one or more thereof;
preferably said carrier component comprises GalNAc or a GalNAc cluster; preferably said carrier component is GalNAc2;
wherein said target sequence comprises at least part of a gene or a mRNA encoding HBx or HBsAg or a naturally-occurring variant thereof;
wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);
AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
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AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852;

wherein the oligomer component of the oligomer conjugate comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides an oligomer for the treatment of a viral disorder, wherein said oligomer comprises at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and wherein said first oligomer region is 12-16 nucleotides in length;

wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;

wherein said first oligomer region is based on a core motif selected from the group consisting of any one or more of:

GCGTAAAGAGAGG (SEQ ID NO: 13);
GCGTAAAGAGAGGT (SEQ ID NO: 11);
AGCGAAGTGCACACG (SEQ ID NO: 20);

AGGTGAAGCGAAGTG (SEQ ID NO: 26);
AGCGAAGTGCACACGG (SEQ ID NO 18);
CGAACCACTGAACA (SEQ ID NO: 7);
GAACCACTGAACAAA (SEQ ID NO 4);
CGAACCACTGAACAAA (SEQ ID NO 5);
CGAACCACTGAACAA (SEQ ID NO 6);
CGAACCACTGAAC (SEQ ID NO 8)
CCGCAGTATGGATCG (SEQ ID NO 9)
CGCAGTATGGATC (SEQ ID NO: 10);
CGCGTAAAGAGAGGT (SEQ ID NO 12);
AGAAGGCACAGACGG (SEQ ID NO 14);
GAGAAGGCACAGACGG (SEQ ID NO 15)
GAAGTGCACACGG (SEQ ID NO 16);
GCGAAGTGCACACGG (SEQ ID NO 17);
CGAAGTGCACACG (SEQ ID NO 19);
AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852;

wherein the oligomer comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

In one aspect, the present invention provides an oligomer suitable for the treatment of a viral disorder, wherein said oligomer comprises at least one first oligomer region capable of modulating a target sequence of Hepatitis B Virus (HBV), preferably HBx or HBsAg of HBV, to treat said viral disorder; and wherein said first oligomer region is 12-16 nucleotides in length;
wherein said first oligomer region is a gapmer, preferably wherein said first oligomer region comprises a 2'-deoxyribonucleotide gap region flanked on each side by a wing, preferably wherein each wing independently comprises one or more LNA units;
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AGGTGAAGCGAAGT (SEQ ID NO 27); and
TAGTAAACTGAGCCA (SEQ ID NO: 852;

wherein the oligomer comprises any one of the motifs: 3-10-3, 3-10-2, 3-9-3, 3-9-2, 3-8-3, 3-8-2 wherein the first number is the number of LNA units in the wing region, the second number is the number of nucleotides in the gap region, and the third number is the number of LNA units in the wing region.

EXAMPLES

Materials and methods

HBsAg and HBeAg detection

Serum HBsAg and HBeAg level were determined in the serum of infected AAV-HBV mouse using the HBsAg chemoluminescence immunoassay (CLIA) and the HBeAg CLIA kit (Autobio diagnostics Co. Ltd., Zhengzhou,China, Cat. no.CL0310-2 and CL0312-2 respectively), according to the manufacturer's protocol. Briefly, 50µl of serum was transferred to the respective antibody coated microtiter plate and 50µl of enzyme conjugate reagent was added. The plate was incubated for 60 min on a shaker at room temperature before all wells were washed six times with washing buffer using an automatic washer. 25µl of substrate A and then 25µl of substrate B was added to each well. The plate was incubated for 10 min at RT before luminescence was measured using an Envision luminescence reader. HBsAg is given in the unit IU/ml; where 1 ng HBsAg =1.14 IU. HBeAg is given in the unit NCU/ml serum.

HBV DNA extraction and qPCR

Initially mice serum was diluted by a factor of 10 (1:10) with Phosphate buffered saline (PBS). DNA was extracted using the MagNA Pure 96 (Roche) robot. 50µl of the diluted serum was mixed in a processing cartridge with 200ul MagNA Pure 96 external lysis buffer (Roche, Cat. no. 06374913001) and incubated for 10 minutes. DNA was then extracted using the "MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit" (Roche, Cat. no. 06543588001) and the "Viral NA Plasma SV external lysis 2.0" protocol. DNA elution volume was 50µl.

Quantification of extracted HBV DNA was performed using a Taqman qPCR machine (ViiA7, life technologies). Each DNA sample was tested in duplicate in the PCR. 5µl of DNA sample was added to 15µl of PCR mastermix containing 10µl TaqMan Gene Expression Master Mix (Applied Biosystems, Cat. no. 4369016), 0.5 µl PrimeTime XL qPCR Primer/Probe (IDT) and 4.5µl distilled water in a 384 well plate and the PCR was performed using the following settings: UDG Incubation (2min, 50°C), Enzyme Activation (10min, 95°C) and PCR (40 cycles with 15sec, 95° for Denaturing and 1min, 60°C for annealing and extension). DNA copy numbers were calculated from C_t values based on a HBV plasmid DNA standard curve by the ViiA7 software.

Sequences for TaqMan primers and probes (IDT):

Forward core primer (F3_core): CTG TGC CTT GGG TGG CTT T

Reverse primer (R3_core): AAG GAA AGA AGT CAG AAG GCA AAA

Taqman probe (P3_core): 56-FAM/AGC TCC AAA /ZEN/TTC TTT ATA AGG GTC GAT GTC CAT G/3IABkFQ

Tissue specific In vitro linker cleavage assay

FAM-labeled oligomers with the physiologically labile linker to be tested (e.g. a DNA phosphodiester linker (PO linker)) are subjected to *in vitro* cleavage using homogenates of the relevant tissues (e.g. liver or kidney) and Serum.

The tissue and serum samples are collected from a suitable animal (e.g. mice, monkey, pig or rat) and homogenized in a homogenisation buffer (0,5% Igepal CA-630, 25 mM Tris pH 8.0, 100 mM NaCl, pH 8.0 (adjusted with 1 N NaOH).The tissue homogenates and Serum are spiked with oligomer to concentrations of 200 µg/g tissue. The samples are incubated for 24 hours at 37°C and thereafter the samples are extracted with phenol - chloroform. The solutions are subjected to AIE HPLC analyses on a Dionex Ultimate 3000 using an Dionex DNApac p-100 column and a gradient ranging from 10mM – 1 M sodium perchlorate at pH 7.5. The content of cleaved and non-cleaved oligomer is determined against a standard using both a fluoresense detector at 615 nm and a uv detector at 260 nm.

S1 nuclease cleavage assay

FAM-labelled oligomers with S1 nuclease susceptible linkers (e.g. a DNA phosphodiester linker (PO linker)) are subjected to *in vitro* cleavage in S1 nuclease extract or Serum.

100 µM of the oligomer are subjected to *in vitro* cleavage by S1 nuclease in nuclease buffer (60 U pr. 100 µL) for 20 and 120 minutes. The enzymatic activity is stopped by adding EDTA to the buffer solution. The solutions are subjected to AIE HPLC analyses on a Dionex Ultimate 3000 using an Dionex DNApac p-100 column and a gradient ranging from 10mM – 1 M sodium perchlorate at pH 7.5. The content of cleaved and non-cleaved oligomer is determined against a standard using both a fluoresense detector at 615 nm and a uv detector at 260 nm.

Example 1 Construction of conjugates

Oligonucleotides were synthesized on uridine universal supports or UnyLinker support from Kinovate using the phosphoramidite approach on a MerMade12 or an OligoMaker DNA/RNA synthesizerat 4 µmol scale. At the end of the synthesis, the oligonucleotides were cleaved from the solid support using aqueous ammonia for 1-2 hours at room temperature, and further deprotected for 16 hours at 65°C. The oligonucleotides were purified by reverse phase HPLC (RP-HPLC) and characterized by UPLC, and the molecular mass was further confirmed by ESI-MS. See below for more details.

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), LNA-T or Amino-C6 linker) was performed by using a solution of 0.1 M of the 5'-O-DMT-protected phosphoramidite in acetonitrile and DCI (4,5–dicyanoimidazole) in acetonitrile (0.25 M) as activator. Thiolation for introduction of

phosphorthioate linkages was carried out using xanthane hydride (0.01 M in acetonitrile/pyridine 9:1). Phosphordiester linkages were introduced using 0.02 M iodine in THF/Pyridine/water 7:2:1. The rest of the reagents were the ones typically used for oligonucleotide synthesis. For post solid phase synthesis conjugation a commercially available C6 aminolinker phorphoramidite was used in the last cycle of the solid phase synthesis and after deprotection and cleavage from the solid support the aminolinked deprotected oligonucleotide was isolated. The conjugate was introduced via activation of the carboxylic acid and subsequent reaction with the amine on the 5'-end of the oligonucleotide using standard synthesis methods.

Purification by RP-HPLC:

The crude compound was purified by preparative RP-HPLC on a Phenomenex Jupiter C18 10µ 150x10 mm column. 0.1 M ammonium acetate pH 8 and acetonitrile was used as buffers at a flow rate of 5 mL/min. The collected fractions were 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

Example 2 Testing in vitro efficacy

Introduction

The HbsAg assay used in the following studies is a standard method. It measures the amount of virus produced. It therefore measures a reduction in virus due to oligomers or oligomer conjugates targeting HBx or HBsAg. In addition, oligomers or oligomer conjugates that target the HBx transcriptet will also target the HbsAg transcript (see also column 3 and 4 in the results table).

Cell lines

HepG2.2.15 cells were cultured in DMEM+Glutamax-I medium (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Invitrogen) and G418 (Invitrogen) at a final concentration of 200 mg/L and maintained in 5% CO_2 at 37°C.

HBsAg Assay

HepG2.2.15 cells (a constitutively HBV-expressing cell line) were seeded in duplicate into white, 96-well plates at 1.5 x 10^4 cells/well. The cells were treated with single concentrations of oligomers or with a three-fold serial dilution series of the compounds in DMSO. The final DMSO concentration in all wells was 1% and DMSO was used as control.

The HBsAg chemiluminescence immunoassay (CLIA) kit (Autobio Diagnostics Co., Zhengzhou, China) was used to measure the levels of secreted HBV antigens semi-quantitatively. For the detection 50uL/well culture supernatant was used and the procedure conducted as directed by manufacturer's instructions. The cytotoxicity was measured using CellTiter-Glo (Promega, Madison, WI, USA, Cat# G7571). Using the E-WorkBook Suite (ID Business Solutions Ltd., Guildford, UK) dose-response curves were generated and the IC_{50} and CC_{50} values extrapolated. The IC_{50} and CC_{50} are defined as the compound concentration (or conditioned media log dilution) at which HBsAg secretion (IC50) and cytotoxicity (CC50), respectively, are reduced by 50% compared to the control. Data may be presented as the EC50 value for an oligomer, when testing at a range of concentrations, or as the absolute level of HBsAg in the supernatant as a percent of the HBsAg levels in the no drug control samples, when testing at a single concentration.

Results from single concentration treatments

A total of 290 oligomers without conjugate were screened in the in vitro efficacy assay using a single dose of 25 µM oligomer. HBV antigen (HBsAg) secretion was measured after 13 days. Table 5 below show the results of the screening. The oligomers of SEQ ID NO 294 to SEQ ID NO 318 all reduced the HBsAg activity to less than 40% of the control. The oligomer of SEQ ID NO 584 corresponds to the oligomer disclosed as SEQ ID NO 16 in US 8,598,334.

An additional 213 oligomers were screened in the in vitro efficacy assay using a single dose of 25 µM oligomer. The results are shown in Figure 5a.

Table 5: HBsAg activity of 25 µM oligomer as % of control.

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 294		X	691	GAAccactgaacAAA	22.07	2.28

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 295		X	691	CGAaccactgaacAAA	18.38	2.08
SeqID 296		X	692	CGAaccactgaaCAA	10.28	0.83
SeqID 297		X	693	CGAaccactgaACA	14.74	1.99
SeqID 298		X	694	CGAaccactgaAC	15.68	1.09
SeqID 299	x		1264	CCGcagtatggaTCG	38.83	4.40
SeqID 300	x		1265	CGCagtatggaTC	26.86	2.15
SeqID 301	x		1530	GCGtaaagagaGGT	34.38	1.52
SeqID 302	x		1530	CGCgtaaagagaGGT	38.30	6.35
SeqID 303	x		1531	GCGtaaagagaGG	37.42	3.29
SeqID 304	x		1551	AGAaggcacagaCGG	25.28	1.62
SeqID 305	x		1551	GAGaaggcacagaCGG	25.02	1.51
SeqID 306	x		1577	GAAgtgcaca^{m}cGG	14.81	3.11
SeqID 307	x		1577	GCGaagtgcacaCGG	21.88	2.55
SeqID 308	x		1577	AGCgaagtgcacaCGG	16.33	2.31
SeqID 309	x		1578	CGAagtgcacaCG	25.64	2.12
SeqID 310	x		1578	AGCgaagtgcacACG	23.45	1.99
SeqID 311	x		1578	AAGmcgaagtgcacACG	31.48	2.85
SeqID 312	x		1580	GAAg^{m}cgaagtgcACA	35.14	0.93
SeqID 313	x		1582	GGTgaag^{m}cgaagtGCA	38.27	2.92
SeqID 314	x		1583	GGTgaag^{m}cgaagTGC	30.58	4.73
SeqID 315	x		1583	AGGtgaag^{m}cgaagTGC	15.21	1.90
SeqID 316	x		1584	AGGtgaag^{m}cgaaGTG	13.27	0.84
SeqID 317	x		1585	AGGtgaag^{m}cgaAGT	13.29	0.75
SeqID 318	x		1588	CAGaggtgaagCGA	32.61	2.06
SeqID 319		X	201	AAAaccc^{m}cgccTGT	72.50	5.31
SeqID 320		X	202	AAAaccc^{m}cgccTG	69.30	7.61
SeqID 321		X	245	ACGagtctagacTCT	99.91	3.04
SeqID 322		X	245	CACgagtctagacTCT	88.47	3.67
SeqID 323		X	246	ACGagtctagaCTC	96.60	5.49
SeqID 324		X	246	CACgagtctagaCTC	94.04	2.94
SeqID 325		X	246	CCA^{m}cgagtctagaCTC	75.51	2.45

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 326		X	247	ACGagtctagaCT	75.87	2.71
SeqID 327		X	247	CACgagtctagACT	85.96	5.46
SeqID 328		X	247	CCA^{m}cgagtctagACT	65.26	8.04
SeqID 329		X	247	ACCa^{m}cgagtctagACT	73.80	4.94
SeqID 330		X	248	ACgagtctagAC	92.49	5.72
SeqID 331		X	248	CACgagtctagAC	83.99	4.30
SeqID 332		X	248	CCA^{m}cgagtctaGAC	72.31	5.85
SeqID 333		X	248	ACCa^{m}cgagtctaGAC	69.87	4.09
SeqID 334		X	248	CACca^{m}cgagtctaGAC	74.68	5.33
SeqID 335		X	249	ACCa^{m}cgagtctAGA	78.52	3.55
SeqID 336		X	249	CACca^{m}cgagtctAGA	78.82	0.55
SeqID 337		X	249	CCAcca^{m}cgagtctAGA	70.68	10.64
SeqID 338		X	250	CCAcca^{m}cgagtcTAG	72.06	4.76
SeqID 339		X	250	TCCacca^{m}cgagtcTAG	96.44	19.49
SeqID 340		X	251	CCAcca^{m}cgagtCTA	62.36	3.29
SeqID 341		X	251	TCCacca^{m}cgagtCTA	74.38	12.81
SeqID 342		X	251	GTCcacca^{m}cgagtCTA	66.96	8.93
SeqID 343		X	252	TCCacca^{m}cgagTCT	88.72	6.91
SeqID 344		X	252	GTCcacca^{m}cgagTCT	81.54	3.34
SeqID 345		X	252	AGTccacca^{m}cgagTCT	73.05	5.43
SeqID 346		X	253	GTCcacca^{m}cgaGTC	86.41	4.93
SeqID 347		X	253	AGTccacca^{m}cgaGTC	68.79	2.19
SeqID 348		X	253	AAGtccacca^{m}cgaGTC	55.97	4.51
SeqID 349		X	254	AGTccacca^{m}cgAGT	74.01	6.04
SeqID 350		X	254	AAGtccacca^{m}cgAGT	64.64	2.31
SeqID 351		X	254	GAAgtccacca^{m}cgAGT	71.86	2.47
SeqID 352		X	255	AAGtccacca^{m}cgAG	74.30	9.08
SeqID 353		X	255	GAAgtccacca^{m}cgAG	72.50	1.71
SeqID 354		X	255	AGAagtccacca^{m}cgAG	70.48	1.43
SeqID 355		X	256	AGAagtccaccaCGA	60.35	2.15
SeqID 356		X	256	GAGaagtccaccaCGA	50.28	3.88
SeqID 357		X	257	GAGaagtccaccACG	67.82	4.14

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 358		X	257	AGAgaagtccaccACG	67.76	2.44
SeqID 359		X	258	GAGagaagtccacCAC	62.90	3.23
SeqID 360		X	259	GAGagaagtccaCCA	74.92	3.15
SeqID 361		X	259	TGAgagaagtccaCCA	73.90	3.92
SeqID 362		X	260	GAGagaagtccACC	87.37	7.72
SeqID 363		X	260	TGAgagaagtccACC	65.36	4.30
SeqID 364		X	261	TGAgagaagtcCAC	109.45	11.99
SeqID 365		X	384	AAAa^{m}cgc^{m}cgcaGA	70.63	10.93
SeqID 366		X	384	TAAaa^{m}cgc^{m}cgcAGA	76.98	11.99
SeqID 367		X	384	ATAaaa^{m}cgc^{m}cgcAGA	67.24	7.92
SeqID 368		X	384	GATaaaa^{m}cgc^{m}cgcAGA	35.91	1.17
SeqID 369		X	385	ATAaaa^{m}cgc^{m}cgCAG	80.52	2.79
SeqID 370		X	385	GATaaaa^{m}cgc^{m}cgCAG	57.46	3.54
SeqID 371		X	385	TGAtaaaa^{m}cgc^{m}cgCAG	62.20	3.88
SeqID 372		X	386	ATAaaa^{m}cgc^{m}cgCA	82.52	1.41
SeqID 373		X	386	GATaaaa^{m}cgc^{m}cGCA	58.35	1.00
SeqID 374		X	386	TGAtaaaa^{m}cgc^{m}cGCA	46.17	4.23
SeqID 375		X	386	ATGataaaa^{m}cgc^{m}cGCA	42.47	2.86
SeqID 376		X	387	ATaaaa^{m}cgc^{m}cGC	86.96	6.36
SeqID 377		X	387	GATaaaa^{m}cgc^{m}cGC	61.13	4.90
SeqID 378		X	387	TGAtaaaa^{m}cgcCGC	55.76	4.38
SeqID 379		X	387	ATGataaaa^{m}cgcCGC	35.38	4.12
SeqID 380		X	388	GAtaaaa^{m}cgcCG	71.71	3.20
SeqID 381		X	388	TGAtaaaa^{m}cgcCG	56.48	9.26
SeqID 382		X	388	ATGataaaa^{m}cgCCG	64.46	2.93
SeqID 383		X	389	TGataaaa^{m}cgCC	87.42	18.65
SeqID 384		X	389	ATGataaaa^{m}cgCC	53.02	0.62
SeqID 385		X	390	ATgataaaa^{m}cGC	103.50	4.41
SeqID 386		X	411	TAGcagcaggaTG	47.43	1.27
SeqID 387		X	411	ATAgcagcaggATG	59.29	9.28
SeqID 388		X	411	CATagcagcaggATG	52.52	5.05
SeqID 389		X	411	GCAtagcagcaggATG	40.12	5.45

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 390		X	412	GCAtagcagcagGAT	33.12	2.79
SeqID 391		X	412	GGCatagcagcagGAT	34.61	1.30
SeqID 392		X	414	GAGgcatagcagcAGG	57.92	6.48
SeqID 393		X	415	TGAggcatagcagCAG	44.07	1.35
SeqID 394		X	416	TGAggcatagcaGCA	59.87	0.84
SeqID 395		X	416	ATGaggcatagcaGCA	57.12	5.36
SeqID 396		X	417	TGAggcatagcAGC	60.89	4.54
SeqID 397		X	417	ATGaggcatagcAGC	53.18	3.00
SeqID 398		X	417	GATgaggcatagcAGC	35.17	4.19
SeqID 399		X	418	GATgaggcatagCAG	51.50	4.39
SeqID 400		X	418	AGAtgaggcatagCAG	30.80	2.00
SeqID 401		X	419	GATgaggcataGCA	56.86	2.93
SeqID 402		X	419	AGAtgaggcataGCA	26.45	3.15
SeqID 403		X	419	AAGatgaggcataGCA	42.05	1.52
SeqID 404		X	422	AAGaagatgaggcATA	50.42	4.13
SeqID 405		X	423	AAGaagatgaggCAT	47.84	4.75
SeqID 406		X	601	TGGgatgggaatACA	127.91	11.95
SeqID 407		X	601	ATGggatgggaatACA	104.41	6.06
SeqID 408		X	602	TGGgatgggaaTAC	97.86	33.35
SeqID 409		X	602	ATGggatgggaaTAC	100.98	11.30
SeqID 410		X	602	GATgggatgggaaTAC	80.86	4.66
SeqID 411		X	603	ATGggatgggaATA	101.56	17.94
SeqID 412		X	603	GATgggatgggaATA	95.71	10.78
SeqID 413		X	604	GATgggatgggAAT	136.89	7.88
SeqID 414		X	691	AACcactgaacAAA	56.01	3.86
SeqID 415		X	695	CGaaccactgAA	67.83	3.12
SeqID 416		X	708	GGGggaaagccCT	61.65	34.06
SeqID 417		X	708	TGGgggaaagcCCT	166.53	7.16
SeqID 418		X	1142	GCAa^{m}cggggtaaAGG	99.66	2.38
SeqID 419		X	1143	GCAa^{m}cggggtaAAG	128.31	18.17
SeqID 420		X	1144	GCAa^{m}cggggtaAA	142.61	5.42
SeqID 421		X	1176	AGCaaacacttgGCA	81.61	3.37

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 422		X	1176	CAGcaaacacttgGCA	59.14	4.84
SeqID 423		X	1177	CAGcaaacacttGGC	47.64	0.61
SeqID 424		X	1177	TCAgcaaacacttGGC	28.43	2.09
SeqID 425		X	1178	TCAgcaaacactTGG	53.37	9.79
SeqID 426	x		1264	GCAgtatggatCG	51.14	8.21
SeqID 427	x		1264	CGCagtatggaTCG	45.10	12.21
SeqID 428	x		1264	TCCgcagtatggaTCG	40.99	6.50
SeqID 429	x		1265	CCGcagtatggATC	144.18	11.10
SeqID 430	x		1265	TCCgcagtatggATC	74.40	9.23
SeqID 431	x		1266	CGcagtatggAT	77.64	7.37
SeqID 432	x		1266	CCGcagtatggAT	86.57	3.84
SeqID 433	x		1266	TCCgcagtatgGAT	86.85	16.10
SeqID 434	x		1267	TCCgcagtatgGA	90.43	0.25
SeqID 435	x		1269	TTc^{m}cgcagtaTG	70.69	5.10
SeqID 436	x		1530	CGTaaagagagGT	65.75	2.97
SeqID 437	x		1530	CCG^{m}cgtaaagagaGGT	62.74	2.15
SeqID 438	x		1531	CGtaaagagaGG	73.84	4.24
SeqID 439	x		1531	CGCgtaaagagAGG	93.28	8.65
SeqID 440	x		1531	CCG^{m}cgtaaagagAGG	64.33	0.96
SeqID 441	x		1532	CGCgtaaagagAG	92.93	7.76
SeqID 442	x		1532	CCG^{m}cgtaaagaGAG	46.68	2.09
SeqID 443	x		1533	CG^{m}cgtaaagaGA	96.70	11.51
SeqID 444	x		1533	CCG^{m}cgtaaagaGA	63.96	2.03
SeqID 445	x		1534	CCg^{m}cgtaaagAG	60.43	4.10
SeqID 446	x		1547	GGCacaga^{m}cgggGAG	105.39	4.49
SeqID 447	x		1547	AGGcacaga^{m}cgggGAG	65.54	1.27
SeqID 448	x		1548	GGCacaga^{m}cggGGA	114.17	2.09
SeqID 449	x		1548	AGGcacaga^{m}cggGGA	63.67	1.22
SeqID 450	x		1548	AAGgcacaga^{m}cggGGA	52.62	1.97
SeqID 451	x		1549	AGGcacaga^{m}cgGGG	117.50	7.77
SeqID 452	x		1549	AAGgcacaga^{m}cgGGG	105.77	3.94
SeqID 453	x		1549	GAAggcacaga^{m}cgGGG	109.39	7.50

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 454	x		1550	AGAaggcacaga^{m}cGGG	60.92	7.17
SeqID 455	x		1552	GAGaaggcacagACG	43.34	2.63
SeqID 456	x		1577	CGAagtgcacaCGG	35.27	4.48
SeqID 457	x		1578	GCGaagtgcacACG	49.25	1.43
SeqID 458	x		1579	GCGaagtgcacAC	65.82	7.11
SeqID 459	x		1579	AGCgaagtgcaCAC	64.47	2.76
SeqID 460	x		1579	AAG^{m}cgaagtgcaCAC	42.82	5.42
SeqID 461	x		1579	GAAg^{m}cgaagtgcaCAC	37.15	4.92
SeqID 462	x		1580	AGCgaagtgcaCA	59.26	7.36
SeqID 463	x		1580	AAG^{m}cgaagtgcACA	56.58	1.80
SeqID 464	x		1580	TGAag^{m}cgaagtgcACA	58.81	5.72
SeqID 465	x		1581	AAG^{m}cgaagtgcAC	61.08	10.94
SeqID 466	x		1581	GAAg^{m}cgaagtgCAC	87.45	24.12
SeqID 467	x		1581	TGAag^{m}cgaagtgCAC	70.27	2.31
SeqID 468	x		1581	GTGaag^{m}cgaagtgCAC	91.50	6.40
SeqID 469	x		1582	AAg^{m}cgaagtgCA	75.07	12.27
SeqID 470	x		1582	GAAg^{m}cgaagtgCA	52.66	2.00
SeqID 471	x		1582	TGAag^{m}cgaagtGCA	55.33	5.23
SeqID 472	x		1582	GTGaag^{m}cgaagtGCA	45.56	1.33
SeqID 473	x		1583	TGAag^{m}cgaagtGC	47.33	2.27
SeqID 474	x		1583	GTGaag^{m}cgaagTGC	48.90	0.54
SeqID 475	x		1584	GTGaag^{m}cgaagTG	44.41	3.30
SeqID 476	x		1584	GGTgaag^{m}cgaaGTG	39.79	2.48
SeqID 477	x		1584	GAGgtgaag^{m}cgaaGTG	45.00	0.65
SeqID 478	x		1585	GTgaag^{m}cgaaGT	62.45	5.15
SeqID 479	x		1585	GGTgaag^{m}cgaaGT	43.08	2.85
SeqID 480	x		1585	GAGgtgaag^{m}cgaAGT	55.57	2.43
SeqID 481	x		1585	AGAggtgaag^{m}cgaAGT	39.17	2.82
SeqID 482	x		1586	AGAggtgaag^{m}cgAAG	41.25	0.88
SeqID 483	x		1586	CAGaggtgaag^{m}cgAAG	53.21	3.02
SeqID 484	x		1587	AGAggtgaag^{m}cGAA	38.81	4.56
SeqID 485	x		1587	CAGaggtgaag^{m}cGAA	42.07	2.56

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 486	x		1587	GCAgaggtgaag^{m}cGAA	56.89	8.91
SeqID 487	x		1588	GCAgaggtgaagCGA	53.71	4.36
SeqID 488	x		1588	TGCagaggtgaagCGA	70.80	5.16
SeqID 489	x		1589	TGCagaggtgaaGCG	92.30	7.94
SeqID 490	x		1589	GTGcagaggtgaaGCG	70.51	10.18
SeqID 491	x		1590	CGTgcagaggtgaAGC	130.05	13.54
SeqID 492	x		1591	CGTgcagaggtgAAG	91.47	26.28
SeqID 493	x		1591	ACGtgcagaggtgAAG	90.23	8.21
SeqID 494	x		1592	CGTgcagaggtGAA	64.85	22.74
SeqID 495	x		1592	ACGtgcagaggtGAA	58.06	4.58
SeqID 496	x		1593	CGTgcagaggtGA	81.44	11.66
SeqID 497	x		1593	ACGtgcagaggTGA	50.58	6.38
SeqID 498	x		1616	CGTtca^{m}cggtgGT	54.29	3.89
SeqID 499	x		1690	CTCaaggt^{m}cggTC	68.75	3.36
SeqID 500	x		1691	CCTcaaggt^{m}cgGT	110.10	6.42
SeqID 501	x		1691	GCCtcaaggt^{m}cGGT	94.30	7.43
SeqID 502	x		1706	ACAgtctttgaaGTA	90.33	13.01
SeqID 503	x		1783	TTTatgcctacAG	98.15	8.26
SeqID 504	x		1784	AATttatgcctACA	115.05	4.58
SeqID 505	x		1785	AATttatgcctAC	126.86	2.63
SeqID 506	x		1787	CCAatttatgcCT	152.55	29.03
SeqID 507	x		1865	GCTtggaggcttGAA	103.91	5.71
SeqID 508	x		1865	AGCttggaggcttGAA	133.58	0.46
SeqID 509	x		1866	GCTtggaggctTGA	79.26	8.08
SeqID 510	x		1866	AGCttggaggctTGA	122.38	3.30
SeqID 511	x		1866	CAGcttggaggctTGA	132.43	18.26
SeqID 512	x		1867	GCTtggaggctTG	81.83	10.69
SeqID 513	x		1867	AGCttggaggcTTG	98.04	9.53
SeqID 514	x		1867	CAGcttggaggcTTG	113.24	9.04
SeqID 515	x		1867	ACAgcttggaggcTTG	145.15	11.39
SeqID 516	x		1868	CACagcttggaggCTT	122.78	2.77
SeqID 517	x		1869	CACagcttggagGCT	123.68	22.48

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 518	x		1869	GCAcagcttggagGCT	145.75	6.22
SeqID 519	x		1870	GCAcagcttggaGGC	146.57	13.30
SeqID 520	x		1870	GGCacagcttggaGGC	127.49	1.31
SeqID 521	x		1871	AGGcacagcttggAGG	126.44	11.99
SeqID 522	x		1872	AGGcacagcttgGAG	113.11	1.53
SeqID 523	x		1872	AAGgcacagcttgGAG	97.13	2.46
SeqID 524	x		1873	AAGgcacagcttGGA	102.44	3.67
SeqID 525	x		1873	CAAggcacagcttGGA	112.11	6.26
SeqID 526	x		1874	AAGgcacagctTGG	111.03	4.15
SeqID 527	x		1874	CAAggcacagctTGG	108.76	6.19
SeqID 528	x		1874	CCAaggcacagctTGG	111.82	10.50
SeqID 529	x		1875	CAAggcacagcTTG	111.24	3.42
SeqID 530	x		1875	CCAaggcacagcTTG	113.32	5.72
SeqID 531	x		1876	CCAaggcacagCTT	93.54	9.51
SeqID 532		X	2272	TGCgaatccacAC	111.18	2.58
SeqID 533		X	2272	GTG^{m}cgaatccaCAC	116.70	3.36
SeqID 534		X	2370	GGAgttcttcttCTA	117.09	3.11
SeqID 535		X	2370	GGGagttcttcttCTA	138.82	2.94
SeqID 536		X	2371	GGGagttcttctTCT	112.95	5.88
SeqID 537		X	2371	AGGgagttcttctTCT	115.73	2.39
SeqID 538		X	2372	AGGgagttcttcTTC	115.60	13.69
SeqID 539		X	2372	GAGggagttcttcTTC	160.52	12.84
SeqID 540		X	2373	AGGgagttcttCTT	187.99	14.17
SeqID 541		X	2373	GAGggagttcttCTT	152.23	3.57
SeqID 542		X	2373	CGAgggagttcttCTT	125.29	10.56
SeqID 543		X	2374	CGAgggagttctTCT	125.58	12.12
SeqID 544		X	2374	GCGagggagttctTCT	127.13	7.13
SeqID 545		X	2375	GCGagggagttcTTC	140.39	2.94
SeqID 546		X	2375	GGCgagggagttcTTC	103.21	15.13
SeqID 547		X	2376	GCGagggagttCTT	104.72	31.01
SeqID 548		X	2376	GGCgagggagttCTT	120.43	4.29
SeqID 549		X	2376	AGG^{m}cgagggagttCTT	126.12	8.49

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 550		X	2377	GCGagggagttCT	99.63	21.49
SeqID 551		X	2377	GGCgagggagtTCT	126.46	5.07
SeqID 552		X	2377	AGG^{m}cgagggagtTCT	124.41	7.46
SeqID 553		X	2377	GAGg^{m}cgagggagtTCT	122.05	4.13
SeqID 554		X	2378	GGCgagggagtTC	93.75	32.13
SeqID 555		X	2378	AGG^{m}cgagggagTTC	97.91	10.99
SeqID 556		X	2378	GAGg^{m}cgagggagTTC	125.97	14.10
SeqID 557		X	2378	CGAgg^{m}cgagggagTTC	114.85	11.32
SeqID 558		X	2379	AGG^{m}cgagggagTT	161.79	17.40
SeqID 559		X	2379	GAGg^{m}cgagggaGTT	112.49	14.53
SeqID 560		X	2379	CGAgg^{m}cgagggaGTT	99.73	6.03
SeqID 561		X	2379	GCGagg^{m}cgagggaGTT	26.82	16.61
SeqID 562		X	2380	GAGg^{m}cgagggaGT	126.11	10.60
SeqID 563		X	2380	CGAgg^{m}cgagggAGT	113.92	2.48
SeqID 564		X	2380	GCGagg^{m}cgagggAGT	114.21	22.83
SeqID 565		X	2380	TGCgagg^{m}cgagggAGT	120.75	7.83
SeqID 566		X	2381	CGAgg^{m}cgagggAG	96.48	7.91
SeqID 567		X	2381	GCGagg^{m}cgaggGAG	111.38	23.08
SeqID 568		X	2381	TGCgagg^{m}cgaggGAG	150.61	9.91
SeqID 569		X	2381	CTG^{m}cgagg^{m}cgaggGAG	115.39	3.45
SeqID 570		X	2382	CGagg^{m}cgaggGA	94.94	9.74
SeqID 571		X	2382	GCGagg^{m}cgaggGA	107.97	15.32
SeqID 572		X	2382	TGCgagg^{m}cgagGGA	90.20	10.52
SeqID 573		X	2382	CTG^{m}cgagg^{m}cgagGGA	117.46	22.79
SeqID 574		X	2382	TCTg^{m}cgagg^{m}cgagGGA	119.40	1.22
SeqID 575		X	2383	TCTg^{m}cgagg^{m}cgaGGG	139.98	6.20
SeqID 576		X	2383	GTCtg^{m}cgagg^{m}cgaGGG	17.32	7.04
SeqID 577		X	2824	GTTcccaagaaTAT	121.37	5.20
SeqID 578		X	2824	TGTtcccaagaaTAT	119.62	5.98
SeqID 579		X	2825	GTTcccaagaaTA	118.81	12.04
SeqID 580		X	2825	TGTtcccaagaATA	119.14	20.21
SeqID 581		X	2825	TTGttcccaagaATA	109.70	3.36
SeqID 582		X	2826	TGTtcccaagaAT	110.02	17.04

SeqID	Oligomers that bind both the HBsAg and the HBx transcript (SEQ ID NO 2)	Oligomers that bind HBsAg transcripts (SEQ ID NO 3), but not HBx transcript	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
SeqID 583		X	2826	TTGttcccaagAAT	132.22	4.26
SeqID 584		X	414	GAGGcatagcagCAGG	79.64	10.52

Table 5a: HBsAg activity of 25 µM oligomer as % of control.

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
585	691	GAAccactgaaCAAA	31.9	2.6
586	691	GAACcactgaacAAA	35.1	1.3
587	691	CGaaccactgaaCAAA	45.5	2.4
588	691	CGAAccactgaacAAA	18.3	2.2
589	691	CGAaccactgaaCAAA	36.2	9.1
590	691	CGAAccactgaacaAA	22.4	1.6
591	691	CGAAccactgaaCAAA	26.3	2.3
592	692	CGAAccactgaacAA	11.8	1.2
593	692	CGAAccactgaaCAA	21.9	8.5
594	692	CGAaccactgaACAA	18.3	0.6
595	693	CGaaccactgAACA	25.8	1.6
596	693	CGAAccactgaaCA	9.5	1.5
597	693	CGAaccactgAACA	23.6	2.0
598	693	CGAAccactgaACA	14.6	0.9
599	694	CGaaccactgAAC	42.1	3.8
600	694	CGAaccactgAAC	25.0	1.7
601	1264	CCgcagtatggATCG	98.7	16.4
602	1264	CCGCagtatggatCG	62.0	2.0
603	1264	CCGCagtatggaTCG	79.6	21.3
604	1264	CCGcagtatggATCG	113.3	12.3
605	1265	CGCAgtatggaTC	43.0	5.9

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
606	1265	CGcagtatggATC	97.7	22.6
607	1265	CGCagtatggATC	80.1	21.3
608	1265	CGcagtatgGATC	110.0	11.2
609	1530	GCGTaaagagagGT	65.2	6.6
610	1530	GCgtaaagagAGGT	43.4	6.1
611	1530	GCGtaaagagAGGT	59.9	5.8
612	1530	GCGTaaagagaGGT	52.7	1.9
613	1530	CGCGtaaagagagGT	96.7	9.7
614	1530	CG^{m}cgtaaagagAGGT	35.9	1.1
615	1530	CGCGtaaagagaGGT	63.1	3.9
616	1530	CGCgtaaagagAGGT	65.0	4.6
617	1531	GCgtaaagagAGG	24.6	1.5
618	1531	GCGtaaagagAGG	32.2	1.3
619	1531	GCgtaaagaGAGG	54.7	4.7
620	1531	GCGTaaagagaGG	59.0	0.4
621	1551	AGaaggcacagACGG	41.2	4.2
622	1551	AGAaggcacagACGG	45.4	10.9
623	1551	AGAAggcacagaCGG	38.3	4.0
624	1551	GAGAaggcacagaCGG	35.0	11.1
625	1551	GAGaaggcacagACGG	50.3	10.2
626	1551	GAGAaggcacagACGG	48.1	2.1
627	1577	GAagtgcacaCGG	21.5	2.1
628	1577	GAAgtgcacaCGG	23.7	1.4
629	1577	GAAGtgcacaCGG	41.2	1.3
630	1577	GAAgtgcacACGG	29.3	1.3
631	1577	GCgaagtgcacaCGG	54.0	19.6
632	1577	GCGaagtgcaca^{m}cGG	49.7	10.7
633	1577	GCGAagtgcaca^{m}cGG	30.3	5.2
634	1577	GCgaagtgcacACGG	46.9	7.3
635	1577	AGCGaagtgcaca^{m}cGG	47.5	10.3
636	1577	AG^{m}cgaagtgcacACGG	38.1	4.2
637	1577	AG^{m}cgaagtgcacaCGG	83.2	37.3
638	1577	AGCgaagtgcaca^{m}cGG	43.1	17.7
639	1578	CGaagtgcaCACG	58.3	6.9
640	1578	CGAagtgcacACG	30.9	2.7
641	1578	CGaagtgcacACG	45.4	2.1
642	1578	AGCgaagtgcaCACG	128.1	7.6
643	1578	AGCGaagtgcacACG	49.6	5.3

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
644	1578	AGCGaagtgcacaCG	47.4	5.3
645	1578	AG^{m}cgaagtgcaCACG	59.1	3.5
646	1578	AAg^{m}cgaagtgcaCACG	91.7	20.2
647	1578	AAGCgaagtgcacaCG	49.5	3.0
648	1578	AAG^{m}cgaagtgcaCACG	63.2	1.9
649	1578	AAGCgaagtgcacACG	41.9	2.1
650	1578	AAGCgaagtgcaCACG	64.7	4.5
651	1580	GAag^{m}cgaagtgCACA	142.9	18.3
652	1580	GAAG^{m}cgaagtgcaCA	61.5	4.1
653	1580	GAAg^{m}cgaagtgCACA	152.1	21.8
654	1580	GAAG^{m}cgaagtgCACA	167.2	17.5
655	1582	GGtgaag^{m}cgaagtGCA	117.8	18.0
656	1582	GGTgaag^{m}cgaagtgCA	108.0	10.4
657	1582	GGTGaag^{m}cgaagtgCA	93.5	24.7
658	1582	GGtgaag^{m}cgaagTGCA	125.1	18.6
659	1583	GGtgaag^{m}cgaagTGC	109.4	18.5
660	1583	GGTgaag^{m}cgaagtGC	104.1	14.0
661	1583	GGTGaag^{m}cgaagtGC	84.4	23.1
662	1583	GGtgaag^{m}cgaaGTGC	60.1	9.7
663	1583	AGgtgaag^{m}cgaagTGC	48.4	5.5
664	1583	AGGtgaag^{m}cgaagtGC	42.3	3.7
665	1583	AGGTgaag^{m}cgaagtGC	71.6	12.3
666	1583	AGgtgaag^{m}cgaaGTGC	50.7	10.7
667	1584	AGGTgaag^{m}cgaagTG	47.7	44.6
668	1584	AGgtgaag^{m}cgaAGTG	27.7	2.1
669	1584	AGGtgaag^{m}cgaAGTG	15.7	2.1
670	1584	AGGTgaag^{m}cgaaGTG	58.8	49.5
671	1585	AGGTgaag^{m}cgaaGT	118.2	14.8
672	1585	AGgtgaag^{m}cgAAGT	31.5	38.6
673	1585	AGGtgaag^{m}cgAAGT	25.8	4.4
674	1585	AGGTgaag^{m}cgaAGT	48.2	37.8
675	1588	CAGAggtgaag^{m}cGA	52.4	4.6
676	1588	CAgaggtgaaGCGA	67.4	0.1
677	1588	CAGaggtgaaGCGA	79.0	9.4
678	670	TAGtaaactgagCCA	31.3	2.7
679	670	TAgtaaactgaGCCA	93.0	12.1
680	670	TAGTaaactgagcCA	15.8	2.4
681	670	TAGtaaactgaGCCA	66.9	6.6

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
682	670	TAGTaaactgagCCA	26.6	4.9
683	670	CTAgtaaactgagCCA	101.6	6.7
684	670	CTagtaaactgaGCCA	158.0	11.6
685	670	CTAGtaaactgagcCA	102.4	34.7
686	671	CTAgtaaactgaGCC	76.3	32.9
687	671	CTagtaaactgAGCC	53.0	13.8
688	671	CTAGtaaactgagCC	41.9	5.1
689	671	CTagtaaactgaGCC	31.8	2.2
690	671	CTAgtaaactgagCC	102.9	10.2
691	674	GCActagtaaacTGA	53.6	4.8
692	674	GCactagtaaaCTGA	74.4	1.5
693	674	GCACtagtaaactGA	81.9	10.6
694	674	GCActagtaaaCTGA	54.5	1.5
695	674	GCACtagtaaacTGA	74.9	4.5
696	674	GGCactagtaaacTGA	42.8	60.5
697	674	GGcactagtaaaCTGA	47.1	38.9
698	674	GGCActagtaaactGA	147.7	7.1
699	1141	CAA^{m}cggggtaaaGGT	187.2	5.6
700	1141	CAa^{m}cggggtaaAGGT	176.2	18.0
701	1141	CAACggggtaaagGT	187.9	3.8
702	1141	CAA^{m}cggggtaaAGGT	142.7	19.7
703	1141	CAACggggtaaaGGT	144.8	31.8
704	1261	CAGtatggat^{m}cgGCA	59.6	19.9
705	1261	CAgtatggat^{m}cGGCA	54.3	4.1
706	1261	CAgtatggat^{m}cgGCA	70.0	8.6
707	1261	CAGtatggat^{m}cggCA	60.3	8.0
708	1265	TTC^{m}cgcagtatggATC	110.7	2.0
709	1265	TTc^{m}cgcagtatgGATC	105.5	4.2
710	1265	TTCCgcagtatggaTC	104.1	6.7
711	1265	TTC^{m}cgcagtatgGATC	107.1	8.8
712	1265	TTCCgcagtatggATC	119.0	9.6
713	1266	TTC^{m}cgcagtatgGAT	99.8	6.8
714	1266	TTc^{m}cgcagtatGGAT	92.3	5.1
715	1266	TTCCgcagtatggAT	104.7	3.6
716	1266	TTC^{m}cgcagtatGGAT	108.7	3.8
717	1266	TTCCgcagtatgGAT	112.0	2.1
718	1266	GTTc^{m}cgcagtatgGAT	85.5	4.5
719	1266	GTtc^{m}cgcagtatGGAT	80.0	6.7

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
720	1266	GTTC^{m}cgcagtatggAT	127.9	16.9
721	1267	GTtc^{m}cgcagtaTGGA	67.2	5.0
722	1267	GTTC^{m}cgcagtatgGA	150.8	5.9
723	1267	GTtc^{m}cgcagtatGGA	78.6	8.0
724	1267	GTTc^{m}cgcagtatgGA	76.5	7.9
725	1267	AGTtc^{m}cgcagtatGGA	72.6	5.2
726	1267	AGTTc^{m}cgcagtatgGA	87.1	5.6
727	1267	AGttc^{m}cgcagtatGGA	83.1	4.3
728	1267	AGTtc^{m}cgcagtatgGA	79.4	5.6
729	1267	AGttc^{m}cgcagtatgGA	89.9	2.0
730	1268	AGTtc^{m}cgcagtaTGG	51.2	4.0
731	1268	AGttc^{m}cgcagtaTGG	63.6	0.5
732	1268	AGTtc^{m}cgcagtatGG	65.9	1.6
733	1268	AGttc^{m}cgcagtatGG	80.9	2.5
734	1268	GAgttc^{m}cgcagtaTGG	49.2	4.7
735	1268	GAGttc^{m}cgcagtatGG	60.1	6.1
736	1268	GAgttc^{m}cgcagtatGG	73.9	1.1
737	1269	GAGTtc^{m}cgcagtaTG	58.6	6.6
738	1269	GAgttc^{m}cgcagtATG	57.1	8.1
739	1269	GAGttc^{m}cgcagtaTG	49.8	6.8
740	1269	GAgttc^{m}cgcagtaTG	60.8	1.6
741	1269	GGAGttc^{m}cgcagtaTG	137.3	2.6
742	1269	GGagttc^{m}cgcagtATG	90.5	22.8
743	1269	GGAgttc^{m}cgcagtaTG	117.5	2.4
744	1269	GGagttc^{m}cgcagtaTG	124.7	6.4
745	1525	TAAagagaggtg^{m}cGCC	71.3	60.7
746	1525	TAaagagaggtgCGCC	73.9	59.0
747	1525	TAAAgagaggtg^{m}cgCC	79.5	45.6
748	1525	TAAagagaggtgCGCC	93.6	4.6
749	1525	TAAAgagaggtg^{m}cGCC	28.0	22.3
750	1526	TAAagagaggtgCGC	96.3	22.2
751	1526	TAaagagaggtGCGC	101.9	73.2
752	1526	TAAagagaggtGCGC	44.3	72.6
753	1526	TAAAgagaggtgCGC	64.1	50.8
754	1526	GTAaagagaggtgCGC	27.0	47.8
755	1526	GTaaagagaggtGCGC	65.6	58.8
756	1527	GTAaagagaggtGCG	23.6	42.9
757	1527	GTaaagagaggTGCG	80.5	2.4

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
758	1527	GTAaagagaggTGCG	81.8	61.8
759	1527	GTAAagagaggtGCG	31.5	35.2
760	1527	CGtaaagagaggTGCG	91.5	62.5
761	1527	CGTAaagagaggtgCG	70.5	60.0
762	1527	CGTaaagagaggTGCG	79.6	60.1
763	1527	CGTAaagagaggtGCG	4.5	4.1
764	1528	CGTaaagagaggTGC	37.0	55.1
765	1528	CGtaaagagagGTGC	89.4	17.7
766	1528	CGTAaagagaggtGC	93.3	17.1
767	1528	CGTaaagagagGTGC	63.4	4.2
768	1528	CGTAaagagaggTGC	34.1	51.7
769	1528	GCGtaaagagaggTGC	50.5	83.8
770	1528	GCgtaaagagagGTGC	43.6	73.2
771	1528	GCgtaaagagaggTGC	31.3	53.0
772	1528	GCGtaaagagaggtGC	43.9	69.9
773	1529	GCGtaaagagagGTG	53.2	67.4
774	1529	GCgtaaagagaGGTG	2.9	3.1
775	1529	GCGTaaagagaggTG	44.0	32.8
776	1529	GCGtaaagagaGGTG	8.8	4.3
777	1529	GCGTaaagagagGTG	-0.6	0.6
778	1529	CGCgtaaagagagGTG	43.2	40.7
779	1529	^{m}cg^{m}cgtaaagagaGGTG	8.3	5.3
780	1529	CGCGtaaagagaggTG	37.6	40.0
781	1529	CGCgtaaagagaGGTG	33.5	40.4
782	1529	CGCGtaaagagagGTG	33.5	54.0
783	1552	TGAgaaggcacagACG	95.2	5.5
784	1552	TGagaaggcacaGACG	54.4	48.4
785	1552	TGAGaaggcacagaCG	67.2	6.3
786	1552	TGAgaaggcacaGACG	49.4	43.8
787	1552	TGAGaaggcacagACG	56.1	9.5
788	1690	GCctcaaggt^{m}cgGTC	78.8	70.1
789	1690	GCCtcaaggt^{m}cggTC	21.6	40.1
790	1690	GCctcaaggt^{m}cggTC	46.7	74.2
791	1778	ATgcctacagccTCC	51.8	1.4
792	1778	ATGcctacagcctCC	51.1	2.2
793	1778	ATgcctacagcctCC	59.4	7.0
794	1785	ACCAatttatgcCTAC	145.3	4.8
795	1785	ACCaatttatgcCTAC	138.6	10.2

SeqID	Oligomer start position U95551 (SEQ ID NO 1)	LNA oligomer sequnces (Upper case letters = beta-D-oxy LNA, C LNA is 5-methyl C LNA, lower case letters = DNA, ^{m}c = 5-methylcytosine DNA, all internucleoside linkages are phosphorothiate internucleoside linkages)	Activity (HBsAg levels in culture supernatant as percent of DMSO treated cells)	standard dev
796	1785	ACCAatttatgccTAC	137.4	0.4
797	1785	ACCaatttatgccTAC	131.3	9.2
798	1785	ACcaatttatgcCTAC	126.0	8.7

Results from multiple concentration treatments

A selection of oligomers from Table 5 were tested using three-fold serial dilutions (25.000, 8.3333, 2.7778, 0.9259, 0.0343, 0.0114, 0.0038, 0.0013 µM oligomer) in the in vitro efficacy assay to assess IC 50 and CC50 values for the oligomers. HBV antigen (HBsAg) secretion was measured after 13 days. Table 6 below show the results of the analysis. The oligomer of SEQ ID NO 585 corresponds to the oligomer disclosed as SEQ ID NO 16 in US 8,598,334.

Table 6

Seq ID	**IC_{50} (µM)**	**CC_{50} (µM)**
SeqID 294	2.07	>25
SeqID 295	2.05	>25
SeqID 296	1.72	<0.0013
SeqID 297	0.45	>25
SeqID 298	0.44	>25
SeqID 300	0.54	>25
SeqID 301	0.96	>25
SeqID 303	2.57	>25
SeqID 304	1.70	<0.0013
SeqID 305	2.05	>25
SeqID 306	1.18	>25
SeqID 307	0.68	>25
SeqID 308	2.62	>25
SeqID 309	2.15	>25
SeqID 310	2.04	>25
SeqID 311	9.75	>25
SeqID 315	1.12	>25
SeqID 316	1.13	>25
SeqID 317	0.80	>25
SeqID 318	5.27	>25
SeqID 368	>25	<0.0013
SeqID 386	12.32	>25
SeqID 389	23.43	>25
SeqID 390	2.57	>25
SeqID 391	5.91	>25
SeqID 393	3.08	>25
SeqID 398	19.84	>25
SeqID 400	3.07	>25
SeqID 402	2.00	>25
SeqID 424	2.43	>25
SeqID 427	0.61	>25
SeqID 442	2.24	>25

SeqID 456	0.78	>25
SeqID 457	6.05	>25
SeqID 473	3.91	>25
SeqID 474	4.67	>25
SeqID 475	6.15	>25
SeqID 476	3.82	>25
SeqID 479	5.88	>25
SeqID 481	6.63	>25
SeqID 482	10.10	>25
SeqID 484	17.04	>25
SeqID 485	4.34	>25
SeqID 584	>25	>25

Example 3 In vivo AAV/HBV Mouse Model:

Anti-HBV LNAs can be evaluated in AAV/HBV mouse model. In this model, mice infected with a recombinant adeno-associated virus (AAV) carrying the HBV genome (AAV/HBV) maintains stable viremia and antigenimia for more than 30 weeks (Dan Yang, et al. 2014 Cellular & Molecular Immunology 11, 71–78).

Male C57BL/6 mice (4-6 weeks old), specific pathogen free, are purchased from SLAC (Shanghai Laboratory Animal Center of Chinese Academy of Sciences) and housed in an animal care facility in individually ventilated cages. Guidelines are followed for the care and use of animals as indicated by WuXi IACUC (Institutional Animal Care and Use Committee, WUXI IACUC protocol number R20131126-Mouse). Mice are allowed to acclimate to the new environment for 3 days and are grouped according to the experimental design.

Recombinant AAV-HBV was diluted in PBS, 200 µL per injection. This recombinant virus carries 1.3 copies of the HBV genome (genotype D, serotype ayw).

On day 0, all mice are injected through tail vein with 200 µL AAV-HBV. On days 6, 13 and 20 after AAV injection, all mice in are submandibularly bled (0.1 ml blood/mouse) for serum collection. On day 22 post injection, mice with stable viremia are treated with vehicle or anti-HBV LNAs dosed intravenously at 5 mg/kg. The LNA oligomers can be unconjugated or GalNAc conjugated.

Mice are dosed biweekly for two weeks. On days 3, 7, 10 and 14 days after first LNA dosing, all mice are submandibularly bled (0.1 ml blood/mouse) for serum collection to monitor HBV surface antigen (HBsAg), HBV e antigen (HBeAg), and HBV genomic DNA in serum.

Example 4 In vivo study of bi-weekly injections at single dose

The AAV/HBV Mouse Model as prepared in Example 3 was used in this study. Ten GalNAc conjugated Anti-HBV LNA oligomers were tested with saline as control in C57BL/6 mice with stable viremia. Some of the oligomers were compared with the standard of care nucleoside analog, Entecavir (ETV), administered as prescribed with 0.03 mg per kilo as a daily oral dosis.

Mice were dosed subcutaneously twice weekly for two weeks on days 0, 3, 7 and 10 or day 0, 3, 6 and 9 with a dose of 2 mg/kg pr injection. HBV surface antigen (HBsAg), HBV e antigen (HBeAg), and HBV genomic DNA in serum was measured at the indicated days using the methods described in the "Materials and methods" section. The mice were followed for 23 – 24 days.

The results are shown in the tables below.

Table 7A-C – Serum level of HBsAg (log_{10}(IU/ml)) following twice-weekly dosages of 2 mg/kg Data are from three independent studies.

A	Saline		SEQID806		SEQID807		SEQID815		SEQID800		SEQID802		ETV	
	HBsAg		HBsAg		HBsAg		HBsAg		HBsAg		HBsAg		HBsAg	
Day		St dev		St dev		St Dev		St Dev		St Dev		St dev		St dev
0	4.23	0.53	4.79	0.04	4.68	0.04	4.06	0.76	4.73	0.01	4.07	0.37	4.49	0.24
3	4.05	0.40	4.48	0.16	4.00	0.12	3.48	0.83	4.08	0.14	3.89	0.24	4.37	0.30
7	4.26	0.22	4.17	0.13	3.37	0.20	2.68	0.71	3.23	0.23	3.57	0.52	4.33	0.33
10	4.38	0.17	4.09	0.14	3.12	0.17	2.60	0.61	2.90	0.30	3.66	0.53	4.43	0.25
14	4.37	0.20	3.86	0.33	2.82	0.21	2.69	0.45	2.67	0.38	3.52	0.52	4.53	0.22
17	4.47	0.12	4.10	0.23	3.03	0.20	2.91	0.36	2.20	0.84	3.62	0.49	3.71	1.52
21	4.55	0.12	4.21	0.22	3.37	0.20	3.36	0.44	2.92	0.24	3.72	0.50	4.64	0.11
24	4.57	0.11	4.36	0.20	3.67	0.08	3.91	0.34	3.21	0.25	4.06	0.32	4.72	0.15

B	Saline		SEQID808		SEQID814		SEQID826		SEQID825	
	HBsAg		HBsAg		HBsAg		HBsAg		HBsAg	
Day		stdev		stdev		stdev		stdev		stdev
0	4.68	0.10	4.64	0.05	4.67	0.07	4.00	0.43	4.50	0.09
3	4.40	0.12	3.99	0.09	3.64	0.16	3.21	0.90	3.44	0.05
6	4.49	0.09	3.56	0.07	2.78	0.20	2.62	1.03	2.59	0.13
9	4.50	0.07	3.24	0.07	2.36	0.16	2.32	1.00	2.16	0.20
13	4.71	0.07	3.16	0.14	2.29	0.15	2.21	1.03	2.03	0.22
16	4.58	0.05	3.10	0.15	2.64	0.08	2.37	1.05	2.27	0.21
20	4.71	0.03	3.37	0.21	3.05	0.14	2.45	1.18	2.47	0.33
23	4.62	0.08	3.47	0.23	3.36	0.12	2.68	1.24	2.54	0.62

C	Saline		SEQID824	
	HBsAg		HBsAg	
day		stdev		stdev
0	4.49	0.13	4.67	0.03
3	4.62	0.13	4.14	0.06

C	Saline		SEQID824	
	HBsAg		HBsAg	
6	4.50	0.14	3.00	0.13
9	4.45	0.21	2.35	0.15
13	4.36	0.38	2.19	0.25
16	4.20	0.66	2.45	0.20
20	4.46	0.09	3.45	0.22
23	3.97	1.05	2.42	0.18

From these data it can be concluded that *in vivo* all GalNAc conjugated anti-HBV antisense oligomers are capable of reducing serum levels of HBV s antigen (HBsAg) to a level that is lower than saline and standard of care. In particular SEQ ID NO 807, SEQ ID NO: 808, SEQ ID NO: 814, SEQ ID NO: 815, SEQ ID NO: 825, SEQ ID NO: 826 can be demonstrated to greatly reduce the serum levels of HBsAg.

Table 8A-C – Serum level of HBeAg (log_{10}(NCU/ml)) following twice-weekly dosages of 2 mg/kg

A	Saline		SEQID806		SEQID807		SEQID815		SEQID800		SEQID802		ETV	
	HBeAg		HBeAg		HBeAg		HBeAg		HBeAg		HBeAg		HBeAg	
Day		St dev		St dev		St dev		St dev		St dev		St dev		St dev
0	3.63	0.05	3.66	0.05	3.62	0.03	3.65	0.04	3.63	0.03	3.63	0.03	3.59	0.05
3	3.54	0.04	3.21	0.06	2.91	0.05	3.04	0.05	2.65	0.03	3.43	0.04	3.62	0.03
7	3.61	0.08	3.00	0.05	2.41	0.14	2.54	0.13	2.19	0.05	3.37	0.03	3.64	0.02
10	3.63	0.05	2.21	1.05	2.21	0.11	2.35	0.93	1.96	0.05	2.29	0.91	3.67	0.02
14	3.63	0.04	2.69	0.34	2.09	0.10	2.95	0.29	1.98	0.10	2.96	0.33	3.67	0.03
17	3.66	0.06	2.90	0.24	2.27	0.10	2.84	0.30	1.73	0.54	2.83	0.25	2.96	1.20
21	3.67	0.04	3.05	0.08	2.45	0.09	2.82	0.10	2.07	0.20	3.33	0.03	3.68	0.01
24	3.70	0.07	3.27	0.03	2.66	0.06	3.12	0.06	2.29	0.03	3.40	0.02	3.74	0.04

B	Saline		SEQID808		SEQID814		SEQID826		SEQID825	
	HBeAg		HBeAg		HBeAg		HBeAg		HBeAg	
Day		stdev		stdev		stdev		stdev		Stdev
0	3.72	0.08	3.63	0.03	3.56	0.03	3.32	0.17	3.51	0.12
3	3.43	0.06	2.74	0.03	2.48	0.08	2.82	0.18	2.31	0.07
6	3.44	0.02	2.35	0.06	1.98	0.07	2.43	0.16	1.94	0.06
9	3.36	0.02	2.03	0.11	1.84	0.07	2.22	0.11	1.76	0.05
13	3.72	0.05	2.25	0.08	1.96	0.03	2.23	0.09	1.95	0.08
16	3.66	0.10	2.32	0.06	2.08	0.04	2.45	0.15	1.98	0.08
20	3.74	0.01	2.58	0.04	2.36	0.05	2.67	0.18	2.21	0.11

B	Saline		SEQID808		SEQID814		SEQID826		SEQID825	
	HBeAg		HBeAg		HBeAg		HBeAg		HBeAg	
23	3.65	0.05	2.60	0.07	2.52	0.08	2.82	0.10	2.33	0.11

C	Saline		SEQID824	
	HBeAg		HBeAg	
day		stdev		stdev
0	3.75	0.02	3.74	0.02
3	3.52	0.09	2.35	0.07
6	3.45	0.06	1.89	0.03
9	3.60	0.06	1.94	0.04
13	3.58	0.10	1.58	0.04
16	3.58	0.15	1.64	0.04
20	3.48	0.13	2.69	0.05
23	3.59	0.12	1.75	0.02

From these data it can be concluded that *in vivo* all GalNAc conjugated anti-HBV antisense oligomers are capable of reducing serum levels of HBeAg to a level that is lower than saline and standard of care. In particular SEQ ID NO: 807, SEQ ID NO: 808, SEQ ID NO: 814, SEQ ID NO: 815, SEQ ID NO: 825, SEQ ID NO: 826 can be demonstrated to greatly reduce the serum levels of HBeAg.

Table 9A-C – Serum level of HBV DNA (by log_{10} copy number) following biweekly dosages of 2 mg/kg

A	Saline		SEQID806		SEQID807		SEQID815		SEQID800		SEQID802		ETV	
	DNA		DNA		DNA		DNA		DNA		DNA		DNA	
Day		St Dev		St Dev		St Dev		St Dev		St Dev		St Dev		St Dev
0	7.08	0.54	7.78	0.08	7.27	0.38	7.06	0.39	7.26	0.35	6.92	0.31	7.30	0.37
3	7.21	0.36	7.28	0.20	6.45	0.54	6.11	0.41	5.99	0.45	6.78	0.33	5.73	0.83
7	6.81	0.54	6.53	0.15	5.16	0.86	4.52	0.38	5.16	0.50	6.43	0.58	5.15	0.50
10	7.64	0.14	6.38	0.37	4.72	0.52	4.23	LLOQ	4.23	LLOQ	6.39	0.60	4.23	LLOQ
14	7.71	0.09	5.97	0.70	4.23	LLOQ	4.23	LLOQ	4.23	LLOQ	6.12	0.46	4.23	LLOQ
17	7.76	0.04	6.13	0.57	4.23	LLOQ	4.56	0.57	4.23	LLOQ	6.07	0.58	4.23	LLOQ
21	7.80	0.08	6.62	0.43	4.29	0.11	4.65	0.49	4.44	0.35	6.54	0.42	4.89	0.41
24	8.01	0.03	6.91	0.34	4.65	0.41	5.60	0.68	4.23	LLOQ	7.01	0.33	5.22	0.57

LLOQ = less than lower level of quantification

B	Saline		SEQID808		SEQID814		SEQID826		SEQID825	
	DNA		DNA		DNA		DNA		DNA	
Day		StDev		StDev		StDev		StDev		StDev
0	6.95	0.35	6.79	0.30	7.13	0.24	6.94	0.40	7.02	0.18
3	7.15	0.26	5.92	0.27	5.88	0.15	6.25	0.50	5.90	0.35
6	7.26	0.22	4.80	0.64	4.44	0.48	5.34	0.70	4.56	0.68
9	7.44	0.23	4.16	LLOQ	4.16	LLOQ	4.78	0.62	4.16	LLOQ
13	7.13	0.26	4.16	LLOQ	4.16	LLOQ	4.35	0.33	4.16	LLOQ
16	7.04	0.44	4.16	LLOQ	4.16	LLOQ	4.27	0.20	4.16	LLOQ
20	7.04	0.36	4.16	LLOQ	4.16	LLOQ	4.40	0.41	4.16	LLOQ
23	7.24	0.14	4.16	LLOQ	4.16	LLOQ	4.77	0.63	4.34	0.30

LLOQ = less than lower level of quantification

C	Saline		SEQID824	
	DNA		DNA	
day		StDev		StDev
0	7.47	0.23	7.33	0.16
3	7.55	0.21	5.99	0.25
6	7.74	0.19	4.89	0.48
9	7.76	0.21	4.51	0.40
13	7.82	0.27	4.32	LLOQ
16	7.60	0.42	4.32	LLOQ
20	7.42	0.16	5.03	0.53
23	7.58	0.57	4.32	LLOQ

LLOQ = less than lower level of quantification

From these data it can be concluded that all GalNAc conjugated anti-HBV antisense oligomers are capable of reducing serum levels of HBV genomic DNA to a level that is lower than saline and or equal to the ETV (the clinical standard of care). In particular SEQ ID NO: 807, SEQ ID NO: 808, SEQ ID NO: 814, SEQ ID NO: 815, SEQ ID NO: 825, SEQ ID NO: 826 can be demonstrated to greatly reduce the serum levels of HBV genomic DNA.

The overall conclusion from these data is that *in vivo* the GalNAc-conjugated, HBV-targeting LNAs can target and reduce expression of HBsAg and HBeAg better than ETV (the clinical standard of care) and the HBV serum DNA with equal or better efficacy as compared to ETV. Given the broader effect on the viral transcriptional program than the nucleoside analog ETV, these data suggest that the use of GalNAc conjugated HBV-targeting LNAs in the clinic is likely

to lead to a much improved outcome, including significantly increasing the cure rate for chronically infected HBV patients. Particularly the reduction of the immune suppressor HBsAg will lead to a recovery of the HBV-directed host immune response.

Example 5 In vivo study of bi-weekly injections at several doses

The AAV/HBV Mouse Model as prepared in Example 3 was used in this study. Seven GalNAc conjugated Anti-HBV LNA oligomers were tested at different doses with saline as control in C57BL/6 mice with stable viremia.

Mice were dosed subcutaneously twice weekly for two weeks on days 0, 3, 7 and 10 or day 0, 3, 6 and 9 with the dose in mg/kg (mpk) pr injection indicated in the tables below. HBV surface antigen (HBsAg), HBV e antigen (HBeAg), and HBV genomic DNA in serum was measured at the indicated days using the methods described in the "Materials and methods" section. The mice were followed for 23 – 24 days.

The results are shown in the tables below.

Table 10A-G – Serum level of HBsAg (log_{10}(IU/ml)) following biweekly dosages at the concentration indicated

A	Saline		SEQID 807					
			7.1 mpk		1.4 mpk		0.28 mpk	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.63	0.12	4.74	0.06	4.71	0.05	4.51	0.24
3	4.67	0.09	3.79	0.19	4.23	0.08	4.23	0.45
6	4.63	0.11	2.68	0.15	3.50	0.08	3.83	0.52
9	4.62	0.10	2.09	0.09	2.95	0.05	3.62	0.42
13	4.64	0.06	1.84	0.09	2.50	0.04	3.54	0.28
16	4.56	0.05	1.72	0.05	2.53	0.13	3.63	0.35
20	4.69	0.03	1.97	0.29	2.92	0.17	3.91	0.25
23	4.67	0.10	1.78	0.11	3.18	0.15	4.08	0.20

B	Saline		SEQID815					
			7.5 mpk		1.5 mpk		0.3 mpk	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.63	0.12	4.74	0.05	4.67	0.18	4.14	0.61
3	4.67	0.09	3.47	0.10	3.55	0.38	3.46	0.99
7	4.63	0.11	2.25	0.20	2.70	0.32	3.02	0.99
10	4.62	0.10	1.89	0.17	2.07	0.27	2.68	1.14
14	4.64	0.06	1.48	0.19	1.81	0.24	2.60	1.07
17	4.56	0.05	1.59	0.19	2.22	0.21	2.79	1.13

B	Saline		SEQID815					
			7.5 mpk		1.5 mpk		0.3 mpk	
21	4.69	0.03	1.68	0.16	2.99	0.16	3.24	1.15
24	4.67	0.10	2.17	0.29	3.54	0.16	3.53	1.11

C	Saline		SEQID814					
			6.15 mg/kg		1.26 mg/kg		0.252 mg/kg	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.49	0.13	4.59	0.07	4.55	0.17	4.48	0.18
3	4.62	0.13	3.56	0.21	4.02	0.29	4.51	0.18
7	4.5	0.14	2.22	0.23	3.26	0.23	4.25	0.23
10	4.45	0.21	1.89	0.24	2.9	0.2	4.21	0.22
14	4.36	0.38	1.69	0.27	2.77	0.24	4.33	0.16
17	4.2	0.66	1.75	0.2	3.02	0.19	4.4	0.09
21	4.46	0.09	2.13	0.21	3.5	0.29	4.31	0.17
24	3.97	1.05	2.45	0.17	3.54	0.36	4.51	0.09

D	Saline		SEQID825					
			7.5 mg/kg		1.5 mg/kg		0.3 mg/kg	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.49	0.13	4.62	0.06	4.51	0.04	4.48	0.25
3	4.62	0.13	3.56	0.18	3.73	0.15	4.47	0.31
7	4.5	0.14	2.36	0.12	2.84	0.16	4.15	0.32
10	4.45	0.21	1.99	0.08	2.43	0.15	3.95	0.35
14	4.36	0.38	1.87	0.09	2.2	0.12	4.01	0.25
17	4.2	0.66	1.93	0.06	2.51	0.12	4.1	0.22
21	4.46	0.09	2.2	0.11	3.04	0.14	4.42	0.13
24	3.97	1.05	2.45	0.19	3.32	0.17	4.49	0.08

E	Saline		SEQID808					
			7.1 mg/kg SC		1.42 mg/kg SC		0.29 mg/kg SC	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.78	0.11	4.58	0.29	4.86	0.08	4.55	0.34
3	4.75	0.1	3.38	0.72	4.27	0.24	4.36	0.43
7	4.85	0.05	2.8	0.55	3.7	0.45	4.25	0.37
10	4.81	0.08	2.31	0.43	3.42	0.35	4.06	0.5
14	4.99	0.02	2.36	0.31	3.51	0.4	4.18	0.68
17	4.91	0.04	2.36	0.31	3.52	0.3	4.11	0.68
21	4.89	0.04	2.26	0.34	3.66	0.35	4.32	0.64

E	Saline		SEQID808					
			7.1 mg/kg SC		1.42 mg/kg SC		0.29 mg/kg SC	
24	4.8	0.06	2.4	0.28	3.87	0.3	4.45	0.47

F	Saline		SEQID824					
			7.4 mg/kg SC		1.5 mg/kg SC		0.3 mg/kg SC	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.78	0.11	4.78	0.05	4.57	0.34	4.7	0.11
3	4.75	0.1	3.86	0.23	4	0.51	4.6	0.15
7	4.85	0.05	2.44	0.32	2.87	0.54	4.15	0.16
10	4.81	0.08	2.38	0.25	2.18	0.58	3.86	0.17
14	4.99	0.02	2.85	0.4	2.21	0.82	3.84	0.28
17	4.91	0.04	2.85	0.41	2.28	0.82	3.49	0.54
21	4.89	0.04			2.25	0.91	3.63	0.74
24	4.8	0.06			2.2	0.76	3.7	0.66

G	Saline		SEQID826					
			7.1 mg/kg		1.42 mg/kg		0.29 mg/kg	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	4.78	0.11	4.61	0.26	4.67	0.06	4.57	0.14
3	4.75	0.1	3.78	0.45	4.42	0.03	4.66	0.2
7	4.85	0.05	2.46	0.51	3.84	0.13	4.52	0.2
10	4.81	0.08	2.02	0.57	3.61	0.11	4.42	0.24
14	4.99	0.02	2.08	0.63	3.65	0.27	4.63	0.21
17	4.91	0.04	1.94	0.55	3.73	0.18	4.57	0.21
21	4.89	0.04	2.54	0.25	4.09	0.13	4.73	0.2
24	4.8	0.06	3.04	0.21	4.23	0.17	4.79	0.24

The above data are also presented in Figure 11.

Table 11A-G – Serum level of HBeAg (log_{10}(NCU/ml)) following biweekly dosages at the concentrations indicated.

A	Saline		SEQID 807					
			7.1 mpk		1.4 mpk		0.28 mpk	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.83	0.04	3.82	0.02	3.78	0.02	3.75	0.03
3	3.74	0.02	2.24	0.04	2.83	0.07	3.39	0.05
6	3.69	0.02	1.67	0.04	2.32	0.06	3.16	0.03
9	3.68	0.03	1.48	0.05	2.04	0.03	3.01	0.02

A	Saline		SEQID 807					
			7.1 mpk		1.4 mpk		0.28 mpk	
13	3.66	0.03	1.53	0.02	1.75	0.03	2.83	0.14
16	3.69	0.03	1.21	0.07	1.85	0.03	2.98	0.04
20	3.66	0.04	1.48	0.07	1.98	0.05	3.12	0.04
23	3.63	0.04	1.34	0.09	2.19	0.06	3.24	0.07

B	Saline		SEQID815					
			7.5 mpk		1.5 mpk		0.3 mpk	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.83	0.04	3.80	0.07	3.77	0.03	3.67	0.15
3	3.74	0.02	2.00	0.08	2.56	0.12	3.37	0.15
7	3.69	0.02	1.60	0.05	2.02	0.08	3.04	0.11
10	3.68	0.03	1.47	0.03	1.85	0.10	2.75	0.38
14	3.66	0.03	1.40	0.39	1.64	0.09	2.81	0.10
17	3.69	0.03	1.29	0.12	1.85	0.09	2.78	0.29
21	3.66	0.04	1.74	0.14	2.27	0.09	3.10	0.23
24	3.63	0.04	1.88	0.07	2.50	0.09	3.19	0.18

C	Saline		SEQID814					
			6.15 mg/kg		1.26 mg/kg		0.252 mg/kg	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.75	0.02	3.8	0.08	3.8	0.05	3.75	0.08
3	3.52	0.09	2.15	0.06	2.97	0.05	3.46	0.09
7	3.45	0.06	1.73	0.05	2.47	0.02	3.22	0.09
10	3.6	0.06	1.7	0.07	2.32	0.05	3.2	0.08
14	3.58	0.1	1.4	0.05	2.15	0.04	3.24	0.12
17	3.58	0.15	1.55	0.05	2.44	0.06	3.34	0.1
21	3.48	0.13	1.75	0.04	2.7	0.05	3.34	0.15
24	3.59	0.12	1.92	0.05	2.8	0.04	3.44	0.13

D	Saline		SEQID825					
			7.5 mg/kg		1.5 mg/kg		0.3 mg/kg	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.75	0.02	3.72	0.08	3.71	0.09	3.93	0.16
3	3.52	0.09	2.22	0.1	2.68	0.06	3.39	0.06
7	3.45	0.06	1.77	0.1	2.36	0.05	3.14	0.12
10	3.6	0.06	1.7	0.05	2.18	0.1	3.17	0.1

D	Saline		SEQID825					
			7.5 mg/kg		1.5 mg/kg		0.3 mg/kg	
14	3.58	0.1	1.47	0.07	1.92	0.07	3.14	0.02
17	3.58	0.15	1.69	0.06	2.17	0.13	3.25	0.04
21	3.48	0.13	1.87	0.08	2.4	0.08	3.38	0.05
24	3.59	0.12	1.98	0.09	2.55	0.06	3.48	0.02

E	Saline		SEQID808					
			7.1 mg/kg SC		1.42 mg/kg SC		0.29 mg/kg SC	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.59	0.06	3.51	0.04	3.56	0.01	3.47	0.07
3	3.59	0.03	2.5	0.06	2.98	0.02	3.4	0.07
7	3.69	0.02	2.05	0.08	2.63	0.03	3.2	0.07
10	3.67	0.04	1.84	0.11	2.46	0.04	3.19	0.06
14	3.81	0.03	1.72	0.07	2.45	0.02	3.27	0.07
17	3.74	0.03	1.68	0.14	2.5	0.02	3.27	0.07
21	3.72	0.03	1.58	0.18	2.64	0.04	3.35	0.08
24	3.73	0.06	1.77	0.19	2.8	0.04	3.55	0.05

F	Saline		SEQID824					
			7.4 mg/kg SC		1.5 mg/kg SC		0.3 mg/kg SC	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.59	0.06	3.5	0.03	3.51	0.02	3.49	0.03
3	3.59	0.03	2.09	0.08	2.47	0.13	3.28	0.03
7	3.69	0.02	1.81	0.05	1.95	0.05	2.88	0.04
10	3.67	0.04	1.88	0.07	1.88	0.03	2.65	0.06
14	3.81	0.03	1.71	0.06	1.72	0.1	2.48	0.05
17	3.74	0.03	1.68	0.06	1.68	0.05	2.56	0.05
21	3.72	0.03			1.8	0.04	2.63	0.08
24	3.73	0.06			2.02	0.06	2.81	0.05

G	Saline		SEQID826					
			7.1 mg/kg		1.42 mg/kg		0.29 mg/kg	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	stdev	Serum level	stdev	Serum level	stdev	Serum level	stdev
0	3.59	0.06	3.44	0.09	3.45	0.06	3.5	0.02
3	3.59	0.03	2.58	0.08	3.17	0.07	3.52	0.02
7	3.69	0.02	1.81	0.08	2.8	0.1	3.4	0.03
10	3.67	0.04	1.45	0.06	2.67	0.09	3.37	0.05
14	3.81	0.03	1.27	0.05	2.54	0.12	3.39	0.07

G	Saline		SEQID826					
			7.1 mg/kg		1.42 mg/kg		0.29 mg/kg	
17	3.74	0.03	1.51	0.05	2.76	0.08	3.45	0.04
21	3.72	0.03	1.83	0.11	2.9	0.08	3.54	0.03
24	3.73	0.06	2.23	0.18	3.18	0.1	3.66	0.03

The above data are also presented in Figure 12.

Table 12A-G – Serum level of log_{10}(HBV DNA) (by copy number) following biweekly dosages at the concentrations indicated.

A	Saline		SEQID 807					
			7.1 mpk		1.4 mpk		0.28 mpk	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	6.64	0.31	6.77	0.57	6.46	0.22	6.80	0.38
3	6.58	0.42	4.86	0.58	4.61	0.49	6.10	0.11
6	7.25	0.49	4.43	0.18	4.32	LLOQ	5.87	0.31
9	7.14	0.23	4.32	LLOQ	4.32	0.01	5.19	0.58
13	7.32	0.33	4.30	LLOQ	4.30	LLOQ	5.23	0.54
16	7.28	0.27	4.30	LLOQ	4.30	LLOQ	5.60	0.27
20	7.23	0.31	4.30	LLOQ	4.40	0.17	6.05	0.19
23	7.43	0.28	4.30	LLOQ	4.30	LLOQ	6.41	0.20

LLOQ = less than lower level of quantification

B	Saline		SEQID815					
			7.5 mpk		1.5 mpk		0.3 mpk	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	6.64	0.31	6.64	0.32	6.48	0.31	6.54	0.42
3	6.58	0.42	4.44	0.21	4.59	0.46	4.95	0.67
7	7.25	0.49	4.32	LLOQ	4.32	LLOQ	4.56	0.41
10	7.14	0.23	4.31	0.01	4.32	LLOQ	4.38	0.11
14	7.32	0.33	4.30	LLOQ	4.30	LLOQ	4.30	LLOQ
17	7.28	0.27	4.30	LLOQ	4.30	LLOQ	4.64	0.58
21	7.23	0.31	4.30	LLOQ	4.30	LLOQ	5.12	0.87
24	7.43	0.28	4.30	LLOQ	4.30	LLOQ	5.37	1.08

LLOQ = less than lower level of quantification

C	Saline	SEQID814		
		6.15 mg/kg	1.26 mg/kg	0.252 mg/kg
	DNA	DNA	DNA	DNA

C	Saline		SEQID814					
			6.15 mg/kg		1.26 mg/kg		0.252 mg/kg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	7.47	0.23	7.55	0.18	7.48	0.22	7.64	0.17
3	7.55	0.21	6.2	0.18	6.44	0.26	7.37	0.14
7	7.74	0.19	5.31	0.25	5.53	0.36	7.31	0.08
10	7.76	0.21	4.46	0.19	4.65	0.49	7.22	0.1
14	7.82	0.27	4.32	0	4.32	0	7.27	0.09
17	7.6	0.42	4.32	0.01	4.38	0.09	7.41	0.14
21	7.42	0.16	4.32	0	4.62	0.56	7.42	0.09
24	7.58	0.57	4.32	0	5.48	0.7	7.66	0.16

D	Saline		SEQID825					
			7.5 mg/kg		1.5 mg/kg		0.3 mg/kg	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	7.47	0.23	7.36	0.28	7.41	0.32	7.5	0.44
3	7.55	0.21	5.89	0.31	5.84	0.67	6.95	0.43
7	7.74	0.19	4.75	0.57	4.92	0.55	6.73	0.43
10	7.76	0.21	4.35	0.13	4.28	0	6.49	0.44
14	7.82	0.27	4.32	0	4.32	0	6.52	0.48
17	7.6	0.42	4.32	0.01	4.31	0.01	6.68	0.26
21	7.42	0.16	4.32	0	4.32	0	6.82	0.57
24	7.58	0.57	4.32	0.01	4.63	0.31	7.19	0.32

E	Saline		SEQID808					
			7.1 mg/kg SC		1.42 mg/kg SC		0.29 mg/kg SC	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	7.77	0.17	7.97	0.26	7.93	0.11	7.93	0.24
3	7.8	0.05	6.29	0.4	6.69	0.21	7.31	0.35
7	7.75	0.15	5.02	0.59	5.67	0.43	7.24	0.35
10	7.79	0.09	4.5	0.36	4.95	0.75	6.98	0.3
14	8.01	0.09	4.29	0	4.56	0.46	6.83	0.55
17	7.89	0.13	4.29	0	4.29	0	6.97	0.46
21	7.94	0.06	4.29	0	4.72	0.47	7.13	0.41
24	7.83	0.08	4.29	0	4.97	0.45	7.37	0.36

F	Saline	SEQID824		
		7.4 mg/kg SC	1.5 mg/kg SC	0.3 mg/kg SC
	DNA	DNA	DNA	DNA

F	Saline		SEQID824					
			7.4 mg/kg SC		1.5 mg/kg SC		0.3 mg/kg SC	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	7.77	0.17	8	0.24	7.98	0.23	7.87	0.17
3	7.8	0.05	6.64	0.22	6.44	0.47	7.05	0.24
7	7.75	0.15	5.58	0.32	5.34	0.75	6.25	0.26
10	7.79	0.09	4.59	0.36	4.87	0.4	5.64	0.3
14	8.01	0.09	4.29	0	4.59	0.2	4.81	0.35
17	7.89	0.13	4.29	0	4.29	0	4.61	0.37
21	7.94	0.06			4.29	0	4.94	0.43
24	7.83	0.08			4.29	0	5.24	0.56

G	Saline		SEQID826					
			7.1 mg/kg		1.42 mg/kg		0.29 mg/kg	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	7.77	0.17	7.86	0.29	7.86	0.18	7.87	0.22
3	7.8	0.05	6.45	0.24	7.05	0.34	7.72	0.18
7	7.75	0.15	5.32	0.47	6.42	0.31	7.57	0.16
10	7.79	0.09	4.76	0.47	6.01	0.48	7.53	0.12
14	8.01	0.09	4.29	0	5.39	0.7	7.53	0.14
17	7.89	0.13	4.29	0	5.47	0.83	7.57	0.16
21	7.94	0.06	4.29	0	6.24	0.5	7.66	0.16
24	7.83	0.08	4.29	0	6.43	0.55	7.74	0.16

The above data are also presented in Figure 13.

From these data it can be concluded that all GalNAc conjugated anti-HBV antisense oligomers are capable of reducing serum levels of HBsAG, HBeAG and HBV genomic DNA to a level that is lower than saline. In particular SEQ ID NO: 807, 814, 815 and 825 show very efficient HBsAG decrease even at the intermediate dose. At the highest dose the antigen reduction is maintained at least 11 days after treatment ended for SEQ ID NO: 807 and 815. SEQ ID 814, 815 and 825 demonstrate the most efficacious knock-down of viral serum DNA, demonstrating a particularly potent effect on the viral polymerase-expressing transcripts.

Example 6 Comparing antiviral efficacy by different route of administrations

The AAV/HBV Mouse Model as prepared in Example 3 was used in this study. A GalNAc conjugated Anti-HBV LNA oligomer was tested at different doses using either subcutaneous (SC) or intravenous (IV) administration routes with saline as control in C57BL/6 mice with stable viremia.

Mice were dosed subcutaneously or intravenously twice weekly for two weeks on days 0, 3, 6 and 9 with the dose in mg/kg (mpk) pr injection indicated in the tables below. HBV surface antigen (HBsAg), HBV e antigen (HBeAg), and HBV genomic DNA in serum was measured at the indicated days using the methods described in the "Materials and methods" section. The mice were followed for 23 days after first dosing.

The results are shown in the tables below.

Table 13A– Serum level of HBsAg (log_{10}(IU/ml)) following biweekly SC dosages at the concentration indicated

A	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	4.64	0.11	4.55	0.08	4.46	0.13	4.69	0.07
3	4.61	0.11	4.44	0.06	3.80	0.22	3.64	0.10
6	4.58	0.07	4.29	0.08	3.33	0.19	2.54	0.12
9	4.57	0.10	4.11	0.05	2.98	0.20	1.83	0.14
13	4.67	0.08	3.93	0.18	2.85	0.23	2.00	0.08
16	4.61	0.08	3.98	0.15	2.93	0.19	2.05	0.09
20	4.52	0.08	4.08	0.11	3.11	0.31	1.98	0.11
23	4.25	0.52	3.97	0.26	3.30	0.33	2.05	0.16

Table 13B – Serum level of HBsAg (log_{10}(IU/ml)) following biweekly IV dosages at the concentration indicated

B	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
	HBsAg		HBsAg		HBsAg		HBsAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	4.64	0.11	4.61	0.09	4.60	0.11	4.63	0.12
3	4.61	0.11	4.39	0.06	4.19	0.12	3.93	0.11
6	4.58	0.07	4.13	0.05	3.69	0.13	3.17	0.13
9	4.57	0.10	3.91	0.12	3.36	0.15	2.73	0.20
13	4.67	0.08	3.72	0.21	3.16	0.02	2.36	0.14
16	4.61	0.08	3.70	0.30	3.14	0.14	2.47	0.16
20	4.52	0.08	3.86	0.28	3.31	0.14	2.57	0.12
23	4.25	0.52	3.99	0.34	3.59	0.13	2.86	0.11

Table 14A – Serum level of HBeAg (log_{10}(IU/ml)) following biweekly SC dosages at the concentration indicated.

A	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	3.57	0.06	3.57	0.04	3.57	0.04	6.85	0.60
3	3.63	0.04	3.43	0.03	2.87	0.07	5.74	0.82
6	3.61	0.04	3.28	0.03	2.42	0.09	5.27	0.60
9	3.63	0.05	3.15	0.03	2.14	0.09	4.57	0.40
13	3.49	0.07	2.84	0.09	2.01	0.05	4.30	LLOQ
16	3.53	0.06	3.06	0.04	2.09	0.06	4.30	LLOQ
20	3.56	0.05	3.21	0.03	2.24	0.08	4.30	LLOQ
23	3.64	0.06	3.29	0.03	2.46	0.09	4.85	0.54

LLOQ = less than lower level of quantification

Table 14B – Serum level of HBeAg (log_{10}(IU/ml)) following biweekly IV dosages at the concentration indicated

B	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
	HBeAg		HBeAg		HBeAg		HBeAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	3.57	0.06	3.65	0.06	3.62	0.04	3.61	0.08
3	3.63	0.04	2.13	0.06	3.37	0.05	2.96	0.03
6	3.61	0.04	1.59	0.05	3.05	0.04	2.52	0.07
9	3.63	0.05	1.39	0.07	2.88	0.06	2.35	0.03
13	3.49	0.07	1.55	0.03	2.63	0.04	2.10	0.04
16	3.53	0.06	1.59	0.04	2.73	0.06	2.18	0.08
20	3.56	0.05	1.60	0.03	3.01	0.06	2.37	0.11
23	3.64	0.06	1.64	0.08	3.22	0.05	2.60	0.07

Table 15A Serum level of HBV DNA (by log_{10} (copy number)) following biweekly SC dosages at the concentrations indicated

A	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	6.50	0.21	6.56	0.19	6.85	0.60	6.48	0.14
3	6.65	0.31	6.30	0.33	5.74	0.82	4.34	LLOQ
6	6.83	0.31	6.20	0.33	5.27	0.60	4.34	LLOQ
9	6.94	0.37	5.90	0.32	4.57	0.40	4.73	0.67
13	7.13	0.21	5.82	0.88	4.30	LLOQ	4.56	0.46

A	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
16	7.12	0.31	6.23	0.38	4.30	LLOQ	4.30	LLOQ
20	7.06	0.17	6.27	0.35	4.30	LLOQ	4.30	LLOQ
23	6.94	0.32	6.42	0.32	4.85	0.54	4.30	LLOQ

LLOQ = less than lower level of quantification

Table 15B Serum level of HBV DNA (by $\log_{10}$ (copy number)) following biweekly IV dosages at the concentrations indicated

A	Saline		SEQID 807					
			0.2 mpk		1.0 mpk		5.0 mpk	
	DNA		DNA		DNA		DNA	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	6.50	0.21	6.71	0.59	6.62	0.23	6.71	0.45
3	6.65	0.31	6.20	0.59	5.50	0.43	5.24	0.69
6	6.83	0.31	5.50	0.90	4.62	0.48	4.34	LLOQ
9	6.94	0.37	5.18	0.85	4.34	LLOQ	4.34	LLOQ
13	7.13	0.21	5.05	0.76	4.30	LLOQ	4.30	LLOQ
16	7.12	0.31	5.13	0.84	4.30	LLOQ	4.30	LLOQ
20	7.06	0.17	5.50	0.80	4.30	LLOQ	4.30	LLOQ
23	6.94	0.32	5.99	0.41	4.30	LLOQ	4.30	LLOQ

LLOQ = less than lower level of quantification

The data are also presented in Figure 14.

From these data it can be concluded that administration of GalNAc conjugated anti-HBV antisense oligomers more efficiently reduces serum levels of HBsAG, HBeAG and HBV genomic DNA to a level that is lower than saline, when dosed by subcutaneous administration, as compared to intravenous administration.

Example 7: Comparison of conjugated and unconjugated oligonucleotides

The AAV/HBV Mouse Model as prepared in Example 3 was used in this study. Unconjugated (SEQ ID NO 308 and 303) and GalNAc conjugated (SEQ ID NO: 807 and 815) Anti-HBV LNA oligomers were tested at equimolar oligomer doses with saline as control in C57BL/6 mice with stable viremia.

Mice were dosed subcutaneously twice weekly for two weeks on days 0, 3, 6 and 9 with the dose in mg/kg (mpk) pr injection indicated in the tables below. HBV surface antigen (HBsAg), HBV e antigen (HBeAg), and HBV genomic DNA in serum was measured at the indicated days using the methods described in the “Materials and methods” section. The mice were followed for 23.

The results are shown in the tables below.

Table 16A-B – Serum level of HBsAg (log_{10}(IU/ml)) following biweekly dosages at equimolarconcentrations

A	Saline		SEQ ID 308		SEQ ID 807	
			5 mpk		7.1 mpk	
	HBsAg		HBsAg		HBsAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	4.63	0.12	4.70	0.05	4.74	0.06
3	4.67	0.09	4.44	0.14	3.79	0.19
6	4.63	0.11	3.91	0.44	2.68	0.15
9	4.62	0.10	3.50	0.44	2.09	0.09
13	4.64	0.06	3.27	0.43	1.84	0.09
16	4.56	0.05	3.20	0.53	1.72	0.05
20	4.69	0.03	3.61	0.54	1.97	0.29
23	4.67	0.10	3.96	0.34	1.78	0.11

B	Saline		SEQ ID 303		SEQ ID 815	
			1 mpk		1.5 mpk	
	HBsAg		HBsAg		HBsAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	4.63	0.28	4.49	0.54	4.62	0.25
3	4.70	0.17	4.58	0.38	3.31	0.09
6	4.84	0.12	4.78	0.17	3.21	0.14
9	4.81	0.13	4.81	0.13	2.64	0.22
13	4.86	0.12	4.91	0.06	2.63	0.58
16	4.83	0.13	4.85	0.10	2.04	0.57
20	4.73	0.18	4.68	0.14	2.26	0.58
23	4.66	0.20	4.48	0.40	2.74	0.64

Table 17A-B – Serum level of HBeAg (log_{10}(IU/ml)) following biweekly dosages at equimolar concentrations

A	Saline		SEQ ID 308		SEQ ID 807	
			5 mpk		7.1 mpk	
	HBeAg		HBeAg		HBeAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	3.83	0.04	3.79	0.05	3.82	0.02
3	3.74	0.02	3.39	0.08	2.24	0.04
6	3.69	0.02	3.07	0.02	1.67	0.04
9	3.68	0.03	2.82	0.04	1.48	0.05
13	3.66	0.03	2.66	0.03	1.53	0.02
16	3.69	0.03	2.75	0.03	1.21	0.07
20	3.66	0.04	3.01	0.02	1.48	0.07

A	Saline		SEQ ID 308		SEQ ID 807	
			5 mpk		7.1 mpk	
23	3.63	0.04	3.10	0.05	1.34	0.09

B	Saline		SEQ ID 303		SEQ ID 815	
			1 mpk		1.5 mpk	
	HBeAg		HBeAg		HBeAg	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	3.82	0.03	3.74	0.04	3.80	0.06
3	3.82	0.03	3.78	0.02	2.48	0.10
6	3.85	0.02	3.83	0.06	2.70	0.05
9	3.81	0.03	3.75	0.02	2.29	0.07
13	3.84	0.02	3.84	0.03	2.45	0.08
16	3.84	0.04	3.80	0.02	2.19	0.02
20	3.78	0.04	3.73	0.04	2.27	0.06
23	3.79	0.03	3.79	0.01	2.60	0.04

Table 18A-B Serum level of HBV DNA (by $\log_{10}$ (copy number)) following biweekly dosages at equimolar concentrations

A	Saline		SEQ ID 308		SEQ ID 807	
	DNA		DNA		DNA	
			5 mpk		7.1 mpk	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	6.64	0.31	6.53	0.26	6.77	0.57
3	6.58	0.42	5.18	0.88	4.86	0.58
6	7.25	0.49	5.05	0.73	4.43	0.18
9	7.14	0.23	4.54	0.37	4.32	LLOQ
13	7.32	0.33	4.30	LLOQ	4.30	LLOQ
16	7.28	0.27	4.41	0.19	4.30	LLOQ
20	7.23	0.31	4.63	0.57	4.30	LLOQ
23	7.43	0.28	5.23	0.93	4.30	LLOQ

LLOQ = less than lower level of quantification

B	Saline		SEQ ID 303		SEQ ID 815	
	DNA		DNA		DNA	
			1 mpk		1.5 mpk	
Day	Serum level	StDev	Serum level	StDev	Serum level	StDev
0	7.89	0.16	7.91	0.42	7.91	0.23
3	8.28	0.13	8.06	0.41	6.54	0.25
6	8.03	0.13	7.94	0.20	5.38	0.63
9	8.19	0.13	8.07	0.14	4.30	LLOQ
13	8.37	0.16	8.31	0.13	4.30	LLOQ

B	Saline		SEQ ID 303		SEQ ID 815	
	DNA		DNA		DNA	
16	8.41	0.19	8.16	0.18	4.30	LLOQ
20	8.23	0.09	7.94	0.19	4.30	LLOQ
23	8.20	0.06	7.89	0.28	4.30	LLOQ

LLOQ = less than lower level of quantification

The data are also presented in Figure 15.

From these data it can be concluded that administration of GalNAc conjugated anti-HBV antisense oligomers more efficiently reduces serum levels of HBsAG and HBeAG to a level that is lower than saline, when dosed by subcutaneous administration, as compared to intravenous administration. Serum HBV DNA is reduced with equal efficacy by the two methods of delivery, although the limitation of the assay precludes discrimination at high doses.

All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference in their entirety and to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein (to the maximum extent permitted by law). All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention. The citation and incorporation of patent documents herein is done for convenience only and does not reflect any view of the validity, patentability, and/or enforceability of such patent documents. This invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law.

CLAIMS

1. An oligomer conjugate selected from the group consisting of:

5'- GN2-C6 caG_S $^{m}C_S$$G_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$a_S$$G_S$G-3' (SEQ ID NO: 815)

5'- GN2-C6 caG_S $^{m}C_S$$G_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$a_S$$G_S$$G_S$T-3' (SEQ ID NO: 814)

5'-GN2-C6 caG_S $^{m}C_S$$G_S$$t_S$$a_S$$a_S$$a_S$$g_S$$a_S$$g_S$$A_S$$G_S$G-3' (SEQ ID NO: 825)

5'- GN2-C6 ca$A_S$$G_S$ $^{m}C_S$$g_S$$a_S$$a_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$A_S$ $^{m}C_S$G-3' (SEQ ID NO: 808)

5'-GN2-C6 ca$A_S$$G_S$$g_S$$t_S$$g_S$$a_S$$a_S$$g_S$ $^{m}c_S$$g_S$$a_S$$A_S$$G_S$$T_S$G-3' (SEQ ID NO: 826); and

5'- GN2-C6 ca$A_S$$G_S$ $^{m}C_S$$g_S$$a_S$$a_S$$g_S$$t_S$$g_S$$c_S$$a_S$$c_S$$a_S$ $^{m}C_S$$G_S$G-3' (SEQ ID NO: 807)

wherein uppercase letters denote beta-D-oxy-LNA units; lowercase letters denote DNA units; the subscript "s" denotes a phosphorothioate linkage; superscript m denotes a a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base; GN2-C6 denotes a GalNAc2 carrier component with a C6 linker.

2. The oligomer conjugate of claim 1, selected from the group consisting of:

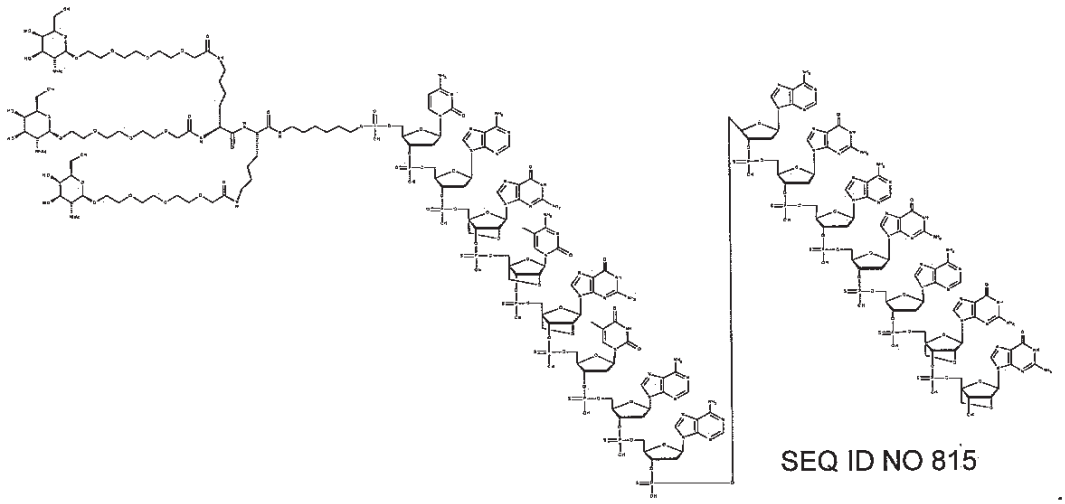
5'- GN2-C6 ca$G_s{}^mC_sG_st_sa_sa_sa_sg_sa_sg_sa_sG_sG$-3' (SEQ ID NO: 815)

5'- GN2-C6 ca$G_s{}^mC_sG_st_sa_sa_sa_sg_sa_sg_sa_sG_sG_sT$-3' (SEQ ID NO: 814); and

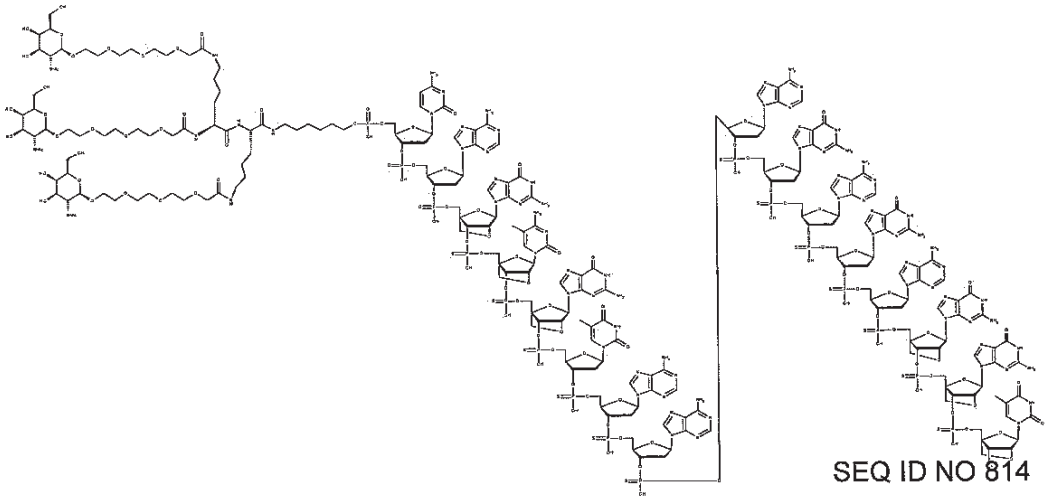
5'-GN2-C6 ca$G_s{}^mC_sG_st_sa_sa_sa_sg_sa_sg_sA_sG_sG$-3' (SEQ ID NO: 825)

wherein uppercase letters denote beta-D-oxy-LNA units; lowercase letters denote DNA units; the subscript "s" denotes a phosphorothioate linkage; superscript m denotes a DNA or beta-D-oxy-LNA unit containing a 5-methylcytosine base; GN2-C6 denotes a GalNAc2 carrier component with a C6 linker.

3. The oligomer conjugate of claim 1, wherein the oligomer conjugate has the following structure:

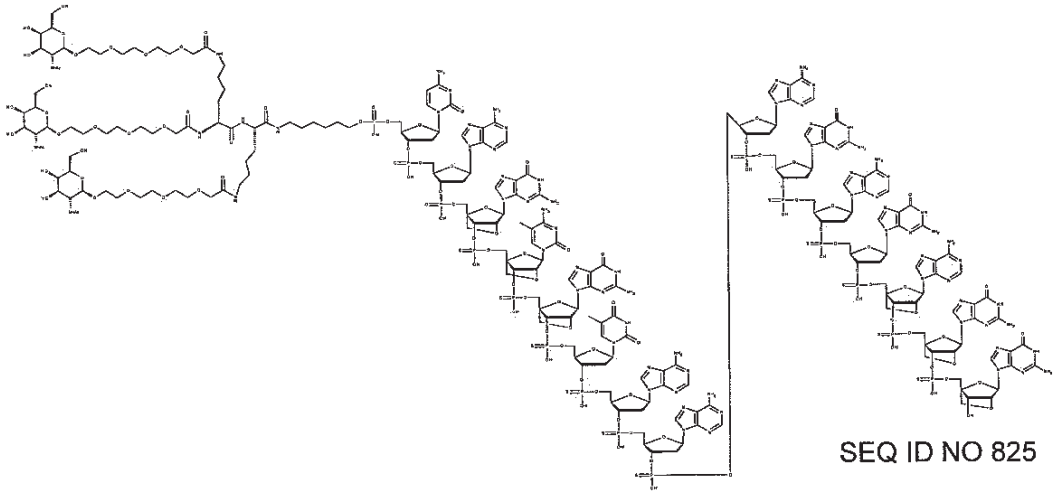


4. The oligomer conjugate of claim 1, wherein the oligomer conjugate has the following structure:



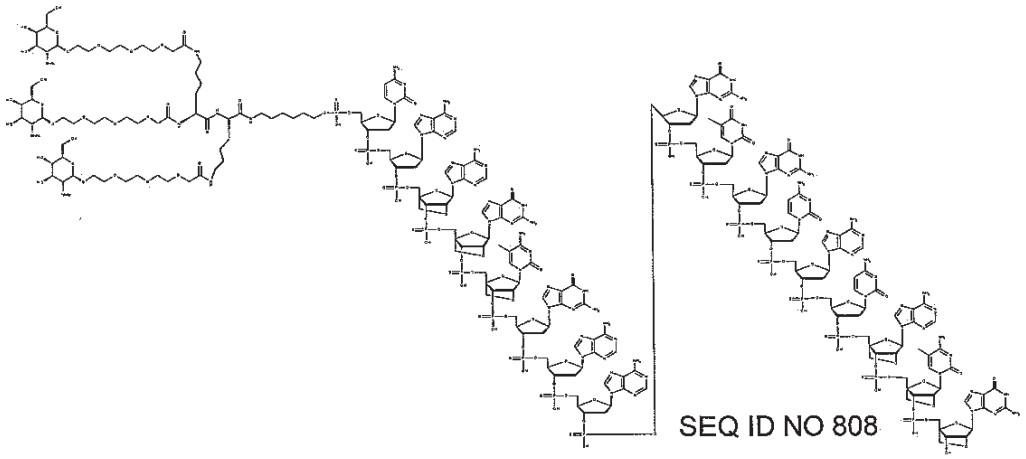
SEQ ID NO 814

5. The oligomer conjugate of claim 1, wherein the oligomer conjugate has the following structure:

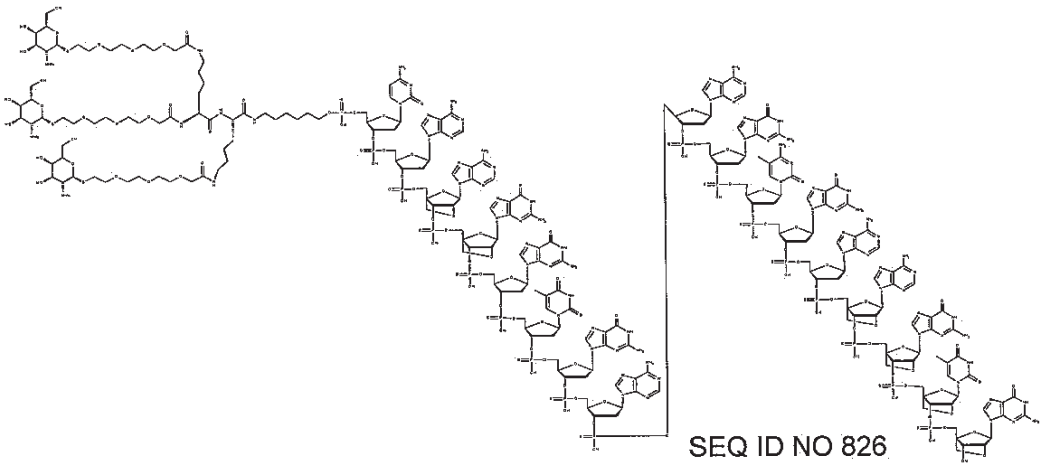


SEQ ID NO 825

6. The oligomer conjugate of claim 1, where the oligomer conjugate has the following structure:



7. The oligomer conjugate of claim 1, where the oligomer conjugate has the following structure:



8. A pharmaceutical composition comprising an oligomer conjugate according to any one of claims 1 to 7; and one or more pharmaceutically acceptable diluents, carriers, salts or adjuvants.

9. An oligomer conjugate for use in the treatment of a viral disorder in the liver, wherein said oligomer conjugate is defined in any one of claims 1 to 7.

10. The oligomer conjugate for use in the treatment of claim 9, wherein the oligomer conjugate is for subcutaneous administration.

11. Use of the oligomer conjugate as defined in any one of claims 1 to 7 in the manufacture of a medicament for the treatment of a viral disorder in the liver.

12. The use of the oligomer of claim 11, wherein the medicament is to be administered subcutaneously.