

ENHANCED OLIGONUCLEOTIDES FOR MODULATING FUBP1 EXPRESSION

FIELD OF INVENTION

The present invention relates to enhanced antisense oligonucleotides that are complementary to the Far Upstream Element-Binding Protein 1 (FUBP1) and are capable of reducing a FUBP1 target nucleic acid, such as FUBP1 mRNA. The invention relates to enhanced antisense oligonucleotides targeting FUBP1 or conjugates thereof for use in treating and/or preventing a hepatitis B virus (HBV) infection, in particular a chronic HBV infection. The invention in particular relates to the use of the enhanced antisense oligonucleotides targeting FUBP1 or conjugates thereof for destabilizing cccDNA, such as HBV cccDNA. The invention further relates to enhanced antisense oligonucleotides targeting FUBP1 or conjugates thereof for use in treating cancer. Also comprised in the present invention is a pharmaceutical composition and its use in the treatment and/or prevention of a HBV infection, or its use in the treatment of cancer.

BACKGROUND

Far Upstream Element-Binding Protein 1 (FUBP1 or FBP1) is a single stranded DNA-binding protein that binds to multiple DNA elements. This protein is also thought to bind RNA and contains 3'-5' helicase activity with *in vitro* activity on both DNA-DNA and RNA-RNA duplexes. FUBP1 is known to activate the transcription of the proto-oncogene c-myc by binding to far upstream element (FUSE) located upstream of c-myc in undifferentiated cells. The protein is primarily present in the nucleus of the cell. Upregulation of FUBP1 has been observed in many types of cancers. Furthermore, FUBP1 can bind to and mediate replication of RNA from Hepatitis C virus and Enterovirus (Zhang and Chen 2013 Oncogene vol 32 p. 2907-2916).

FUBP1 has also been identified in Hepatocellular carcinoma (HCC) where it has been suggested to be involved in HCC tumorigenesis (Ramdzan et al 2008 Proteomics Vol 8 p. 5086-5096) and that FUBP1 is required for HCC tumour growth as illustrated using lentivirus-expressed shRNA targeting FUBP1 (Rabenhorst et al 2009 Hepatology vol 50 p 1121-1129).

It has been demonstrated that knock-down of FUBP1 with lentivirus-expressed shRNA's enhances treatment response in ovarian cancer (Zhang et al 2017 Oncology Letters Vol 14 p. 5819-5824).

WO 2004/027061 disclose a screening method, which involves the step of analyzing whether or not a test substance inhibits FBP (FBP is now referred to as FUBP) and a medicinal composition for treating a proliferative disease, which contains as the active ingredient(s) a substance inhibiting FBP.

Poly(U) Binding Splicing Factor 60 (PUF60) is a potentially regulator of both transcriptional and post-transcriptional steps of HBV pregenome expression. PUF60 is known to form a complex with FUBP1 in relation to c-myc repression. However, FUBP1 does not participate in the PUF60 dependent regulation of HBV pregenome expression (Sun et al 2017 Scientific Reports 7:12874).

HBV infection remains a major health problem worldwide affecting an estimated 350 million chronically infected carriers. Approximately 25% of carriers ultimately die from chronic hepatitis, cirrhosis, or liver cancer. 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 respectively. 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 replicative cycle of the HBV genome 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. The RC DNA may stem from an infecting viral particle, or as an intracellular replication intermediate.

HBsAg quantification is a significant biomarker for prognosis and treatment response in chronic hepatitis B with the loss of circulating HBsAg in the chronically infected patient seen as a key event in achieving cure. However, the achievement of HBsAg loss and seroconversion (functional cure) is rarely observed in chronically infected patients. Hepatitis B e-antigen (also called HBV envelope antigen or HBeAg) is a viral protein that is secreted by hepatitis B infected cells. HBeAg is associated with chronic hepatitis B infections and is used as a marker of active viral disease and a patient's degree of infectiousness.

Accordingly, reducing secretion of HBeAg in addition to secretion of HBsAg would lead to an improved inhibition of development of a chronic HBV infection as compared to the inhibition of secretion of HBsAg alone.

Current therapy such as nucleos(t)ide analogues are molecules that inhibit HBV DNA synthesis but are not directed at reducing HBsAg level. Most therapies currently under development aim to reach a

functional cure, defined as a durable HBsAg loss with or without anti-HBs seroconversion, with undetectable serum DNA, and with cccDNA in a transcriptionally inactive state, but do not address cccDNA persistence. In contrast, complete cure of HBV infection is defined as cccDNA loss in combination with durable HBV DNA and HBsAg loss. The persistence of cccDNA in infected hepatocytes is the main barrier for eradicating the virus in chronic hepatitis B virus (CHB) patients, and there is an urgent need to develop new therapies for the HBV complete cure that eliminates cccDNA.

In WO 2019/193165, it was shown that inhibition of FUBP1 functionality, either using a small molecule, a siRNA or a LNA antisense oligonucleotide, resulted in reduction of HBV cccDNA. In the Examples section of WO 2019/193165, single stranded LNA gapmer oligonucleotides were analyzed, which were able to inhibit FUBP1 expression.

There is a need for therapeutic agents, which can inhibit FUBP1 specifically. We have screened more than 2000 antisense oligonucleotides targeting human FUBP1 and identified sequences and compounds, which are particularly potent and effective to specifically target human FUBP1. Specifically, nine alternating flank gapmers were identified, which conferred a strong down-regulation of human FUBP1 *in vitro*. Eight compounds target a region within exon 14 of human FUBP1, one compound targets a region within exon 20 (CMP ID 18_1).

OBJECTIVE OF THE INVENTION

The present invention provides antisense oligonucleotides and conjugates thereof, which modulate FUBP1 expression. We identified a specific target sequence present in exon 14 or exon 20 of the human FUBP1 pre-mRNA, which may be targeted by antisense oligonucleotides, or conjugates thereof, to give effective FUBP1 inhibition. In particular, targeting position 16184-16205 of SEQ ID NO: 1 is advantageous in terms of reducing FUBP1.

Furthermore, we identified a specific target sequence present in exon 20 of the human FUBP1 pre-mRNA, which may be targeted by antisense oligonucleotides, or conjugates thereof, to give effective FUBP1 inhibition. In particular, targeting position 30536-30553 of SEQ ID NO: 1 is advantageous in terms of reducing FUBP1.

Accordingly, an objective of the present invention is to provide enhanced antisense oligonucleotides targeting FUBP1 or conjugates thereof, wherein the antisense oligonucleotides or conjugates thereof are capable of inhibiting the expression of FUBP1 *in vitro* and *in vivo*, thereby reducing cccDNA in an

HBV infected cell. The enhanced antisense oligonucleotides targeting FUBP1 or the conjugate thereof can be used in the treatment and/or prevention of an HBV infection, or in the treatment of cancer.

SUMMARY OF INVENTION

The invention relates to antisense oligonucleotides, or conjugates thereof, which target a FUBP1 (Far upstream element-binding protein 1) nucleic acid, such as a mammalian FUBP1 nucleic acid, and which are capable of inhibiting the expression of said nucleic acid in a cell expressing said nucleic acid, and their use in medicine. Said antisense oligonucleotides are complementary to a mammalian FUBP1 nucleic acid, such as human FUBP1.

The invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is complementary to, such as fully complementary to a region from nucleotides 16184 to 16205 of the human FUBP1 pre-mRNA (as illustrated in SEQ ID NO: 1).

Also, the invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is complementary to, such as fully complementary to a region from nucleotides 30536-30553 of the human FUBP1 pre-mRNA (as illustrated in SEQ ID NO: 1).

In some embodiments, the antisense oligonucleotide or the contiguous nucleotide sequence is complementary to, such as fully complementary to a region from nucleotides 16184 to 16200 of SEQ ID NO: 1.

In some embodiments, the antisense oligonucleotide or the contiguous nucleotide sequence is complementary to, such as fully complementary to a region from nucleotides 16186 to 16203 of SEQ ID NO: 1.

In some embodiments, the antisense oligonucleotide or the contiguous nucleotide sequence is complementary to, such as fully complementary to a region from nucleotides 30536-30553 of SEQ ID NO: 1.

In some embodiments, the antisense oligonucleotide or the contiguous nucleotide sequence is complementary to, such as fully complementary to a region from nucleotides 16188 to 16205 of SEQ ID NO: 1. In some embodiments, the antisense oligonucleotide or the contiguous nucleotide sequence is complementary to, such as fully complementary to a region from nucleotides 16189 to 16205 of SEQ ID NO: 1.

The antisense oligonucleotide of the invention is typically 12 – 30, such as 12 to 22, such as 16 to 20 nucleotides in length, and comprises a contiguous nucleotide sequence of at least 12 nucleotides, such as of 13, 14, 15, 16, 17 or 18 nucleotides, which is complementary to, such as fully complementary to a region of the human FUBP1 pre-mRNA (as illustrated in SEQ ID NO: 1), selected from a region from nucleotides 16184-16205, 16184-16200, 16186-16203, 16188-16205, 16189-16205 and 30536-30553 of SEQ ID NO: 1.

The invention provides for an antisense oligonucleotide, 12-22 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 12 – 22 nucleotides in length, wherein the contiguous nucleotide sequence is complementary, such as fully complementary, to SEQ ID NO 10.

The invention provides for an antisense oligonucleotide, 12-20 nucleotides in length (such as 15, 16, 17, or 18 nucleotides in length), wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 12 – 18 nucleotides in length (such as 15, 16, 17, or 18 nucleotides in length), wherein the contiguous nucleotide sequence is complementary, such as fully complementary, to SEQ ID NO 11.

The invention provides for an antisense oligonucleotide, 12-20 nucleotides in length (such as 15, 16, 17, or 18 nucleotides in length), wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 12 – 18 nucleotides in length (such as 15, 16, 17, or 18 nucleotides in length), wherein the contiguous nucleotide sequence is complementary, such as fully complementary, to SEQ ID NO 19.

The invention provides for an antisense oligonucleotide 10 to 30 nucleotides in length, which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length, wherein the contiguous nucleotide sequence is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9 and 18; or at least 14 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide 10 to 30 nucleotides in length, which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length, wherein the contiguous nucleotide sequence is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9 and 18, or at least 15 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide 10 to 30 nucleotides in length, which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length, wherein the contiguous

nucleotide sequence is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9 and 18, or at least 16 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide which comprises a contiguous nucleotide sequence, which is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9 and 18, or 14, 15, 16, or 17 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide, which comprises (or consists of) a contiguous nucleotide sequence selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9 and 18.

The invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is 100% identical to SEQ ID NO: 6 (CTTATGCTTTTATGGT), or 14, 15 or 16 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is 100% identical to SEQ ID NO: 7 (CTTATGCTTTTATGGTT), or 14, 15, 16 or 17 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is 100% identical to SEQ ID NO: 8 (GCTTTTATGGTTTCAC), or 14, 15 or 16 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is 100% identical to SEQ ID NO: 9 (TATGCTTTTATGGTTTC), or 14, 15, 16 or 17 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide, which comprises a contiguous nucleotide sequence, which is 100% identical to SEQ ID NO: 18 (ACCAATTTTCATTTCTAC), or 14, 15, 16 or 17 contiguous nucleotides thereof.

The invention provides for an antisense oligonucleotide selected from

CTTatGcttttatgGT (SEQ ID NO: 6, Compound ID No 6_1),
CTTaTgcttttatgGT (SEQ ID NO: 6, Compound ID No 6_2),
CTtATgcttttatgGTT (SEQ ID NO: 7, Compound ID No 7_1),
CTtAtgcttttatgGTT (SEQ ID NO: 7, Compound ID No 7_2),
CTtAtgcttttatGgTT (SEQ ID NO: 7, Compound ID No 7_3),

CTtAtgcttttatGGTT (SEQ ID NO: 7, Compound ID No 7_4),
 GcttTttatggtTtCAC (SEQ ID NO: 8, Compound ID No 8_1),
 TATgcTtttatggtTTC (SEQ ID NO: 9, Compound ID No 9_1), and
 AcCAAAttttcatttCtAC (SEQ ID NO: 18, Compound ID No 18_1)

wherein capital letters are beta-D-oxy LNA nucleosides, lowercase letters are DNA nucleosides, all LNA Cs are LNA 5-methyl cytosine, and all internucleoside linkages are phosphorothioate internucleoside linkages.

The present invention also provides for a pharmaceutically acceptable salt of the antisense oligonucleotide of the present invention.

The invention provides for an antisense oligonucleotide selected from the group listed in Table 1, or a pharmaceutically acceptable salt thereof.

Table 1. Compound Table (Exemplary antisense oligonucleotides of the present invention) – HELM Annotation Format

SEQ ID Number	Compound ID Number #	HELM Annotation Written 5' – 3'.	Comprised by conjugate shown in Fig.
6	6_1	{[LR]([5meC])[sP].[LR](T)[sP].[LR](T)[sP].[dR](A)[sP].[dR](T)[sP].[LR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)}	1
6	6_2	{[LR]([5meC])[sP].[LR](T)[sP].[LR](T)[sP].[dR](A)[sP].[LR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)}	2
7	7_1	{[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[LR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)[sP].[LR](T)}	3

7	7_2	{[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)[sP].[LR](T)}	4
7	7_3	{[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[LR](G)[sP].[dR](G)[sP].[LR](T)[sP].[LR](T)}	5
7	7_4	{[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[LR](G)[sP].[LR](G)[sP].[LR](T)[sP].[LR](T)}	6
8	8_1	{[LR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[LR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](G)[sP].[dR](T)[sP].[LR](T)[sP].[dR](T)[sP].[LR]([5meC])[sP].[LR](A)[sP].[LR]([5meC])}	7
9	9_1	{[LR](T)[sP].[LR](A)[sP].[LR](T)[sP].[dR](G)[sP].[dR](C)[sP].[LR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](G)[sP].[dR](T)[sP].[LR](T)[sP].[LR](T)[sP].[LR]([5meC])}	8
18	18_1	{[LR](A)[sP].[dR](C)[sP].[LR]([5meC])[sP].[LR](A)[sP].[LR](A)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](C)[sP].[dR](A)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[LR]([5meC])[sP].[dR](T)[sP].[LR](A)[sP].[LR]([5meC])}	8.1

Helm Annotation Key:

[LR](G) is a beta-D-oxy-LNA guanine nucleoside,
 [LR](T) is a beta-D-oxy-LNA thymine nucleoside,
 [LR](A) is a beta-D-oxy-LNA adenine nucleoside,
 [LR]([5meC]) is a beta-D-oxy-LNA 5-methyl cytosine nucleoside,

[dR](G) is a DNA guanine nucleoside,
[dR](T) is a DNA thymine nucleoside,
[dR](A) is a DNA adenine nucleoside,
[dR](C) is a DNA cytosine nucleoside,
[sP] is a phosphorothioate internucleoside linkage,
P is a phosphodiester internucleoside linkage.

The invention thus provides for an antisense oligonucleotide selected from the group consisting of compound ID Nos # 6_1, 6_2, 7_1, 7_2, 7_3, 7_4; 8_1 and 9_1.

The invention further provides for an antisense oligonucleotide with compound ID No: 18_1.

In an embodiment, the antisense oligonucleotide is not an antisense oligonucleotide compound ID Nos 53_1 or 54_1 as disclosed in WO 2019/193165 (see also Table 7 in the Examples section).

In an embodiment, the antisense oligonucleotide is not an antisense oligonucleotide compound ID Nos 78_1 and 79_1 as disclosed in WO 2019/193165 (see also Table 7 in the Examples section).

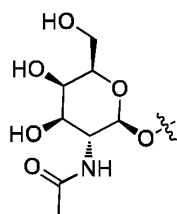
The present invention further provides a conjugate comprising the antisense oligonucleotide of the present invention and at least one conjugate moiety covalently attached to said antisense oligonucleotide.

In some embodiments, the conjugate moiety is capable of binding to the asialoglycoprotein receptor, such as the human asialoglycoprotein receptor. For example, the conjugate moiety may comprise at least one asialoglycoprotein receptor-targeting moiety selected from group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N-isobutanoylgalactosamine.

In some embodiments, the asialoglycoprotein receptor-targeting moiety is N-acetylgalactosamine (GalNAc). Thus, the antisense oligonucleotide of the present invention may be conjugated to at least one conjugate moiety comprising at least one N-Acetylgalactosamine (GalNAc) moiety, such as at least one conjugate moiety comprising at least one N-Acetylgalactosamine (GalNAc) moiety as described below. According to one aspect of the invention, the conjugate moiety is a GalNAc residue R as described hereinunder.

In some embodiments, the conjugate moiety is an at least trivalent, such as a divalent, trivalent or tetravalent GalNAc residue R. Preferably the conjugate moiety is a trivalent GalNAc residue R.

The term “trivalent GalNAc residue” as used herein refers to a residue comprising three N-Acetylgalactosamine moieties, i.e. preferably three moieties of formula



The conjugate moiety or the GalNAc residue R, respectively, and the antisense oligonucleotide may be linked together via a linker L, such as a biocleavable linker L. Thus, the conjugate compound may comprise a linker L, which is positioned between the antisense oligonucleotide and the conjugate moiety or GalNAc residue R, respectively.

In some embodiments, the linker L comprises between 1 and 10 linked nucleosides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 linked nucleosides, such as between 2 and 6 linked nucleosides, such as between 2 and 5 linked nucleosides, such as between 2 and 4 linked nucleosides. In some embodiments, the linker comprises two linked nucleotides. Thus, the nucleosides may be DNA nucleosides. Typically, the nucleosides are linked via phosphodiester internucleoside linkages. Moreover, the linker L may be linked to the antisense compound via a phosphodiester internucleoside linkage.

Exemplary conjugates are provided in Table 2 (in HELM Annotation format) as well as in Fig. 1 to 8, Fig. 8.1 and Fig. 10.

The invention provides for a conjugate selected from the group of conjugates listed in Table 2, or a pharmaceutically acceptable salt thereof.

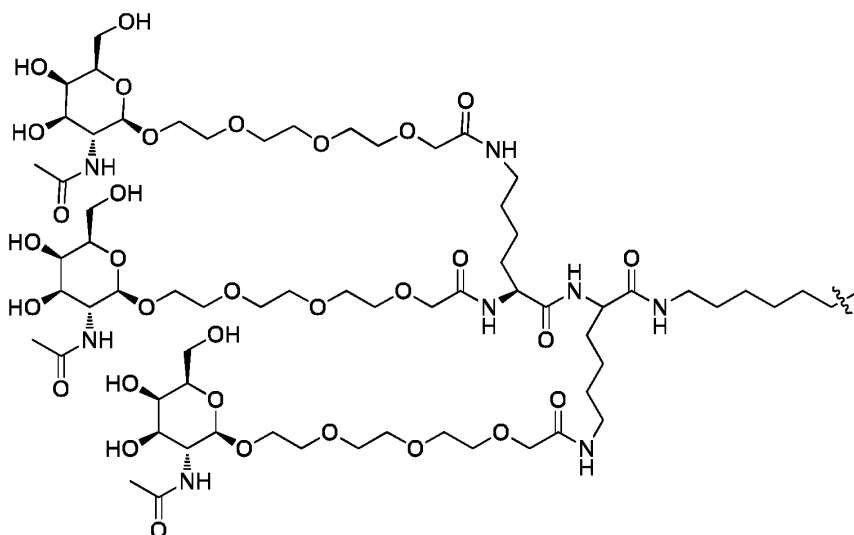
Table 2. Compound Table (Exemplary conjugates of the present invention) – HELM Annotation Format (for the annotation on the HELM annotation, see explanations for Table 1).

SEQ ID Number	Oligo Compound ID Number # (acc. to Table 1 above)	HELM Annotation Written 5' – 3'.	Exemplary compound – see Figure

6	6_1	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR]([5meC])[sP].[LR](T)[sP].[LR](T)[sP].[dR](A)[sP].[dR](T)[sP].[LR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)}	1
6	6_2	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR]([5meC])[sP].[LR](T)[sP].[LR](T)[sP].[dR](A)[sP].[LR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)}	2
7	7_1	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[LR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)[sP].[LR](T)}	3
7	7_2	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[LR](G)[sP].[LR](T)[sP].[LR](T)}	4
7	7_3	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[LR](G)[sP].[dR](G)[sP].[LR](T)[sP].[LR](T)}	5
7	7_4	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR]([5meC])[sP].[LR](T)[sP].[dR](T)[sP].[LR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[LR](G)[sP].[LR](G)[sP].[LR](T)[sP].[LR](T)}	6
8	8_1	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR](G)[sP].[dR](C)[sP].[dR](T)[sP].[dR](T)[sP].[LR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](G)[sP].[dR](T)[sP].[LR](T)[sP].[dR](T)[sP].[LR]([5meC])[sP].[LR](A)[sP].[LR]([5meC])}	7
9	9_1	{{5gn2c6}P.[dR](C)P.[dR](A)P.[LR](T)[sP].[LR](A)[sP].[LR](T)[sP].[dR](G)[sP].[dR](C)[sP].[LR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP]	8

		.[dR](T)[sP].[dR](A)[sP].[dR](T)[sP].[dR](G)[sP].[dR](G)[sP].[dR](T)[sP].[LR](T)[sP].[LR](T)[sP].[LR]([5meC])}	
18	18_1	{[5gn2c6]}P.[dR](C)P.[dR](A)P.[LR](A)[sP].[dR](C)[sP].[LR]([5meC])[sP].[LR](A)[sP].[LR](A)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[dR](C)[sP].[dR](A)[sP].[dR](T)[sP].[dR](T)[sP].[dR](T)[sP].[LR]([5meC])[sP].[dR](T)[sP].[LR](A)[sP].[LR]([5meC])}	8.1

In the above Table, [5gn2c6] is a GalNAc residue R having the formula:



It is to be understood that R as shown in the figure above and as used in the above table is a mixture of the two stereoisomers shown in Fig. 9D1 and 9D2.

According to a further aspect of the invention, R as shown in the figure above and as used in the above table is the stereoisomer as shown in to Fig 9D1.

According to a further aspect of the invention R as shown in the figure above and as used in the above table is the stereoisomer as shown in Fig 9D1. The structures of the conjugates provided in Table 2 are shown in Figures 1 to 8, and 8.1.

The invention provides for the conjugate of Figure 1, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 6_1, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 2, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 6_2, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 3, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 7_1, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 4, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 7_2, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 5, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 7_3, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 6, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 7_4, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 7, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 8_1, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 8, or a pharmaceutically acceptable salt thereof.

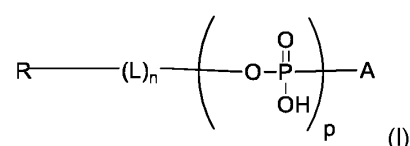
The invention provides for the antisense oligonucleotide of Compound ID Number 9_1, or a pharmaceutically acceptable salt thereof.

The invention provides for the antisense oligonucleotide of Compound ID Number 18_1, or a pharmaceutically acceptable salt thereof.

The invention provides for the conjugate of Figure 8.1, or a pharmaceutically acceptable salt thereof.

The compound of formula (I)

The present invention also provides for compounds of the following formula (I)



wherein

n is 0 or 1

p is 0 or 1

with the proviso that in case n is 1, p is preferably 1,

and with the proviso that in case n is 0 and p is 0, R is preferably H,

L is a linker, preferably L is a linker comprising or consisting of 2-10 nucleosides, such as 2-5 nucleosides,

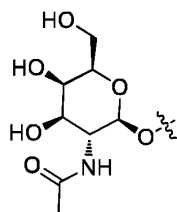
R is a GalNAc residue, preferably a trivalent GalNAc residue,

and A is an antisense oligonucleotide residue according to the present invention.

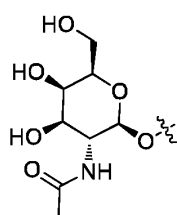
The term "antisense oligonucleotide residue" refers to an antisense oligonucleotide according to the present invention which is attached via its 5' end to residue R via $-(L)_n-(O-P(=O)(-OH))_p-$, such as an antisense oligonucleotide as shown in Table 6. Preferred antisense oligonucleotide residues are depicted in Fig. 1A, 2A, 3A, 4A, 5A, 6A, 7A and 8A. A further preferred antisense oligonucleotide residue is depicted in Fig. 8.1A.

The GalNAc residue R

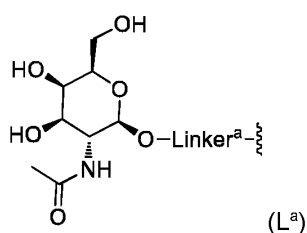
R is a GalNAc residue, preferably a trivalent GalNAc residue. The term "GalNAc residue" as used herein refers to a residue comprising at least one N-Acetylgalactosamine (GalNAc) moiety, i.e. at least one moiety of formula



The term “trivalent GalNAc residue” as used herein refers to a residue comprising three N-acetylgalactosamine (GalNAc) moieties, i.e. preferably three moieties of formula



Preferably, the GalNAc residue comprises at least one, preferably three GalNAc building blocks (L^a) having the following structure,



wherein Linker^a is selected from alkyl groups, alkyl-oxy-alkyl groups, alkyl groups comprising at least one phosphodiester linkage, alkyl groups comprising at least one amide linkage, alkyl-oxy-alkyl groups comprising at least one phosphodiester linkage and alkyl-oxy-alkyl groups comprising at least one amide linkage.

The term “alkyl” refers to substituted or unsubstituted, linear or branched, alkyl groups, such as C1 to C20 alkyl groups, preferably, C2 to C8, such as C2, C3, C4, C5, C6, C7 or C8, alkyl groups.

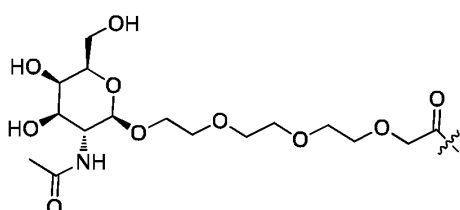
Preferably the alkyl groups are unsubstituted, more preferably linear and unsubstituted, alkyl groups.

The term “alkyl-oxy-alkyl” groups refers to at least two alkyl groups linked via an oxygen, preferably to ethyl-oxy-ethyl groups, such as $-(\text{CH}_2\text{-O})_x$ groups with integer x preferably being in the range of from 2 to 20, more preferably in the range of from 2 to 6, such as 2, 3, 4, 5 or 6, more preferably x is 3 or 5.

According to an aspect of the invention, the GalNAc building block (L^a) is selected from the group of the following structures (L^a).

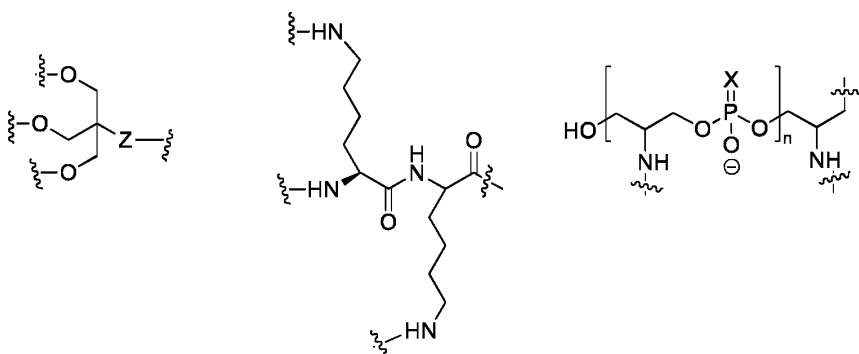
If more than one residue (L^a) is present in a GalNAc residue, such as the three residues in the trivalent GalNAc residue, then all residues are preferably the same.

Most preferably, L^a has the structure



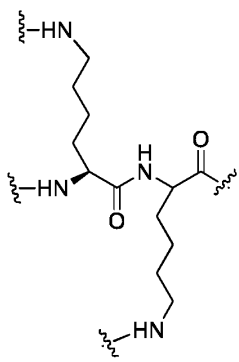
In case, the conjugate moiety R comprises multiple, such as preferably three, GalNAc moieties, R comprises besides the GalNAc building blocks (L^a), a multivalent, preferably a tetravalent, building block (L^b), to which the building blocks (L^a) are preferably being attached, to the antisense oligonucleotide residue A via $-(L)_n-(O-P(=O)(OH))_p-$.

L^b is preferably selected from one of the following structures:

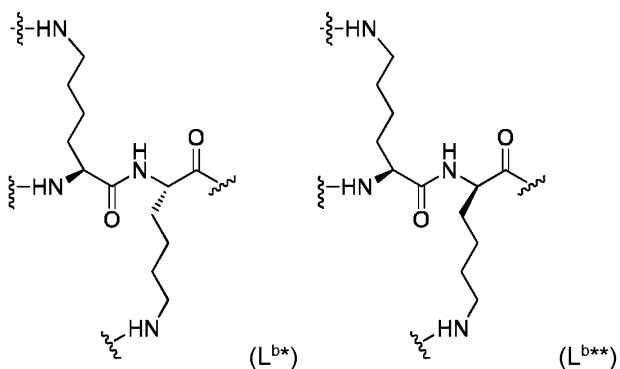


with X being O or S, and with Z being O or NH, and wherein n is of from 1 to 4, preferably 2 or 3, more preferably 2.

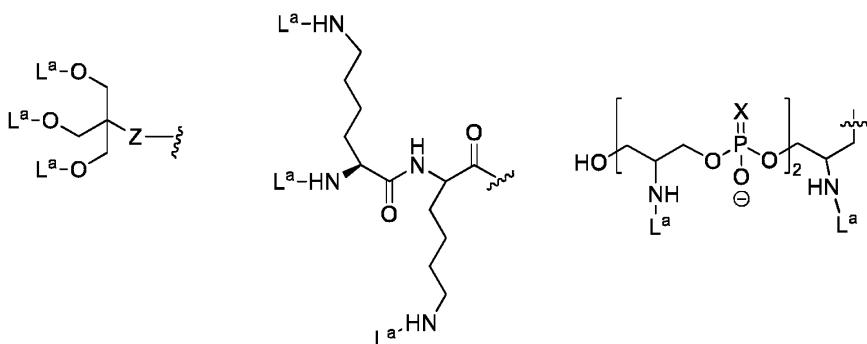
More preferably, L^b has the structure



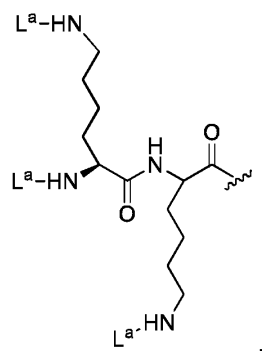
It is to be understood that, L^b has either the structure L^{b*} or the structure L^{b**} or is a mixture thereof. According to a preferred aspect L^b is a mixture of L^{b*} and L^{b**} .



Thus, the conjugate moiety R preferably comprises a structure $(L^a)_3-L^b$, more preferably R comprises one of the following structures

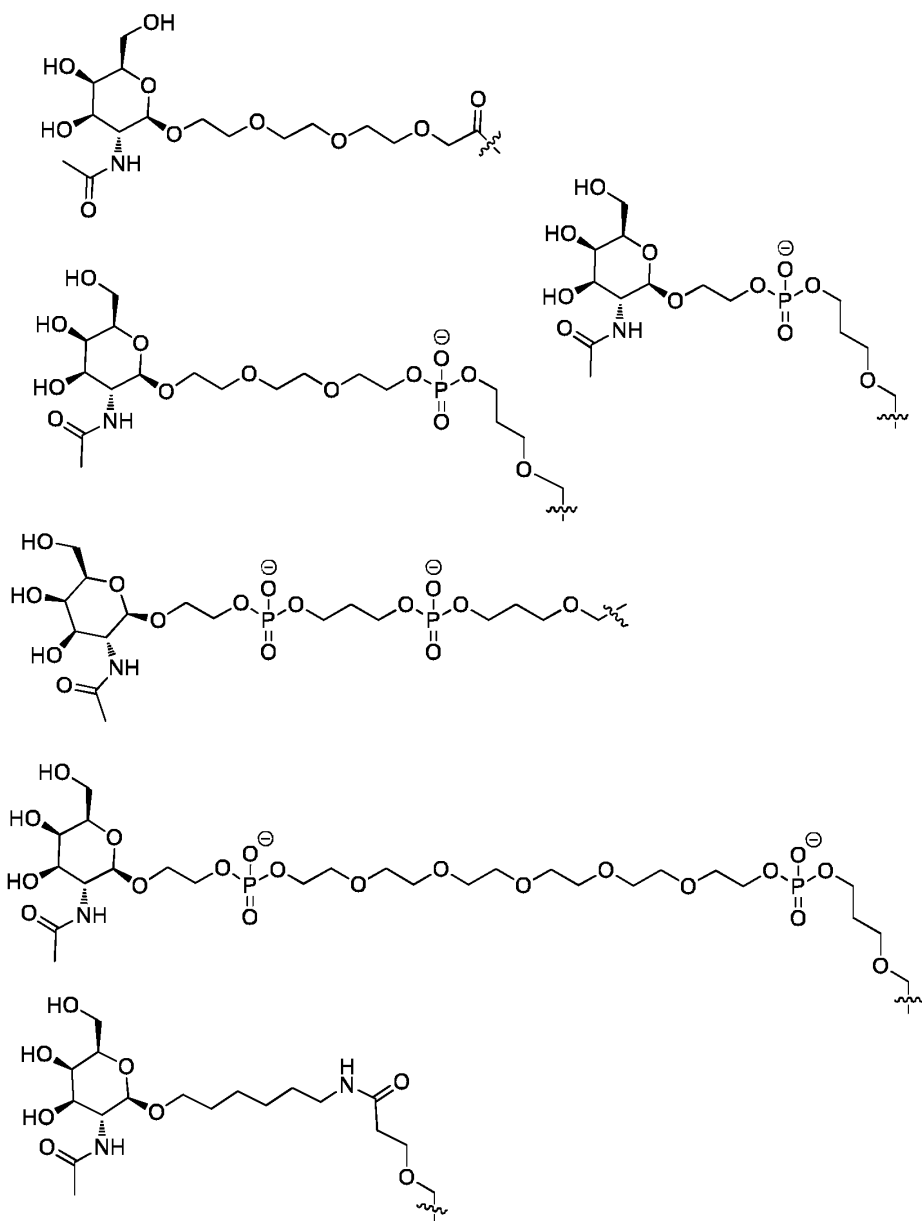


more preferably, the structure



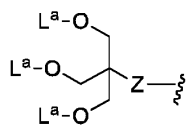
wherein L^b is preferably a mixture of L^{b*} and L^{b**} ,

and wherein X is O or S, and with Z being O or NH, and wherein n is of from 1 to 3, preferably 2, and with L^a being as described above, preferably with L^a being selected from the group consisting of

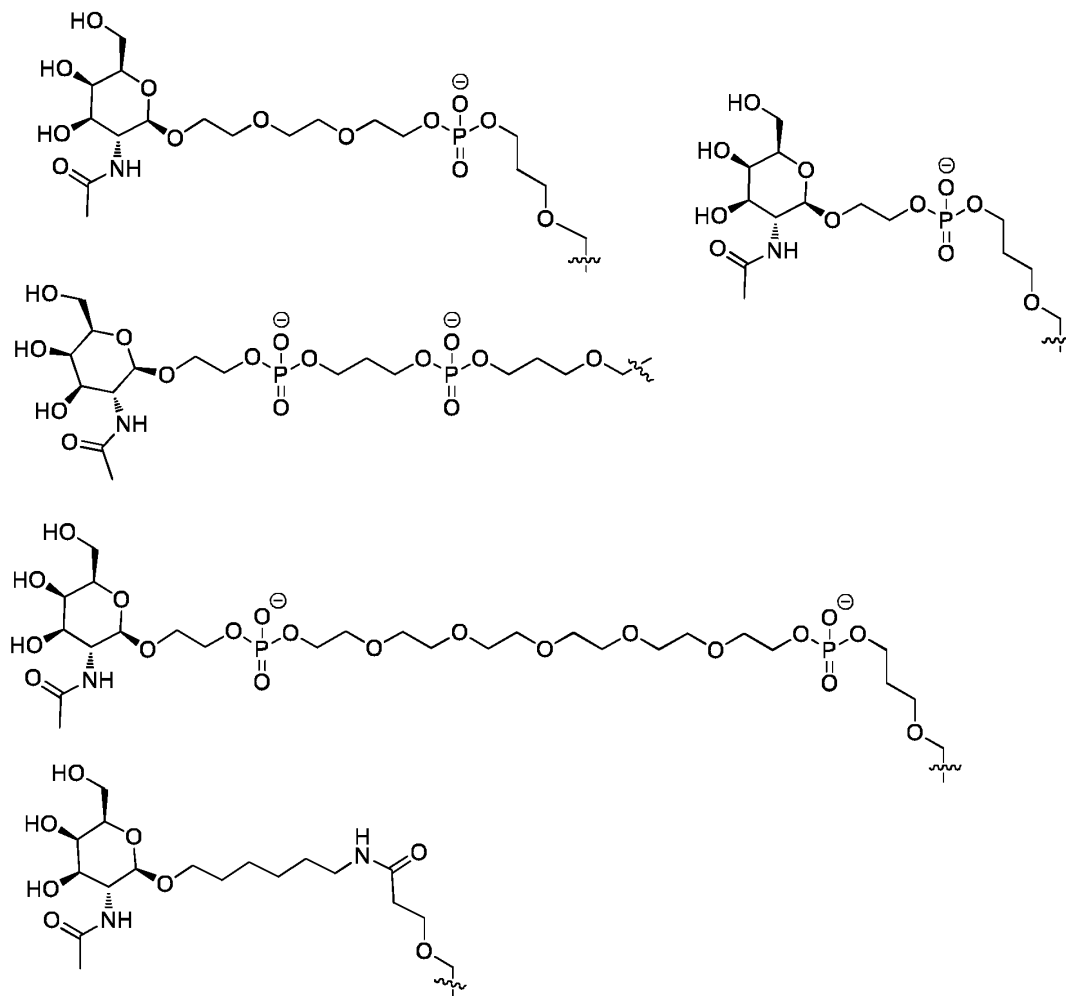


and mixtures thereof, wherein preferably all residues (L^a) within the GalNAc residue are the same.

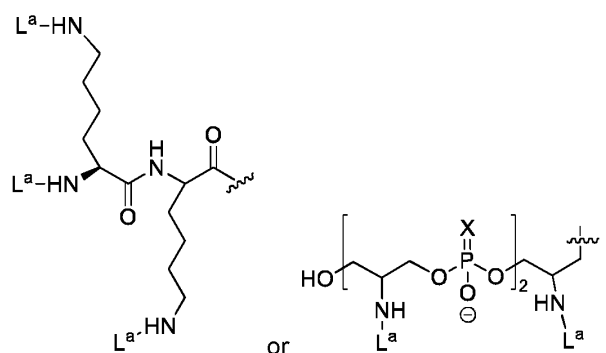
In case (L^a)₃- L^b is



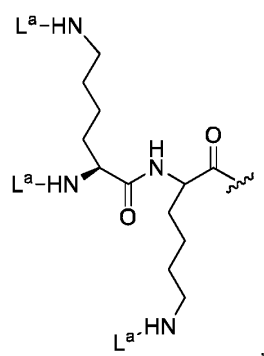
L^a is more preferably selected from the group consisting of



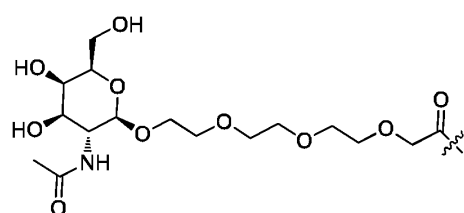
In case $(L^a)_3-L^b$ is



preferably



L^a is preferably

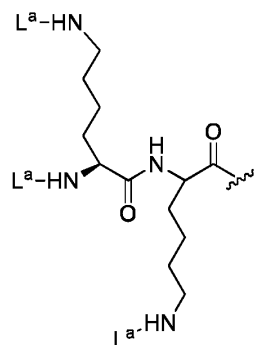


Optionally, the conjugate moiety R additionally comprises a linker L^c . Thus, R preferably has the structure $(L^a)_3-L^b-(L^c)_c-$ with integer c being 1 or 0.

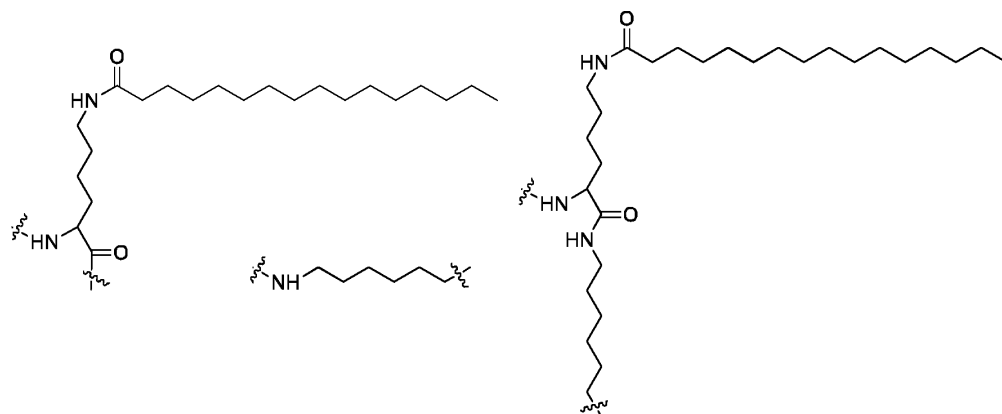
Such linker compounds are known those skilled in the art and are suitably chosen to attach $(L^a)_3-L^b$ to the remaining part of the compound, i.e. to the antisense oligonucleotide residue via $-(L)_n-(O-P(=O)(OH))_p-$.

Depending on the structure of L^b , L^c is selected from the group consisting of alkyl, alkyl-oxy-alkyl, amino-alkyl (-NH-alkyl-), amino-alkyl-oxy-alkyl, unnatural amino acid residues, and natural amino acid residues. According to one aspect of the invention, L^c is a substituted or unsubstituted lysine group.

According to one aspect of the invention, R is $(L^a)_3-L^b-(L^c)_c$ with $c = 1$ and $(L^a)_3-L^b$ is



L^c is preferably an amino-alkyl group or an amino acid, such as a substituted or unsubstituted lysine group, in particular L^c is e.g. selected from the group consisting of

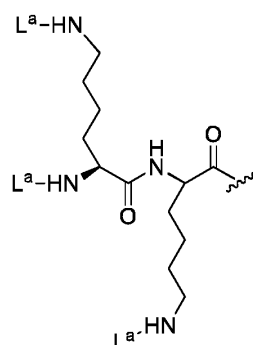


with the amino group being attached to the carbonyl group of L^b thereby forming an amide bond.

Preferred residues R according to this aspect are depicted in Fig. 9A1, 9A2; 9C1, 9C2, 9D1, 9D2.

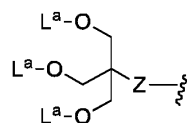
Thus, according to one aspect of the invention, R is selected from the group consisting of the residues depicted in Fig. 9A1, 9A2; 9C1, 9C2, 9D1 and 9D2.

According to a further aspect of the invention, R has the structure $(L^a)_3-L^b-(L^c)_c$ with c being 0, and wherein $(L^a)_3-L^b$ is



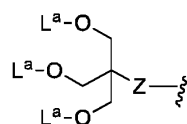
Preferred residues R according to this aspect of the invention are depicted in Fig. 9B1 and 9B2.

According to a further aspect of the invention, R has the structure $(L^a)_3-L^b-(L^c)_c$ with $(L^a)_3-L^b$ being

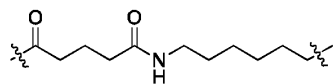


and with Z being O. In this case, c is preferably 1 and L^c is preferably an alkyl group, more preferably a C3 to C6 alkyl group, more preferably a propyl group, most preferably a n-propyl group. Preferred residues R according to this aspect are depicted in Fig. 9E1, 9F1, 9G1 and 9H1. Thus, according to one aspect of the invention, R is selected from the group consisting of the residues depicted in Fig. 9E1, 9F1, 9G1 and 9H1

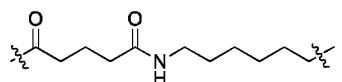
According to a further aspect of the invention, R has the structure $(L^a)_3-L^b-(L^c)_c$ with $(L^a)_3-L^b$ being



and with Z being NH. In this case c is preferably 1 and L^c is preferably an alkyl group, a namino acid comprising group or a group having the following structure:

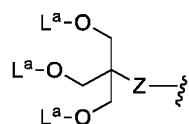


In particular, in this case L^c is



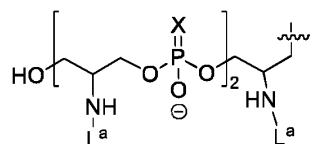
A preferred residue R according to this aspect of the invention is depicted in Fig. 9J1.

According to a further aspect of the invention, R has the structure $(L^a)_3-L^b-(L^c)_c$ with $(L^a)_3-L^b$ being



and with Z being NH and c being 0. A preferred residue R according to this aspect of the invention is depicted in Fig. 9I1.

According to one aspect of the invention, R is $(L^a)_3-L^b-(L^c)_c$ with $c = 0$ and wherein $(L^a)_3-L^b$ is



Preferred residues R according to this aspect of the invention are depicted in Fig. 9L1 and 9L2.

Thus, R is preferably selected from the residues depicted in Fig. 9A1, 9A2; 9C1, 9C2, 9D1, 9D2, 9E1, 9F1, 9G1, 9H1, 9I1, 9J1, 9L1 9L2, and mixtures thereof, such as stereoisomeric mixtures of 9A1 and 9A2; of 9C1 and 9C2 or of 9D1 and 9D2, more preferably R is selected from the residues depicted in 9D1, 9D2 and a mixture thereof, more preferably R is a mixture of the residues depicted in 9D1 and 9D2, such as a mixture having a molar ratio of 9D1 to 9D2 in the range of from 10: 90 to 90:10, such as in the range of from 30:70 to 70:30, such as in the range of from 45:55 to 55:45 .

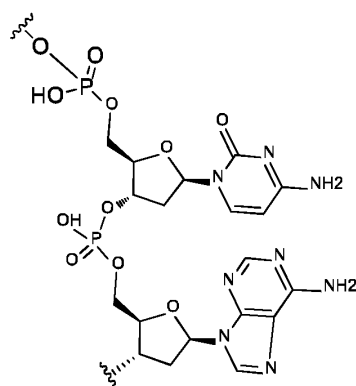
Thus, compound (I) is preferably selected from the compounds depicted in Fig. 10A1, 10A2; 10C1, 10C2, 10D1, 10D2, 10E1, 10F1, 10G1, 10H1, 10I1, 10J1, 10L1, 10L2 and mixtures thereof, such as stereoisomeric mixtures of 10A1 and 10A2; of 10C1 and 10C2 or of 10D1 and 10D2, depicted in 10D1, 10D2, and mixtures thereof, more preferably compound (I) is a mixture of the compounds depicted in 10D1 and 10D2, such as a mixture having a molar ratio of 10D1 to 10 D2 in the range of from 10: 90 to 90:10, such as in the range of from 30:70 to 70:30, such as in the range of from 45:55 to 55:45 .

The linker L

In the above formula, L is a linker as defined herein, preferably L is a linker comprising or consisting of 2-10 nucleosides, such as 2-5 nucleosides, such as 2 nucleosides, wherein optionally the nucleosides are phosphodiester linked nucleosides.

As the linker L comprises between 1 and 10 linked nucleosides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 linked nucleosides, such as between 2 and 6 linked nucleosides, such as between 2 and 5 linked nucleosides, such as between 2 and 4 linked nucleosides. In some embodiments, the linker comprises two linked nucleotides. Thus, the nucleosides may be DNA nucleosides. Typically, the nucleosides are linked via phosphodiester internucleoside linkages. Moreover, the linker L may be linked to the antisense compound via a phosphodiester internucleoside linkage. Further, the linker L is linked to conjugate moiety R via a suitable function group, such as eg, via an amide, an amine, an ether, an ester, a phosphodiester (-O-P(=O)(-OH)-O-) or thiophosphodiester (-O-P(=S)(-OH)-O-) linkage. It is to be understood that L may optionally additionally comprise alkyl groups or alkyl-oxy-alkyl groups between the nucleosides and the functional group linking L to R. In this case, the nucleosides are preferably linked via a phosphodiester bond to the alkyl groups or alkyl-oxy-alkyl group which in turn is linked to R via a suitable function group, such as eg, via an amide, an amine, an ether, an ester, a phosphodiester (-O-P(=O)(-OH)-O-) or thiophosphodiester (-O-P(=S)(-OH)-O-) bond

According to a preferred embodiment L is

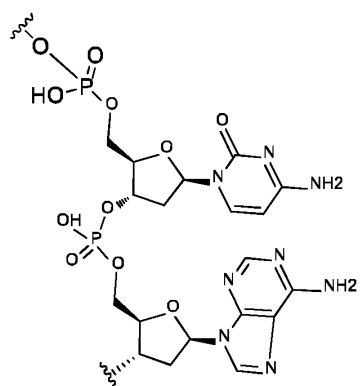


The antisense (A) oligonucleotide residue

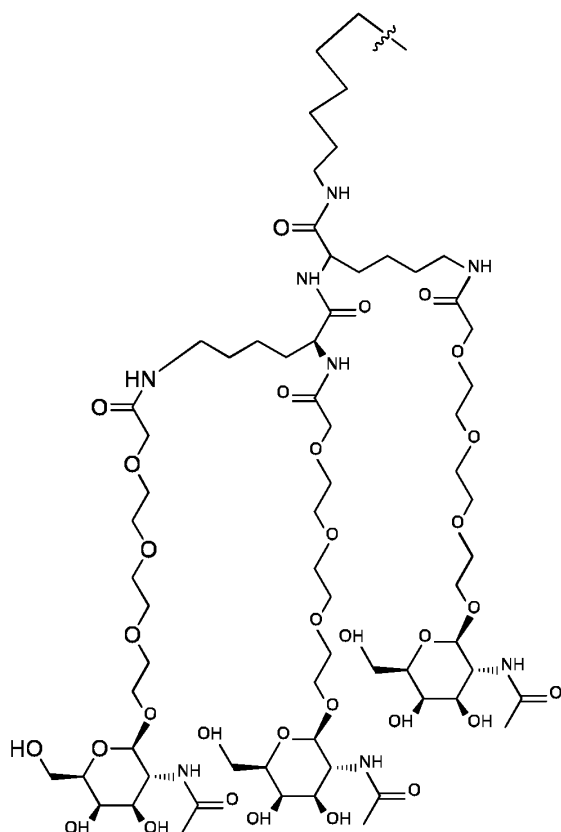
A is an antisense oligonucleotide residue according to the present invention, such an antisense oligonucleotide shown in Table 6, being attached via its 5'prime end to R via $-(L)_n-(O-P(=O)(-OH)-)_p$. Preferably, A is an antisense oligonucleotide residue selected from the residues depicted in Fig. 1A, 2A, 3A, 4A, 5A, 6A, 7A and 8A or depicted in Fig. 1A, 2A 3A, 4A, 5A, 6A, 7A, 8A and 8.1A.

According to a further aspect of the invention, A is the antisense oligonucleotide residue depicted in Fig. 8.1A.

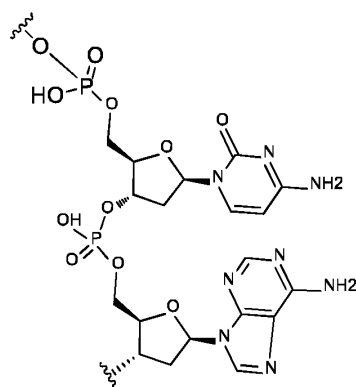
Thus, compound (I) is preferably selected from the compounds depicted in Fig. 10A1, 10A2; 10C1, 10C2, 10D1, 10D2, 10E1, 10F1, 10G1, 10H1, 10I1, 10J1, 10L1 10L2, and mixtures thereof, such as stereoisomeric mixtures of 10A1 and 10A2; of 10C1 and 10C2 or of 10D1 and 10D2, more preferably compound (I) is selected from the compounds depicted in 10D1 and 10D2, and a mixture thereof, more preferably compound (I) is a mixture of compound 10D1 and 10D2, preferably with A being selected from the antisense oligonucleotide shown in Table 6, preferably with A being an antisense oligonucleotide residue selected from the residues depicted in Fig. 1A, 2A 3A, 4A, 5A, 6A, 7A and 8A. and with L is a linker comprising or consisting of 2-10 nucleosides, such as 2-5 nucleosides, such as 2 nucleosides, wherein optionally the nucleosides are phosphodiester linked nucleosides, more preferably wherein L is



In a further aspect, R is a residue having the structure (I)



L is a linker as defined herein, preferably L is a linker comprising or consisting of 2-10 nucleosides, such as 2-5 nucleosides, such as 2 nucleosides, wherein optionally the nucleosides are phosphodiester linked nucleosides, more preferably L is



and

A is an antisense oligonucleotide according to the present invention, such as an antisense oligonucleotide shown in Table 6.

According to one aspect of the invention, A is an antisense oligonucleotide residue selected from the residues depicted in Fig. 1A, 2A 3A, 4A, 5A, 6A, 7A and 8A or depicted in Fig. 1A, 2A 3A, 4A, 5A, 6A, 7A 8A, and 8.1A.

According to a further aspect of the invention, A is the antisense oligonucleotide residue depicted in Fig. 8.1A.

The invention provides pharmaceutical compositions comprising the antisense oligonucleotide of the invention or the conjugate of the present invention, and a pharmaceutically acceptable diluents, carriers, salts and/or adjuvants.

The invention provides for a pharmaceutically acceptable salt of the antisense oligonucleotide of the invention or the conjugate thereof. In some embodiments, the pharmaceutically acceptable salt is selected from the group consisting of a sodium salt, a potassium salt and an ammonium salt.

The invention provides for a pharmaceutical solution of the antisense oligonucleotide of the invention or the conjugate thereof, wherein the pharmaceutical solution comprises the antisense oligonucleotide of the invention or the conjugate thereof and a pharmaceutically acceptable solvent, such as phosphate buffered saline. Alternative, the solvent is water or a sodium chloride solution.

The invention provides for the antisense oligonucleotide of the invention or the conjugate thereof in solid powdered form, such as in the form of a lyophilized powder.

The invention provides for a pharmaceutically acceptable salt of the antisense oligonucleotide of the invention or the conjugate thereof.

The invention provides for a pharmaceutically acceptable salt of the antisense oligonucleotide according to the invention, or the conjugate of the invention, wherein the pharmaceutically acceptable salt is a sodium salt. Alternatively, the salt is a potassium salt.

The invention provides for a pharmaceutical composition comprising the antisense oligonucleotide of the invention, or the conjugate of the invention, or the salt of the invention and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

The invention provides for a method for inhibiting FUBP1 expression in a target cell, which is expressing FUBP1, said method comprising administering an antisense oligonucleotide of the invention, or the conjugate of the invention, or the salt of the invention, or the composition of the invention in an effective amount to said cell. The method may be an *in vivo* method or an *in vitro* method.

The invention provides for a method for treating and/or preventing an HBV infection in a subject such as a human, comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide of the invention, or the conjugate of the invention, or the salt of the invention, or the composition of the invention, such as to treat and/or prevent a disease selected from the group consisting of HBV infection, such as chronic HBV infection and proliferative diseases such as cancer, in particular hepatocellular carcinoma.

In some embodiments, the antisense oligonucleotide of the invention, or the conjugate of the invention, or the salt of the invention, or the pharmaceutical composition of the invention, is for the use in the treatment and/or prevention of an HBV infection, such as a chronic HBV infection.

The invention provides the antisense oligonucleotide of the invention, or the conjugate of the invention, or the pharmaceutical composition, or the salt of the invention for use in medicine. In a further aspect, the invention provides methods for inhibition of FUBP1 expression in a target cell, which is expressing FUBP1, by administering an antisense oligonucleotide of the invention, or conjugate of the invention in an effective amount to said cell. In a further aspect, the invention

provides methods for *in vivo* or *in vitro* method for inhibition of FUBP1 expression in a target cell, which is expressing FUBP1, by administering an antisense oligonucleotide, or the conjugate of the invention in an effective amount to said cell. The cell may for example be a human cell, such as a liver cell, such as a hepatocyte. In one embodiment, the cell is a hepatocellular carcinoma cell.

In a further aspect, the invention provides methods for reducing cccDNA in an HBV infected cell, by administering an antisense oligonucleotide of the invention, or conjugate of the invention in an effective amount to said cell.

In a further aspect, the invention provides methods for *in vivo* or *in vitro* method for reducing cccDNA in an HBV infected cell, by administering an antisense oligonucleotide of the invention, or the conjugate of the invention in an effective amount to said cell.

In a further aspect, the invention provides methods for treating and/or preventing a disease selected from the group consisting of HBV infection, such as chronic HBV infection and proliferative diseases such as cancer, in particular hepatocellular carcinoma.

In a further aspect, the invention provides the antisense oligonucleotide, or the conjugate of the invention, or the pharmaceutical composition of the invention, for use in the manufacture of a medicament for the treatment and/or prevention of a disease selected from the group consisting of HBV infection, such as chronic HBV infection and proliferative diseases such as cancer, in particular hepatocellular carcinoma.

In a further aspect, the invention provides the antisense oligonucleotide, or the conjugate of the invention, or the pharmaceutical composition of the invention, for use in the manufacture of an antiviral drug.

In a further aspect, the invention provides the antisense oligonucleotide, or the conjugate of the invention, or the pharmaceutical composition of the invention, for use in the manufacture of an antitumor drug.

The invention provides for the antisense oligonucleotide of the invention, or the conjugate of the invention, or the pharmaceutical composition of the invention, for use in the treatment and/or prevention of a disease selected from the group consisting of HBV infection, such as chronic HBV infection and proliferative diseases such as cancer, in particular hepatocellular carcinoma.

SEQUENCE LISTING

The sequence listing submitted with this application is hereby incorporated by reference. In the event of a discrepancy between the sequence listing and the specification or figures, the information disclosed in the specification (including the figures) shall be deemed to be correct.

BRIEF DESCRIPTION OF FIGURES

- Figure 1** Compound 6_1 (SEQ ID NO: 6) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 1A** Residue A of Compound 6_1 (SEQ ID NO: 6)
- Figure 2** Compound 6_2 (SEQ ID NO: 6) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 2A** Residue A of Compound 6_2 (SEQ ID NO: 6)
- Figure 3** Compound 7_1 (SEQ ID NO: 7) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 3A** Residue A of Compound 7_1 (SEQ ID NO: 7)
- Figure 4** Compound 7_2 (SEQ ID NO: 7) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 4A** Residue A of Compound 7_2 (SEQ ID NO: 7)
- Figure 5** Compound 7_3 (SEQ ID NO: 7) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 5A** Residue of Compound 7_3 (SEQ ID NO: 7)
- Figure 6** Compound 7_4 (SEQ ID NO: 7) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 6A** Residue A of Compound 7_4 (SEQ ID NO: 7)
- Figure 7** Compound 8_1 (SEQ ID NO: 8) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 7A** Residue A of Compound 8_1 (SEQ ID NO: 8)

- Figure 8** Compound 9_1 (SEQ ID NO: 9) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 8A** Residue A of Compound 9_1 (SEQ ID NO: 9)
- Figure 8.1** Compound 18_1 (SEQ ID NO: 18) conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide
- Figure 8.1A** Residue A of Compound 18_1 (SEQ ID NO: 18)
- Figure 9** Figure 9 illustrates exemplary GalNAc moieties. The compound in Fig. 9L is composed of monomeric GalNAc phosphoramidites added to the oligonucleotide while still on the solid support as part of the synthesis, X is S or O, Y is S or O, and n = 1-3 (see WO 2017/178656). Fig. 9B and Fig. 9D are also termed GalNAc2 or GN2 herein, without and with C6 linker respectively.
- Figure 10** Figure 10 illustrates exemplary antisense oligonucleotide conjugates. Compounds in Fig. 10A-D comprise a di-lysine brancher molecule, a PEG3 spacer and three terminal GalNAc carbohydrate moieties. In the compounds in Fig. 10A and Fig. 10B the oligonucleotide is preferably attached directly to the asialoglycoprotein receptor-targeting conjugate moiety without a linker. In the compounds in Fig. 10C and Fig. 10D the oligonucleotide is attached to the asialoglycoprotein receptor-targeting conjugate moiety via a C6 linker. The compounds in Fig. 10E-J comprise a commercially available trebler brancher molecule and spacers of varying length and structure and three terminal GalNAc carbohydrate moieties. The compound in Fig. 10L is composed of monomeric GalNAc phosphoramidites added to the oligonucleotide while still on the solid support as part of the synthesis, X= S or O, Y is S or O, and n = 1-3 (see WO 2017/178656).
- Figure 11** Figure 11 illustrates the results of an analysis of the *in vitro* efficacy of anti-FUBP1 compounds in Hela cells. FUBP1 mRNA levels are normalized and shown as % of control.
- Figure 12** Target engagement: FUBP1 mRNA. As described in Example 3, four antisense oligonucleotide compounds have been tested in HBV infected PHH cells. Each compound has been delivered to cells at a concentration of 10 μ M once per week for three weeks. FUBP1 mRNA target KD has been evaluated one week after the last

treatment. Total RNA has been extracted from cells using a MagNA Pure robot and the MagNA Pure 96 Cellular RNA Large Volume Kit according to the manufacturer's protocol and FUBP1 mRNA quantified by TaqMan qPCR. The figure shows the residual expression of the Target mRNA compared to negative control (NDC=1) with oligos tested at 10 μ M. Data are normalized to the human GUS B reference gene and the mean + SD from two biological replicates are reported for each oligo tested. FC of 50% and 20% are highlighted on the graph. CMP ID NO: 7_3 shows the best FUBP1 mRNA KD with 80% reduction mRNA expression respectively at 10 μ M. CMP ID NO: 18_1 shows the strongest effect in reducing FUBP1 mRNA compared to the prior art oligos (CMP ID Nos: 35_1 and 50_1), equally to the oligonucleotide with CMP ID NO: 7_3. They both reduce target mRNA expression at 10 μ M by about 80% compared to the NDC (see Example 3 in more detail).

Figure 13: Assessment of *in vivo* liver PK/PD correlation of the oligonucleotides with CMP ID Nos: 7_3 and 18_1 conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide was assessed in a single-dose mouse study (Conj. = Conjugate, see Example 4 for more details).

DEFINITIONS

HBV infection

The term "hepatitis B virus infection" or "HBV infection" is commonly known in the art and refers to an infectious disease that is caused by the hepatitis B virus (HBV) and affects the liver. A HBV infection can be an acute or a chronic infection. Chronic hepatitis B virus (CHB) infection is a global disease burden affecting 248 million individuals worldwide. Approximately 686,000 deaths annually are attributed to HBV-related end-stage liver diseases and hepatocellular carcinoma (HCC) (GBD 2013; Schweitzer et al., 2015). WHO projected that without expanded intervention, the number of people living with CHB infection will remain at the current high levels for the next 40-50 years, with a cumulative 20 million deaths occurring between 2015 and 2030 (WHO 2016). CHB infection is not a homogenous disease with singular clinical presentation. Infected individuals have progressed through several phases of CHB-associated liver disease in their life; these phases of disease are also the basis for treatment with standard of care (SOC). Current guidelines recommend treating only selected CHB-infected individuals based on three criteria - serum ALT level, HBV DNA level, and severity of

liver disease (EASL, 2017). This recommendation was due to the fact that SOC i.e. nucleos(t)ide analogs (NAs) and pegylated interferon-alpha (PEG-IFN), are not curative and must be administered for long periods of time thereby increasing their safety risks. NAs effectively suppress HBV DNA replication; however, they have very limited/no effect on other viral markers. Two hallmarks of HBV infection, hepatitis B surface antigen (HBsAg) and covalently closed circular DNA (cccDNA), are the main targets of novel drugs aiming for HBV cure. In the plasma of CHB individuals, HBsAg subviral (empty) particles outnumber HBV virions by a factor of 10³ to 10⁵ (Ganem & Prince, 2014); its excess is believed to contribute to immunopathogenesis of the disease, including inability of individuals to develop neutralizing anti-HBs antibody, the serological marker observed following resolution of acute HBV infection.

In some embodiments, the term "HBV infection" refers to "chronic HBV infection".

Further, the term encompasses infection with any HBV genotype.

In some embodiments, the patient to be treated is infected with HBV genotype A.

In some embodiments, the patient to be treated is infected with HBV genotype B.

In some embodiments, the patient to be treated is infected with HBV genotype C (which was tested in the Examples section, Example 3)

In some embodiments, the patient to be treated is infected with HBV genotype D.

In some embodiments, the patient to be treated is infected with HBV genotype E.

In some embodiments, the patient to be treated is infected with HBV genotype F.

In some embodiments, the patient to be treated is infected with HBV genotype G.

In some embodiments, the patient to be treated is infected with HBV genotype H.

In some embodiments, the patient to be treated is infected with HBV genotype I.

In some embodiments, the patient to be treated is infected with HBV genotype J.

cccDNA (covalently closed circular DNA)

cccDNA is the viral genetic template that resides in the nucleus of infected hepatocytes, where it gives rise to all HBV RNA transcripts needed for productive infection and is responsible for viral persistence

during natural course of chronic HBV infection (Locarnini & Zoulim, 2010 Antivir Ther. 15 Suppl 3:3-14. doi: 10.3851/IMP1619). Acting as a viral reservoir, cccDNA is the source of viral rebound after cessation of treatment, necessitating long term, often, lifetime treatment. PEG-IFN can only be administered to a small subset of CHB due to its various side effects.

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

Compound

Herein, the term “compound” means any molecule capable of inhibition FUBP1 expression or activity. Particular compounds of the invention are nucleic acid molecules, such as antisense oligonucleotides according to the invention or any conjugate comprising such a nucleic acid molecule. For example, herein the compound may be a nucleic acid molecule targeting FUBP1, in particular an antisense oligonucleotide.

Oligonucleotide

The term “oligonucleotide” as used herein is defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers. Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification and isolation. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The oligonucleotide of the invention is man-made, and is chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides such as 2' sugar modified nucleosides. The oligonucleotide of the invention may comprise one or more modified internucleoside linkages, such as one or more phosphorothioate internucleoside linkages.

Antisense oligonucleotides

The term “antisense oligonucleotide” or “ASO” as used herein is defined as oligonucleotides capable of modulating expression of a target gene by hybridizing to a target nucleic acid, in particular to a contiguous sequence on a target nucleic acid. Antisense oligonucleotides are not essentially double stranded and are therefore not siRNAs or shRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded. It is understood that single stranded oligonucleotides of the present invention can form hairpins or intermolecular duplex structures (duplex between two

molecules of the same oligonucleotide), as long as the degree of intra or inter self-complementarity is less than 50% across of the full length of the oligonucleotide.

In some embodiments, the single stranded antisense oligonucleotide of the invention may not contain RNA nucleosides.

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

Contiguous Nucleotide Sequence

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

Nucleotides and nucleosides

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

Modified nucleoside

The term "modified nucleoside" or "nucleoside modification" as used herein refers to nucleosides modified as compared to the equivalent DNA or RNA nucleoside by the introduction of one or more modifications of the sugar moiety or the (nucleo)base moiety. Advantageously, one or more of the modified nucleosides of the antisense oligonucleotide of the invention comprise a modified sugar

moiety. The term “modified nucleoside” may also be used herein interchangeably with the term “nucleoside analogue” or modified “units” or modified “monomers”. Nucleosides with an unmodified DNA or RNA sugar moiety are termed DNA or RNA nucleosides herein. Nucleosides with modifications in the base region of the DNA or RNA nucleoside are still generally termed DNA or RNA if they allow Watson Crick base pairing.

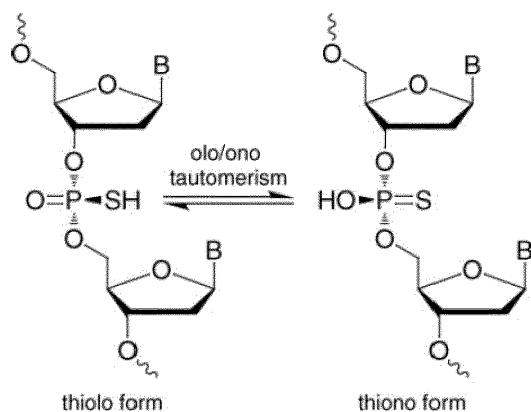
Modified internucleoside linkage

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

In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate, such as at least 60%, such as at least 70%, such as at least 75%, such as at least 80% or such as at least 90% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate.

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

Phosphorothioate linkages may exist in different tautomeric forms, for example as illustrated below:



It is recognized that, as disclosed in EP 2 742 135, antisense oligonucleotides may comprise other internucleoside linkages (other than phosphodiester, phosphorothioate and phosphorodithioate), for example alkyl phosphonate/methyl phosphonate internucleoside, which according to EP 2 742 135 may for example be tolerated in an otherwise DNA phosphorothioate the gap region.

Nucleobase

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

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

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

Modified oligonucleotide

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

Complementarity

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

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

to form Watson Crick base pairing is retained (e.g. 5¹-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

The term “fully complementary”, refers to 100% complementarity.

Identity

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

Hybridization

The term “hybridization”, “hybridizing” or “hybridizes” as used herein is to be understood as two nucleic acid strands (e.g. an oligonucleotide and a target nucleic acid) forming hydrogen bonds between base pairs on opposite strands thereby forming a duplex. The affinity of the binding between two nucleic acid strands is the strength of the hybridization. It is often described in terms of the melting temperature (T_m) defined as the temperature at which half of the oligonucleotides are duplexed with the target nucleic acid. At physiological conditions T_m is not strictly proportional to the affinity (Mergny and Lacroix, 2003, *Oligonucleotides* 13:515–537). The standard state Gibbs free energy ΔG° is a more accurate representation of binding affinity and is related to the dissociation constant (K_d) of the reaction by $\Delta G^\circ = -RT \ln(K_d)$, where R is the gas constant and T is the absolute temperature. Therefore, a very low ΔG° of the reaction between an oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. ΔG° is the energy associated with a reaction where aqueous concentrations are 1M, the pH is 7, and the temperature is 37°C. The hybridization of oligonucleotides to a target nucleic acid is a spontaneous reaction and for

spontaneous reactions ΔG° is less than zero. ΔG° can be measured experimentally, for example, by use of the isothermal titration calorimetry (ITC) method as described in Hansen et al., 1965, Chem. Comm. 36–38 and Holdgate et al., 2005, Drug Discov Today. The skilled person will know that commercial equipment is available for ΔG° measurements. ΔG° can also be estimated numerically by using the nearest neighbor model as described by SantaLucia, 1998, Proc Natl Acad Sci USA. 95: 1460–1465 using appropriately derived thermodynamic parameters described by Sugimoto et al., 1995, Biochemistry 34:11211–11216 and McTigue et al., 2004, Biochemistry 43:5388–5405. In order to have the possibility of modulating its intended nucleic acid target by hybridization, oligonucleotides of the present invention hybridize to a target nucleic acid with estimated ΔG° values below -10 kcal for oligonucleotides that are 10-30 nucleotides in length. In some embodiments the degree or strength of hybridization is measured by the standard state Gibbs free energy ΔG° . The oligonucleotides may hybridize to a target nucleic acid with estimated ΔG° values below the range of -10 kcal, such as below -15 kcal, such as below -20 kcal and such as below -25 kcal for oligonucleotides that are 8-30 nucleotides in length. In some embodiments the oligonucleotides hybridize to a target nucleic acid with an estimated ΔG° value of -10 to -60 kcal, such as -12 to -40, such as from -15 to -30 kcal or -16 to -27 kcal such as -18 to -25 kcal.

The Target

The term “target” as used herein refers to the mammalian protein “Far Upstream Element-Binding Protein 1”, alternatively known as “FUBP1” or “FBP” or “FUBP” or “hDH V”. The *homo sapiens* FUBP1 gene is located at chromosome 1, 77944055..77979435, complement (NC_000001.11, Gene ID 1462). The FUBP1 gene encodes a ssDNA binding protein that activates the far upstream element of c-myc and stimulates expression of c-myc in undifferentiated cells. Regulation of FUSE by FUBP occurs through single-strand binding of FUBP to the non-coding strand. The FUBP1 protein has ATP-dependent DNA helicase activity. The amino acid sequence of human FUBP1 is known in the art and can be assessed via UniProt, see e.g. UniProt entry Q96AE4 for human FUBP1, hereby incorporated by reference.

Target Nucleic Acid

According to the present invention, the target nucleic acid is a nucleic acid, which encodes mammalian FUBP1 and may for example be a gene, a RNA, an mRNA, and pre-mRNA, a mature mRNA or a cDNA sequence. The target may therefore be referred to as a FUBP1 target nucleic acid.

Suitably, the target nucleic acid encodes a FUBP1 protein, in particular mammalian FUBP1, such as the human FUBP1 gene encoding pre-mRNA or mRNA sequences provided herein as SEQ ID NO: 1, 2 and/or 3. SEQ ID NO: 1 is sequence of the human FUBP1 pre-mRNA. SEQ ID NO: 2 and 3 are sequences of human FUBP1 mRNAs.

Table 3 lists predicted exon and intron regions of SEQ ID NO: 1.

Table 3. Exon and intron regions in the human FUBP1 pre-mRNA.

Exonic regions in the human FUBP1 premRNA (SEQ ID NO 1)			Intronic regions in the human FUBP1 premRNA (SEQ ID NO 1)		
ID	start	end	ID	start	end
E1	19	226	I1	227	9095
E2	9096	9186	I2	9187	10907
E3	10908	10946	I3	10947	11444
E4	11445	11484	I4	11485	12009
E5	12010	12062	I5	12063	12155
E6	12156	12227	I6	12228	12359
E7	12360	12417	I7	12418	13879
E8	13880	14042	I8	14043	14142
E9	14143	14241	I9	14242	14363
E10	14364	14465	I10	14466	14754
E11	14755	14857	I11	14858	14948
E12	14949	15049	I12	15050	15395
E13	15396	15537	I13	15538	16180
E14	16181	16341	I14	16342	18615
E15	18616	18767	I15	18768	18847
E16	18848	18927	I16	18928	22410
E17	22411	22539	I17	22540	23781
E18	23782	23856	I18	23857	29810

Exonic regions in the human FUBP1 premRNA (SEQ ID NO 1)			Intronic regions in the human FUBP1 premRNA (SEQ ID NO 1)		
E19	29811	29956	I19	29957	30196
E20	30197	30706			

In some embodiments, the target nucleic acid may be a cynomolgus monkey FUBP1 nucleic acid, such as an mRNA or pre-mRNA.

In some embodiments, the target nucleic acid may be a mouse FUBP1 nucleic acid, such as a mRNA or pre-mRNA.

Table 4 provides an overview on the genomic sequences of human, cyno monkey and mouse FUBP1. Table 5 provides an overview on pre-mRNA sequences for human, monkey and mouse FUBP1 and for on mature mRNAs for human FUBP1.

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, and/or 5, or naturally occurring variants thereof (e.g. sequences encoding a mammalian FUBP1).

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 1, 2 and/or 3, or naturally occurring variants thereof (e.g. sequences encoding a mammalian FUBP1).

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 1 and 4 and 5, or naturally occurring variants thereof (e.g. sequences encoding a mammalian FUBP1).

Table 4. Genome and assembly information for FUBP1 across species.

Species	Chr.	Strand	Genomic coordinates		Assembly	ensembl gene_id
			Start	End		
Human	1	Rv	77944055	77979110	GRCh38.p10	ENSG00000162613
Cyno monkey	1	Fwd	149243675	149283374	Macaca_fascicularis_5.0	ENSMFAG00000031825
Mouse	3	Fwd	152210422	152236826	GRCm38.p5	ENSMUSG

Species	Chr.	Strand	Genomic coordinates		Assembly	ensembl gene_id
			Start	End		
						00000028034

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

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

For *in vivo* or *in vitro* application, the therapeutic antisense oligonucleotide of the invention is typically capable of inhibiting the expression of the FUBP1 target nucleic acid in a cell, which is expressing the FUBP1 target nucleic acid. The contiguous sequence of nucleobases of the antisense oligonucleotide of the invention is typically complementary to a conserved region of the FUBP1 target nucleic acid, as measured across the length of the antisense oligonucleotide, optionally with the exception of one or two mismatches, and optionally excluding nucleotide based linker regions which may link the antisense oligonucleotide to an optional functional group such as a conjugate, or other non-complementary terminal nucleotides.

The target nucleic acid may be a messenger RNA, such as a pre-mRNA which encodes mammalian FUBP1 protein, such as human FUBP1, e.g. the human FUBP1 pre-mRNA sequence, such as that disclosed as SEQ ID NO: 1, the cynomolgus monkey FUBP1 pre-mRNA sequence, such as that disclosed as SEQ ID NO: 4, or the mouse FUBP1 pre-mRNA sequence, such as that disclosed as SEQ ID NO: 5, or a mature FUBP1 mRNA, such as a human mature mRNA disclosed as SEQ ID NO: 2 and 3. SEQ ID NOs: 1-5, 10, 11, 15 and 19 are DNA sequences – it will be understood that target RNA sequences have uracil (U) bases in place of the thymidine bases (T).

Further information on exemplary target nucleic acids is provided in Table 5.

Table 5. Sequence details for FUBP1 across species.

Species	RNA type	Length (nt)	SEQ ID NO
Human	Pre-mRNA	35056	1
Human	Mature mRNA, variant 1	1968	2
Human	Mature mRNA, variant t	1935	3

Cyno monkey	Pre-mRNA	39750	4
Mouse	Pre-mRNA	26405	5

Note: SEQ ID NO: 4 comprises regions of multiple NNNNs, where the sequencing has been unable to accurately refine the sequence, and a degenerate sequence is therefore included. For the avoidance of doubt, the compounds of the invention are complementary to the actual target sequence and are not therefore degenerate compounds.

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

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

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

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

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

In some embodiments, the target nucleic acid is SEQ ID NO: 1, 2 and 3.

In some embodiments, the target nucleic acid is SEQ ID NO: 1 and 4. Thus, the antisense oligonucleotide may target both human and cyno monkey FUBP1.

In some embodiments, the target nucleic acid is SEQ ID NO: 1 and 5. Thus, the antisense oligonucleotide targets both human and mouse FUBP1.

In some embodiments, the target nucleic acid is SEQ ID NO: 1, 4 and 5. Thus, the antisense oligonucleotide may target human, cyno monkey and mouse FUBP1

Target Sequence

The term "target sequence" as used herein refers to a sequence of nucleotides present in the target nucleic acid, which comprises the nucleobase sequence, which is complementary to the oligonucleotide or nucleic acid molecule of the invention. In some embodiments, the target sequence consists of a region on the target nucleic acid with a nucleobase sequence that is complementary to the contiguous nucleotide sequence of the antisense oligonucleotide of the invention (i.e. a sub-sequence). This region of the target nucleic acid may interchangeably be referred to as the target nucleotide sequence, target sequence or target region. In some embodiments, the target sequence is longer than the complementary sequence of a single antisense oligonucleotide, and may, for example

represent a preferred region of the target nucleic acid, which may be targeted by several antisense oligonucleotides of the invention.

In one embodiment, the target sequence is a region within exon 14 of human FUBP1 mRNA (see Table 3 above).

In another embodiment, the target sequence is a region within exon 20 of human FUBP1 mRNA (see Table 3 above).

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

Provided herein below are target sequence regions, as defined by regions of the human FUBP1 pre-mRNA (using SEQ ID NO 1 as a reference) which may be targeted by the oligonucleotides of the invention.

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

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

Target Sequence Regions

The inventors have identified particularly effective sequences of the FUBP1 target nucleic acid, which may be targeted by the oligonucleotide of the invention.

In some embodiments, the target sequence is SEQ ID NO 10.

In some embodiments, the target sequence is SEQ ID NO 11.

In some embodiments, the target sequence is SEQ ID NO 15.

In some embodiments, the target sequence is SEQ ID NO 19.

SEQ ID NO 10: GTGAAACCATAAAAAGCATAAG

SEQ ID NO 11: AACCATAAAAAGCATAAG

SEQ ID NO 15: GTGAAACCATAAAAAGCATA

SEQ ID NO 19: GTAGAAATGAAAATTGGT

SEQ ID NO: 10, 11, 15 and 19 are DNA sequences – it will be understood that target RNA sequences have uracil (U) bases in place of the thymidine bases (T).

In some embodiments, the target sequence is the region from nucleotides 16184 to 16200 of SEQ ID NO: 1.

In some embodiments, the target sequence is the region from nucleotides 16186 to 16203 of SEQ ID NO: 1.

In some embodiments, the target sequence is the region from nucleotides 16188 to 16205 of SEQ ID NO: 1.

In some embodiments, the target sequence is the region from nucleotides 16189 to 16205 of SEQ ID NO: 1.

In some embodiments, the target sequence is the region from nucleotides 30536-30553 of SEQ ID NO: 1.

Target Cell

The term a “target cell” as used herein refers to a cell, which is expressing the target nucleic acid. In some embodiments, the target cell may be *in vivo* or *in vitro*. In some embodiments, the target cell is a mammalian cell such as a rodent cell, such as a mouse cell or a rat cell, or a primate cell such as a monkey cell or a human cell.

Typically, the target cell expresses the FUBP1 mRNA, such as the FUBP1 pre-mRNA or FUBP1 mature mRNA. For example, the target cell expresses the human FUBP1 pre-mRNA, e.g. SEQ ID NO 1, or human FUBP1 mature mRNA comprising exon 14 (or exon 20), such as SEQ ID NO: 2 or 3). For experimental evaluation a target cell may be used which expresses a nucleic acid which comprises a target sequence. The poly A tail of FUBP1 mRNA is typically disregarded for antisense oligonucleotide targeting.

The antisense oligonucleotide of the invention is typically capable of inhibiting the expression of the FUBP1 target nucleic acid in a target cell which is expressing the FUBP1 target nucleic acid, for example either *in vivo* or *in vitro*.

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

In one embodiment, the target cell may be infected with HBV. Further, the target cell may comprise HBV cccDNA. Thus, the target cell preferably comprises FUBP1 mRNA, such as the FUBP1 pre-mRNA or FUBP1 mature mRNA, and HBV cccDNA.

Further, the target cell may be a cancer cell, such as a hepatocellular carcinoma cell.

Naturally occurring variant

The term “naturally occurring variant” refers to variants of FUBP1 gene or transcripts which originate from the same genetic loci as the target nucleic acid, but may differ for example, by virtue of degeneracy of the genetic code causing a multiplicity of codons encoding the same amino acid, or due to alternative splicing of pre-mRNA, or the presence of polymorphisms, such as single nucleotide polymorphisms (SNPs), and allelic variants. Based on the presence of the sufficient complementary sequence to the oligonucleotide, the oligonucleotide of the invention may therefore target the target nucleic acid and naturally occurring variants thereof.

In some embodiments, the naturally occurring variants have at least 95% such as at least 98% or at least 99% homology to a mammalian FUBP1 target nucleic acid, such as a target nucleic acid selected from the group consisting of SEQ ID NO 1, 2, 3, 4 or 5. In some embodiments, the naturally occurring variants have at least 99% homology to the human FUBP1 target nucleic acid of SEQ ID NO: 1.

Inhibition of expression

The term “Inhibition of expression” as used herein is to be understood as an overall term for an oligonucleotide’s ability to inhibit the amount or the activity of FUBP1 in a target cell. Inhibition of activity may be determined by measuring the level of FUBP1 pre-mRNA or FUBP1 mRNA, or by measuring the level of FUBP1 or FUBP1 activity in a cell. Inhibition of expression may therefore be determined *in vitro* or *in vivo*.

Typically, inhibition of expression is determined by comparing the inhibition of activity due to the administration of an effective amount of the antisense oligonucleotide to the target cell and comparing that level to a reference level obtained from a target cell without administration of the antisense oligonucleotide (control experiment), or a known reference level (e.g. the level of expression prior to administration of the effective amount of the antisense oligonucleotide, or a predetermine or otherwise known expression level).

For example a control experiment may be an animal or person, or a target cell treated with a saline composition or a reference oligonucleotide (often a scrambled control).

The term inhibition or inhibit may also be referred as down-regulate, reduce, suppress, lessen, lower, the expression of FUBP1.

The inhibition of expression may occur e.g. by degradation of pre-mRNA or mRNA (e.g. using RNase H recruiting oligonucleotides, such as gapmers).

High affinity modified nucleosides

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

Sugar modifications

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

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

Such modifications include those where the ribose ring structure is modified, e.g. by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradicle bridge between the C2 and C4

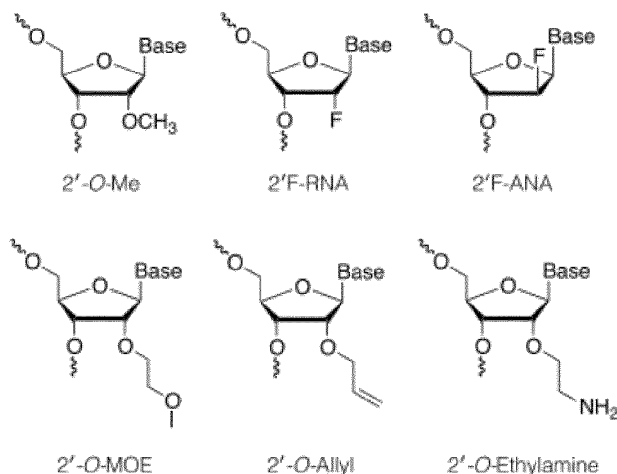
carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g. UNA). Other sugar modified nucleosides include, for example, bicyclohexose nucleic acids (WO2011/017521) or tricyclic nucleic acids (WO2013/154798). Modified nucleosides also include nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

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

2' sugar modified nucleosides

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

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



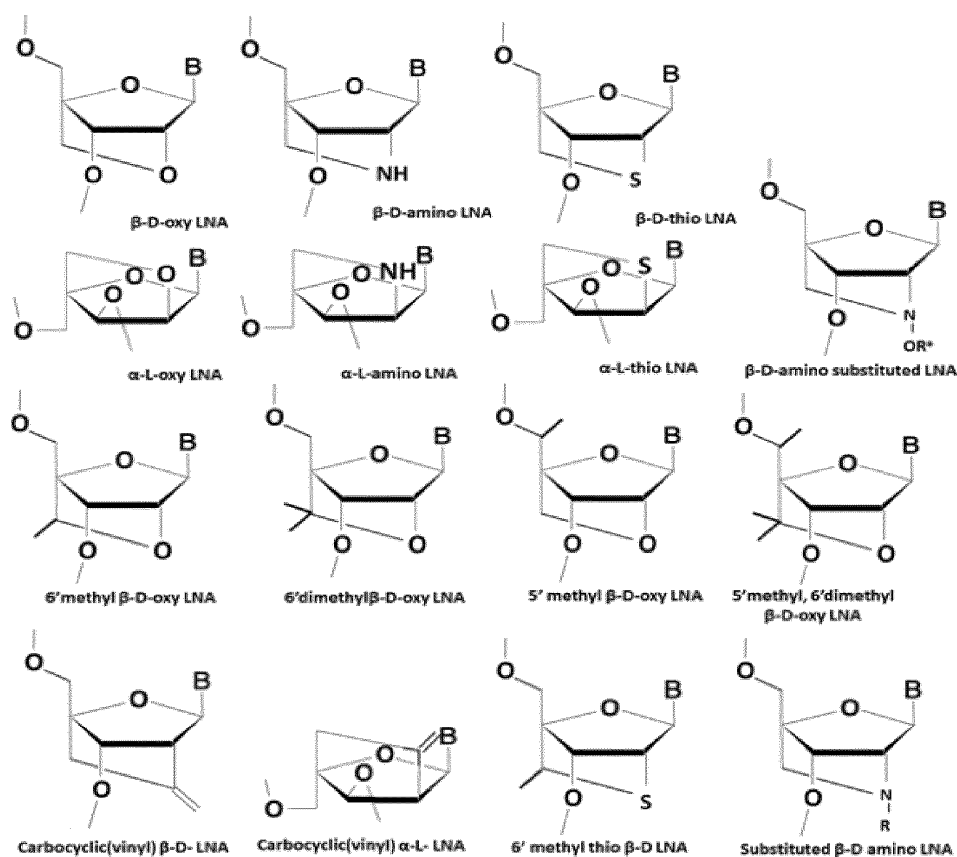
In relation to the present invention 2' substituted sugar modified nucleosides does not include 2' bridged nucleosides like LNA.

Locked Nucleic Acid Nucleosides (LNA nucleoside)

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

Non limiting, exemplary LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352 , WO 2004/046160, WO 00/047599, WO 2007/134181, WO 2010/077578, WO 2010/036698, WO 2007/090071, WO 2009/006478, WO 2011/156202, WO 2008/154401, WO 2009/067647, WO 2008/150729, Morita et al., Bioorganic & Med.Chem. Lett. 12, 73-76, Seth et al. J. Org. Chem. 2010, Vol 75(5) pp. 1569-81, and Mitsuoka et al., Nucleic Acids Research 2009, 37(4), 1225-1238, and Wan and Seth, J. Medical Chemistry 2016, 59, 9645–9667. Further non limiting, exemplary LNA nucleosides are disclosed in Scheme 1.

Scheme 1:



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

Nuclease Mediated Degradation

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

In some embodiments, the oligonucleotide may function via nuclease mediated degradation of the target nucleic acid, where the oligonucleotides of the invention are capable of recruiting a nuclease, particularly an endonuclease, preferably endoribonuclease (RNase), such as RNase H. Examples of oligonucleotide designs which operate via nuclease mediated mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 consecutive DNA nucleosides and are flanked on one side or both sides by affinity enhancing nucleosides, for example gapmers.

RNase H Activity and Recruitment

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

Gapmer

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

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

Regions F-G-F' form a contiguous nucleotide sequence. Antisense oligonucleotides of the invention, or the contiguous nucleotide sequence thereof, may comprise a gapmer region of formula F-G-F'. In some embodiments, all internucleoside linkages between the nucleosides of the gapmer region of formula F-G-F' are phosphorothioate internucleoside linkages.

The overall length of the gapmer design F-G-F' may be, for example 12 to 32 nucleosides, such as 13 to 24, such as 14 to 22 nucleosides, such as from 15 to 20 such as 16 to 18 nucleosides. In some embodiments, the overall length is 17 nucleosides. In some embodiments, the overall length is 17 nucleosides.

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

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

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

$F_{4-8}-G_{7-12}-F'_{2-8}$, or

$F_{4-6}-G_{7-11}-F'_{2-6}$

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

In an embodiment, the gapmer oligonucleotide of the present invention can be represented by the following formula:

$F_{4-6}-G_{7-11}-F'_{2-6}$

preferably wherein the overall length of the gapmer regions F-G-F' is at least 16 nucleotides, such as 17 or 18 nucleotides.

In an aspect of the invention the antisense oligonucleotide or contiguous nucleotide sequence thereof consists of or comprises a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise or consist of 1-8 nucleosides, of which 1-4 are 2' sugar modified and defines the 5' and 3' end of the F and F' region, and G is a region between 6 and 16 nucleosides, such as 7 to 12 nucleosides, which are capable of recruiting RNase H.

In some embodiments, all the modified nucleosides of region F and F' are beta-D-oxy LNA nucleosides. Further, region F or F', or F and F' may optionally comprise DNA nucleosides. Optionally, the flanking region F or F', or both flanking regions F and F' may comprise one or more DNA nucleosides (an alternating flank, see definition of the alternating flank for more details).

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

Gapmer - Region G

Region G (gap region) of the gapmer is a region of nucleosides which enables the oligonucleotide to recruit RNaseH, such as human RNase H1, typically DNA nucleosides. RNase H is a cellular enzyme which recognizes the duplex between DNA and RNA, and enzymatically cleaves the RNA molecule. Suitably gapmers may have a gap region (G) of at least 5 or 6 contiguous DNA nucleosides, such as 5 – 16 contiguous DNA nucleosides, such as 6 – 15 contiguous DNA nucleosides, such as 7-14 contiguous DNA nucleosides, such as 8 – 12 contiguous DNA nucleotides, such as 8 – 12 contiguous DNA nucleotides in length. The gap region G may, in some embodiments consist of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous DNA nucleosides.

In some embodiments, the gap region G may consist of 12 or less contiguous DNA nucleosides, such as of 7, 8, 9, 10, or 11 contiguous DNA nucleosides, such as 9, 10 or 11 contiguous DNA nucleosides. One or more cytosine (C) DNA in the gap region may in some instances be methylated (e.g. when a DNA c is followed by a DNA g). Such residues are either annotated as 5-methyl-cytosine (^{me}C). In some embodiments, the gap region G may consist of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous phosphorothioate linked DNA nucleosides.

In some embodiments, all internucleoside linkages in the gap are phosphorothioate linkages.

Gapmer - flanking regions, F and F'

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

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

Region F is 1 – 8 contiguous nucleotides in length, such as 2-6, such as 4-6 contiguous nucleotides in length. In some embodiments, the length of region F is 4 contiguous nucleotides. In some embodiments, the length of region F is 5 contiguous nucleotides. In some embodiments, the length of region F is 6 contiguous nucleotides.

Advantageously the 5' most nucleoside of region F is a sugar modified nucleoside. In some embodiments the two 5' most nucleosides of region F are sugar modified nucleosides. In some embodiments the 5' most nucleoside of region F is an LNA nucleoside. In some embodiments the two 5' most nucleosides of region F are LNA nucleosides.

Region F' is 1 – 8 contiguous nucleotides in length, such as 2-6, such as 2-5 contiguous nucleotides in length. In some embodiments, the length of region F' is 2 contiguous nucleotides. In some embodiments, the length of region F' is 3 contiguous nucleotides. In some embodiments, the length of region F' is 4 contiguous nucleotides. In some embodiments, the length of region F' is 5 contiguous nucleotides.

Advantageously, the 3' most nucleoside of region F' is a sugar modified nucleoside. In some embodiments, the two 3' most nucleosides of region F' are sugar modified nucleosides. In some embodiments, the two 3' most nucleosides of region F' are LNA nucleosides. In some embodiments the 3' most nucleoside of region F' is an LNA nucleoside.

It should be noted that when the length of region F is one, it is advantageously an LNA nucleoside. Further, it is noted that when the length of region F and/or F' is two, both nucleosides of region F and/or F' are advantageously LNA nucleosides.

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

In some embodiments, all the nucleosides of region F or F', or F and F' are LNA nucleosides, such as beta-D-oxy LNA nucleosides. In an alternative embodiment, all the sugar modified nucleosides of region F and F' are LNA nucleosides, such as beta-D-oxy LNA nucleosides, wherein region F or F', or both regions F and F' may comprise DNA nucleosides (an alternating flank, see definition of these for more details).

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

In some embodiments, the internucleoside linkage between region F and region G and/or the internucleoside linkage between region F' and region G is a phosphorothioate internucleoside linkage.

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

LNA Gapmer

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

In some embodiments, the LNA gapmer is of formula: [LNA]₁₋₅-[region G]-[LNA]₁₋₅, wherein region G is or comprises a region of contiguous DNA nucleosides which are capable of recruiting RNase H.

MOE Gapmers

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

Mixed Wing Gapmer

A mixed wing gapmer is an LNA gapmer wherein one or both of region F and F' comprise a 2' substituted nucleoside, such as a 2' substituted nucleoside independently selected from the group consisting of 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units, such as a MOE nucleoside. In some embodiments wherein at least one of region F and F', or both region F and F' comprise at least one LNA nucleoside, the remaining nucleosides of region F and F' are independently selected from the group consisting of MOE and LNA. In some embodiments wherein at least one of region F and F', or both region F and F' comprise at least two LNA nucleosides, the remaining nucleosides of region F and F' are independently selected from the group consisting of MOE and LNA. In some mixed wing embodiments, one or both of region F and F' may further comprise one or more DNA nucleosides.

Alternating Flank Gapmers

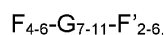
Flanking regions may comprise both LNA and DNA nucleoside and are referred to as "alternating flanks" as they comprise an alternating motif of LNA-DNA-LNA nucleosides. Gapmers comprising at least one alternating flank are referred to as "alternating flank gapmers". "Alternative flank gapmers" are thus LNA gapmer oligonucleotides, where at least one of the flanks (F or F') comprises one or

more DNA nucleotides in addition to the LNA nucleoside(s). In some embodiments at least one of region F or F', or both region F and F', comprise both LNA nucleosides and DNA nucleosides. In such embodiments, the flanking region F or F', or both F and F' comprise at least three nucleosides, wherein the 5' and 3' most nucleosides of the F and/or F' region are LNA nucleosides. Alternating flank LNA gapmers are disclosed in WO2016/127002.

An alternating flank region may comprise up to 3 contiguous DNA nucleosides, such as 1 to 2 or 1 or 2 or 3 contiguous DNA nucleosides.

The alternating flank regions can be annotated as a series of integers, representing a number of LNA nucleosides (L) followed by a number of DNA nucleosides (D), for example [L]₁₋₃-[D]₁₋₃-[L]₁₋₃ or [L]₁₋₂-[D]₁₋₂-[L]₁₋₂-[D]₁₋₂-[L]₁₋₂. In oligonucleotide designs these will often be represented as numbers such that 2-2-1 represents 5' [L]₂-[D]₂-[L] 3', and 1-1-1-1-1 represents 5' [L]-[D]-[L]-[D]-[L] 3'. The length of the flank (region F and F') in oligonucleotides with alternating flanks may be as described herein above for these regions, such as 4 to 8, such as 5 to 6 nucleosides, such as 4, 5, 6 or 7 modified nucleosides. It may be advantageous to have at least two LNA nucleosides at the 3' end of the 3' flank (F'), to confer additional exonuclease resistance.

In an embodiment, the gapmer oligonucleotide of the present invention can be represented by the following formula:



wherein F has a design of [L]₁₋₃-[D]₁₋₃-[L]₁₋₃ and F' has a design of [L]₁₋₂-[D]₁₋₂-[L]₂₋₄, or [L]₂₋₆

with the proviso that the overall length of the gapmer regions F-G-F' is at least 16 nucleotides, such as 17 or 18 nucleotides in length.

Thus, the gapmer oligonucleotide of the present invention may comprise at least one alternating flank. Typically, at least the F region is an alternating flank. In some embodiments, the both the F and the F' regions are alternating flanks. In some embodiments, the F region is an alternating flank and the F' region is a uniform flank (i.e. F' consists of only one type of sugar modified nucleosides, such as only beta-D-oxy LNA).

In some embodiments, the design of region F is selected from a design of 3-2-1 (i.e. LLLDDL), 3-1-1 (i.e. LLLDL), 2-1-2 (LLDLL), 2-1-1 (LLDL) and 1-3-1 (i.e. LDDDL).

In some embodiments, the design of region F' is 1-1-3 (i.e. LDLLL) or 1-1-2 (i.e. LDLL). In some embodiments, the design of region F is LL, LLL or LLLL.

Region D' or D'' in an oligonucleotide

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

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

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

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

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

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

$F-G-F'$; in particular $F_{1-8}-G_{5-16}-F'_{2-8}$, such as $F_{4-6}-G_{7-11}-F'_{2-6}$

$D'-F-G-F'$, in particular $D'_{1-3}-F_{1-8}-G_{5-16}-F'_{2-8}$, such as $D'_{1-3}-F_{4-6}-G_{7-11}-F'_{2-6}$

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

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

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

Conjugate

The term conjugate as used herein refers to an oligonucleotide, which is covalently linked to a non-nucleotide moiety (conjugate moiety or region C or third region). The conjugate moiety may be covalently linked to the antisense oligonucleotide, optionally via a linker group, such as region D' or D''.

Oligonucleotide conjugates and their synthesis has also been reported in comprehensive reviews by Manoharan in *Antisense Drug Technology, Principles, Strategies, and Applications*, S.T. Crooke, ed., Ch. 16, Marcel Dekker, Inc., 2001 and Manoharan, *Antisense and Nucleic Acid Drug Development*, 2002, 12, 103.

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

Exemplary conjugate moieties include those capable of binding to the asialoglycoprotein receptor (ASGPR). In particular, tri-valent N-acetylgalactosamine conjugate moieties are suitable for binding to the ASGPR, see for example WO 2014/076196, WO 2014/207232 and WO 2014/179620. Such conjugates serve to enhance uptake of the oligonucleotide to the liver.

In some embodiments, the conjugate is an antibody or an antibody fragment which has a specific affinity for a transferrin receptor, for example as disclosed in WO 2012/143379 hereby incorporated by

reference. In some embodiments, the non-nucleotide moiety is an antibody or antibody fragment, such as an antibody or antibody fragment that facilitates delivery across the blood-brain-barrier, in particular an antibody or antibody fragment targeting the transferrin receptor.

Linkers

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

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

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

In one embodiment, the linker between the oligonucleotide and the conjugate moiety is a physiologically labile linker composed of 2 to 5 consecutive phosphodiester linked nucleosides

comprising at least two consecutive phosphodiester linkages at the 5' or 3' terminal of the contiguous nucleotide sequence of the antisense oligonucleotide.

In some embodiments, the physiologically labile linker comprises or consists of a DNA dinucleotide with a sequence selected from the group consisting of AA, AT, AC, AG, TA, TT, TC, TG, CA, CT, CC, CG, GA, GT, GC, or GG, where there is a phosphodiester linkage between the two DNA nucleosides and at least one further phosphodiester at the 5' or 3' end of the dinucleotide linking either the oligonucleotide of the nucleic acid molecule to the dinucleotide or the conjugate moiety to the dinucleotide. For example, the linker may be a CA dinucleotide. In some embodiments, the physiologically labile linker comprises or consists of a DNA 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, or GGG, where there are phosphodiester linkages between the DNA nucleosides and potentially a further phosphodiester at the 5' or 3' end of the trinucleotide. Phosphodiester containing biocleavable linkers are described in more detail in WO 2014/076195 (hereby incorporated by reference). In a conjugate compound with a biocleavable linker at least about 50% of the conjugate moiety is cleaved from the oligonucleotide, 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 85% cleaved, such as at least about 90% cleaved, such as at least about 95% of the conjugate moiety is cleaved from the oligonucleotide cleaved when compared against a standard.

Region Y refers to linkers that are not necessarily biocleavable but primarily serve to covalently connect a conjugate moiety (region C or third region), to an oligonucleotide (region A or first region). The region Y linkers may comprise a chain structure or an oligomer of repeating units such as ethylene glycol, amino acid units or amino alkyl groups.

The oligonucleotide conjugates of the present invention can be constructed of the following regional elements A-C, A-B-C, A-B-Y-C, A-Y-B-C or A-Y-C. In some embodiments, the linker (region Y) is an amino alkyl, such as a C2 – C36 amino alkyl group, including, for example C6 to C12 amino alkyl groups. In some embodiments, the linker (region Y) is a C6 amino alkyl group.

Pharmaceutically acceptable salts

The term "pharmaceutically acceptable salts" refers to those salts which retain the biological effectiveness and properties of the free bases or free acids, which are not biologically or otherwise

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

Treatment

The terms "treatment", "treating", "treats" or the like as used herein generally means obtaining a desired pharmacological and/or physiological effect. This effect is therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a subject and includes: (a) inhibiting the disease; or (b) ameliorating (i.e. relieving) the disease, i.e. causing regression of the disease. A compound that ameliorates and/or inhibits a HBV infection is a compound that treats a HBV infection. Preferably, the term "treatment" as used herein relates to medical intervention of an already manifested disorder, like the treatment of an already defined and manifested HBV infection or cancer.

Prevention

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

prevention of HBV infection in children from HBV infected mothers are contemplated. Also contemplated is the prevention of an acute HBV infection turning into a chronic HBV infection.

Patient

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

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention is an enhanced antisense oligonucleotide targeting FUBP1, or a conjugate thereof for use in the treatment and/or prevention of a disease selected from the group consisting of HBV infection, such as chronic HBV infection and proliferative diseases such as cancer, in particular hepatocellular carcinoma.

An embodiment of the invention is an antisense oligonucleotide of the invention or conjugate thereof, which is capable of reducing HBV DNA, such as cccDNA, and HBV RNA transcripts, such as pgRNA, in an infected cell, such as an HBV infected cell.

In a further embodiment, the antisense oligonucleotide of the invention or conjugate thereof is capable of reducing HBsAg and/or HBeAg *in vivo* in an HBV infected individual.

Another aspect of the present invention is the use of the antisense oligonucleotides of the invention or the conjugate thereof in the treatment and/or prevention of Hepatitis B virus (HBV) infection, in particular a chronic HBV infection or in the treatment of cancer where FUBP1 is over-expressed.

The antisense oligonucleotide of the invention

The enhanced antisense oligonucleotides of the invention or conjugates thereof are potentially excellent FUBP1 inhibitors since they can target the FUBP1 transcript and may promote its degradation either via RNase H cleavage.

One aspect of the present invention is an enhanced antisense oligonucleotide or conjugates thereof for use in treatment and/or prevention of HBV infection, or in the treatment of cancer.

The present section describes enhanced antisense oligonucleotides or conjugates thereof suitable for use in treatment and/or prevention of HBV infection, or in the treatment of cancer.

The antisense oligonucleotides of the present invention or conjugates thereof are capable of inhibiting expression of FUBP1 *in vitro* and *in vivo*. The inhibition is achieved by hybridizing an antisense oligonucleotide to a target nucleic acid encoding FUBP1 or which is involved in the regulation of FUBP1. The target nucleic acid may be a mammalian FUBP1 sequence, such as a sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 and/or 5.

The oligonucleotide of the invention is thus an antisense oligonucleotide, which targets FUBP1

In some embodiments, the antisense oligonucleotide of the invention or conjugates thereof is capable of modulating the expression of the target by inhibiting or down-regulating it. Preferably, such modulation produces an inhibition of expression of at least 20% compared to the normal expression level of the target, more preferably at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% inhibition compared to the normal expression level of the target. In some embodiments, the antisense oligonucleotide of the invention or conjugates thereof may be capable of inhibiting expression levels of FUBP1 mRNA by at least 50% or 60% *in vitro* using 25 μ M in PXB-PHH cells. In some embodiments, the antisense oligonucleotide of the invention or conjugates thereof may be capable of inhibiting expression levels of FUBP1 protein by at least 50% *in vitro* using 25 μ M in PXB-PHH cells, this range of target reduction is advantageous in terms of selecting antisense oligonucleotides with good correlation to the cccDNA reduction. Suitably, the examples provide assays, which may be used to measure FUBP1 RNA inhibition (e.g. Example 1 or 2). The target inhibition is triggered by the hybridization between a contiguous nucleotide sequence of the antisense oligonucleotide and the target nucleic acid. In some embodiments, the antisense oligonucleotide of the invention comprises mismatches between the antisense oligonucleotide and the target nucleic acid. Despite mismatches hybridization to the target nucleic acid may still be sufficient to show a desired inhibition of FUBP1 expression. Reduced binding affinity resulting from mismatches may advantageously be compensated by increased number of nucleotides in the oligonucleotide and/or an increased number of modified nucleosides capable of increasing the binding affinity to the target, such as 2' sugar modified nucleosides, including LNA, present within the antisense oligonucleotide sequence.

An aspect of the present invention relates to an enhanced antisense oligonucleotide of 12 to 30, such as 12 to 22, such as 16 to 20 nucleotides in length, which comprises a contiguous nucleotide sequence of at least 12 nucleotides in length, such as 14, 15, 16, or 17 nucleotides in length, with at least 90% complementarity, such as 100% complementarity, a target sequence from nucleotides 16184-16205, such as a target sequence selected from 16184-16200, 16186-16203, 16188-16205 and 16189-16205 of SEQ ID NO: 1. In particular, antisense oligonucleotides which are capable of inhibiting the expression of FUBP1, i.e. are capable of reducing a FUBP1 nucleic acid such as FUBP1 mRNA are considered part of the present invention.

In some embodiments, the antisense oligonucleotide of the present invention comprises a contiguous nucleotide sequence of 12 to 22 nucleotides, such as of 15 to 20 nucleotides, with at least 90% complementarity, such as fully complementary, to the target nucleic acid of SEQ ID NO: 10.

In some embodiments, antisense oligonucleotide comprises a contiguous nucleotide sequence of 15 to 18 nucleotides, such as of 17 or 18 nucleotides, with at least 90% complementarity, such as fully complementary, to the target nucleic acid of SEQ ID NO: 11.

In some embodiments, antisense oligonucleotide comprises a contiguous nucleotide sequence of 15 to 18 nucleotides, such as of 17 or 18 nucleotides, with at least 90% complementarity, such as fully complementary, to the target nucleic acid of SEQ ID NO: 18.

In some embodiments, the antisense oligonucleotide comprises a contiguous nucleotide sequence of 15 to 22 nucleotides, such as of 15 to 18 nucleotides, such as of 17 or 18 nucleotides with at least 90% complementarity, such as fully complementary, to the target nucleic acid selected from the following regions of SEQ ID NO: 1: 16184-16205, 16184-16200, 16186-16203, 16188-16205 and 16189-16205 of SEQ ID NO: 1. Moreover, it may comprise a contiguous nucleotide sequence of 15 to 22 nucleotides, such as of 15 to 18 nucleotides, such as of 17 or 18 nucleotides with at least 90% complementarity, such as fully complementary, to the target nucleic acid selected from the following region of SEQ ID NO: 1: 30536-30553.

In some embodiments, the antisense oligonucleotide comprises a contiguous sequence of 12 to 30 nucleotides in length, which is at least 90% complementary, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as

at least 97%, such as at least 98%, or 100% complementary with a region of the target nucleic acid or a target sequence.

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

In some embodiments, the antisense oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid of SEQ ID NO: 1.

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

In some embodiments, the antisense oligonucleotide comprises a contiguous nucleotide sequence of 15 to 22 nucleotides in length with at least 90% complementary, such as 100% complementarity, to a corresponding target sequence present in SEQ ID NO: 1, wherein the target sequence is selected from nucleotides 16184-16205, 16184-16200, 16186-16203, 16188-16205, 16189-16205 and 30536-30553 of SEQ ID NO: 1.

In some embodiments, the contiguous nucleotide sequence of the antisense oligonucleotide is at least 90% complementary, advantageously 100% complementary, to a target site sequence of SEQ ID NO: 10.

In some embodiments, the contiguous nucleotide sequence of the antisense oligonucleotide is at least 90% complementary, advantageously 100% complementary, to a target site sequence of SEQ ID NO: 11.

In some embodiments, the contiguous nucleotide sequence of the antisense oligonucleotide is at least 90% complementary, advantageously 100% complementary, to a target site sequence of SEQ ID NO: 15.

In some embodiments, the contiguous nucleotide sequence of the antisense oligonucleotide is at least 90% complementary, advantageously 100% complementary, to a target site sequence of SEQ ID NO: 19.

In some embodiments, the contiguous nucleotide sequence comprises a sequence of nucleobases selected from the group consisting of SEQ ID NO: 6, 7, 8, 9 and 18, or at least 14 contiguous nucleotides thereof, such as 17 or 18 contiguous nucleotides thereof.

In some embodiments, the antisense oligonucleotide of the invention or contiguous nucleotide sequence thereof, comprises or consists of 10 to 30 nucleotides in length, such as from 12 to 25, such as 11 to 22, such as from 12 to 20, such as from 14 to 18 or 16 to 18 contiguous nucleotides in length.

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

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 12 – 24 nucleotides in length, such as 12 – 18 nucleotides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 12, such as at least 13, such as at least 14, such as at least 15 or at least 16 contiguous nucleotides present in SEQ ID NO: 6.

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 12 – 24 nucleotides in length, such as 12 – 18 nucleotides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 12, such as at least 13, such as at least 14, such as at least 15 or at least 16 contiguous nucleotides present in SEQ ID NO: 7

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 12 – 24 nucleotides in length, such as 12 – 18 nucleotides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 12, such as at least 13, such as at least 14, such as at least 15 or at least 16 contiguous nucleotides present in SEQ ID NO: 8.

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 12 – 24 nucleotides in length, such as 12 – 18 nucleotides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 12, such

as at least 13, such as at least 14, such as at least 15 or at least 16 contiguous nucleotides present in SEQ ID NO: 9.

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 12 – 24 nucleotides in length, such as 12 – 18 nucleotides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 12, such as at least 13, such as at least 14, such as at least 15, at least 16, at least 17 or 18 contiguous nucleotides present in SEQ ID NO: 18.

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

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence thereof comprises or consists of a sequence selected from SEQ ID NO: 6, 7, 8, 9 and 18.

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

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

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

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

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

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

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

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

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

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

In some embodiments, all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

In some embodiments, at least 75% the internucleoside linkages within the antisense oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate internucleoside linkages.

In some embodiments, all the internucleoside linkages within the antisense oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate internucleoside linkages.

In an advantageous embodiment of the invention the antisense oligonucleotide of the invention is capable of recruiting RNase H, such as RNase H1. In some embodiments, the antisense oligonucleotide of the invention, or the contiguous nucleotide sequence thereof is a gapmer.

In some embodiments, the antisense oligonucleotide, or contiguous nucleotide sequence thereof, consists or comprises a gapmer of formula 5'-F-G-F'-3'.

In some embodiments, region G consists of 6 – 16 DNA nucleosides, such as 7 to 12 DNA nucleosides. In some embodiments, region F comprises 4 to 6 nucleosides and/or region F' comprises 2 to 6 nucleosides.

In some embodiments, region F and F' each comprise at least one LNA nucleoside.

In some embodiments of the oligonucleotide of the present invention, all LNA nucleosides are beta-D-oxy LNA nucleosides.

In some embodiments, the oligonucleotide of the present invention is a LNA gapmer with uniform flanks.

In some embodiments of the invention, the LNA gapmer is an alternating flank LNA gapmer. In some embodiments, the alternating flank LNA gapmer comprises at least one alternating flank (such as flank F). In some embodiments, the alternating flank LNA gapmer comprises one alternating flank (such as flank F) and one uniform flank (such as flank F'). In some embodiments, the alternating flank LNA gapmer comprises two alternating flanks. For example, the LNA gapmer may have a design selected from the following designs: 3-2-1-9-2, 3-1-1-10-2, 2-1-2-10-3, 2-1-1-11-3, 2-1-1-10-1-1-2, 2-1-1-10-4, 1-3-1-7-1-1-3, and 3-2-1-9-3. Alternatively, the LNA gapmer may have the following design: 1-1-3-9-1-1-2.

Table 6 lists preferred designs for each motif sequence.

The invention provides the following oligonucleotide compounds (Table 6):

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

SEQ ID NO	Motif sequence	position on SEQ ID NO: 1		Design	CMP ID NO	Oligonucleotide Compound
		Start	end			
6	CTTATGCTTTTTATGGT	16189	16205	3-2-1-9-2	6_1	CTTatGcttttatgGT
6	CTTATGCTTTTTATGGT	16189	16205	3-1-1-10-2	6_2	CTTaTgcttttatgGT
7	CTTATGCTTTTTATGGT T	16188	16205	2-1-2-10-3	7_1	CTtAtgcttttatgGTT
7	CTTATGCTTTTTATGGT T	16188	16205	2-1-1-11-3	7_2	CTtAtgcttttatgGTT
7	CTTATGCTTTTTATGGT T	16188	16205	2-1-1-10-1-1-2	7_3	CTtAtgcttttatGgTT
7	CTTATGCTTTTTATGGT T	16188	16205	2-1-1-10-4	7_4	CTtAtgcttttatGGTT
8	GCTTTTTATGGTTTCAC	16184	16200	1-3-1-7-1-1-3	8_1	GcttTttatggtTtCAC
9	TATGCTTTTTATGGTTT C	16186	16203	3-2-1-9-3	9_1	TATgcTtttatggtTTC
18	ACCAATTTTCATTTCTAC	30536	30553	1-1-3-9-1-1-2	18_1	AcCAAAttttcatttCtAC

The heading "Oligonucleotide compound" in the table represents specific designs of a motif sequence. Capital letters are beta-D-oxy LNA nucleosides, lowercase letters are DNA nucleosides, all LNA C are 5-methyl cytosine, all internucleoside linkages are phosphorothioate internucleoside linkages. The heading "Designs" refers to the gapmer design, F-G-F'. In gapmers with alternating flank designs the flanks of the oligonucleotide are annotated as a series of integers, representing a number of beta-D-oxy LNA nucleosides (L) followed by a number of DNA nucleosides (D). For example, a flank with a 2-1 motif represents LLDDL. Both flanks have a beta-D-oxy LNA nucleoside at the 5' and 3' terminal. The gap region (G), which is constituted of a number of DNA nucleosides is located between the flanks.

For some embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP ID NO: 6_1, 6_2, 7_1, 7_2, 7_3, 7_4; 8_1 and 9_1 (see Table 6). For example, the compound may be the compound with CMP ID NO: 7_3.

In an alternative embodiment, the oligonucleotide is oligonucleotide the compound with CMP ID NO: 18_1 (see Table 6).

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

Conjugates

Since HBV infection primarily affects the hepatocytes in the liver it is advantageous to conjugate the enhanced antisense oligonucleotide of the invention to a conjugate moiety that will increase the delivery of the antisense oligonucleotide to the liver compared to the unconjugated antisense oligonucleotide. In one embodiment, liver targeting moieties are selected from moieties comprising

cholesterol or other lipids or conjugate moieties capable of binding to the asialoglycoprotein receptor (ASGPR).

In some embodiments, the invention provides a conjugate comprising an antisense oligonucleotide of the invention covalently attached to a conjugate moiety.

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

In one embodiment, the conjugate moiety comprises at least one asialoglycoprotein receptor-targeting moiety selected from group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N-isobutanoylgalactosamine. Advantageously the asialoglycoprotein receptor-targeting moiety is N-acetylgalactosamine (GalNAc).

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

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

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

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

In one embodiment, the conjugate moiety is a tri-valent N-acetylgalactosamine (GalNAc), such as those shown in Figure 9A1, 9A2; 9C1, 9C2, 9D1, 9D2, 9E1, 9F1, 9G1, 9H1, 9I1, 9J1, 9L1 and 9L2, or the conjugate moiety is a mixture of 9A1 and 9A2; a mixture of 9C1 and 9C2 or a mixture of 9D1 and 9D2. in particular a tri-valent Nacetylgalactosamine (GalNAc), as shown in Figure 9D1 or 9D2 or a mixture thereof.

In some embodiments, the conjugate is selected from the group consisting of

5'-GN2-C6₀C₀a₀ ^mC_sT_sT_sa_st_sG_sC_st_st_st_st_sa_st_sG_sT_s,
 5'-GN2-C6₀C₀a₀ ^mC_sT_sT_sa_sT_sG_sC_st_st_st_st_sa_st_sG_sT_s,
 5'-GN2-C6₀C₀a₀ ^mC_sT_st_sA_sT_sG_sC_st_st_st_st_sa_st_sG_sT_sT_s,
 5'-GN2-C6₀C₀a₀ ^mC_sT_st_sA_st_sG_sC_st_st_st_st_sa_st_sG_sT_sT_s,
 5'-GN2-C6₀C₀a₀ ^mC_sT_st_sA_st_sG_sC_st_st_st_st_sa_st_sG_sT_sT_s,
 5'-GN2-C6₀C₀a₀ ^mC_sT_st_sA_st_sG_sC_st_st_st_st_sa_st_sG_sT_sT_s,
 5'-GN2-C6₀C₀a₀ G_sC_st_st_sT_st_st_sa_st_sG_st_sT_s^mC_sA_s^mC, and
 5'-GN2-C6₀C₀a₀ T_sA_sT_sG_sC_sT_st_st_st_sa_st_sG_st_sT_s^mC,
 5'-GN2-C6₀C₀a₀ A_sC_s^mC_sA_sA_st_st_st_st_sC_sa_st_st_s^mC_sA_s^mC

wherein a capital letter represents a beta-D-oxy LNA nucleoside, a lower case letter represents a DNA nucleoside, wherein each LNA cytosine is 5-methyl cytosine, and wherein subscript s represents a phosphorothioate internucleoside linkage, and a subscript o represents a phosphodiester internucleoside linkage, and GN2-C6 is tri-valent N-acetylgalactosamine (GalNAc) as shown in Figure 9D, such as a tri-valent N-acetylgalactosamine (GalNAc) as shown in Figure 9D-1 or Figure 9D2, or a mixture of both, preferably bound via a phosphodiester linkage at the 5' end of the oligonucleotide. Chemical drawings representing some of the molecules are shown in figures 1 to 8 and 8.1.

In some embodiments, the conjugate is the conjugate as shown in Fig. 1.

In some embodiments, the conjugate is the conjugate as shown in Fig. 2.

In some embodiments, the conjugate is the conjugate as shown in Fig. 3.

In some embodiments, the conjugate is the conjugate as shown in Fig. 4.

In some embodiments, the conjugate is the conjugate as shown in Fig. 5.

In some embodiments, the conjugate is the conjugate as shown in Fig. 6.

In some embodiments, the conjugate is the conjugate as shown in Fig. 7.

In some embodiments, the conjugate is the conjugate as shown in Fig. 8.

In some embodiments, the conjugate is the conjugate as shown in Fig. 8.1.

The compounds illustrated in figures 1-8, and 8.1 are shown in the protonated form - the S atom on the phosphorothioate linkage is protonated – it will be understood that the presence of the proton will depend on the acidity of the environment of the molecule, and the presence of an alternative cation (e.g. when the oligonucleotide is in salt form). Protonated phosphorothioates exist in tautomeric forms.

Pharmaceutically acceptable salts

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

In a further aspect, the invention provides a pharmaceutically acceptable salt of the antisense oligonucleotide or a conjugate thereof, such as a pharmaceutically acceptable sodium salt, ammonium salt or potassium salt.

Method of manufacture

In a further aspect, the invention provides methods for manufacturing the oligonucleotides of the invention comprising reacting nucleotide units and thereby forming covalently linked contiguous nucleotide units comprised in the oligonucleotide. Preferably, the method uses phosphoramidite chemistry (see for example Caruthers et al, 1987, Methods in Enzymology vol. 154, pages 287-313). In a further embodiment, the method further comprises reacting the contiguous nucleotide sequence with a conjugating moiety (ligand) to covalently attach the conjugate moiety to the oligonucleotide. In a further aspect, a method is provided for manufacturing the composition of the invention, comprising mixing the oligonucleotide or conjugated oligonucleotide of the invention with a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

Pharmaceutical Composition

In a further aspect, the invention provides pharmaceutical compositions comprising any of the aforementioned oligonucleotides and/or oligonucleotide conjugates or salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS) and pharmaceutically acceptable salts include, but are not limited to, sodium, ammonium and potassium salts. In some embodiments the pharmaceutically acceptable diluent is sterile phosphate buffered saline. Alternatively, the diluent may be water or a sodium chloride solution. In some embodiments, the oligonucleotide is used in the pharmaceutically acceptable diluent at a concentration of 50 – 300 μ M solution.

Suitable formulations for use in the present invention are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (Science 249:1527-1533, 1990). WO 2007/031091 provides further suitable and preferred examples of pharmaceutically acceptable diluents, carriers and adjuvants (hereby incorporated by reference). Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations are also provided in WO2007/031091.

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

In some embodiments, the antisense oligonucleotide of the invention or conjugate thereof may be mixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

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

Applications

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

In research, such antisense oligonucleotides may be used to specifically modulate the synthesis of FUBP1 protein in cells (e.g. *in vitro* cell cultures) and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention. Typically, the target modulation is achieved by degrading or inhibiting the mRNA producing the protein, thereby prevent protein formation or by degrading or inhibiting a modulator of the gene or mRNA producing the protein.

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

Also encompassed by the present invention is an *in vivo* or *in vitro* method for modulating FUBP1 expression in a target cell, which is expressing FUBP1, said method comprising administering an antisense oligonucleotide, a conjugate thereof or pharmaceutical composition of the invention in an effective amount to said cell.

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

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

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

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

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

Due to the reduction of FUBP1 levels the antisense oligonucleotide, conjugate thereof or pharmaceutical compositions of the present invention can be used to inhibit development of or in the treatment of HBV infection. In particular, the destabilization and reduction of the cccDNA, the antisense oligonucleotide, conjugate thereof or pharmaceutical compositions of the present invention more efficiently inhibits development of or treats a chronic HBV infection as compared to a compound that only reduces secretion of HBsAg.

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

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

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

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

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

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

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

Combination Therapy

In some embodiments, the enhanced antisense oligonucleotide, conjugate thereof or the pharmaceutical composition of the invention is for use in a combination treatment with another therapeutic agent. The therapeutic agent can for example be the standard of care for the diseases or disorders described above.

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

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

In particular, related embodiments, the additional HBV agent may be interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated), ribavirin; an HBV RNA replication inhibitor; a second antisense oligomer; an HBV therapeutic vaccine; an HBV prophylactic vaccine; lamivudine (3TC); entecavir (ETV); tenofovir disoproxil fumarate (TDF); telbivudine (LdT); adefovir; or an HBV antibody therapy (monoclonal or polyclonal).

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

Administration

The enhanced antisense oligonucleotide, conjugate thereof, or pharmaceutical composition of the invention is formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular mammal being treated, the clinical condition of the individual patient, the site of delivery of the agent, the method of administration, the scheduling of administration, the age and sex of the patients and other factors known to medical practitioners. Herein, an "effective amount" (also known as "(therapeutically) effective dose") means the amount of a compound that will elicit the biological or medical response of a subject that is being sought by a medical doctor or other clinician. The "effective amount" of an oligonucleotide, conjugate compound or pharmaceutical composition of the invention, will be governed by such considerations, and is the minimum amount necessary to inhibit HBsAg and/or HBeAg. For example, such amount may be below the amount that is toxic to the cells of the recipient, or to the mammal as a whole.

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

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

In a preferred embodiment, the antisense oligonucleotide, conjugate thereof or pharmaceutical compositions of the present invention are administered by a parenteral route including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion. In one embodiment, the active oligonucleotide or oligonucleotide conjugate is administered intravenously. With GalNAc conjugated compounds it may be advantageous to administer subcutaneously in order to delay saturation of the ASGP receptor.

Embodiments of the invention

The following embodiments of the present invention may be used in combination with any other embodiments described herein. The definitions and explanations provided herein above, in particular in the sections "SUMMARY OF INVENTION", "DEFINITIONS" and DETAILED DESCRIPTION OF THE INVENTION" apply *mutatis mutandis* to the following.

1. An antisense oligonucleotide which comprises a contiguous nucleotide sequence, which is at least 90% complementary, such as fully complementary to a FUBP1 nucleic acid, wherein the antisense oligonucleotide is capable of inhibiting the expression of FUBP1, such as human FUBP1, in a cell.
2. The antisense oligonucleotide of embodiment 1, wherein
 - a) the contiguous nucleotide sequence is at least 90% complementary, such as fully complementary to a region within exon 14 of human FUBP1 (see Table 3), or
 - b) the contiguous nucleotide sequence is at least 90% complementary, such as fully complementary to a region within exon 20 of human FUBP1 (see Table 3).
3. The antisense oligonucleotide of embodiments 1 and 2, wherein
 - a) the contiguous nucleotide sequence is fully complementary to a region from nucleotides 16184 to 16205 of the human FUBP1 pre-mRNA as shown in in SEQ ID NO: 1, such as to a region selected from a region from nucleotides 16184 to 16200, from nucleotides 16186 to 16203, from nucleotides 16188 to 16205, and from nucleotides 16189 to 16205 of SEQ ID NO: 1, or
 - b) the contiguous nucleotide sequence is fully complementary to a region from nucleotides 30536-30553 of the human FUBP1 pre-mRNA as shown in in SEQ ID NO: 1
4. The antisense oligonucleotide of any one of embodiments 1 to 3, wherein a) the contiguous nucleotide sequence is fully complementary to SEQ ID NO 10 and/or SEQ ID NO: 11 or b) the contiguous nucleotide sequence is fully complementary to SEQ ID NO: 19.
5. The antisense oligonucleotide of any one of embodiments 1 to 4, wherein the antisense oligonucleotide is 12 – 30 nucleotides in length, such as 12 to 22 nucleotides in length, such as 16 to 20 nucleotides in length.

6. The antisense oligonucleotide of any one of embodiments 1 to 4, wherein the contiguous nucleotide sequence is a contiguous sequence of at least 12 nucleotides, such as of 14, 15, 16, 17 or 18 nucleotides.
7. The antisense oligonucleotide of embodiment 6, wherein the contiguous nucleotide sequence is a contiguous sequence of 17 or 18 nucleotides.
8. The antisense oligonucleotide of any one of embodiments 1 to 7, wherein the contiguous nucleotide sequence is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9 and 18, or at least 15 contiguous nucleotides thereof.
9. The antisense oligonucleotide of any one of embodiments 1 to 8, comprising one or more modified nucleosides in the contiguous nucleotide sequence.
10. The antisense oligonucleotide of embodiment 9, wherein the one or more modified nucleosides in the contiguous nucleotide sequence are 2' sugar modified nucleosides.
11. The antisense oligonucleotide of embodiment 10, wherein the one or more 2' sugar modified nucleosides are independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA and LNA nucleosides.
12. The antisense oligonucleotide of any one of embodiments 9 to 11, wherein the one or more modified nucleosides are LNA nucleosides, such as oxy-LNA with the following 2'-4' bridge $-O-CH_2-$.
13. The antisense oligonucleotide of embodiment 12, wherein the one or more modified nucleosides are beta-D-oxy-LNA.
14. The antisense oligonucleotide of any one of embodiments 1 to 13, wherein at least one internucleoside linkage in the contiguous nucleotide sequence is a phosphorothioate internucleoside linkage.
15. The antisense oligonucleotide of any one of embodiments 1 to 14, wherein at least one internucleoside linkage in the contiguous nucleotide sequence is a phosphorodithioate internucleoside linkage.

16. The antisense oligonucleotide of any one of embodiments 1 to 15, wherein at least one internucleoside linkage in the contiguous nucleotide sequence is a phosphodiester internucleoside linkage.
17. The antisense oligonucleotide of embodiment 16, wherein all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.
18. The antisense oligonucleotide of any one of embodiments 1 to 17, wherein the antisense oligonucleotide is an antisense oligonucleotide which is capable of recruiting RNase H, such as RNase H1.
19. The antisense oligonucleotide of embodiment 18, wherein the antisense oligonucleotide, or contiguous nucleotide sequence thereof, consists of or comprises a gapmer of formula 5'-F-G-F'-3'.
20. The antisense oligonucleotide according to embodiment 19, wherein region G has a length of 6 to 16 DNA nucleosides, such as 7 to 12 DNA nucleosides, such as 7 to 11 DNA nucleosides.
21. The antisense oligonucleotide according to any one of embodiments 18 to 20, wherein region F and F' each comprise at least one LNA nucleoside, for example wherein region F and F' each comprise at least one LNA nucleoside.
22. The antisense oligonucleotide according to any one of embodiments 18 to 21, wherein region F has a length of 1 to 8 DNA nucleosides, such as 4 to 6 DNA nucleosides.
23. The antisense oligonucleotide according to any one of embodiments 18 to 22, wherein region F' has a length of 1 to 8 DNA nucleosides, such as 2 to 6 DNA nucleosides.
24. The antisense oligonucleotide according to any one of embodiments 18 to 23, wherein the antisense oligonucleotide, or contiguous nucleotide sequence thereof, consists or comprises a gapmer of formula $F_{4-6}-G_{7-11}-F'_{2-6}$, and preferably, wherein the gapmer comprises at least one alternating flank.
25. The antisense oligonucleotide according to any one of embodiments 1 to 24, wherein the antisense oligonucleotide is selected from the group of antisense oligonucleotides consisting of CTTatGcttttatgGT (SEQ ID NO: 6),

CTTaTgcttttatgGT (SEQ ID NO: 6),
 CTtATgcttttatgGTT (SEQ ID NO: 7),
 CTtAtgcttttatgGTT (SEQ ID NO: 7),
 CTtAtgcttttatGgTT (SEQ ID NO: 7),
 CTtAtgcttttatGGTT (SEQ ID NO: 7),
 GcttTttatggtTtCAC (SEQ ID NO: 8),
 TATgcTtttatggtTTC (SEQ ID NO: 9), and
 AcCAAttttcatttCtAC (SEQ ID NO: 18)

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

26. A conjugate comprising the antisense oligonucleotide according to any one of embodiments 1 to 25, and at least one conjugate moiety covalently attached to said antisense oligonucleotide.
27. The conjugate of embodiment 27, wherein the conjugate moiety comprises at least one asialoglycoprotein receptor-targeting moiety selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N-isobutanoylgalactosamine.
28. The conjugate compound of embodiment 27, wherein the asialoglycoprotein receptor-targeting moiety is N-acetylgalactosamine (GalNAc).
29. The conjugate compound of embodiment 27 or 28, wherein the conjugate moiety is mono-valent, di-valent, tri-valent or tetra-valent with respect to asialoglycoprotein receptor-targeting moieties.
30. The conjugate compound of embodiment 29, wherein the conjugate moiety consists of two to four terminal GalNAc moieties and a spacer linking each GalNAc moiety to a brancher molecule that can be conjugated to the antisense compound.
31. The conjugate compound of embodiment 30, wherein the spacer is a PEG spacer.

32. The conjugate compound of any one of embodiments 26 to 31, wherein the conjugate moiety is a tri-valent N-acetylgalactosamine (GalNAc) moiety.
33. The conjugate compound of any one of embodiments 26 to 32, wherein the conjugate moiety is selected from one of the trivalent GalNAc moieties in Figure 9A1, 9A2; 9C1, 9C2, 9D1, 9D2, 9E1, 9F1, 9G1, 9H1, 9I1, 9J1, 9L1 and 9L2.
34. The conjugate compound of embodiment 33, wherein the conjugate moiety is the trivalent GalNAc moiety in Figure 9D1, or 9D2 or a mixture thereof
35. The conjugate compound of any one of embodiments 26 to 34, comprising a linker which is positioned between the antisense oligonucleotide and the conjugate moiety.
36. The conjugate compound of embodiment 35, wherein the linker comprises or consists of 2 to 5 consecutive phosphodiester linked nucleosides, such as 2 consecutive phosphodiester linked nucleosides, such as phosphodiester linked nucleosides ca.
37. The conjugate according to any one of embodiments 26 to 36, wherein the conjugate is selected from the group consisting of

5'-GN2-C6_oC_oa_o ^mC_sT_sT_sa_st_sG_sc_st_st_st_sa_st_sG_sT_s,
 5'-GN2-C6_oC_oa_o ^mC_sT_sT_sa_sT_sg_sc_st_st_st_sa_st_sG_sT_s,
 5'-GN2-C6_oC_oa_o ^mC_sT_st_sA_sT_sg_sc_st_st_st_sa_st_sG_sT_sT_s,
 5'-GN2-C6_oC_oa_o ^mC_sT_st_sA_st_sg_sc_st_st_st_sa_st_sG_sT_sT_s,
 5'-GN2-C6_oC_oa_o ^mC_sT_st_sA_st_sg_sc_st_st_st_sa_st_sG_sg_sT_sT_s,
 5'-GN2-C6_oC_oa_o ^mC_sT_st_sA_st_sg_sc_st_st_st_sa_st_sG_sG_sT_sT_s,
 5'-GN2-C6_oC_oa_o G_sc_st_sT_st_sa_st_sg_sg_sT_st_s^mC_sA_s^mC,
 5'-GN2-C6_oC_oa_o T_sA_sT_sg_sT_st_st_sa_st_sg_sg_sT_s^mC, and
 5'-GN2-C6_oC_oa_o A_sc_s^mC_sA_sA_st_st_st_sc_sa_st_st_s^mC_st_sA_s^mC

preferably, wherein a capital letter represents a beta-D-oxy LNA nucleoside, a lower case letter represents a DNA nucleoside, wherein each LNA cytosine is 5-methyl cytosine, and ^mc is 5-methyl cytosine DNA, and wherein subscript s represents a phosphorothioate internucleoside linkage, and a subscript o represents a phosphodiester internucleoside linkage, and GN2-C6 is a tri-valent N-acetylgalactosamine (GalNAc) as shown in Figure 9D, such as a tri-valent N-acetylgalactosamine (GalNAc) as shown in Figure 9D-1 or Figure 9D2, or a mixture of both, preferably bound via a phosphodiester linkage at the 5' end of the oligonucleotide.

38. A conjugate as shown in Fig. 1.
39. A conjugate as shown in Fig. 2.
40. A conjugate as shown in Fig. 3.
41. A conjugate as shown in Fig. 4.
42. A conjugate as shown in Fig. 5.
43. A conjugate as shown in Fig. 6.
44. A conjugate as shown in Fig. 7.
45. A conjugate as shown in Fig. 8.
46. A conjugate as shown in Fig. 8.1.
47. A pharmaceutically acceptable salt of the oligonucleotide of any one of embodiments 1 to 25, or the conjugate according to any one of embodiments 26 to 46.
48. A pharmaceutical composition comprising the antisense oligonucleotide of any one of embodiments 1 to 25, the conjugate of any one of embodiments 26 to 46, or the pharmaceutically acceptable salt of embodiment 48, and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.
49. An in vivo or in vitro method for modulating FUBP1 expression in a target cell which is expressing FUBP1, said method comprising administering the antisense oligonucleotide of any one of embodiments 1 to 25, the conjugate of any one of embodiments 26 to 46, the pharmaceutically acceptable salt of embodiment 48, or the pharmaceutical composition of embodiment 48 in an effective amount to said cell.
50. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of the antisense oligonucleotide of any one of embodiments 1 to 25, the conjugate of any one of embodiments 26 to 46, the pharmaceutically acceptable salt of embodiment 47, or the pharmaceutical composition of embodiment 48 to a subject suffering from or susceptible to the disease, wherein the disease is hepatitis B virus (HBV) infection and/or cancer.

51. The antisense oligonucleotide of any one of embodiments 1 to 25, the conjugate of any one of embodiments 26 to 46, the pharmaceutically acceptable salt of embodiment 47, or the pharmaceutical composition of embodiment 48 for use in medicine.
52. The antisense oligonucleotide of any one of embodiments 1 to 25, the conjugate of any one of embodiments 26 to 46, the pharmaceutically acceptable salt of embodiment 47, or the pharmaceutical composition of embodiment 48 for use in the treatment or prevention of hepatitis B virus (HBV) infection and/or cancer.
53. Use of antisense oligonucleotide of any one of embodiments 1 to 25, the conjugate of any one of embodiments 26 to 46, the pharmaceutically acceptable salt of embodiment 47, or the pharmaceutical composition of embodiment 48, for the preparation of a medicament for treatment or prevention of a hepatitis B virus (HBV) infection and/or cancer.
54. The method of embodiment 50, the antisense oligonucleotide, conjugate, pharmaceutical composition, or the pharmaceutically acceptable salt for use of embodiment 52, or the use of embodiment 53, wherein the disease is hepatitis B virus (HBV) infection, such as chronic HBV infection.
55. The method of embodiment 50, the antisense oligonucleotide, conjugate, pharmaceutical composition, or the pharmaceutically acceptable salt for use of embodiment 52, or the use of embodiment 53, wherein the disease is cancer, such as hepatocellular carcinoma.
56. The antisense oligonucleotide according to any one of embodiments 1 to 25, the conjugate of any one of claims 26 to 33 and 45, the pharmaceutically acceptable salt of embodiment 47, or the pharmaceutical composition of embodiment 48, the use of claim 53, or the method of claims 54 and 54, wherein the antisense oligonucleotide is AcCAAttttcatttCtAC (SEQ ID NO: 18).
57. The antisense oligonucleotide according to any one of embodiments 1 to 25, the conjugate of any one of claims 26 to 33 and 42, the pharmaceutically acceptable salt of embodiment 47, or the pharmaceutical composition of embodiment 48, the use of claim 53, or the method of claims 54 and 54, wherein the antisense oligonucleotide is CTtAtgctttttatGgTT (SEQ ID NO: 7).

EXAMPLES

Introduction

Overexpression of and mutations in FUBP1 has been known to be associated with cancers for many years. In particular, strong overexpression of FUBP1 in human hepatocellular carcinoma (HCC) supports tumor growth and correlates with poor patient prognosis.

HBV cccDNA in infected hepatocytes is responsible for persistent chronic infection and reactivation, being the template for all viral subgenomic transcripts and pre-genomic RNA (pgRNA) to ensure both newly synthesized viral progeny and cccDNA pool replenishment via intracellular nucleocapsid recycling.

In WO 2019/193165, it was shown that FUBP1 is associated with cccDNA stability. This knowledge allows for the opportunity to destabilize cccDNA in HBV infected subjects which in turn opens the opportunity for a complete cure of chronically infected HBV patients.

In the the present study, more than 2000 antisense oligonucleotides targeting human FUBP1 were screened. In this screening, compounds were identified which are particularly potent and effective to target human FUBP1. Specifically, nine alternating flank gapmer LNA oligonucleotides were identified which target a region within exon 14 of human FUBP1 and which conferred a strong down-regulation of human FUBP1 *in vitro*. Furthermore, one alternating flank gapmer LNA oligonucleotide was identified which targets a region within exon 20 of human FUBP1 and which conferred a strong down-regulation of human FUBP1 as well. An overview on the identified nine compounds is provided in Table 6 above.

The target sequence of the identified compounds overlaps with the target sequence of CMP ID NO 53_1 and 54_1 as disclosed in WO 2019/193165. These two compounds inhibit FUBP1 in HeLa cells to around ~70% at 5 μ M. However, the nine identified compounds are clearly more efficacious, as they inhibit FUBP1 in HeLa cells down to about ~25% to 35% at 3.3 μ M or to ~27% at 5 μ M (CMP ID NO: 18_1. In addition, they are more efficacious in targeting FUBP1 in HeLa cells than CMP ID NO 50_1, which is the best compound of WO 2019/193165 (see Example 1).

An overview on the prior art compounds 35_1, 50_1, 53_1, 54_1, 78_1 and 79_1 of WO 2019/193165 is provided in Table 7 below. The compounds are gapmers with uniform flanks. CMP ID NO: 50_1 was the best compound in PHH cells, CMP ID NO: 35_1 was the best compound in HeLa cells. CMP ID NO 53_1 and 54_1 are the closest compounds for CMP ID Nos: 6_1, 6_2, 7_1, 7_2, 7_3, 7_4; 8_1 and 9_1. CMP ID NO 78_1 and 79_1 are the closest compounds for CMP ID NO: 18_1.

Table 7: list of control oligonucleotide compounds (as disclosed in WO 2019/193165)

SEQ ID NO	Motif sequence	position on SEQ ID NO: 1		Design	CMP ID NO	Oligonucleotide Compound
		Start	end			
22	CCCATAACCATAGTCAT	9142	9157	3-12-2	35_1	CCCaataaccatagtcAT
12	CCATTTCTTCCTATTACAA	14783	14801	3-14-2	50_1	CCAtttcttcctattacAA
13	GCTTTTTATGGTTTCACC	16183	16200	1-15-2	53_1	GcttttatggtttcaCC
14	ATGCTTTTTATGGTTTCACC	16183	16202	1-17-2	54_1	AtgcttttatggtttcaCC
20	atattaacctcctatcagt	30511	30530	1-15-3	78_1	AtattaacctcctatcAGT
21	aatattaacctcctatcag	30512	30531	3-13-3	79_1	AAAtattaacctcctatCAG

For Compounds: Capital letters represent LNA nucleosides (beta-D-oxy LNA nucleosides were used), all LNA cytosines are 5-methyl cytosine, lower case letters represent DNA nucleosides. All internucleoside linkages are phosphorothioate internucleoside linkages.

Example 1: Testing in vitro efficacy of antisense oligonucleotides targeting human FUBP1 mRNA in HeLa cells

Antisense oligonucleotides targeting *FUBP1* were tested for their ability to reduce *FUBP1* mRNA expression in human HeLa cells acquired from ECACC (Catalog No. 93021013).

HeLa cells were grown in cell culturing media (EMEM [Sigma, cat.no M2279], supplemented with 10% Fetal Bovine Serum [Sigma, cat.no F7524], 2mM Glutamine [Sigma, G7513], 0.1mM NEAA [Sigma, M7145] and 0.025mg/ml Gentamicin [Sigma, cat.no G1397]). Cells were trypsinized every 5 days, by washing with Phosphate Buffered Saline (PBS), [Sigma cat.no 14190-094] followed by addition of 0.25% Trypsin-EDTA solution (Sigma, T3924), 2-3 minutes incubation at 37°C, and trituration before cell seeding.

For experimental use, 2500 cells per well were seeded in 96 well plates (Nunc cat.no 167008) in 190 µL growth media. ASO dissolved in PBS was added approximately 24 hours after the cells were seeded to reach final custom concentrations. Cells were incubated for 3 days without any media change.

After incubation, cells were harvested by removal of media followed by addition of 125 µL RLT Lysis buffer (Qiagen 79216) and 125 µL 70% ethanol. RNA was purified according to the manufacturer's

instruction (Qiagen RNeasy 96 kit) and eluted in a final volume of 200 µL DNase/RNase free Water (Gibco).

The RNA was heat shocked for 40 seconds at 90°C to melt RNA:LNA duplexes, moved directly to ice and spun down before use. For one-step qPCR reaction qPCR-mix (qScript™XLE 1-step RT-qPCR TOUGHMIX®Low ROX from QauntaBio, cat.no 95134-500) was mixed with two IDT probes (final concentration 1X) to generate the mastermix. Taqman probes were acquired from IDT: FUBP1: Hs.PT.58.26883775 (primer-probe ratio 2, FAM) or ThermoFisher Scientific: GUSB: 4326320E. Mastermix (6 µL) and RNA (4 µL, 1-2 ng/µL) were then mixed in a qPCR plate (MICROAMP®optical 384 well, 4309849). After sealing, the plate was given a quick spin, 1000g for 1 minute at RT, and transferred to a Viia™ 7 system (Applied Biosystems, Thermo), and the following PCR conditions used: 50°C for 15 minutes; 95°C for 3 minutes; 40 cycles of: 95°C for 5 sec followed by a temperature decrease of 1.6°C/sec followed by 60°C for 45 sec. The data was analyzed using the QuantStudio™ Real_time PCR Software.

The qPCR data was captured and raw data quality control done in Quantstudio7 software.

The data were then imported into E-Workbook where a BioBook template was used to capture and analyze the data. The data were analyzed using the following steps:

1. Quantity calculated by the delta delta Ct method ($\text{Quantity} = 2^{-(\Delta\text{Ct})} \times 1000000000$)
2. Quantity normalized to the calculated quantity for the housekeeping gene assay run in the same well. $\text{Relative Target Quantity} = \text{QUANTITY_target} / \text{QUANTITY_housekeeping}$
3. The RNA knockdown was calculated for each well by division with the mean of all PBS-treated wells on the same plate. $\text{Normalised Target Quantity} = (\text{Relative Target Quantity} / [\text{mean}] \text{Relative Target Quantity}_{\text{pbs_wells}}) \times 100$
4. The final data are shown as a percentage of untreated (PBS) wells.
5. For concentration-response experiments, a curve was fitted from the RNA knockdown values (step 3-4) for each compound [either 8 or 10 concentrations, depending on the dilution model]. Curves are fitted using a 4 Parameter Sigmoidal Dose-Response Model in Biobook.

The relative FUBP1 mRNA expression levels are shown in Table 8 as % of control, i.e. the lower the value the larger the inhibition. Further, the results are shown in Figure 11.

Table 8: *In vitro* efficacy of anti-FUBP1 compounds in Hela cells. FUBP1 mRNA levels are normalized to GUSB and shown as % of control.

CMP ID NO	FUBP1 Residual mRNA level, % of ctrl	
	3.3 μ M	5 μ M
6_1	34	<i>nd</i>
6_2	26	<i>nd</i>
7_1	29	<i>nd</i>
7_2	29	<i>nd</i>
7_3	34	<i>nd</i>
7_4	26	<i>nd</i>
8_1	<i>nd</i>	15
9_1	33	<i>nd</i>
18_1	<i>nd</i>	27.8
17_1**	54	34
16_1**	59	32
50_1*	<i>nd</i>	62
53_1*	<i>nd</i>	70
54_1*	<i>nd</i>	78
78_1*	<i>nd</i>	68.4***
79_1*	<i>nd</i>	51.4***

*Control compounds, *nd*: not determined

**CMP ID NO: 17_1 is as follows: ATgctTtttatggttCA (SEQ ID NO: 17), CMP ID NO: 16_1 is as follows: TTAtgcttttatggTTT (SEQ ID NO: 16), wherein capital letters are beta-D-oxy LNA nucleosides, lowercase

letters are DNA nucleosides, all LNA C are 5-methyl cytosine, all internucleoside linkages are phosphorothioate internucleoside linkages. CMP ID NO: 17 targets nt 16185 to 16202 of SEQ ID NO: 1. CMP ID NO: 16 targets nt 16187 of 16204 of SEQ ID NO: 1.

***Data are from WO 2019/193165

Experiments with the control compounds were carried out separately

Example 2: Testing in vitro efficacy of antisense oligonucleotides targeting human *FUBP1* mRNA in Primary Human Hepatocytes (PXB-PHH)

Fresh primary human hepatocytes (PXB-PHH) harvested from humanized mice (uPA/SCID mice) - herein called PHH - were obtained from PhoenixBio Co., Ltd (Japan) in 96-well format and cultured in modified hepatocyte clonal growth medium (dHCGM). dHCGM is a DMEM medium containing 100 U/ml Penicillin, 100 µg/ml Streptomycin, 20 mM Hepes, 44 mM NaHCO₃, 15 µg/ml L-proline, 0.25 µg/ml Insulin, 50 nM Dexamethazone, 5 ng/ml EGF, 0.1 mM Asc-2P, 2% DMSO and 10% FBS (Ishida et al., 2015).

Cells were cultured at 37°C, in a humidified atmosphere with 5% CO₂. Culture medium was replaced 2 times per week until harvest.

Non-infected cells received a single treatment at 5 µM and were harvested 7 days later. In all treatments cells were dosed with oligonucleotide compounds in a final volume of 120 µl/well of dHCGM Medium. The experiments for RNA measurement were performed in biological duplicated.

Afterwards a real-time PCR for *FUBP1* RNA was carried out. Total mRNA was extracted from the cells using a MagNA Pure robot and the MagNA Pure 96 Cellular RNA Large Volume Kit (Roche, #05467535001) according to the manufacturer's protocol. The mRNA expression levels were quantified in technical duplicates by qPCR using a QuantStudio 12K Flex (Applied Biosystems), the TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems, #4392938), and human GusB endogenous control (Applied Biosystems, #Hs00939627_m1). The mRNA expression was analyzed using the comparative cycle threshold 2-ΔΔCt method normalized to the reference gene GusB and to non-treated cells. TaqMan primers used for GusB RNA and *FUBP1* RNA quantification are listed in the table below:

Table 9: Primers for *GusB* RNA and *FUBP1* RNA quantification

Parameter	Source
FUBP1	ThermoFisher – Assay ID: Hs00900762_m1

GusB	ThermoFisher – Assay ID: <u>Hs00939627_m1</u>
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The relative FUBP1 mRNA expression levels of 8 compounds (CMP ID Nos: 6_1, 6_2, 7_1, 7_2, 7_3, 7_4; 8_1 and 9_1. CMP ID NO 78_1 and 79_1) in PXB-PHH cells are shown in Table 10 as % of control, i.e. the lower the value the larger the inhibition. The FUBP1 mRNA expression levels of CMP ID NO: 18_1) in PXB-PHH cells is analyzed in Example 3.

Table 10: *In vitro* efficacy of anti-FUBP1 compounds in PXB-PHH cells. FUBP1 mRNA levels are normalized to GUSB and shown as % of control.

SEQ ID NO	CMP ID NO	Rel. mRNA level PXB-PHH at 25µM	SD	Rel. mRNA PXB-PHH at 5µM	SD
6	6_1	37	3	46	4
6	6_2	33	4	45	4
7	7_1	31	1	52	15
7	7_2	21	1	43	2
7	7_3	33	1	49	5
7	7_4	26	0	41	0
8	8_1	33	1	59	5
9	9_1	22	2	60	38

Conclusions drawn from Examples 1 and 2

The data in Examples 1 and 2 show that targeting FUBP1 with an LNA ASO as shown in Table 6 leads to an efficient reduction of FUBP1.

Example 3: Further analysis of CMP IDs NO: 7_1 and 18_1

In the following, additional experiments with two of the nine identified compounds are described: CMP IDs NO: 7_3 and 18_1. In these experiments, the two compounds were compared to two prior compounds which gave the best results in WO 2019/193165.

Materials and Methods**Primary Human Hepatocytes (PXB-PHH)**

Fresh primary human hepatocytes (PXB-PHH) were cultivated as described in Example 2, except that 24-well format was used.

ASOs sequences and compounds

Table 11 provides an overview on the compounds tested in Example 3:

Table 11: Human FUBP1 sequences targeted by the ASOs

Description	Sequence	CMP ID
Compound according to invention	5'- CTTATGCTTTTATGGTT- 3' (SEQ ID NO: 7)	7_3*
Control compound (best prior art compound in HeLa cells)	5'- CCCATAACCATAGTCAT-3' (SEQ ID NO: 22)	35_1**
Compound according to invention	5'- ACCAATTTTCATTTCTAC- 3' (SEQ ID NO: 18)	18_1*

Control compound (best prior art compound in PHH cells)	5'- CCATTTCTTCCTATTACAA- 3' (SEQ ID NO: 12)	50_1**
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*see Table 6, compounds according to the invention

**see Table 7: control compounds as disclosed in WO 2019/193165

HBV infection and oligonucleotide treatment

Upon arrival, PHH were infected with an MOI 110 using chronic patient-derived purified inoculum (genotype C) by incubating the PHH cells with HBV in 4% (v/v) PEG in PHH medium for 16 hours. The cells were then washed three times with PBS and cultured in a humidified atmosphere with 5% CO₂ in fresh PHH medium. Four days post-infection the cells were treated with FUBP1 LNAs (see Table 11) at a final concentration of 10 µM in duplicate or with PBS as no drug control (NDC). On the day of the treatment, the old medium was removed from the cells and replaced by 400 µl/well of fresh PHH medium. Per well, 100 µL of each FUBP1 LNA at 50 µM or PBS as NDC were added to the 400 µL PHH medium. The same treatment was repeated 3 times on days 4, 11 and 18 post-infection. Cell culture medium was changed with fresh one every three days on days 7, 14 and 21 post-infection.

Real-time PCR for intracellular HBV pgRNA and FUBP1 mRNA

Following cell viability determination the cells were washed with PBS once. Total RNA was extracted from the cells using a MagNA Pure robot and the MagNA Pure 96 Cellular RNA Large Volume Kit (Roche, #05467535001) according to the manufacturer's protocol. The FUBP1 mRNA and the viral pgRNA expression levels were quantified in technical duplicates by qPCR using a QuantStudio 12K Flex (Applied Biosystems), the TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems, #4392938), and human GusB endogenous control (Applied Biosystems, #Hs00939627_m1) have been used. The FUBP1 mRNA and the viral pgRNA relative expressions were analyzed using the comparative cycle threshold 2- $\Delta\Delta C_t$ method normalized to the reference gene GusB and non-treated cells. TaqMan primers used for GusB RNA, FUBP1 RNA and HBV pgRNA quantifications are listed in Table 12.

Table 12: TaqMan primers used for GusB gene, FUBP1 RNA and HBV pgRNA quantifications

Parameter	Source
FUBP1	ThermoFisher – Assay ID: Hs00900762_m1
HBV pgRNA	custom: AILJKX5
GusB	ThermoFisher – Assay ID: Hs00939627_m1

Results

The relative FUBP1 mRNA expression levels of the tested compounds are shown in Table 13 and Figure 12. As can be derived from the table and Figure 13, both compounds of the invention (CMP ID NO: 7_3 and 18_1) reduce target mRNA expression by about 80% compared to the NDC. Their effect on the FUBP1 mRNA level is much stronger than the effect of the prior art compounds (CMP ID NO: 50_1 and 35_1).

Table 13: *In vitro* efficacy of anti-FUBP1 compounds in PXB-PHH cells. FUBP1 mRNA levels are normalized to GUSB and shown as % of control.

		Best naked Prio Art in HeLa				Best naked Prio Art in PHH	
CMP ID 7_3		CMP ID 35_1		CMP ID 18_1		CMP ID 50_1	
Residual Expression Rel to NDC (=100)	SD	Residual Expression Rel to NDC (=100)	SD	Residual Expression Rel to NDC (=100)	SD	Residual Expression Rel to NDC (=100)	SD
23	4	46	5	22	0	38	3

Table 14 shows the pgRNA in HBV infected PHH cells treated with different concentrations of antisense compounds. As can be derived from the table, the down-regulation was related to the concentration of antisense compounds. At a concentration of 10 μ M, the lowest pgRNA level was observed for CMP ID NO: 7_3. Moreover, the highest pgRNA level was observed for the prior art compound with CMP ID NO: 35_1. CMP ID NO: 18_1 downregulated HBV pgRNA in a similar manner as prior art compound CMP ID NO: 50_1.

Table 14: *In vitro* efficacy of anti-FUBP1 compounds in HBV infected PHH cells: pgRNA. pgRNA levels are normalized to untreated cells (NDC) and are shown as % of control.

	CMP ID NO: 7_3			CMP ID NO: 35_1			CMP ID NO: 18_1			CMP ID NO: 50_1		
Concentration (μ M)	Residual Expression Rel to NDC (=100)	S	N	Residual Expression Rel to NDC (=100)	S	N	Residual Expression Rel to NDC (=100)	S	N	Residual Expression Rel to NDC (=100)	S	N
10	34	21	2	62	15	2	54	1	2	52	3	2
2	65	11	2	68	21	2	88	6	2	97	13	2
4	88	23	2	95	1	2	84	19	2	81	14	2
0.08	113	8	2	117	15	2	87	17	2	99	12	2

The cells were also tested at a concentration of 2 μ M once per week for three weeks. At 2 μ M, CMP ID NO: 7_3 showed the best FUBP1 mRNA KD with 50% reduction of mRNA expression. Thus, the effect depends on the concentration (since 80% reduction was observed at 2 μ M). Moreover, CMP ID NO: 18_1 showed a similar effect on target mRNA expression level compared to the prior art oligos (at 2 μ M).

EXAMPLE 4: FUBP1 ASO IN VIVO PK/PD

The *in vivo* liver PK/PD correlation of the oligonucleotides with CMP ID Nos: 7_3 and 18_1 conjugated to a GalNAc moiety via a phosphodiester linked DNA dinucleotide was assessed in a single-dose mouse study using C57BL/6 mice (for the structure of the conjugates, see e.g. Figures 5 and 8.1). The mice were dosed 3 mg/kg subcutaneously and terminated at different time points. Fubp1 mRNA knockdown, compound exposure and PKPD was measured as described below

MATERIALS AND METHODS

TISSUE SAMPLE PROCESSING

Material	Vendor	Catalog No.
Eppendorf 2 ml tubes	Eppendorf	0030 123.344
Tungsten carbide beads 5mm	Qiagen	69989
MagNaPure LC RNA Isolation Tissue buffer	Roche Applied Science	03604721001

Liver samples were received frozen in 2 ml round bottom Eppendorf tubes and homogenized in MagNa pure buffer (Roche) on a TissueLyser II (Qiagen) for 2 x 1.5 minutes after addition of a 5mm homogenization bead. After complete sample homogenization, the homogenate was left for 30 minutes at room temperature (RT) to complete the tissue lysis. All steps of the homogenization process were carried out in a flow hood due to the buffer thiocyanate salt and mercaptoethanol contents. After lysis, the homogenates were centrifuged for 3 minutes at 17.000 g.

Homogenates were diluted to approx. 20 mg tissue per 400 µL to avoid overloading the MagNa pure instrument. 350 µL of the homogenate was used for RNA extraction on a MagNA pure 96 instrument for subsequent qPCR analysis. The remaining aliquot of the homogenate was used for hELISA analysis

HYBRIDIZATION ELISA

The following oligos and (all-LNA phospho-diester) ELISA probes were used for the hELISA analysis, all designed, synthesized and qualified at Roche Innovation Center Copenhagen A/S.

Conjugate	Biotinylated capture probe	Digoxigenin-conjugated detection probe
Conjugate of CMP ID NO 18_1	5'-bio-GTAGAAA-3'	5'-GAAAATTGG-dig-3'
Conjugate of CMP ID NO 7_3	5'-bio-AACCATAAAA-3'	5'-AGCATAAG-dig-3'

Materials	Vendor	Catalog No.
Polypropylene 96-well plate with round bottom (dilution plate)	Thermo Scientific	267334
Roche StreptaWell High Bind, 96-well plate clear	Roche Applied Science	11989685001
Substrate (AP) Blue Phos Substrate	KPL	50-88-00
Anti-Digoxigenin-AP, Fab fragments	Roche Applied Science	11093274910

Buffers	Comments
5 x SSCT buffer, pH 7.0	750 mM NaCl, and 75 mM sodium citrate, containing 0.05 % (v/v) Tween-20
2 x SSCT buffer, pH 7.0	300 mM NaCl, and 30 mM sodium citrate, containing 0.05 % (v/v) Tween-20

PBST, pH 7.2	Phosphate buffered saline, containing 0.05 % (v/v) Tween-20
Conjugate of CMP ID NO 18_1 Capture – detection solution	A solution of capture probe 5 nM and detection probe 5 nM in 5xSSCT buffer
Conjugate of CMP ID NO 7_3 Capture – detection solution	A solution of capture probe 35 nM and detection probe 35 nM in 5xSSCT buffer

Before hELISA analysis, the homogenates were brought to RT and vortexed before use. The samples were diluted at least 10-fold in 5 × SSCT buffer.

Appropriate standards matching sample matrix and dilution factor were run on every plate and prepared in parallel with the samples using the relevant oligo (from a quality and identity checked formulation). The standard for each compound was spiked in to a sample pool from un-dosed samples. The spike-in concentrations were made so they were within ~10 fold of the sample oligo content.

Samples and standards were added to a dilution plate in the desired setup, and dilution series were made. 300 µL sample/standard plus capture-detection solution was added to the first wells and 150 µL capture-detection solution in the remaining wells.

A two-fold dilution series of standards and samples was made by transferring 150 µL liquid sequentially. 2–4 wells were kept for blanks (capture-detection solution only). A two-fold sample dilution series of at least 6 wells is recommended for optimal results.

The samples in the dilution plate were incubated for 30 minutes at RT. 100 µL of liquid was transferred from the dilution plate to a streptavidin plate. The plate was incubated for 1 hour at RT with gentle agitation (plate shaker). The wells were aspirated and washed three times with 300 µL of 2 × SSCT buffer.

100 μ L anti-DIG-AP diluted 1:4000 in PBST (made on the same day) were added to each well and incubated for 1 hour at RT under gentle agitation. The wells were aspirated and washed three times with 300 μ L of 2 x SSCT buffer.

100 μ L of substrate (AP) solution (freshly prepared) were added to each well. The intensity of the color was measured spectrophotometrically at 615 nm after a 30-minute incubation with gentle agitation.

Raw data were exported from the readers (Gen5 2.0 software) to excel format and further analysed in excel. Standard curves were generated using GraphPad Prism 8 software and a logistic 4PL regression model.

Data points were reported as the mean value of the technical replicates.

RNA PURIFICATION

All samples were purified using the MagNA Pure 96 Instrument (Roche) using the manufacturer's protocol.

Material	Material	Catalog No.
MagNaPure LC RNA Isolation Tissue buffer	Roche	No. 03 604 721 001
MagNa Pure 96 Cellular RNA Large Volume Kit (elution buffer)	Roche	05467535001
MagNA Pure 96 Processing Cartridge	Roche	06241603001
Sealing foil	Roche	5435307001

350 μ L of the tissue homogenate was transferred to a MagNaPure 96 Processing Cartridge. Remaining lysate was stored for later analysis of oligonucleotide exposure analysis. RNA was purified using the MagNa Pure 96 with the kit Cellular RNA Large Volume Kit, and using the protocol "RNA Tissue FF Standard LV 3.1". RNA was eluted in 50 μ L elution buffer (from kit, 05467535001).

The RNA concentration and A260/280 ratio of ~2.0 of all samples was determined using an Eon Microplate Spectrophotometer (BioTek Instruments). Based on these concentrations, samples were normalized to 25 ng/μL by dilution in DNase/RNase free water and further diluted down to a working concentration of 2.5 ng/μL.

The samples were then used as input for one-step qPCR analysis. The assay details are shown below.

qPCR ANALYSIS

qPCR was run as a one-step qPCR format using the following materials:

Material	Vendor	Catalog No.	Comments
RNA dilution plate	Thermo Scientific	#AB0900	–
qScript™ XLT One-Step RT-qPCR ToughMix®, Low ROX™	Quanta Bioscience	95134-500	Assay buffer
Fubp1 probe/primer sets	IDT	Mm.PT.58.7603777, Dye Quencher Mod. 6-FAM/ZEN/IBFQ (Primer to Probe ratio 3,5) Mm.PT.58.11399179, Dye Quencher Mod. 6-FAM/ZEN/IBFQ (Primer to Probe ratio 3,5)	Mouse Fubp1 assays

Control probe/primer sets	Thermo (_m1) IDT (rest)	Mm 01197698_m1, Dye Quencher Mod. VIC-MGB_PL Mm.PT.58.43894205 Dye Quencher Mod. HEX/ZEN/IBFQ (Primer to Probe ratio 2) Mm.PT.5821577577 Dye Quencher Mod. HEX/ZEN/IBFQ (Primer to Probe ratio 2) Mm.PT.39a.22214839 Dye Quencher Mod. HEX/ZEN/IBFQ (Primer to Probe ratio 2)	Housekeeping control assays (mouse Gusb, Rplp0, Rps29, Tbp respectively)
MicroAmp Optical 384-well plate	Applied Biosystems	4309849	qPCR plate
Quantstudio v.1.3	Applied Biosystems	–	Software for qPCR analysis.
MicroAmp Optical Adhesive Film	Applied Biosystems	4311971	–

Preparation of RNA for qPCR analysis

The reaction was kept cool for all steps of this protocol to avoid unwanted RT enzyme activity. The diluted RNA was then heat shocked for 40 seconds at 90°C to dissociate RNA:ASO duplexes and placed on ice. Prior to analyses the RNA samples were spun to the bottom of the wells.

A standard curve was run on each plate and used for quantification and amplification efficiency measurement. 4 uL of a 10 ng/uL PBS sample was used as input in a 10 uL reaction. A 2-fold dilution series was prepared in RNase free water, to form a 7-point standard curve.

2 separate mouse Fubp1 assays and 4 control assays were run in duplex reactions with two technical replicates for each animal.

For the qPCR the following steps were followed:

For each qPCR well, a stock mastermix was prepared containing 5 µL XLT One-Step mix, 0.5 µL Probe mix1 (20x), 0.5 µL Probe mix2 (20x). From the stock mastermix, 6 µL was added to each well in a 384-well plate (MicroAmp Optical 384-well plate - Applied Biosystems 4309849).

From the RNA dilution plate, 4 µL of diluted RNA (2.5 ng/uL) was added to each well of master mix. Plates were then sealed and vortexed. The plates were then centrifuged at high speed for 3 minutes. The qPCR reactions were kept cold until transferring to the qPCR instrument (Life Technology Viia7; software: QuantStudio v. 1.3) set to run the following program: 15 minutes at 50°C and then 3 minutes at 95°C, with a set temperature change rate to 1.9°C/s. This was followed by 40 cycles of 95°C for 5 seconds and 60°C for 45 seconds with a set temperature change rate to 1.6°C/s.

All samples were analyzed in the same run limiting technical variability to a minimum.

qPCR DATA PROCESSING

qPCR data were reviewed in the Quantstudio software (Applied Biosystems). Based on irregularities in the amplification curve possible outlier wells were identified and removed. Following this review of each plate, an export file was generated with quantities calculated from the ct-values of each sample based on the standard curves for each qPCR assay and analysed using Excel.

In general, the standard curves were of high quality with efficiencies between the recommended 95-105%, indicating high performing assays.

Four different HK genes (Gusb, Rplp0, Rps29, and Tbp) were assayed and a geometric mean of these used for normalization. The stability of the HK genes was assessed before inclusion using the method

published by Vandesompele et al. (Vandesompele et al., 2002). By using four HK genes the pairwise HK gene variation is below the recommended threshold of 0.15 for all tissues.

The “% remaining Fubp1” was calculated as follows: Quantities from each of the Fubp1 qPCR assays were normalized to the geometric mean of the HK assays and further divided by the mean of the untreated group to give a % mRNA remaining. A mean of the two % Fubp1 mRNA remaining results was used as a final readout.

PKPD PLOTTING AND CALCULATIONS

Liver tissue exposure values were calculated as nmol compound per g tissue (nmol/g). They were further log10-transformed and plotted against the % Fubp1 mRNA remaining (figure 13). GraphPad Prism 8 was used to fit a non-linear regression curve (4PL regression model, constrained at top=100). The best-fit estimated PKPD IC50 was calculated by the software (Regression IC50: Conjugate of CMP ID 18_1: 0.092 nmol/g; Conjugate of CMP ID 7_3: 0.068 nmol/g).

Results: Both tested conjugates have a good PK profile. The Conjugate of CMP ID 7_3 is slightly superior to the Conjugate of CMP ID 18_1 in term of early onset on target KD.

CLAIMS

1. An antisense oligonucleotide selected from the group of antisense oligonucleotides consisting of
CTtAtgctttttatGgTT (SEQ ID NO: 7),
AcCAAttttcatttCtAC (SEQ ID NO: 18),
CTTatGctttttatgGT (SEQ ID NO: 6),
CTTaTgctttttatgGT (SEQ ID NO: 6),
CTtATgctttttatgGTT (SEQ ID NO: 7),
CTtAtgctttttatgGTT (SEQ ID NO: 7),
CTtAtgctttttatGGTT (SEQ ID NO: 7),
GcttTttatggtTtCAC (SEQ ID NO: 8), and
TATgcTttttatggtTTC (SEQ ID NO: 9),
wherein capital letters are beta-D-oxy LNA nucleosides, lowercase letters are DNA nucleosides,
all LNA C are 5-methyl cytosine, and all internucleoside linkages are phosphorothioate
internucleoside linkages.
2. A conjugate comprising the antisense oligonucleotide of claim 1, and at least one conjugate
moiety covalently attached to said antisense oligonucleotide.
3. The conjugate of claim 2, wherein the at least one conjugate moiety is capable of binding to the
asialoglycoprotein receptor.
4. The conjugate of claims 2 or 3, wherein the conjugate moiety is selected from one of the trivalent
GalNAc moieties in FIG. 9.
5. The conjugate of claim 4, wherein the conjugate moiety is the trivalent GalNAc moiety in Figure
9D1, or 9D2 or a mixture thereof.
6. The conjugate of any one of claims 1 to 5, comprising a linker positioned between the antisense
oligonucleotide and the conjugate moiety.
7. The conjugate of claim 6, wherein the linker comprises or consists of 2 to 5 consecutive
phosphodiester linked nucleosides.

8. A conjugate selected from the group of conjugates shown in Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8 and Fig. 8.1.
9. A pharmaceutically acceptable salt of the oligonucleotide of claim 1, or the conjugate according to any one of claims 2 to 8.
10. A pharmaceutical composition comprising the antisense oligonucleotide of claim 1, the conjugate of any one of claims 2 to 8, or the pharmaceutically acceptable salt of claim 9, and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.
11. An *in vivo* or *in vitro* method for modulating FUBP1 expression in a target cell which is expressing FUBP1, said method comprising administering the antisense oligonucleotide of claim 1, the conjugate of any one of claims 2 to 8, the pharmaceutically acceptable salt of claim 9, or the pharmaceutical composition of claim 10 in an effective amount to said cell.
12. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of the antisense oligonucleotide of claim 1, the conjugate of any one of claims 2 to 8, the pharmaceutically acceptable salt of claim 9, or the pharmaceutical composition of claim 10 to a subject suffering from or susceptible to the disease, wherein the disease is hepatitis B virus (HBV) infection and/or cancer.
13. The antisense oligonucleotide of claim 1, the conjugate of any one of claims 2 to 8, the pharmaceutically acceptable salt of claim 9, or the pharmaceutical composition of claim 10 for use in medicine.
14. The antisense oligonucleotide of claim 1, the conjugate of any one of claims 2 to 8, the pharmaceutically acceptable salt of claim 9, or the pharmaceutical composition of claim 10 for use in the treatment or prevention of hepatitis B virus (HBV) infection and/or cancer.
15. Use of antisense oligonucleotide of claim 1, the conjugate of any one of claims 2 to 8, the pharmaceutically acceptable salt of claim 9, or the pharmaceutical composition of claim 10, for the preparation of a medicament for treatment or prevention of a hepatitis B virus (HBV) infection and/or cancer.
16. The method of claim 12, the antisense oligonucleotide, conjugate, pharmaceutical composition, or the pharmaceutically acceptable salt for use of claim 14, or the use of claim 15, wherein the disease is hepatitis B virus (HBV) infection, such as chronic HBV infection.

17. The method of claim 12, the antisense oligonucleotide, conjugate, pharmaceutical composition, or the pharmaceutically acceptable salt for use of claim 14, or the use of claim 15, wherein the disease is cancer, such as hepatocellular carcinoma.