

NUCLEIC ACID MOLECULES FOR REDUCTION OF PAPD5 OR PAPD7 mRNA FOR TREATING HEPATITIS B INFECTION

FIELD OF THE INVENTION

The present invention relates to a method for identifying a compound that prevents, ameliorates
5 and/or inhibits a hepatitis B virus (HBV) infection, wherein a compound that reduces the
expression and/or activity of PAP associated domain containing 5 (PAPD5) or PAP associated
domain containing 7 (PAPD7) is identified as a compound that prevents, ameliorates and/or
inhibits a HBV infection. The invention also provides for inhibitors of PAPD5 or PAPD7 for use
in treating and/or preventing a HBV infection. Specifically the present invention identifies nucleic
10 acid molecules, such as antisense oligonucleotides or RNAi agents as inhibitors of PAPD5 or
PAPD7 as well as a combined preparation of these comprising an inhibitor of PAPD5 and an
inhibitor of PAPD7 for simultaneous or sequential use in the treatment or prevention of a HBV
infection. Also comprised in the present invention is a pharmaceutical composition for use in the
treatment and/or prevention of a HBV infection, and a method for monitoring the therapeutic
15 success during the treatment of a HBV infection.

BACKGROUND

The hepatitis B virus (HBV) is an enveloped, partially double-stranded DNA virus. The compact
3.2 kb HBV genome consists of four overlapping open reading frames (ORF), which encode for
the core, polymerase (Pol), envelope and X-proteins. The Pol ORF is the longest and the
20 envelope ORF is located within it, while the X and core ORFs overlap with the Pol ORF. The
lifecycle of HBV has two main events: 1) generation of closed circular DNA (cccDNA) from
relaxed circular (RC DNA), and 2) reverse transcription of pregenomic RNA (pgRNA) to produce
RC DNA. Prior to the infection of host cells, the HBV genome exists within the virion as RC
DNA. It has been determined that HBV virions are able to gain entry into host cells by non-
25 specifically binding to the negatively charged proteoglycans present on the surface of human
hepatocytes (Schulze, *Hepatology*, 46, (2007), 1759-68) and via the specific binding of HBV
surface antigens (HBsAg) to the hepatocyte sodium-taurocholate cotransporting polypeptide
(NTCP) receptor (Yan, *J Virol*, 87, (2013), 7977-91). The control of viral infection needs a tight
surveillance of the host innate immune system which could respond within minutes to hours
30 after infection to impact on the initial growth of the virus and limit the development of a chronic
and persistent infection. Despite the available current treatments based on IFN and
nucleos(t)ide analogues, the HBV infection remains a major health problem worldwide which
concerns an estimated 350 million chronic carriers who have a higher risk of liver cirrhosis and
hepatocellular carcinoma.

35 The secretion of antiviral cytokines in response to a HBV infection by the hepatocytes and/or
the intra-hepatic immune cells plays a central role in the viral clearance of the infected liver.

However, chronically infected patients only display a weak immune response due to various escape strategies adopted by the virus to counteract the host cell recognition systems and the subsequent antiviral responses.

Many observations showed that several HBV viral proteins could counteract the initial host cellular response by interfering with the viral recognition signaling system and subsequently the interferon (IFN) antiviral activity. Among these, the excessive secretion of HBV empty subviral particles (SVPs, HBsAg) are thought to participate to the maintenance of the immunological tolerant state observed in chronically infected patients (CHB). The persistent exposure to HBsAg and other viral antigens can lead to HBV-specific T-cell deletion or to progressive functional impairment (Kondo, *Journal of Immunology* (1993), 150, 4659–4671; Kondo, *Journal of Medical Virology* (2004), 74, 425–433; Fiscaro, *Gastroenterology*, (2010), 138, 682-93;). Moreover HBsAg has been reported to suppress the function of immune cells such as monocytes, dendritic cells (DCs) and natural killer (NK) cells by direct interaction (Op den Brouw, *Immunology*, (2009b), 126, 280-9; Woltman, *PLoS One*, (2011), 6, e15324; Shi, *J Viral Hepat.* (2012), 19, e26-33; Kondo, *ISRN Gastroenterology*, (2013), Article ID 935295).

HBsAg quantification is a significant biomarker for prognosis and treatment response in chronic hepatitis B. However the achievement of HBsAg loss and seroconversion is rarely observed in chronically infected patients but remains one of the ultimate goals of therapy. Current therapy such as Nucleos(t)ide analogues are molecules that inhibit HBV DNA synthesis but are not directed at reducing HBsAg level. Nucleos(t)ide analogs, even with prolonged therapy, only show weak HBsAg clearance comparable to those observed naturally (between -1%-2%) (Janssen, *Lancet*, (2005), 365, 123-9; Marcellin, *N. Engl. J. Med.*, (2004), 351, 1206-17; Buster, *Hepatology*, (2007), 46, 388-94).

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.

The function of the hepatitis B virus precore or HBeAg is not completely known. However HBeAg is well known to play a key role in viral persistence. HBeAg is thought to promote HBV chronicity by functioning as an immunoregulatory protein. In particular, the HBeAg is a secreted accessory protein, which appears to attenuate the host immune response to the intracellular nucleocapsid protein (Walsh, *Virology*, 2011, 411(1):132-141). The HBeAg acts as an immune tolerogen contributing to HBV persistence, and possibly functions in utero considering that soluble HBeAg traverses the placenta (Walsh, *Virology*, 2011, 411(1):132-141). Furthermore, HBeAg downregulates: i) cellular genes controlling intracellular signaling; and ii) the Toll-like receptor 2 (TLR-2) to dampen the innate immune response to viral infection (Walsh, *Virology*, 2011, 411(1):132-141). In the absence of HBeAg, HBV replication is associated with upregulation of the TLR2 pathway (Walsh, *Virology*, 2011, 411(1):132-141). Accordingly,

HBeAg has a significant role in modulating virus/host interactions to influence the host immune response (Walsh, *Virology*, 2011, 411(1):132-141). Thus, reducing HBeAg in HBeAg positive patient population may lead to reversal of HBV specific immunedysfunction (Milich, 1997, *J. Viral. Hep.* 4: 48–59; Milich, 1998, *J. Immunol.* 160: 2013–2021). In addition, the secreted HBeAg is significantly more efficient than the intracellular hepatitis core antigen (HBcAg) at eliciting T-cell tolerance, and the split T-cell tolerance between the HBeAg and the HBcAg and the clonal heterogeneity of HBc/HBeAg-specific T-cell tolerance may have significant implications for natural HBV infection and especially for precore-negative chronic hepatitis (Chen, 2005, *Journal of Virology*, 79: 3016-3027).

- 10 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. In addition, the highest rates of transmission of an acute infection to chronic (>80%) have been reported in cases of materno-fetal and neonatal HBV transmission from HBeAg-positive mothers (Liaw, *Lancet*, 2009, 373: 582-592; Liaw, *Dig. Dis. Sci.*, 2010, 55: 2727-2734; and Hadziyannis, 2011, *Journal of hepatology*, 55: 183-191). Therefore, reducing HBeAg in an expected mother may not only reduce the patient's degree of infectiousness, but may also inhibit the development of a chronic HBV infection of her child.

- Therefore, in the therapy of HBV there is an unmet medical need to inhibit viral expression, particularly to inhibit secretion of HBsAg and HBeAg (Wieland, S. F. & F. V. Chisari. *J Virol*, 20 (2005), 79, 9369-80; Kumar et al. *J Virol*, (2011), 85, 987-95; Woltman et al. *PLoS One*, (2011), 6, e15324; Op den Brouw et al. *Immunology*, (2009b), 126, 280-9).

- WO 03/022987 discloses for example in Table 7A 1298 genes that are upregulated in hepatitis C-positive tissue. One of the mentioned genes is topoisomerase-related function protein 4 (TRF4, AF089897). AF089897 is also called TRF4-2, which is quite similar to position 880 to 2340 of SEQ ID NO: 4 herein. The observation that a fragment of PAPD5 is upregulated slightly in hepatitis C positive cells does not provide any indication that inhibiting PAPD5 represents an effective therapy. WO 03/022987A2 does not disclose any hint that fragments of PAPD5 plays any critical role during hepatitis C infection at all. In addition, HCV and HBV are two completely different viruses leading to two completely different diseases with different etiologies, different progression and different medication. This is in line with the observation of the present inventors that the PAPD5 and PAPD7 inhibitors DHQ and THP are inactive against hepatitis C virus (HCV) or other viruses beside HBV (data not shown).

- In WO 2010/040571 PAPD5 has been suggested in a long list of other genes as having a potential role in cell proliferation in metabolic and tumorous disease without the provision of any actual evidence.

In WO 2013/166264 PAPD5 has been suggested in a long list of other genes as having a potential role in increasing viral replication without the provision of any actual evidence.

In WO 2017/066712 down regulation of PAPD5 in relation to the treatment and diagnosis of telomere diseases has been described. Five shRNA structures for this purpose have been described.

5 To our knowledge the expression of PAPD5 or PAPD7 has never been associated with HBV infection, nor has modified single stranded antisense oligonucleotides been made against these targets.

OBJECTIVE OF THE INVENTION

Thus, the technical problem underlying the present invention is the identification and provision of ameliorated means and methods for treating and/or preventing a HBV infection.

10 The technical problem is solved by the provision of the embodiments described herein and characterized in the claims.

BRIEF DESCRIPTION OF THE FIGURES

The Figures show:

15 **Figure 1:** Pictures from 1-by-1 experiment with HBX129653/HBX129654 chemical probes and the 3 prey fragments.

Figure 2: Pictures from 1-by-1 experiment with HBX129653/HBX129654 chemical probes and PAPD5/7 full length proteins.

Figure 3: Pictures from competition assay using HBX129653 (DHQ) and MOL653/654 for competition

20 **Figure 4:** Pictures from competition assay using HBX129654 (THP) and MOL653/654 for competition

Figure 5: Pictures from competition assay using HBX129653 (DHQ) and INACT653/INACT654 for competition. MOL653 was included as positive control.

25 **Figure 6:** Pictures from competition assay using HBX129654 (THP) and INACT653/INACT654 for competition. MOL653 was included as positive control.

30 **Figure 7: (A)** SiRNA knock-down (KD) of PAPD5 and PAPD7 in HBV-infected dHepaRG leads to reduction in HBV expression. Differentiated HepaRG cells were infected with HBV and treated with siRNA against either PAPD5, PAPD7 or both (25nM each) one day prior to HBV infection and on day 4 post infection. Supernatant were harvested on day 11, levels of HBsAg and HBeAg secreted in the supernatant were measured by ELISA and normalized to non-treated control. Cell toxicity and inhibition of gene expression was measured subsequently and also normalized to the non-treated control. **(B)** The same experiment as described in (A) was performed, with the exception that only the level of HBsAg secreted in the supernatant was measured.

Figure 8: Representation of the ability of the oligonucleotides, tested in example 4, to reduce the expression of PAPD5 in HeLa cell cultures. Each oligonucleotide is represented by a dot indicating its location on the PAPD5 mRNA by chromosomal position. The oligonucleotide concentrations were 5 and 25 microM as indicated in the right hand side each plot.

- 5 **Figure 9:** Representation of the ability of the oligonucleotides, tested in example 5, to reduce the expression of PAPD7 in HeLa cell cultures. Each oligonucleotide is represented by a dot indicating its location on the PAPD7 mRNA by chromosomal position. The oligonucleotide concentrations were 5 and 25 microM as indicated in the right hand side each plot.

- 10 **Figure 10:** Representation of the inhibition of HBsAg in HBV infected HepaRG cells with combinations oligonucleotides targeting PAPD5 and PAPD7 (20 µM each) compared to the inhibition obtained using a single oligonucleotide (20 µM) present in the combination. The assay was conducted with gymnosis.

- 15 **Figure 11:** Representation of the inhibition of HBsAg in HBV infected HepaRG cells with combinations oligonucleotides targeting PAPD5 and PAPD7 (500 nM each) compared to the inhibition obtained using a single oligonucleotide (500 nM) from the combination. The assay was conducted with transfection.

SUMMARY OF THE INVENTION

- One aspect of the present invention relates to a composition comprising a nucleic acid molecule for use in the treatment and/or prevention of Hepatitis B virus infection, wherein said nucleic acid molecule inhibits expression and/or activity of PAPD5. In particular a composition
20 comprising a combined preparation of a nucleic acid molecule inhibits expression and/or activity of PAPD5 and another nucleic acid molecule inhibits expression and/or activity of PAPD7 for use in the treatment and/or prevention of Hepatitis B virus infection.

- A further aspect of the present invention relates to nucleic acid molecules that inhibit expression and/or activity of PAPD7. In particular single stranded antisense, siRNA and shRNA molecules.
25

A further aspect of the present invention relate to single stranded antisense oligonucleotides that inhibit expression and/or activity of PAPD5. In particular modified antisense oligonucleotides comprising 2'sugar modified oligonucleotide and phosphorothioate internucleoside linkages.

- 30 Further aspects of the invention are conjugates of nucleic acid molecules of the invention, combined preparations of nucleic acid molecules capable of inhibiting expression and/or activity of both PAPD5 and PAPD7 and pharmaceutical compositions comprising the molecules of the invention.

- A further aspect of the invention is a method for identifying a compound or composition that
35 prevents, ameliorates and/or inhibits a hepatitis B virus (HBV) infection, comprising:

- a. contacting a test compound or composition with a cell expressing PAPD5 and/or PAPD7;
- b. measuring the expression and/or activity of PAPD5 and/or PAPD7 in the presence and absence of said test compound or composition; and
- c. identifying a compound or composition that reduces the expression and/or activity of PAPD5 and/or PAPD7 as a compound that prevents, ameliorates and/or inhibits a HBV infection.

DETAILED DESCRIPTION OF THE INVENTION

PAPD5 and PAPD7 are non-canonical poly(A)-polymerases that belong to the superfamily of polymerase β -like nucleotidyl transferases. In context of the present invention it has surprisingly been shown that a compound that is useful for the therapeutic intervention of a HBV infection can successfully be identified by analysing whether a test compound inhibits PAPD5 or PAPD7. Or, in other words, inhibition of PAPD5 or PAPD7, or the inhibition of both, was identified in the appended examples as being an indicator for the efficacy of a compound to inhibit a HBV infection. The appended examples demonstrate that a dihydroquinolizinone compound having the formula (III) shown in the Materials and Methods section, herein called DHQ, and a tetrahydropyridopyrimidine compound having the formula (IV) as shown in the Materials and Methods section, herein called THP, bind to PAPD5 and PAPD7 polypeptides (SEQ ID NO: 1 and 2 respectively). These compounds have the capacity to inhibit production of HBV surface antigen (HBsAg) and the expression of HBV RNA during HBV infection (WO 2015/113990 A1 and WO2016/177655). In addition, the appended examples show that inhibition of PAPD5 or PAPD7 or both by using pools of siRNA leads to an inhibition of viral expression, particularly of the secretion of HBsAg and HBeAg as well as of the production of intracellular HBV mRNA. These results directly indicate that by reducing the amount and/or activity (e.g. the amount) of PAPD5 and/or PAPD7 an HBV infection (e.g. a chronic HBV infection) can be prevented or treated (i.e. ameliorated and/or inhibited).

Screening methods of the invention

Thus, the present invention relates to a screening method, wherein a compound that reduces the expression and/or activity (e.g. the expression) of PAPD5 or PAPD7 (or combinations of compounds that reduce PAPD5 and PAPD7) is identified as a compound that prevents and/or treats (i.e. ameliorates and/or inhibits) a HBV infection. In a preferred embodiment of the present invention the compound is a RNAi molecule, in particular a nucleic acid molecule, such as a siRNA, shRNA or antisense oligonucleotide. Using the screening method of the invention LNA modified antisense oligonucleotides targeting either PAPD5 or PAPD7 mRNA have been screened for their ability to reduce the expression of PAPD5 or PAPD7, or both using combinations of compounds. Some of these have further been tested to confirm their ability to ameliorate and/or inhibits a HBV infection, either alone or in combination.

One aspect of the invention is a method for identifying a compound or composition that prevents, ameliorates and/or inhibits a hepatitis B virus (HBV) infection, comprising:

- a) contacting a test compound with a cell expressing PAP associated domain containing 5 (PAPD5) and/or PAP associated domain containing 7 (PAPD7);
- 5 b) measuring the expression and/or activity of PAPD5 and/or PAPD7 in the presence and absence of said test compound or composition; and
- c) identifying a compound that reduces the expression and/or activity of PAPD5 or PAPD7 as a compound or composition that prevents, ameliorates and/or inhibits a HBV infection; optionally
- 10 d) testing combinations of compounds to reduce the expression and/or activity of PAPD5 and PAPD7.

It has been found in context of the present invention that a compound (or composition) that reduces PAPD5 or PAPD7 or combinations of compounds that reduce PAPD5 and PAPD7 in combination leads to inhibition of HBV gene expression and replication; and thus, prevents, ameliorates and/or inhibits a HBV infection. Such a compound may lead to a reduction of the PAPD5 or PAPD7 expression and/or activity of 10-100%, preferably of 20-100%, more preferably of 30-100%, even more preferably of 40-100%, even more preferable of 50-100%, even more preferably of 60-100%, even more preferably of 70-100%, even more preferably of 80-100%, and most preferably of 90-100%.

20 In the herein provided screening method it is envisaged that the expression of PAPD5 and/or PAPD7 is measured (i.e. analyzed/determined) by using in step (a) a cell expressing PAPD5 and/or PAPD7, such as a HeLa or a HepaRG cell line. The expression and/or activity of PAPD5 and/or PAPD7 may be measured (i.e. analyzed/determined) by either (i) determining PAPD5 and/or PAPD7 polypeptide; or (ii) determining transcript levels in a cell expressing PAPD5 and/or PAPD7.

In one aspect of the invention, a compound that reduces the expression of PAPD5 or PAPD7 (e.g. of PAPD5, or preferably combinations of compounds that reduce both PAPD5 and PAPD7) is identified as a compound(s) that prevents, ameliorates and/or inhibits (i.e. treats) HBV infection. In another aspect of the invention a compound that reduces the activity of PAPD5 or PAPD7 polypeptide (e.g. of PAPD5, or preferably combinations of compounds that reduce both PAPD5 and PAPD7) is identified as a compound(s) that prevents, ameliorates and/or inhibits (i.e. treats) a HBV infection. It is prioritized that a compound that reduces the expression and/or activity of PAPD5 or combinations of compounds that reduce both molecules, PAPD5 and PAPD7, is identified as compounds that prevents, ameliorates and/or inhibits a HBV infection.

35 Most preferably, a combination of compounds that reduces the expression and/or activity of both molecules, PAPD5 and PAPD7, is identified as a composition that prevents, ameliorates and/or inhibits a HBV infection.

The above described screening method lead to the identification of a compound or combination of compounds, that prevents, ameliorates and/or inhibits a HBV infection. It is prioritized that said compounds ameliorates and/or inhibits (i.e. treats) a HBV infection. Thus, the herein provided screening method is useful in the identification of a compound that treats a HBV infection.

In the context of the present invention, PAPD5 may be the PAPD5 polypeptide or the PAPD5 mRNA. It is prioritized in context of the screening methods provided herein that PAPD5 is the PAPD5 mRNA.

One aspect of the present invention relates to the herein provided screening method, wherein the cells expressing PAPD5 contain a PAPD5 target nucleic acid comprising or consisting of

- (i) a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1 or 2;
- (ii) a nucleotide sequence of SEQ ID NO: 4, 5 or 10 or natural variants thereof;
- (iii) a nucleotide sequence encoding an amino acid sequence having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to SEQ ID NO: 1 or 2, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function;
- (iv) a nucleotide sequence having at least 80% identity, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to the nucleotide sequence of (ii), wherein the polypeptide expressed from the nucleotide sequence has poly-A polymerase function;
- (v) a nucleotide sequence encoding an enzymatically active fragment of SEQ ID NO: 1 or 2, such as a nucleotide sequence encoding SEQ ID NO: 7 or 8;
- (vi) a nucleotide sequence encoding an amino acid sequence having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to an amino acid sequence of an enzymatically active fragment of SEQ ID NO: 1 or 2, such as SEQ ID NO: 7 or 8,, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function; or
- (vii) a nucleotide sequence comprising or consisting of SEQ ID NO: 4, 5 or 10.

In preferred embodiments, the PAPD5 target nucleic acid is a mRNA, such as a pre-mRNA or mature mRNA. In further embodiments the PAPD5 target nucleic acid is a polynucleotide comprising or consisting of the nucleotide sequence of SEQ ID NO: 4, 5 or 10 or natural variants thereof. However, the PAPD5 mRNA may also be a polynucleotide comprising or consisting of a nucleotide sequence having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to SEQ ID NO: 4, 5 or 10, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function.

In context of the present invention PAPD7 may be the PAPD7 polypeptide or the PAPD7 mRNA. It is prioritized in context of the screening methods provided herein that PAPD7 is the PAPD7 mRNA.

- One aspect of the present invention relates to the herein provided screening methods, wherein
- 5 the cells expressing PAPD7 contain a PAPD7 target nucleic acid comprising or consisting of
- (i) a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 3;
 - (ii) a nucleotide sequence of SEQ ID NO: 6 or 11 or natural variants thereof;
 - (iii) a nucleotide sequence encoding an amino acid sequence having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more
 - 10 preferably at least 99% identity to SEQ ID NO: 3, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function;
 - (iv) a nucleotide sequence having at least 80% identity, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to an nucleic acid sequence of (ii), wherein the polypeptide expressed from the nucleic acid
 - 15 sequence has poly-A polymerase function;
 - (v) a nucleotide sequence encoding an enzymatically active fragment of SEQ ID NO: 3, such as a nucleotide sequence encoding SEQ ID NO: 9; or
 - (vi) a nucleotide sequence encoding an amino acid sequence having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more
 - 20 preferably at least 99% identity to an amino acid sequence of an enzymatically active fragment of SEQ ID NO: 3, such as SEQ ID NO: 9, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function; or
 - (vii) a nucleotide sequence comprising or consisting of SEQ ID NO: 6 or 11.

- In preferred embodiments, the PAPD7 target nucleic acid is a mRNA, such as a pre-mRNA or
- 25 mature mRNA. In further embodiments the PAPD7 target nucleic acid is a polynucleotide comprising or consisting of the nucleotide sequence of SEQ ID NO: 6 or 11, or natural variants thereof. However, the PAPD7 mRNA may also be a polynucleotide comprising or consisting of a nucleotide sequence having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to SEQ
- 30 ID NO: 6 or 11, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function.

- In context of the present invention the cell used for screening may be a eukaryotic cell. For example, said cell may be a yeast cell or a vertebrate cell. Vertebrate cells include fish, avian, reptilian, amphibian, marsupial, and mammalian cells. Preferably, the cell is a mammalian cell,
- 35 most preferably, a human cell. Mammalian cells also include feline, canine, bovine, equine, caprine, ovine, porcine murine, such as mice and rat, and rabbit cells. In the herein provided screening methods, the "cell" may endogenously express PAPD5 and/or PAPD7 or overexpress

PAPD5 and/or PAPD7. For overexpressing PAPD5 and/or PAPD7 the cell may comprise the nucleotide sequence encoding the PAPD5 polypeptide and/or the PAPD7 polypeptide within an expression vector. In preferred embodiments the cell comprises a nucleotide sequence encoding the PAPD5 polypeptide and a nucleotide sequence encoding the PAPD7 polypeptide.

- 5 The cell of the herein provided screening methods may be comprised in a non-human animal, e.g. a mouse, rat, rabbit or ferret. The cells provided for the screening method described herein may also be termed target cells.

- In the herein provided screening method wherein the activity of PAPD5 polypeptide and/or PAPD7 polypeptide is measured, said activity of PAPD5 and PAPD7 is preferably the poly-A polymerase function (i.e. the poly-A polymerase activity). The poly-A polymerase function/activity of a polypeptide (e.g. of PAPD5 or PAPD7) may be measured, e.g. by monitoring the *in vitro* polyadenylation of mRNA, e.g. as described in Rammelt, RNA, 2011, 17:1737–1746. This method can also be used to measure the poly-A polymerase function of PAPD5 and/or PAPD7 in the presence and absence of a test compound. In brief, a ribo-
- 10 oligonucleotide A₁₅ may be incubated with recombinant PAPD5 protein expressed in *Escherichia coli* in the presence of ATP(A), CTP (C), GTP(G), UTP(U), or a mixture of all four dNTPs, respectively.

The expression of PAPD5 and/or PAPD7 in a cell in the presence and absence of the test compound may be measured, e.g. by using (q)PCR, western blot, or MassSpec.

- 20 A compound that inhibits the propagation of HBV may be a compound that reduces the expression of viral RNA, that reduces the production of viral DNA (HBV DNA) derived from viral RNA (HBV RNA), that reduces the production of new viral particles (HBV particles), and/or that produces production and/or secretion of HBsAg and/or HBeAg. Thus, one aspect of the present invention relates to the herein provided screening methods, wherein the compound that inhibits
- 25 propagation of HBV inhibits secretion of HBsAg, inhibits secretion of HBeAg, and/or inhibits production of intracellular HBV mRNA or HBV DNA. Preferably, a compound that inhibits the propagation of HBV is a compound that inhibits secretion of HBsAg, secretion of HBeAg and production of intracellular HBV mRNA or HBV DNA.

- For example, a compound that inhibits propagation of HBV may reduce the expression of viral RNA (HBV RNA), the production of viral DNA (HBV DNA) deriving from viral RNA, the production of new viral particles (HBV particles), the production and/or secretion of HBsAg and/or HBeAg by 10-100%, preferably by 20-100%, more preferably by 30-100%, even more preferably by 40-100%, even more preferable by 50-100%, even more preferably by 60-100%, even more preferably by 70-100%, even more preferably by 80-100%, and most preferably by
- 30 90-100%, when compared the untreated cells or animals or cell or animal treated with an appropriate control.

- Inhibition of propagation of HBV may be measured, e.g., by measuring whether the test compound has the activity to inhibit secretion of HBsAg and/or of HBeAg, and/or to inhibit production of intracellular HBV mRNA or HBV DNA. 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. Inhibition of production of intracellular HBV mRNA may be measured by real-time PCR, e.g. as described in the appended examples. Further methods for evaluating whether a test compound inhibits propagation of HBV are measuring secretion of HBV DNA by RT-qPCR e.g. as described in WO 2015/173208 or as described in the appended examples; Northern Blot; in-situ hybridization, or immuno-fluorescence.
- 10 The herein provided screening methods may additionally comprise the step of comparing the test compound to a control. Said control may be an inactive test compound, wherein said inactive test compound is a compound that does not reduce the expression and/or activity of PAPD5 or PAPD7.

- This inactive test compound has no activity against HBV, e.g. it does not lead to inhibition of secretion of HBsAg and HBeAg and to inhibition of production of intracellular HBV mRNA. For example, the inactive test compound may have an IC_{50} value in the inhibition of HBsAg of more than 6 μ M. In the herein provided screening method, the inactive test compound may be a non-targeting antisense oligonucleotide, siRNA or shRNA. In the screening method wherein expression and/or activity of PAPD5 and/or PAPD7 is measured, the test compound as defined above in (i) may be used. An inactive compound can be designed from an active one, e.g., by chemical modification and/or functional interruption.
- 20

- For performing the herein provided screening methods publicly or commercially available molecule libraries may be used. Thus, in context of the invention the said test compound may be a screening library of nucleic acid molecules selected from
- 25 (i) single stranded antisense oligonucleotides, preferably comprising at least on 2' modified nucleoside; or
- (ii) siRNA molecules; or
- (iii) shRNA molecules.

- The appended examples demonstrate that by inhibiting PAPD5 and/or PAPD7 polypeptide or mRNA, the secretion of HBsAg and HBeAg as well as production of intracellular HBV mRNA can effectively be inhibited. These data demonstrate that an inhibitor of PAPD5 and/or PAPD7 can be used to prevent and/or treat a HBV infection.
- 30

- Several small molecule compounds that have a certain efficacy in the treatment of a HBV infection have been described in the art (see, e.g. WO 2015/113990 A1 and WO 2016/177655).
- 35 The appended examples demonstrate for the first time a clear correlation between activity of the small molecule compound against a HBV infection and binding affinity towards PAPD5 and PAPD7. This realization opened for design of nucleic acid molecules targeting PAPD5 or

PAPD7 mRNA leading to particularly high anti-HBV efficacy. The nucleic acid molecules can be targeted directly to the liver using conjugates capable of binding to the asialoglycoprotein receptor (ASGPr). Compared to systemically administered small molecules the nucleic acid molecules will have a different PK/PD profile and toxicity profile. Furthermore, the present invention shows for the first time that a compound(s) that inhibits PAPD5 or PAPD7, or particularly PAPD5 and PAPD7 has an extraordinary high activity in terms of inhibition of secretion of HBsAg and HBeAg as well as of production of intracellular HBV mRNA. Reduction of secretion of HBsAg and HBeAg inhibits development of chronic HBV infection more effectively as compared to the reduction of secretion of HBsAg alone. In addition, inhibition of secretion of HBsAg and HBeAg reduces the infectiousness of a HBV infected person. Furthermore, reducing HBeAg in an expected mother may also inhibit the development of a chronic HBV infection of her child. Thus, the present invention unexpectedly demonstrates that selectively using compounds that inhibit PAPD5 or PAPD7 target nucleic acids or combinations of compounds that inhibit both PAPD5 and PAPD7 target nucleic acids, leads to an improved therapeutic success in the treatment of a HBV infection in terms of a considerably more effective reduction of HBsAg and HBeAg.

Accordingly, an aspect of the present invention is using one or more inhibitors capable of reducing PAPD5 or PAPD7 target nucleic acids or combinations of compounds that inhibit expression of both PAPD5 and PAPD7 target nucleic acids, in the treatment of HBV infection, in particular a chronic HBV infection. In a further embodiment the invention relates to the use of at least two inhibitors capable of reducing PAPD5 and/or PAPD7 target nucleic acids, in reduction of the viral antigens HBsAg and HBeAg.

Thus, the present invention relates to an inhibitor or a combination of inhibitors of PAPD5 and/or PAPD7 for use in treating and/or preventing a HBV infection, wherein said inhibitor(s) are independently selected from the group consisting of:

- (a) one or more RNA interference (RNAi) molecules against PAPD5 or PAPD7;
- (b) a genome editing machinery, comprising:
 - (i) a site-specific DNA nuclease or a polynucleotide encoding a site-specific DNA nuclease; and
 - (ii) a guide RNA or a polynucleotide encoding a guide RNA.

The RNAi molecules may independently be selected from the group consisting of:

- a) a single stranded antisense oligonucleotide;
 - b) a siRNA molecule; and
 - c) a shRNA molecule;
- The inhibitor of the present invention may also be a PAPD5 or PAPD7 specific locked nucleic acid (LNA) molecule.

It is envisaged that the inhibitor of the invention is used for treating (e.g. ameliorating) a HBV infection.

The inhibitor may be a molecule that specifically inhibits PAPD7. Preferably, the inhibitor is a molecule that specifically inhibits PAPD5. More preferably, the inhibitors are combined such that they inhibit both, PAPD5 and PAPD7. Thus, it is prioritized that the inhibitors of the present invention either inhibits PAPD5 or are combined such that they inhibit both PAPD5 and PAPD7. Most preferably, the inhibitors of the present invention are combined such that they inhibit PAPD5 and PAPD7. In one aspect of the invention the inhibitors of the present invention are combined such that they inhibit both PAPD5 and PAPD7 and lead to a reduction of secretion of HBsAg and/or HBeAg of at least 50% as compared to the no drug control (i.e. compared to cells or subjects to which no drug has been administrated).

The inhibitor of the present invention may have an IC_{50} value in the inhibition of HBsAg and HBeAg of below 6 μ M, preferably of below 5 μ M, preferably of below 4 μ M, preferably of below 3 μ M, preferably of below 2 μ M, more preferably below 1 μ M, more preferably below 0.5 μ M, and most preferably below 0.1 μ M.

Genome editing by using a site-specific DNA nuclease (such as Cas9 or Cpf1) and a guide RNA is commonly known in the art and described, e.g., in "CRISPR-Cas: A Laboratory Manual", 2016, edited by Jennifer Doudna, ISBN 978-1-621821-31-1.

For example, if said site-specific DNA nuclease is a Cas9 nuclease, then the genome editing machinery preferably further comprises:

- (i) at least one guide RNA consisting of at least one target sequence specific CRISPR RNA (crRNA) molecule and at least one trans-activating crRNA (tracrRNA) molecule;
- (ii) a polynucleotide encoding the RNA molecules of (i);
- (iii) at least one guide RNA, which is a chimeric RNA molecule comprising at least one target sequence specific crRNA and at least one tracrRNA; or
- (iv) a polynucleotide encoding the chimeric RNA of (iii).

In an alternative example the site-specific DNA nuclease is a Cpf1 nuclease, and the genome editing machinery preferably further comprises:

- (i) at least one guide RNA comprising a target sequence specific CRISPR RNA (crRNA) molecule; or
- (ii) a polynucleotide encoding the RNA molecules of (i).

The herein provided inhibitors of PAPD5 or PAPD7 may also be a genome editing machinery that comprises at least one pre-assembled Cas9 protein-guide RNA ribonucleoprotein complex (RNP).

Herein, the guide RNA is designed to target the genomic PAPD5 or PAPD7 DNA. Alternatively, several guide RNAs are used, so that the genomic DNA of PAPD5 and of PAPD7 can be

targeted. Inhibition of PAPD5 and/or PAPD7 may be achieved by introducing frame-shift knockout mutations into the genomic PAPD5 and/or PAPD7 DNA through non-homologous end-joining (NHEJ), or by modifying the genomic PAPD5 and/or PAPD7 DNA through homology-directed repair (HDR). How these mechanisms can be induced is commonly known in the art and described, e.g., in Heidenreich, 2016, Nat Rev Neurosci 17 36-44.

The inhibitor of the present invention of the present invention is preferably a non-naturally occurring molecule. The inhibitor of the invention may be a nucleic acid molecule, selected from RNAi agents, including siRNA, shRNA, Crisper RNA and single stranded antisense oligonucleotides. Preferably the RNAi molecules comprise at least one non-naturally occurring nucleotide, such as a oligonucleotide thiophosphate, a substituted ribo-oligonucleotide, a 2' sugar modified nucleoside, a LNA nucleoside, a PNA nucleoside, a GNA (glycol nucleic acid) molecule, a TNA (threose nucleic acid) molecule, a morpholino nucleotide, or a nucleic acid with a modified backbone such as polysiloxane, 2'-O-(2-methoxy) ethyl-phosphorothioate, or a nucleic acid with a substituent, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleoside, or a reporter molecule to facilitate its detection. The inhibitor of the invention may also be naturally occurring or a non-naturally occurring small molecule or genome editing machinery.

In context of the present invention, the herein provided inhibitor inhibits expression and/or activity of PAPD5 or PAPD7.

For example, the inhibitor of the present invention may bind to PAPD5 target nucleic acid and inhibit activity of PAPD5 polypeptide. In another example, the inhibitor of the present invention binds to PAPD7 target nucleic acid and inhibits activity of PAPD7 polypeptide. It is prioritized herein that the inhibitors are combined to target both, PAPD5 and PAPD7 mRNA and inhibits the activity of both, PAPD5 and PAPD7 polypeptide. The inhibitor of the present invention may inhibit the expression of PAPD5 or PAPD7; or a combination of inhibitors may inhibit the expression of both, PAPD5 and PAPD7.

Compounds of the invention

As described above, the inhibitor of the present invention may be a nucleic acid molecule.

In one aspect of the invention, the inhibitor of the present is a RNAi molecule against PAPD5 or PAPD7. Said RNAi molecule may be a siRNA or a shRNA.

For example, the inhibitor of the present invention may be a siRNA that is directed against PAPD5, wherein said siRNA is any one of or a combination of the following siRNAs:

PAPD5 siRNA pool (L-010011-00-0010; ON-TARGETplus Human PAPD5):

siRNA – 1 – J-010011-05 – Target Sequence: CAUCA AUGCUUUAUAUCGA (SEQ ID NO: 252)
siRNA – 2 – J-010011-06 – Target Sequence: GGACGACACUCAAUUAUU (SEQ ID NO: 253)

siRNA – 3 – J-010011-07 – Target Sequence: GAUAAAGGAUGGUGGUUCA (SEQ ID NO: 254)

siRNA – 4 – J-010011-08 – Target Sequence: GAAUAGACCUGAGCCUUCA (SEQ ID NO: 255)

The inhibitor of the present invention may also be a siRNA that is directed against PAPD7,
5 wherein said siRNA is any one of or a combination of the following siRNAs:

PAPD7 siRNA pool (L-009807-00-0005; ON-TARGETplus Human PAPD7):

siRNA – 1 – J-009807-05 – Target Sequence: GGAGUGACGUUGAUUCAGA (SEQ ID NO: 256)

siRNA – 2 – J-009807-06 – Target Sequence: CGGAGUUCAUCAAGAAUUA (SEQ ID NO: 257)

10 siRNA – 3 – J-009807-07 – Target Sequence: CGGAGUUCAUCAAGAAUUA (SEQ ID NO: 258)

siRNA – 4 – J-009807-08 – Target Sequence: GCGAAUAGCCACAUGCAAU (SEQ ID NO: 259)

Above, target sequences of suitable siRNAs are shown. The sequences of the corresponding siRNAs are directly complementary to these target sequences.

It is envisaged in context of the present invention that a combined preparation may comprise (a)
15 siRNA(s) directed against PAPD5 is combined with (b) siRNA(s) directed against PAPD7, in order to inhibit expression of both, PAPD5 and PAPD7.

It is also envisaged in context of the present invention that a combined preparation may comprise (a) shRNA directed against PAPD5 is combined with (b) shRNA directed against PAPD7, in order to inhibit expression of both, PAPD5 and PAPD7. In this context the shRNA
20 molecule in (a) may be one or more of the following shRNA molecules

CCGGGCCACATATAGAGATTGGATACTCGAGTATCCAATCTCTATATGTGGCTTTTTTG (SEQ ID NO: 260)

CCGGCCAACAAATCTCAGCATGGATCTCGAGATCCATGCTGAGATTTGTTGGTTTTTG (SEQ ID NO: 261)

25 CCGGCGCCTGTAATCCCAGCACTTTCTCGAGAAAGTGCTGGGATTACAGGCGTTTTTG (SEQ ID NO: 262)

CCGGGCCTGTAATCCCAGCACTTTACTCGAGTAAAGTG CTG GGATTACAGGCTTTTTTG (SEQ ID NO: 263)

30 CCGGCGATGTTGGAAGGAGTTCATACTCGAGTATGAACTCCTTCCAACATCGTTTTTG (SEQ ID NO: 264)

In a further aspect the invention the RNAi molecule is an antisense oligonucleotide capable of inhibiting expression of PAPD5 or PAPD7. The modulation is achieved by hybridizing to a target nucleic acid encoding PAPD5 or PAPD7. The target nucleic acid may be a mammalian PAPD5, such as a sequence selected from the group consisting of SEQ ID NO: 4, 5 and 10, or natural
35 variants thereof.

The target nucleic acid may be a mammalian PAPD7, such as a sequence selected from SEQ ID NO: 6 or 11 or natural variants thereof.

The oligonucleotide of the invention is an antisense oligonucleotide which targets PAPD5 or PAPD7.

- 5 In some embodiments the antisense oligonucleotide of the invention is capable of modulating the expression of the target by inhibiting or down-regulating it. Preferably, such modulation produces an inhibition of expression of at least 20% compared to the normal expression level of the target, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% inhibition compared to the normal expression level of the target. In some embodiments oligonucleotides
10 of the invention may be capable of inhibiting expression levels of PAPD5 or PAPD7 mRNA by at least 60% or 70% *in vitro* using HeLa cells or HepaRG cells. In some embodiments compounds of the invention may be capable of inhibiting expression levels of PAPD5 or PAPD7 protein by at least 50% *in vitro* using HeLa cells or HepaRG cells. Suitable, assays which may be used to measure PAPD5 or PAPD7 RNA or protein inhibition are described under the screening
15 methods above. The target modulation is triggered by the hybridization between a contiguous nucleotide sequence of the oligonucleotide and the target nucleic acid.

- An aspect of the present invention relates to an antisense oligonucleotide which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 90% complementarity to PAPD5. The antisense oligonucleotide
20 is capable of reducing expression of PAPD5. Preferably the antisense oligonucleotide comprise at least one 2' sugar modified nucleoside.

- Another aspect of the present invention relates to an antisense oligonucleotide which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 90% complementarity to PAPD7. The antisense oligonucleotide
25 is capable of reducing expression of PAPD7. Preferably the antisense oligonucleotide comprise at least one 2' sugar modified nucleoside.

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

- In a preferred embodiment the oligonucleotide of the invention, or contiguous nucleotide sequence thereof is fully complementary (100% complementary) to a region of the target nucleic acid, or in some embodiments may comprise one or two mismatches between the oligonucleotide and the target nucleic acid.
- 35 In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementary, such as fully (or 100%)

complementary, to a target nucleic acid region, such as a target sequence, present in SEQ ID NO: 4, 5 or 10. In some embodiments the oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid region present in SEQ ID NO: 10. In some embodiments the oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid region present SEQ ID NO: 4, 5 or 10.

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementary, such as fully (or 100%) complementary, to a target nucleic acid region, such as a target sequence, present in SEQ ID NO: 6 or 11. In some embodiments the oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid region present in SEQ ID NO: 11. In some embodiments the oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid region present SEQ ID NO: 6 or 11.

In some embodiments, the oligonucleotide of the invention comprises or consists of 10 to 35 nucleotides in length, such as from 10 to 30, such as 11 to 22, such as from 12 to 20, such as from such as from 14 to 20, such as from 14 to 18 such as from 14 to 16, such as from 16 to 20 contiguous nucleotides in length.

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

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting of sequences listed in table 3, in the materials and method section.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 12 to 131 (see motif sequences listed in table 3).

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 15, 18, 23, 25, 26, 30, 32, 39, 54, 56, 58, 65, 80, 88, 92, 93, 111, 115, 116 and 118 (see motif sequences listed in table 3).

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably

100% identity, to a sequence selected from the group consisting of SEQ ID NO: 23, 26, 54, 56, 80, 93 and 115.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting of sequences listed in table 4, in the materials and method section.

- 5 In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 132 to 251 (see motif sequences listed in table 4).
- 10 In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 153, 155, 168, 171, 172, 174, 183, 184, 188, 190, 191, 194, 195, 197, 221, 224, 229, 232, 239, and 244 (see motif sequences listed in table 4).
- 15 In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 172, 188, 190, 229 and 239.

In a further aspect the invention relates to a combined preparation comprising a) a nucleic acid molecule which inhibits expression and/or activity of PAPD5; and b) a nucleic acid molecule which inhibits expression and/or activity of PAPD7. In particular embodiments the nucleic acid molecules are independently selected from siRNA, shRNA and antisense oligonucleotides described herein.

In some embodiments the combined preparation comprises a) one of more siRNA molecules targeting a PAPD5 target sequence selected from one or more of SEQ ID NO: 252, 253, 254 and 255; and b) one of more siRNA molecules targeting a PAPD7 target sequence selected from one or more of SEQ ID NO: 256, 257, 258 and 259.

In some embodiments the combined preparation comprises a) one of more siRNA molecules targeting a PAPD5 target sequence selected from one or more of SEQ ID NO: 252, 253, 254 and 255; and b) one of more antisense oligonucleotides targeting a PAPD7 target sequence selected from the group consisting of SEQ ID NO: 153, 155, 168, 171, 172, 174, 183, 184, 188, 190, 191, 194, 195, 197, 221, 224, 229, 232, 239, and 244.

In some embodiments the combined preparation comprises a) one of more antisense oligonucleotide molecules targeting a PAPD5 target sequence selected from the group consisting of SEQ ID NO: 15, 18, 23, 25, 26, 30, 32, 39, 54, 56, 58, 65, 80, 88, 92, 93, 111, 115,

116 and 118; and b) one of more siRNA molecules targeting a PAPD7 target sequence selected from one or more of SEQ ID NO: 256, 257, 258 and 259.

In some embodiments the combined preparation comprises a) one of more shRNA molecules targeting a PAPD5 target sequence selected from one or more of SEQ ID NO: 260, 261, 262, 263 and 264; and b) one of more siRNA molecules targeting a PAPD7 target sequence selected from one or more of SEQ ID NO: 256, 257, 258 and 259.

In some embodiments the combined preparation comprises a) one of more shRNA molecules targeting a PAPD5 target sequence selected from one or more of SEQ ID NO: 260, 261, 262, 263 and 264; and b) one of more antisense oligonucleotides targeting a PAPD7 target sequence selected from the group consisting of SEQ ID NO: 153, 155, 168, 171, 172, 174, 183, 184, 188, 190, 191, 194, 195, 197, 221, 224, 229, 232, 239, and 244.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 15, 18, 23, 25, 26, 30, 32, 39, 54, 56, 58, 65, 80, 88, 92, 93, 111, 115, 116 and 118 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 153, 155, 168, 171, 172, 174, 183, 184, 188, 190, 191, 194, 195, 197, 221, 224, 229, 232, 239, and 244.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 18, 23, 25, 26, 32, 39, 54, 56, 80, 92, 93, 116 and 118 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 153, 155, 172, 174, 183, 188, 190, 195, 197, 221, 224, 229, 232 and 244.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 18 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 221.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 23 and b) an antisense

oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 172 or 188.

5 In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 25 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 174 or 183.

10 In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 26 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ
15 ID NO: 183.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 39 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30
20 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 229.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 54 and b) an antisense
25 oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 190 or 232.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 56 and b) an antisense
30 oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 153 or 244.

In some embodiments the combined preparation comprises a) an antisense oligonucleotide or
35 contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 80 and b) an antisense

oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 153 or 244.

5 In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 92 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ ID NO: 190 or 232.

10 In some embodiments the combined preparation comprises a) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence SEQ ID NO: 116 and b) an antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence of SEQ
15 ID NO: 155 or 195.

Oligonucleotide design

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

25 In an embodiment, the oligonucleotide comprises at least 1 modified nucleoside, such as from 1 to 8 modified nucleosides, such as from 2 to 8 modified nucleosides, such as from 3 to 7 modified nucleosides, such as from 4 to 6 modified nucleosides.

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

In a further embodiment the oligonucleotide comprises at least one modified internucleoside
35 linkage. In a preferred embodiment all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate or boranophosphate internucleoside linkages. In

some embodiments all the internucleotide linkages in the contiguous sequence of the oligonucleotide are phosphorothioate linkages.

In some embodiments, the oligonucleotide of the invention comprises at least one LNA nucleoside, such as from 1 to 8 LNA nucleosides, such as from 2 to 8 LNA nucleosides, such as
5 from 3 to 7 LNA nucleosides, such as from 4 to 6 LNA nucleosides.

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

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

10 ***Gapmer design***

In a preferred embodiment the oligonucleotide of the invention has a gapmer design or structure also referred herein merely as "Gapmer". In a gapmer structure the oligonucleotide comprises at least three distinct structural regions a 5'-flank, a gap and a 3'-flank, F-G-F' in '5 -> 3' orientation. In this design, flanking regions F and F' (also termed wing regions) comprise a
15 contiguous stretch of modified nucleosides, which are complementary to the PAPD5 or PAPD7 target nucleic acid, while the gap region, G, comprises a contiguous stretch of nucleotides which are capable of recruiting a nuclease, preferably an endonuclease such as RNase, for example RNase H, when the oligonucleotide is in duplex with the target nucleic acid. In preferred embodiments the gap region consists of DNA nucleosides. Regions F and F', flanking
20 the 5' and 3' ends of region G, preferably comprise non-nuclease recruiting nucleosides (nucleosides with a 3' endo structure), more preferably one or more affinity enhancing modified nucleosides. In some embodiments, the 3' flank comprises at least one LNA nucleoside, preferably at least 2 LNA nucleosides. In some embodiments, the 5' flank comprises at least one LNA nucleoside. In some embodiments both the 5' and 3' flanking regions comprise a LNA
25 nucleoside. In some embodiments all the nucleosides in the flanking regions are LNA nucleosides. In other embodiments, the flanking regions may comprise both LNA nucleosides and other nucleosides (mixed flanks), such as DNA nucleosides and/or non-LNA modified nucleosides, such as 2' substituted nucleosides. In this case the gap is defined as a contiguous sequence of at least 5 RNase H recruiting nucleosides (nucleosides with a 2' endo structure,
30 preferably DNA) flanked at the 5' and 3' end by an affinity enhancing modified nucleoside, preferably LNA, such as beta-D-oxy-LNA. Consequently, the nucleosides of the 5' flanking region and the 3' flanking region which are adjacent to the gap region are modified nucleosides, preferably non-nuclease recruiting nucleosides or high affinity nucleosides.

Region F

35 Region F (5' flank or 5' wing) is attached to the 5' end of region G and comprises, contains or consists of at least one modified nucleoside such as at least 2, at least 3, or at least 4 modified

nucleosides. In an embodiment region F comprises or consists of from 1 to 4 modified nucleosides, such as from 2 to 4 modified nucleosides, such as from 1 to 3 modified nucleosides, such as 1, 2, 3 or 4 modified nucleosides. The F region is defined by having at least one modified nucleoside at the 5' end and at the 3' end of the region.

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

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

In another embodiment region F comprises DNA and/or RNA nucleosides in addition to the 2' modified nucleosides. Flanks comprising DNA and/or RNA are characterized by having a 2' modified nucleoside in the 5' end and the 3' end (adjacent to the G region) of the F region. The DNA nucleosides in the flanks should preferably not be able to recruit RNase H. The length of the 5' flank (region F) in oligonucleotides with DNA and/or RNA nucleotides in the flanks may be longer, maintaining the number of 2' modified nucleotides at 1 to 4 as described above. In a further embodiment one or more of the 2' modified nucleosides in region F are selected from 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, LNA units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units.

15 In some embodiments the F region comprises both LNA and a 2' substituted modified nucleoside. These are often termed mixed wing or mixed flank oligonucleotides.

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

Region G

Region G (gap region) preferably comprise, contain or consist of from 4 to 18, or from 5 to 17, or from 6 to 16 or from 8 to 12 consecutive nucleotide units capable of recruiting RNase H nuclease.

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

is unlocked nucleic acid, typically where the bond between C2 and C3 of the ribose has been removed, forming an unlocked "sugar" residue.

In some embodiments, region G consists of 100% DNA units.

In further embodiments the region G may consist of a mixture of DNA and other nucleosides capable of mediating RNase H cleavage.

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

Region F'

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

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

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

In another embodiment region F' comprises DNA and/or RNA nucleosides in addition to the 2' modified nucleosides. Flanks comprising DNA and/or RNA are characterized by having a 2' modified nucleoside in the 5' end and the 3' end (adjacent to the G region) of the F' region. The DNA nucleosides in the flanks should preferably not be able to recruit RNase H. The length of the 3' flank (region F') in oligonucleotides with DNA and/or RNA nucleotides in the flanks may be longer, maintaining the number of 2' modified nucleotides at 1 to 4 as described above. In a further embodiment one or more of the 2' modified nucleosides in region F' are selected from 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, LNA units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units.

In some embodiments the F' region comprises both LNA and a 2' substituted modified nucleoside. These are often termed mixed wing or mixed flank oligonucleotides.

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

Region D' and D''

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

- Region D' or D'' may independently comprise 1, 2, 3, 4 or 5 additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. In this respect the oligonucleotide of the invention, may in some embodiments comprise a contiguous nucleotide sequence capable of modulating the target which is flanked at the 5' and/or 3' end by additional nucleotides. Such additional nucleotides may serve as a nuclease susceptible biocleavable linker (see definition of linkers). In some embodiments the additional 5' and/or 3' end nucleotides are linked with phosphodiester linkages, and may be DNA or RNA. In another embodiment, the additional 5' and/or 3' end nucleotides are modified nucleotides which may for example be included to enhance nuclease stability or for ease of synthesis. In one embodiment the oligonucleotide of the invention comprises a region D' and/or D'' in addition to the contiguous nucleotide sequence.
- The gapmer oligonucleotide of the present invention can be represented by the following formulae:

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

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

- In some embodiments the oligonucleotide is a gapmer consisting of 14-20 nucleotides in length, wherein each of regions F and F' independently consists of 1, 2, 3 or 4 modified nucleoside units and region G consists of 6-17 nucleoside units, capable of recruiting nuclease when in duplex with the PAPD5 or PAPD7 target nucleic acid and wherein the oligonucleotide is complementary to the PAPD5 or PAPD7 target nucleic acid.

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

In each design, the preferred modified nucleoside is LNA.

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

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

5 For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 12_1 to 131_1 (see oligonucleotides listed in table 3).

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 15_1, 18_1, 23_1, 25_1, 26_1, 30_1, 32_1, 39_1, 54_1, 56_1, 58_1, 65_1, 80_1, 88_1, 92_1, 93_1, 111_1, 115_1, 116_1 and 118_1 (see
10 oligonucleotides listed in table 3).

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 23_1, 26_1, 54_1, 56_1, 80_1, 93_1 and 115_1.

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 132_1 to 251_1 (see oligonucleotides listed in
15 table 4).

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 153_1, 155_1, 168_1, 171_1, 172_1, 174_1, 183_1, 184_1, 188_1, 190_1, 191_1, 194_1, 195_1, 197_1, 221_1, 224_1, 229_1, 232_1, 239_1, and 244_1 (see oligonucleotides listed in table 4).

20 For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 172_1, 188_1, 190_1, 229_1 and 237_1.

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide is selected from the group of oligonucleotide compounds with CMP ID NO: 18_1, 25_1, 26_1, 32_1, 39_1, 54_1, 56_1, 80_1, 92_1, 93_1, 116_1 and 118_1 and b) the oligonucleotide is
25 selected from the group of oligonucleotide compounds with CMP ID NO: 153_1, 155_1, 168_1, 171_1, 172_1, 174_1, 183_1, 184_1, 188_1, 190_1, 191_1, 194_1, 195_1, 197_1, 221_1, 224_1, 229_1, 232_1, 23_19, and 244_1.

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 18_1 and b) the oligonucleotide compound with CMP ID NO:
30 221_1.

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 23_1 and b) the oligonucleotide compound with CMP ID NO: 172_1 or 188_1.

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 25_1 and b) the oligonucleotide compound with CMP ID NO: 174_1 or 183_1.

5 An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 26_1 and b) the oligonucleotide compound with CMP ID NO: 183_1.

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 39_1 and b) the oligonucleotide compound with CMP ID NO: 229_1.

10

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 54_1 and b) the oligonucleotide compound with CMP ID NO: 190_1 or 232_1.

15 An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 56_1 and b) the oligonucleotide compound with CMP ID NO: 153_1 or 244_1.

An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 80_1 and b) the oligonucleotide compound with CMP ID NO: 153_1 or 244_1.

20 An embodiment of the invention is a combined preparation comprising a) the oligonucleotide compound with CMP ID NO: 116_1 and b) the oligonucleotide compound with CMP ID NO: 155_1 or 195_1.

Applications

25 In context of the present invention it has surprisingly been shown that the combined inhibition of PAPD5 and PAPD7 leads to a synergistic effect in the inhibition of HBV propagation. The appended examples show that reduction of the expression of PAPD5 alone leads to a reduction of the secretion of HBsAg and HBeAg of around 50%, likewise intracellular HBV mRNA was reduced using a PAPD5 inhibitor. Reduction of the expression of PAPD7 alone leads to a reduction of the secretion of HBsAg and HBeAg of not more than 15%. Simultaneous knock-
30 down of PAPD5 and PAPD7 leads to a synergistic effect in the reduction of secretion of HBsAg and HBeAg that lies above the sum of the single knock-downs. Without being bound by theory, this synergistic effect may be due to a compensatory effect of PAPD5 and PAPD7 since both proteins have high sequence homology and same enzymatic functions. Due to the reduction of HBsAg secretion the inhibitor of the present invention inhibits development of chronic HBV
35 infection. In particular, due to inhibition of HBeAg secretion, the inhibitor of the present invention

more efficiently inhibits development of a chronic HBV infection as compared to a compound that only reduces secretion of HBsAg. In addition, reducing HBeAg in an expected mother may also inhibit the development of a chronic HBV infection of her child. Thus, due to the reduction of HBeAg secretion the inhibitor of the present invention inhibits development of a chronic HBV infection (such as development of a chronic HBV infection in the offspring of an HBV infected mother) and reduces the infectiousness of a HBV infected person. Accordingly, one aspect of the present invention related to the herein provided inhibitor, wherein the inhibitor reduces secretion of HBsAg and HBeAg. In line with this, a further aspect of the invention relates to the herein provided inhibitor, in particular a nucleic acid molecule or a combination of nucleic acid molecules, wherein the inhibitor inhibits development of chronic HBV infection and reduces the infectiousness of a HBV infected person. In a particular aspect of the invention, the herein provided inhibitor inhibits development of a chronic HBV infection in the offspring of a HBV infected mother. This mother is preferably HBeAg positive.

The subject to be treated with the inhibitor of the invention (or which prophylactically receives the inhibitor 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. Said human patient may be an expected mother, e.g. an expected mother who is HBeAg positive and/or HBsAg positive, more preferably an expected mother who is HBeAg positive and HBsAg positive.

One embodiment of the present invention relates a PAPD5 inhibitor, in particular a nucleic acid molecule that inhibits the expression and/or activity of PAPD5, for use in the treatment and/or prevention of a HBV infection, in particular a chronic HBV infection. A further embodiment of the present invention relates to a combined preparation comprising an inhibitor of PAPD5 and an inhibitor of PAPD7 for use in the treatment and/or prevention of a HBV infection, in particular a chronic HBV infection. In a preferred embodiment the combined composition for use in treatment and/or prevention of a HBV infection comprises a) a nucleic acid molecule which inhibits expression and/or activity PAPD5; and b) a nucleic acid molecule which inhibits expression and/or activity of PAPD7. Thus, the present invention relates to a combined preparation comprising an inhibitor of PAPD5 and an inhibitor of PAPD7 for simultaneous or sequential use in the treatment and/or prevention of a HBV infection.

The present invention also relates to a combined preparation comprising a) a nucleic acid molecule which inhibits expression and/or activity PAPD5; and b) a nucleic acid molecule which inhibits expression and/or activity of PAPD7. It is envisaged in context of the invention that said combined preparation is used for treating (e.g. ameliorating) a HBV infection. The definitions disclosed herein in connection with the inhibitor of the present invention apply, mutatis mutandis, to the combined preparation of the present invention. The combined preparation may comprise a molecule that is a PAPD5 inhibitor and a separate molecule that is a PAPD7

inhibitor (e.g. two separate RNAi molecules, such as siRNA molecules, shRNA and antisense oligonucleotides, or two separate small molecules). These two separate inhibitors may be formulated within one unit, e.g., within one pill or vial. Alternatively, these two separate inhibitors may be formulated separately, in separate units, e.g. separate pills or vials. The two separate inhibitors may be administered together, (i.e. simultaneously) or separately (i.e. sequentially) provided that the synergistic effect of the two inhibitors is achieved. In one aspect of the invention the combined preparation leads to a reduction of secretion of HBsAg and HBeAg of at least 50% as compared to the no drug control (i.e. compared to cells or subjects to which no drug is administered).

- 10 The present invention also relates to a pharmaceutical composition for use in the treatment and/or prevention of a HBV infection, wherein the pharmaceutical composition comprises (i) the inhibitor of the invention; or the combined preparation of the invention; and (ii) optionally a pharmaceutically acceptable carrier.

Accordingly, the present invention relates to a method of treating and/or preventing a HBV infection, wherein the method comprises administering an effective amount of the inhibitor of the invention, in particular a nucleic acid molecule, a conjugate of the inhibitor, the pharmaceutical composition of the invention, or of the combined preparation of the invention to a subject in need of such a treatment.

The invention also provides for the use of the inhibitor of the invention, in particular a nucleic acid molecule, a conjugate of the inhibitor, the pharmaceutical composition of the invention, or of the combined preparation of the invention for the manufacture of a medicament. In preferred embodiments the medicament is manufactured in a dosage form for subcutaneous administration and for the combined preparation the ratio of the PAPD5 inhibitor and the PAPD7 inhibitor is 1:1 by weight.

25 The invention also provides for the use of the inhibitor of the invention, in particular a nucleic acid molecule, a conjugate of the inhibitor, the pharmaceutical composition of the invention, or of the combined preparation of the invention as described for the manufacture of a medicament wherein the medicament is in a dosage form for intravenous administration and for the combined preparation the ratio of the PAPD5 inhibitor and the PAPD7 inhibitor is 1:1 by weight.

30 The inhibitor of the invention, the combined preparation of the invention, or the pharmaceutical composition of the invention may be used in a combination therapy. For example, the inhibitor of the invention, the combined preparation of the invention, or the pharmaceutical composition of the invention may be combined with other anti-HBV agents such as interferon alpha-2b, interferon alpha-2a, and interferon alphacon-1 (pegylated and unpegylated), ribavirin,

35 lamivudine (3TC), entecavir, tenofovir, telbivudine (LdT), adefovir, or other emerging anti-HBV agents such as a HBV RNA replication inhibitor, a HBsAg secretion inhibitor, a HBV capsid inhibitor, an antisense oligomer (e.g. as described in WO2012/145697 and WO 2014/179629), a

siRNA (e.g. described in WO 2005/014806, WO 2012/024170, WO 2012/2055362, WO 2013/003520, WO 2013/159109, WO 2017/027350 and WO2017/015175), a HBV therapeutic vaccine, a HBV prophylactic vaccine, a HBV antibody therapy (monoclonal or polyclonal), or TLR 2, 3, 7, 8 or 9 agonists for the treatment and/or prophylaxis of HBV.

- 5 The appended examples demonstrate that down regulation of PAPD5 and/or PAPD7 goes along with a reduction in the production of HBsAg and HBeAg as well as of intracellular HBV mRNA in HBV infected cells. These results indicate that the amount and/or activity of PAPD5 and/or PAPD7 can be used for monitoring therapeutic success during the treatment of a HBV infection, e.g. if treatment with an inhibitor of PAPD5 and/or PAPD7 is ongoing or has been
- 10 performed. Thus, the present invention relates to a method for monitoring the therapeutic success during the treatment of a HBV infection, wherein the method comprises:
- (a) analyzing in a sample obtained from a test subject the amount and/or activity of PAPD5 and/or PAPD7;
- (b) comparing said amount and/or activity with reference data corresponding to the amount
- 15 and/or activity of PAPD5 and/or PAPD7 of at least one reference subject; and
- (c) predicting therapeutic success based on the comparison step (b).

In the monitoring method of the invention the test subject may be a human being who receives medication for a HBV infection or has received medication for a HBV infection. The medication may comprise anti-HBV agents as described above. The medication may also comprise an

20 inhibitor of PAPD5 and/or PAPD7.

In the monitoring method of the invention the reference data may correspond to the amount and/or activity of PAPD5 and/or PAPD7 in a sample of at least one reference subject. Said sample may be blood or a liver biopsy.

One aspect of the invention relates to the monitoring method of the invention, wherein the at

25 least one reference subject has a HBV infection but did not receive medication for a HBV infection; and wherein in step (c) a decreased amount and/or activity of PAPD5 and/or PAPD7 of the test subject as compared to the reference data indicates therapeutic success in the treatment of a HBV infection. For example, said decreased amount and/or activity of PAPD5 and/or PAPD7 may mean that the amount and/or activity of PAPD5 and/or PAPD7 in the

30 sample of the test subject is 0 to 90% of the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the at least one reference subject. For example, said decreased amount and/or activity of PAPD5 and/or PAPD7 may be 0 to 80%, preferably 0 to 70%, more preferably 0 to 60%, even more preferably 0 to 50%, even more preferably 0 to 40%, even more preferably 0 to 30, even more preferably 0 to 20%, and most preferably 0 to 10% of the amount and/or activity

35 of PAPD5 and/or PAPD7 in the sample of the at least one reference subject.

Another aspect of the invention relates to the monitoring method of the invention, wherein the at least one reference subject has a HBV infection and has received medication for a HBV

infection; and wherein in step (c) an identical or similar amount and/or activity of PAPD5 and/or PAPD7 of the test subject as compared to the reference data indicates therapeutic success in the treatment of a HBV infection. A further aspect of the invention relates to the monitoring method of the invention, wherein the at least one reference subject does not have a HBV

5 infection; and wherein in step (c) an identical or similar amount and/or activity of PAPD5 and/or PAPD7 of the test subject as compared to the reference data indicates therapeutic success in the treatment of a HBV infection. An identical or similar amount and/or activity of PAPD5 and/or PAPD7 may mean that the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the test subject is 90-110% of the amount and/or activity of PAPD5 and/or PAPD7 in the sample of

10 the at least one reference subject. For example, said identical or similar amount and/or activity of PAPD5 and/or PAPD7 may be 95-105% of the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the at least one reference subject.

Also encompassed by the present invention is a cell or a non-human animal (e.g. a mouse, rat, ferret or rabbit) with increased, reduced or absent PAPD5 and/or PAPD7 expression that can be

15 used for identifying and/or characterizing a compound that prevents and/or treats (e.g. ameliorates) a HBV infection. For example, said cell or non-human animal may comprise an exogenous nucleotide sequence encoding PAPD5 and/or PAPD7, e.g. cloned into an expression vector and operable linked to an exogenous promoter. Said cell or non-human animal may overexpress PAPD5 and/or PAPD7, preferably PAPD5 and PAPD7. Alternatively,

20 said cell or non-human animal may have a knock-down of PAPD5 and/or PAPD7, preferably of PAPD5 and PAPD7.

Embodiments of the invention

Thus, the present invention relates to the following items:

1. A method for identifying a compound that prevents, ameliorates and/or inhibits a
- 25 hepatitis B virus (HBV) infection, comprising
 - a. contacting a test compound with a cell expressing PAPD5 and/or PAPD7
 - b. measuring the expression and/or activity of PAPD5 and/or PAPD7 in the presence and absence of said test compound; and
 - c. identifying a compound that reduces the expression and/or activity of PAPD5 or
 - 30 PAPD7 as a compound that prevents, ameliorates and/or inhibits a HBV infection.
2. The method of item 1, further comprising the step of testing the ability of combinations of compounds identified in c) to reduce the expression and/or activity of PAPD5 and PAPD7.
3. The method of item 1 or 2, wherein PAPD5 is a PAPD5 target nucleic acid.
4. The method of item 3, wherein the PAPD5 target nucleic acid comprises or consists of
- 35 a. a nucleotide sequence of SEQ ID NO: 4 or 5 or 10, or natural variant thereof;

- b. a nucleotide sequence having at least 80% identity to a nucleotide sequence of a.), wherein the polypeptide expressed from the nucleic acid sequence has poly-A polymerase function;
- c. a nucleotide sequence comprising or consisting of SEQ ID NO: 4, 5 or 10;
- 5 d. a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1 or 2;
- e. a nucleotide sequence encoding an amino acid sequence having at least 80% identity to SEQ ID NO: 1 or 2, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function;
- f. a nucleotide sequence encoding an enzymatically active fragment of SEQ ID NO: 1 or 10 2, such as SEQ ID NO: 7 or 8; or
- g. a nucleotide sequence encoding an amino acid sequence having at least 80% identity to an amino acid sequence of an enzymatically active fragment of SEQ ID NO: 1 or 2, such as SEQ ID NO: 7 or 8, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function;
- 15 5. The method of item 1 or 2, wherein PAPD7 is a PAPD7 target nucleic acid.
6. The method of item 5, wherein the PAPD7 target nucleic acid comprises or consists of
- a. a nucleotide sequence of SEQ ID NO: 6 or 11, or natural variant thereof;
- b. a nucleotide sequence having at least 80% identity to a nucleotide sequence of (a.), wherein the polypeptide expressed from the nucleic acid sequence has poly-A 20 polymerase function;
- c. a nucleotide sequence comprising or consisting of SEQ ID NO: 6 or 11;
- d. the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 3;
- e. a nucleotide sequence encoding an amino acid sequence having at least 80% identity to SEQ ID NO: 3, wherein the polynucleotide encodes a polypeptide that has poly-A 25 polymerase function;
- f. the nucleotide sequence encoding an enzymatically active fragment of SEQ ID NO: 3, such as SEQ ID NO: 9; or
- g. a nucleotide sequence encoding an amino acid sequence having at least 80% identity to an amino acid sequence of an enzymatically active fragment of SEQ ID NO: 3, 30 such as SEQ ID NO: 9, wherein the polynucleotide encodes a polypeptide that has poly-A polymerase function.
7. The method of any one of items 1 to 6, wherein said cell is an eukaryotic cell.
8. The method of any one of items 1 to 7, wherein the compound that inhibits propagation of HBV inhibits secretion of HBV surface antigen (HBsAg), inhibits secretion of HBV envelope 35 antigen (HBeAg), and/or inhibits production of intracellular HBV mRNA or HBV DNA.
9. The method of any one of items 1 to 8, wherein the test compound is a screening library of nucleic acid molecules selected from

- a. single stranded antisense oligonucleotides, or
 - b. siRNA molecules; and
 - c. shRNA molecules.
10. The method of any one of items 1 to 9, wherein the compound identified in step c. of
5 item 1 reduce PAPD5 or PAPD7 mRNA expression by at least 50%.
11. The method of any one of items 1 to 10, wherein the test compound is a combined preparation of a nucleic acid molecule capable of reducing PAPD5 and a nucleic acid molecule capable of reducing PAPD7.
12. The method of item 11, wherein the combined preparation reduce HBV surface antigen
10 (HBsAg), HBV envelope antigen (HBeAg), and/or intracellular HBV mRNA or HBV DNA by at least 70%.
13. The method of any one of items 1 to 12, which additionally comprises the step of comparing the test compound to a control.
14. The method of item 13, wherein said control is an inactive test compound that does not
15 reduce the expression and/or activity of PAPD5 or PAPD7.
15. The method of any one of items 1 to 14, wherein the activity of PAPD5 and PAPD7 is the poly-A polymerase function.
16. An inhibitor of PAPD5 or PAPD7 for use in treating and/or preventing a HBV infection, wherein said inhibitor is
- 20 a. a RNA interference (RNAi) molecule against PAPD5 or PAPD7; or
- b. a genome editing machinery, comprising:
- i. a site-specific DNA nuclease or a polynucleotide encoding a site-specific DNA nuclease; and
 - ii. a guide RNA or a polynucleotide encoding a guide RNA.
- 25 17. The inhibitor for the use according to item 16, wherein the inhibitor is an RNAi molecule selected from the group consisting of:
- a. a single stranded antisense oligonucleotide;
 - b. a siRNA molecule; and
 - c. a shRNA molecule.
- 30 18. The inhibitor for the use according to item 16 or 17, wherein the inhibitor is a combined preparation comprising
- a. a RNAi molecule which inhibits expression and/or activity of PAPD5; and
 - b. a RNAi molecule which inhibits expression and/or activity of PAPD7.
19. The inhibitor for the use according to any one of items 16 to 18, wherein the inhibitor
35 reduces secretion of HBsAg and HBeAg.

20. The inhibitor for the use according to item any one of items 16 to 18, wherein the inhibitor reduces production of intracellular HBV mRNA or HBV DNA.
21. The inhibitor for the use according to any one of items 16 to 20, wherein the inhibitor inhibits development of chronic HBV infection and/or reduces the infectiousness of a HBV infected person.
22. An antisense oligonucleotide or siRNA molecule which comprises or consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 80% complementarity to PAPD5 target nucleic acid and the antisense oligonucleotide capable of reducing expression of PAPD5.
23. A nucleic acid molecule which comprises or consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 80% complementarity to PAPD7 target nucleic acid and the antisense oligonucleotide capable of reducing expression of PAPD7.
24. The nucleic acid molecule of item 23, wherein the nucleic acid molecule is a single stranded antisense oligonucleotide.
25. The antisense oligonucleotide of item 22, wherein the oligonucleotide is capable of hybridizing to a target nucleic acid of selected from the group consisting of SEQ ID NO: 4, 5 and 10 with a ΔG° below -10 kcal.
26. The antisense oligonucleotide of item 23 or 24, wherein the oligonucleotide is capable of hybridizing to a target nucleic acid of selected from SEQ ID NO: 6 or 11 with a ΔG° below -10 kcal.
27. The antisense oligonucleotide of any one of items 22 to 26, wherein the target nucleic acid is RNA.
28. The antisense oligonucleotide of item 27, wherein the RNA is mRNA.
29. The antisense oligonucleotide of item 28, wherein the mRNA is pre-mRNA or mature mRNA.
30. The antisense oligonucleotide of any one of items 22-29, wherein the contiguous nucleotide sequence comprises or consists of from 12 to 22 nucleotides.
31. The antisense oligonucleotide of item 30, wherein the contiguous nucleotide sequence comprises or consists of from 14-20 nucleotides.
32. The antisense oligonucleotide of any one of items 22-31, wherein the antisense oligonucleotide comprises or consists of 12 to 25 nucleotides in length.
33. The antisense oligonucleotide of any one of items 22-32, wherein the oligonucleotide or contiguous nucleotide sequence is single stranded.

34. The antisense oligonucleotide of any one of items 22-33 wherein the oligonucleotide is neither siRNA nor self-complementary.
35. The antisense oligonucleotide of any one of items 22 or 25, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from SEQ ID NO: 12-131.
- 5 36. The antisense oligonucleotide of item 35, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from SEQ ID NO: 15, 18, 23, 25, 26, 30, 32, 39, 54, 56, 58, 65, 80, 88, 92, 93, 111, 115, 116 and 118.
37. The nucleic acid molecule or antisense oligonucleotide of any one of item 23, 24 or 26, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from
10 SEQ ID NO: 132-151.
38. The antisense oligonucleotide of item 37, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from SEQ ID NO: 153, 155, 168, 171, 172, 174, 183, 184, 188, 190, 191, 194, 195, 197, 221, 224, 229, 232, 239, and 244.
39. The antisense oligonucleotide molecule of any one of items 22-38, wherein the
15 contiguous nucleotide sequence has zero to three mismatches compared to the target nucleic acid it is complementary to.
40. The antisense oligonucleotide of item 39, wherein the contiguous nucleotide sequence has one mismatch compared to the target nucleic acid.
41. The antisense oligonucleotide of item 39, wherein the contiguous nucleotide sequence is
20 fully complementary to the target nucleic acid sequence.
42. The antisense oligonucleotide of any one of items 22-41, comprising one or more modified nucleosides.
43. The antisense oligonucleotide of item 42, wherein the one or more modified nucleoside is a high-affinity modified nucleoside.
- 25 44. The antisense oligonucleotide of any one of items 22-43, wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage.
45. The antisense oligonucleotide of item 44, wherein the modified internucleoside linkage is nuclease resistant.
46. The antisense oligonucleotide of item 44 or 45, wherein at least 50% of the
30 internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages or boranophosphate internucleoside linkages.
47. The antisense oligonucleotide of item 44 or 45, wherein all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

48. The antisense oligonucleotide of any one of items 22-47, wherein the oligonucleotide is capable of recruiting RNase H.
49. The antisense oligonucleotide of item 48, wherein the oligonucleotide is a gapmer.
50. The antisense oligonucleotide of item 48 or 49, wherein the oligonucleotide is a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise or consist of 1 - 4 modified nucleosides and G is a region between 6 and 17 nucleosides which are capable of recruiting RNaseH.
51. The antisense oligonucleotide of any one of items 42-44 or 50, wherein the modified nucleoside is a 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.
52. The antisense oligonucleotide of item 50 or 51, wherein one or more of the modified nucleosides in region F and F' is a LNA nucleoside.
53. The antisense oligonucleotide of item 52, wherein all the modified nucleosides in region F and F' are LNA nucleosides.
54. The antisense oligonucleotide of item 53, wherein region F and F' consist of LNA nucleosides.
55. The antisense oligonucleotide of any one of items 51-54, wherein the LNA nucleoside is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA, alpha-L-amino-LNA, beta-D-thio-LNA, alpha-L-thio-LNA, (S)cET, (R)cET beta-D-ENA and alpha-L-ENA.
56. The oligonucleotide of any one of items 51-54, wherein the LNA nucleoside is oxy-LNA.
57. The antisense oligonucleotide of any one of items 51-56, wherein the LNA nucleoside is beta-D-oxy-LNA.
58. The antisense oligonucleotide of any one of items 51-54, wherein the LNA nucleoside is thio-LNA.
59. The antisense oligonucleotide of any one of items 51-54, wherein the LNA nucleoside is amino-LNA.
60. The antisense oligonucleotide of any one of items 51-54, wherein the LNA nucleoside is cET.
61. The antisense oligonucleotide of any one of items 51-54, wherein the LNA nucleoside is ENA.
62. The antisense oligonucleotide of item 52, wherein at least one of region F or F' further comprises at least one 2' substituted 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 and 2'-fluoro-DNA.

63. The antisense oligonucleotide of any one of items 52-62, wherein the RNaseH recruiting nucleosides in region G are independently selected from DNA, alpha-L-LNA, C4' alkylated DNA, ANA and 2' F-ANA and UNA.
64. The antisense oligonucleotide of item 50 or 63, wherein the nucleosides in region G are DNA nucleosides.
65. The antisense oligonucleotide of any one of items 22 or 25, wherein the antisense oligonucleotide is selected from CMP ID NO: 12_1 to 131_1.
66. The antisense oligonucleotide of item 65, wherein the antisense compound are selected from CMP ID NO: 15_1, 18_1, 23_1, 25_1, 26_1, 30_, 32_1, 39_1, 54_1, 56_1, 58_1, 65_1, 80_1, 88_1, 92_1, 93_1, 111_1, 115_1, 116_1 and 118_1.
67. The antisense oligonucleotide of items 24 wherein the antisense oligonucleotide is selected from CMP ID NO: 132_ to 151_1.
68. The antisense oligonucleotide of item 67, wherein the antisense compound are selected from CMP ID NO: 153_1, 155_1, 168_1, 171_1, 172_1, 174_1, 183_1, 184_1, 188_1, 190_1, 191_1, 194_1, 195_1, 197_1, 221_1, 224_1, 229_1, 232_1, 239_1, and 244_1.
69. The siRNA molecule of item 22, wherein the siRNA molecule is targeting a PAPD5 target sequence selected from one or more of SEQ ID NO: 252, 253, 254 and 255
70. The nucleic acid molecule of item 23, wherein the nucleic acid molecule is an siRNA molecule targeting a PAPD7 target sequence selected from one or more of SEQ ID NO: 256, 257, 258 and 259.
71. A conjugate comprising the antisense oligonucleotide or siRNA according to any one of claims 22-70, and at least one conjugate moiety covalently attached to said oligonucleotide.
72. The conjugate of item 71, wherein the conjugate moiety is selected from carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins, vitamins, viral proteins or combinations thereof.
73. The conjugate of item 71 or 72, wherein the conjugate moiety is capable of binding to the asialoglycoprotein receptor.
74. The conjugate of any one of item 71-73, comprising a linker which is positioned between the antisense oligonucleotide and the conjugate moiety.
75. The conjugate of item 74, wherein the linker is a physiologically labile linker.
76. The conjugate of item 75, wherein the physiologically labile linker is nuclease susceptible linker.

77. The conjugate of item 75 or 76, wherein the oligonucleotide has the formula D'-F-G-F' or F-G-F'-D'', wherein F, F' and G are as defined in any one of items 52-65 and D' or D'' comprises 1, 2 or 3 DNA nucleosides with phosphorothioate internucleoside linkages.
78. A combined preparation comprising:
- 5 a. a RNAi molecule which inhibits expression and/or activity of PAPD5; and
 - b. a RNAi molecule which inhibits expression and/or activity of PAPD7.
79. The combined preparation of item 78, wherein the RNAi molecules are selected from items 22-70 or a conjugate of any one of items 71-77.
80. The combined preparation of item 78, wherein the RNAi molecule in a) is an antisense
10 compounds of item 36 or 66 and where the RNAi molecule in b) is an antisense compounds of item 38 or 68.
81. A pharmaceutical composition comprising the antisense oligonucleotide or siRNA molecule of any one of items 22-70, or a conjugate of any one of items 71-77, or a combined preparation of item 78-80 and optionally a pharmaceutically acceptable diluent, carrier, salt
15 and/or adjuvant.
82. An *in vivo* or *in vitro* method for modulating PAPD5 and/or PAPD7 expression in a target cell which is expressing PAPD5 and/or PAPD7, said method comprising administering an antisense oligonucleotide or siRNA molecule of item 22-70 or a conjugate of item 71-77, or a combined preparation of item 78 or 79, or the pharmaceutical composition of item 81 in an
20 effective amount to said cell.
83. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide or siRNA molecule of item 22-70 or a conjugate of item 71-77 or a combined preparation of item 78-80, or the pharmaceutical composition of item 81 to a subject suffering from or susceptible to the disease.
- 25 84. The antisense oligonucleotide or siRNA molecule of item 22-70 or a conjugate of item 71-77 or a combined preparation of item 78-80, or the pharmaceutical composition of item 81, for use as a medicament for treatment or prevention of a disease in a subject.
85. Use of the oligonucleotide or oligonucleotide or siRNA molecule of item 22-70 or a conjugate of item 71-77 or a combined preparation of item 78-80, for the preparation of a
30 medicament for treatment or prevention of a disease in a subject.
86. The method, the antisense oligonucleotide or the use of any one of items 83 - 85, wherein the PAPD5 and/or PAPD7 is reduced by at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95% compared to the expression without the antisense oligonucleotide or siRNA molecule of item
35 22-70 or a conjugate of item 71-77, or a combined preparation of item 78-80.

87. The method, the antisense oligonucleotide or the use of items 83 - 85, wherein the disease is selected from HBV infection, in particular chronic HBV infection.
88. A method for monitoring the therapeutic success during the treatment of a HBV infection, wherein the method comprises:
- 5 a. analyzing in a sample obtained from a test subject the amount and/or activity of PAPD5 and/or PAPD7;
 - b. comparing said amount and/or activity with reference data corresponding to the amount and/or activity of PAPD5 and/or PAPD7 of at least one reference subject; and
 - c. predicting therapeutic success based on the comparison step (b).
- 10 89. The monitoring method of item 88, wherein the test subject is a human being who receives medication for a HBV infection or has received medication for a HBV infection.
90. The monitoring method of item 88 or 89, wherein the reference data corresponds to the amount and/or activity of PAPD5 and/or PAPD7 in a sample of at least one reference subject.
91. The monitoring method of any one of items 88 to 90, wherein the at least one reference
15 subject has a HBV infection but did not receive medication for a HBV infection; and wherein in step (c) a decreased amount and/or activity of PAPD5 and/or PAPD7 of the test subject as compared to the reference data indicates therapeutic success in the treatment of a HBV infection.
92. The monitoring method of item 91, wherein said decreased amount and/or activity of
20 PAPD5 and/or PAPD7 means that the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the test subject is 0 to 90% of the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the at least one reference subject.
93. The monitoring method of any one of items 88 to 90, wherein the at least one reference
25 subject has a HBV infection and has received medication for a HBV infection; and wherein in step (c) an identical or similar amount and/or activity of PAPD5 and/or PAPD7 of the test subject as compared to the reference data indicates therapeutic success in the treatment of a HBV infection.
94. The monitoring method of any one of items 88 to 90, wherein the at least one reference
30 subject does not have a HBV infection; and wherein in step (c) an identical or similar amount and/or activity of PAPD5 and/or PAPD7 of the test subject as compared to the reference data indicates therapeutic success in the treatment of a HBV infection.
95. The monitoring method of item 93 or 94, wherein said identical or similar amount and/or
35 activity of PAPD5 and/or PAPD7 means that the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the test subject is 90-110% of the amount and/or activity of PAPD5 and/or PAPD7 in the sample of the at least one reference subject.

Pharmaceutical Compositions

As described above, the invention relates to a composition comprising an inhibitor of PAPD5 alone or in combination with a PAPD7 inhibitor for use in treating and/or preventing a HBV infection. The inhibitor is preferably a nucleic acid molecule as defined herein. Specifically, a
5 combined preparation comprising an inhibitor of PAPD5 and an inhibitor of PAPD7 for use in the treatment and/or prevention of a HBV infection is contemplated; and a pharmaceutical composition comprising said inhibitor composition or said combined preparation. Said pharmaceutical composition (i.e. medicament) optionally comprises a pharmaceutically acceptable carrier. Said pharmaceutical composition may further comprise a therapeutically
10 acceptable diluent, salt, excipient and/or adjuvant.

A typical pharmaceutical composition is prepared by mixing a PAPD5 inhibitor alone or with a PAPD7 inhibitor and a carrier or excipient. Suitable carriers and excipients are well known to those skilled in the art and are described in detail in, e.g., Ansel, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Philadelphia: Lippincott, Williams & Wilkins, 2004;
15 Gennaro, Remington: The Science and Practice of Pharmacy, Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Handbook of Pharmaceutical Excipients, Chicago, Pharmaceutical Press, 2005. The formulations may also include one or more buffers, stabilizing agents, surfactants, wetting agents, lubricating agents, emulsifiers, suspending agents, preservatives, antioxidants, opaquing agents, glidants, processing aids, colorants, sweeteners, perfuming
20 agents, flavoring agents, diluents and other known additives to improve appearance of the drug or aid in the manufacturing of the pharmaceutical product (i.e., medicament). For example, the pharmaceutical composition of the invention may be formulated by mixing an inhibitor of PAPD5 and/or an inhibitor of PAPD7 at ambient temperature at an appropriate pH, and with the desired degree of purity, with physiologically acceptable carriers, i.e., carriers that are non-toxic to
25 recipients at the dosages and concentrations employed into a suitable administration form. The pharmaceutical composition of the invention may be sterile.

For nucleic acid molecules suitable formulations 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
30 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.

The compounds according to the present invention may exist in the form of their
35 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.

The 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 the inhibitor of the invention, the combined preparation of the invention, or the 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.

For example, if the PAPD5 inhibitor or the PAPD7 inhibitor is/an antisense oligonucleotide, then the pharmaceutically effective amount administered is a dose of 0.1 – 15 mg/kg, such as from 0.2 – 10 mg/kg, such as from 0.25 – 5 mg/kg. The administration can be once a week, every 2nd week, every third week or even once a month.

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

In a preferred embodiment the nucleic acid molecule or pharmaceutical compositions of the present invention are administered by a parenteral route including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intrathecal or intracranial, e.g. intracerebral or intraventricular, intravitreal administration. In one embodiment the active

oligonucleotide or oligonucleotide conjugate is administered intravenously. In another embodiment the active nucleic acid molecule or nucleic acid molecule conjugate is administered subcutaneously.

- 5 The inhibitor of the invention, the combined preparation of the invention, or the pharmaceutical composition of the invention is useful in the prevention and/or treatment of an HBV infection. They preferably inhibit secretion of HBsAg and/or HBeAg, most preferably of HBsAg and HBeAg.

Definitions

Nucleotide sequence

- 10 The term “nucleotide sequence” or “polynucleotide” is commonly known in the art and comprises molecules comprising or consisting of naturally occurring molecules such as DNA and RNA as well as nucleic acid analogues such as, e.g., oligonucleotides thiophosphates, substituted ribo-oligonucleotides, LNA molecules, PNA molecules, GNA (glycol nucleic acid) molecules, TNA (threose nucleic acid) molecules, morpholino polynucleotides, or nucleic acids
15 with modified backbones such as polysiloxane, and 2'-O-(2-methoxy) ethyl-phosphorothioate, or a nucleic acid with substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection. Furthermore, the term “nucleotide sequence” is to be construed equivalently with the term “nucleic acid molecule” in context of the present invention and may inter alia refer to DNA, RNA,
20 PNA or LNA or hybrids thereof or any modification thereof that is known in the art (see, e.g., US 5,525,711, US 4,711,955, US 5,792,608 or EP 302175 for examples of modifications). Nucleic acid residues comprised by the nucleic acid sequence described and provided herein may be naturally occurring nucleic acid residues or artificially produced nucleic acid residues. Examples for nucleic acid residues are adenine (A), guanine (G), cytosine (C), thymine (T), uracil (U),
25 xanthine (X), and hypoxanthine (HX). As understood by the person of skill in the art, thymine (T) and uracil (U) may be used interchangeably depending on the respective type of polynucleotide. For example, as the skilled person is aware of, a thymine (T) as part of a DNA corresponds to an uracil (U) as part of the corresponding transcribed mRNA. The polynucleotides described and provided herein may be single- or double-stranded, linear or circular, natural or synthetic.
- 30 The nucleotide sequences provided herein may be cloned into a vector. The term “vector” as used herein includes plasmids, cosmids, viruses, bacteriophages and other vectors commonly used in genetic engineering. In a preferred embodiment, these vectors are suitable for the transformation of cells, like mammalian cells or yeast cells. Herein, the vector may be an expression vector. Generally, expression vectors have been widely described in the literature.
- 35 They may comprise a selection marker gene and a replication-origin ensuring replication in the host, a promoter, and a termination signal for transcription. Between the promoter and the termination signal there may be at least one restriction site or a polylinker which enables the

insertion of a nucleic acid sequence desired to be expressed. Non-limiting examples for the vector into which a nucleotide sequence provided herein may be cloned are adenoviral, adeno-associated viral (AAV), lentiviral, HIV-based lentiviral, nonviral minicircle-vectors, or other vectors for bacterial and eukaryotic expression systems.

5 ***Nucleic acid molecule***

The term “nucleic acid molecule” or “therapeutic nucleic acid molecule” as used herein is defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides (i.e. a nucleotide sequence). The nucleic acid molecule(s) referred to in the method of the invention are generally therapeutic oligonucleotides below 50
10 nucleotides in length. The nucleic acid molecules may be or comprise an antisense oligonucleotide, or may be another oligomeric nucleic acid molecule, such as a CRISPR RNA, a siRNA, shRNA, an aptamer, or a ribozyme. Nucleic acid molecules are compositions that are commonly made in the laboratory by solid-phase chemical synthesis followed by purification. When referring to a sequence of the nucleic acid molecule, reference is made to the sequence
15 or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The nucleic acid molecule of the invention is man-made, and is chemically synthesized, and is typically purified or isolated. The nucleic acid molecule of the invention may comprise one or more modified nucleosides or nucleotides.

In some embodiments, the nucleic acid molecule of the invention comprises or consists of 8 to
20 40 nucleotides in length, such as from 9 to 35, such as from 10 to 30, such as from 11 to 22, such as from 12 to 20, such as from 13 to 18 or 14 to 16 contiguous nucleotides in length.

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

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

30 The nucleic acid molecule(s) are typically for modulating the expression of a target nucleic acids in a mammal. In some embodiments the nucleic acid molecules, such as for siRNAs, shRNAs and antisense oligonucleotides, are typically for inhibiting the expression of a target nucleic acid. The nucleic acid molecules, when combined, may therefore be effective at modulating the expression of one or more target nucleic acids in a mammal.

In one embodiment of the invention the nucleic acid molecule is selected from a RNAi agent, such as a siRNA, shRNA or an antisense oligonucleotide. In preferred embodiments the nucleic acid molecule is a high affinity modified antisense oligonucleotide.

In some embodiments the nucleic acid molecule is a phosphorothioate nucleic acid molecule.

- 5 In some embodiments the nucleic acid molecule comprises phosphorothioate internucleoside linkages.

In some embodiments the nucleic acid molecule(s) may be conjugated to non-nucleosidic moieties (conjugate moieties).

- 10 A library of nucleic acid molecules is to be understood as a collection of variant nucleic acid molecules. The purpose of the library of nucleic acid molecules can vary. In some embodiments, the library of nucleic acid molecules is composed of oligonucleotides with different nucleobase sequences, for example it may be a library of nucleic acid molecules which are designed across a target nucleic acid (e.g. a RNA sequence), for example a library of antisense oligonucleotides or siRNA molecules may be generated by a mRNA gene-walk with
- 15 the purpose of identifying regions on the target nucleic acid where nucleic acid molecules efficiently modulate the target nucleic acid. In some embodiments, the library of nucleic acid molecules is composed of oligonucleotides with overlapping nucleobase sequence targeting a specific region on the target nucleic acid with the purpose of identifying the most potent sequence within the library of nucleic acid molecules. In some embodiments, the library of
- 20 nucleic acid molecules is a library of nucleic acid molecule design variants (child nucleic acid molecules) of a parent or ancestral nucleic acid molecule, wherein the nucleic acid molecule design variants retaining the core nucleobase sequence of the parent nucleic acid molecule.

Oligonucleotide

- 25 The term "oligonucleotide" as used herein is defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers.

- Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently
- 30 linked nucleotides or nucleosides. The oligonucleotide of the invention is man-made, and is chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides. An antisense oligonucleotide is a single stranded oligonucleotide with minimal or no internal duplex formation. A siRNA molecule generally consists of 2 complementary oligonucleotide stands (a sense strand and an
- 35 antisense strand) that forms a double stranded molecule. A shRNA molecule is an oligonucleotide which is generally longer than antisense oligonucleotides and which form an internal duplex (hairpin) structure within the molecule.

Antisense oligonucleotides

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

RNAi

Herein, the term "RNA interference (RNAi) molecule" refers to any molecule inhibiting RNA expression or translation, including the nucleic acid molecules defined herein. A small
 10 interfering RNA (siRNA) is a double-stranded RNA molecule that, by binding complementary mRNA after transcription, leads to their degradation and loss in translation. A small hairpin RNA (shRNA) is an artificial RNA molecule with a hairpin structure which upon expression is able to reduce mRNA via the DICER and RNA reducing silencing complex (RISC). RNAi molecules can be designed on the base of the RNA sequence of the gene of interest. Corresponding RNAi can
 15 then be synthesized chemically or by in vitro transcription, or expressed from a vector or PCR product

siRNA and shRNA molecules are generally between 20 and 50 nucleotides in length, such as between 25 and 35 nucleotides in length, and interacts with the endonuclease known as Dicer which is believed to process dsRNA into 19-23 base pair short interfering RNAs with
 20 characteristic two base 3' overhangs which are then incorporated into an RNA-induced silencing complex (RISC). Effective extended forms of Dicer substrates have been described in US 8,349,809 and US 8,513,207, hereby incorporated by reference. Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing. RNAi agents may be chemically modified using modified internucleotide
 25 linkages and high affinity nucleosides, such as 2'-4' bicyclic ribose modified nucleosides, including LNA and cET.

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
 30 "contiguous nucleobase sequence" and the term "oligonucleotide motif sequence". In some embodiments all the nucleotides of the oligonucleotide constitute the contiguous nucleotide sequence. In some embodiments the oligonucleotide comprises the contiguous nucleotide sequence and may optionally comprise further nucleotide(s), for example a nucleotide linker region which may be used to attach a functional group to the contiguous nucleotide sequence.
 35 The nucleotide linker region may or may not be complementary to the target nucleic acid.

Nucleotides

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

Modified nucleoside

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

Modified internucleoside linkage

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

In an embodiment, the nucleic acid molecule, e.g. antisense oligonucleotide, shRNA or siRNA, comprises one or more internucleoside linkages modified from the natural phosphodiester to a linkage that is for example more resistant to nuclease attack. Nuclease resistance may be determined by incubating the oligonucleotide in blood serum or by using a nuclease resistance assay (e.g. snake venom phosphodiesterase (SVPD), both are well known in the art.

Internucleoside linkages which are capable of enhancing the nuclease resistance of an oligonucleotide are referred to as nuclease resistant internucleoside linkages. In some embodiments at least 50% of the internucleoside linkages in the antisense oligonucleotide, or

contiguous nucleotide sequence thereof, are modified, such as at least 60%, such as at least 70%, such as at least 80 or such as at least 90% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are modified. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are modified. It will be recognized that, in some embodiments the nucleosides which link the oligonucleotide of the invention to a non-nucleotide functional group, such as a conjugate, may be phosphodiester. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are nuclease resistant internucleoside linkages.

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

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

A phosphorothioate internucleoside linkage is particularly useful due to nuclease resistance, beneficial pharmacokinetics and ease of manufacture. In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate, such as at least 60%, such as at least 70%, such as at least 80 or such as at least 90% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate.

In some embodiments, the oligonucleotide comprises one or more neutral internucleoside linkage, particularly a internucleoside linkage selected from phosphotriester, methylphosphonate, MMI, amide-3, formacetal or thioformacetal.

Further internucleoside linkages are disclosed in WO2009/124238 (incorporated herein by reference). In an embodiment the internucleoside linkage is selected from linkers disclosed in WO2007/031091 (incorporated herein by reference). Particularly, the internucleoside linkage may be selected from -O-P(O)₂-O-, -O-P(O,S)-O-, -O-P(S)₂-O-, -S-P(O)₂-O-, -S-P(O,S)-O-, -S-P(S)₂-O-, -O-P(O)₂-S-, -O-P(O,S)-S-, -S-P(O)₂-S-, -O-PO(R^H)-O-, -O-PO(OCH₃)-O-, -O-PO(NR^H)-O-, -O-PO(OCH₂CH₂S-R)-O-, -O-PO(BH₃)-O-, -O-PO(NHR^H)-O-, -O-P(O)₂-NR^H-, -NR^H-P(O)₂-O-, -NR^H-CO-O-, -NR^H-CO-NR^H-, and/or the internucleoside linker may be selected from the group consisting of: -O-CO-O-, -O-CO-NR^H-, -NR^H-CO-CH₂-, -O-CH₂-CO-NR^H-, -O-CH₂-CH₂-NR^H-, -CO-NR^H-CH₂-, -CH₂-NR^H-CO-, -O-CH₂-CH₂-S-, -S-CH₂-CH₂-O-, -S-CH₂-CH₂-S-, -CH₂-SO₂-CH₂-, -CH₂-CO-NR^H-, -O-CH₂-CH₂-NR^H-CO-, -CH₂-NCH₃-O-CH₂-, where R^H is selected from hydrogen and C1 -4-alkyl.

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

Each of the design regions may however comprise internucleoside linkages other than phosphorothioate, such as phosphodiester linkages, in particular in regions where modified nucleosides, such as LNA, protect the linkage against nuclease degradation. Inclusion of phosphodiester linkages, such as one or two linkages, particularly between or adjacent to modified nucleoside units (typically in the non-nuclease recruiting regions) can modify the bioavailability and/or bio-distribution of an oligonucleotide – see WO2008/113832, incorporated herein by reference.

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

Nucleobase

The term nucleobase includes the purine (e.g. adenine and guanine) and pyrimidine (e.g. uracil, thymine and cytosine) moiety present in nucleosides and nucleotides which form hydrogen bonds in nucleic acid hybridization. In the context of the present invention the term nucleobase also encompasses modified nucleobases which may differ from naturally occurring nucleobases, but are functional during nucleic acid hybridization. In this context “nucleobase” refers to both naturally occurring nucleobases such as adenine, guanine, cytosine, thymine, 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-thiazolocytosine, 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 or modified nucleic acid molecule describes an oligonucleotide or nucleic acid molecule comprising one or more sugar-modified nucleosides and/or modified internucleoside linkages. The term “chimeric” is a term that has been used in the literature to describe oligonucleotides or nucleic acid molecules with modified nucleosides, in particular gapmer oligonucleotides.

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 number of nucleotides in percent of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which, at a given position, are complementary to (*i.e.* form Watson Crick base pairs with) a contiguous nucleotide sequence, at a given position of a separate nucleic acid molecule (e.g. the target nucleic acid). The percentage is calculated by counting the number of aligned bases that form pairs between the two sequences (when aligned with the target sequence 5'-3' and the oligonucleotide sequence from 3'-5'), dividing by the total number of nucleotides in the oligonucleotide and multiplying by 100. In such a comparison a nucleobase/nucleotide which does not align (form a base pair) is termed a mismatch. Preferably, insertions and deletions are not allowed in the calculation of % complementarity of a contiguous nucleotide sequence.

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

The following is an example of an oligonucleotide (SEQ ID NO: 12) that is fully complementary to a region of a target nucleic acid (SEQ ID NO: 10).

```

1651 cccttagttaaacaacatctacccttgacca 1680 (Pos. 1651-1690 of SEQ ID NO: 10)
      |||||
1    --3' -TCAATTTGTGTAGATGGGA-5' --- 19 (SEQ ID NO: 12)

```

Identity

In context of the present invention, the term “identity” or “percent identity” means that amino acid or nucleotide sequences have identities of at least 80%, preferably at least 90%, more

preferably at least 95%, even more preferably at least 98%, and even more preferably at least 99% identity to the sequences shown herein, e.g. those of SEQ ID NO: 1, 2, or 3, wherein the higher identity values are preferred upon the lower ones. In accordance with the present invention, the term “identity/identities” or “percent identity/identities” in the context of two or
5 more nucleic acid or amino acid sequences, refers to two or more sequences that are the same, or that have a specified percentage of amino acid residues or nucleotides that are the same (e.g., at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity with the amino acid sequences of, e.g., SEQ ID NO: 1, 2, 3, 7, 8 or 9 or with the nucleotide sequences of, e.g., SEQ ID NO: 4, 5, 6, 10 or 11), when compared and aligned for maximum
10 correspondence over a window of comparison, or over a designated region as measured using a sequence comparison algorithm as known in the art, or by manual alignment and visual inspection.

For amino acid sequences, preferably the described identity exists over a region that is at least about 50 amino acids, preferably at least 100 amino acids, more preferably at least 400 amino
15 acids, more preferably at least 500 amino acids, more preferably at least 600 amino acids and most preferably all amino acids in length.

In case of nucleotide sequences, the described identity most preferably exists over a region that is at least 100 nucleotides, preferably at least 1,000 nucleotides, more preferably at least 2,000 nucleotides and most preferably all nucleotides in length. However, for nucleic acid molecules,
20 which generally are below 50 nucleotides, the identity can be assessed over a significantly shorter region. Generally, the percentage identity of nucleic acid molecules is calculated by counting the number of aligned bases that are identical between the two sequences dividing by the total number of nucleotides in the nucleic acid molecule and multiplying by 100. Percent Identity = (Matches x 100)/Length of aligned region. Preferably, insertions and deletions are not
25 allowed in the calculation of % identity of a contiguous nucleotide sequence in a nucleic acid molecule.

Those having skills in the art will know how to determine percent identity between/among sequences using, for example, algorithms such as those based on CLUSTALW computer program (Thompson, 1994, Nucl Acids Res, 2: 4673-4680) or FASTDB (Brutlag, 1990, Comp
30 App Biosci, 6: 237-245), as known in the art. Also available to those having skills in this art are the BLAST and BLAST 2.0 algorithms (Altschul, 1997, Nucl Acids Res 25: 3389-3402; Altschul, 1993, J Mol Evol, 36: 290-300; Altschul, 1990, J Mol Biol 215: 403-410). For example, BLAST 2.0, which stands for Basic Local Alignment Search Tool BLAST (Altschul, 1997, loc. cit.; Altschul, 1993, loc. cit.; Altschul, 1990, loc. cit.), can be used to search for local sequence
35 alignments. BLAST, as discussed above, produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying similar sequences.

Analogous computer techniques using BLAST (Altschul, 1997, loc. cit.; Altschul, 1993, loc. cit.; Altschul, 1990, loc. cit.) are used to search for identical or related molecules in nucleotide databases such as GenBank or EMBL.

Hybridization

5 The term “hybridizing” or “hybridizes” as used herein is to be understood as two nucleic acid strands (e.g. an oligonucleotide and a target nucleic acid) forming hydrogen bonds between base pairs on opposite strands thereby forming a duplex. The affinity of the binding between two nucleic acid strands is the strength of the hybridization. It is often described in terms of the melting temperature (T_m) defined as the temperature at which half of the oligonucleotides are
 10 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
 15 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
 20 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
 25 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
 30 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
 35 kcal or -16 to -27 kcal such as -18 to -25 kcal.

Target nucleic acid

According to the present invention, the target nucleic acid is a nucleic acid which encodes mammalian PAPD5 or PAPD7 and may for example be a gene, a RNA, a mRNA, and pre-mRNA, a mature mRNA or a cDNA sequence. The target may therefore be referred to as a PAPD5 or PAPD7 target nucleic acid. The oligonucleotide or nucleic acid molecule of the invention may for example target exon regions of a mammalian PAPD5 or PAPD7, or may for example target intron region in the PAPD5 or PAPD7 pre-mRNA. Suitably, the target nucleic acid encodes a PAPD5 or PAPD7 protein, in particular mammalian PAPD5 or PAPD7, such as human PAPD5 or PAPD7 (See for example tables 1A and B and Table 2A and B) which provides the mRNA and pre-mRNA sequences for human, monkey, rat and pig PAPD5 or PAPD7).

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 4, 5 or 10 or naturally occurring variants thereof (e.g. sequences encoding a mammalian PAPD5).

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 6 or 11 or naturally occurring variants thereof (e.g. sequences encoding a mammalian PAPD7).

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

For *in vivo* or *in vitro* application, the oligonucleotide of the invention is typically capable of inhibiting the expression of the PAPD5 or PAPD7 target nucleic acid in a cell which is expressing the PAPD5 or PAPD7 target nucleic acid. The contiguous sequence of nucleobases of the oligonucleotide of the invention is typically complementary to the PAPD5 or PAPD7 target nucleic acid, as measured across the length of the oligonucleotide, optionally with the exception of one or two mismatches, and optionally excluding nucleotide based linker regions which may link the oligonucleotide to an optional functional group such as a conjugate, or other non-complementary terminal nucleotides (e.g. region D' or D''). The target nucleic acid may, in some embodiments, be a RNA or DNA, such as a messenger RNA, such as a mature mRNA or a pre-mRNA. In some embodiments the target nucleic acid is a RNA or DNA which encodes mammalian PAPD5 or PAPD7 protein, such as human PAPD5 or PAPD7, e.g. the human PAPD5 mRNA sequence, such as that disclosed as SEQ ID NO 4, 5 or 10 or the human PAPD7 mRNA sequence, such as that disclosed as SEQ ID NO 6 or 11. Further information on exemplary target nucleic acids is provided in tables 1A and B and Table 2A and B.

Table 1A. Genome and assembly information for PAPD5 across species.

Species	Chr.	Band	Strand	Genomic coordinates		ensembl_gene_id	Assembly
				Start	End		
Human	16	q12.1	fwd	50152918	50235310	ENSG00000121274	GRCh38.p7

mouse	8	C3	fwd	88199213	88259722	ENSMUSG00000036779	GRCm38.p5
Rat	19	p11	rev	19771677	19832812	ENSRNOG00000024212	Rnor_6.0

Table 1B. Genome and assembly information for PAPD7 across species.

Species	Chr	Band	Strand	Genomic coordinates Start End		ensembl_gene_id	Assembly
Human	5	p15.31	fwd	6713007	6757048	ENSG00000112941	GRCh38.p7
mouse	13	B3	rev	69497959	69534617	ENSMUSG00000034575	GRCm38.p5
Rat	1	p11	fwd	36400443	36433238	ENSRNOG00000017613	Rnor_6.0

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

5 **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 which is complementary to the contiguous nucleotide sequence of the oligonucleotide of the invention (i.e. a sub-sequence).

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

The target nucleic sequence to which the oligonucleotide is complementary to or hybridizes to generally comprises a stretch of contiguous nucleobases of at least 10 nucleotides. The contiguous nucleotide sequence is between 10 to 50 nucleotides, such as 12-30, such as 13 to 25, such as 14 to 20, such as 15 to 18 contiguous nucleotides.

Naturally occurring variant

The term “naturally occurring variant” refers to variants of PAPD5 or PAPD7 gene or transcripts which originate from the same genetic loci as the target nucleic acid, but may differ for example, by virtue of degeneracy of the genetic code causing a multiplicity of codons encoding the same amino acid, or due to alternative splicing of pre-mRNA, or the presence of polymorphisms, such as single nucleotide polymorphisms, and allelic variants. Based on the presence of the sufficient complementary sequence to the oligonucleotide, the oligonucleotide of the invention may therefore target the target nucleic acid and naturally occurring variants thereof.

In some embodiments, the naturally occurring variants have at least 95% such as at least 98% or at least 99% homology to a mammalian PAPD5 target nucleic acid, such as a target nucleic acid selected from the group consisting of SEQ ID NO: 4, 5 or 10.

In some embodiments, the naturally occurring variants have at least 95% such as at least 98% or at least 99% homology to a mammalian PAPD5 target nucleic acid, such as a target nucleic acid selected from the group consisting of SEQ ID NO: 6 or 11.

- 5 Numerous single nucleotide polymorphisms are known in the PAPD5 or PAPD7 gene, for example those disclosed in Table 2A (human premRNA start/reference sequence is SEQ ID NO: 10) and Table 2B human premRNA start/reference sequence is SEQ ID NO: 11).

Table 2A: PAPD5 polymorphisms (naturally occurring variants)

minor allele	Minor allele frequency	Start on SEQ ID NO: 10
G	0,00399361	29
G	0,000199681	34
T	0,000399361	39
A	0,000599042	62
A	0,000599042	97
G	0,000199681	141
A	0,000199681	142
T	0,000199681	158
A	0,0241613	235
A	0,00239617	279
-	0,214058	370
G	0,000798722	450
CAGCA	0,000798722	603
A	0,0223642	1028
C	0,000199681	1044
A	0,0189696	1068
T	0,000199681	1181
T	0,0249601	1199
T	0,000998403	1258
A	0,000199681	1261
T	0,000599042	1441
T	0,000199681	1443
C	0,000599042	1469
A	0,000399361	1535

Table 2B: PAPD7 polymorphisms (naturally occurring variants)

minor allele	Minor allele frequency	Start on SEQ ID NO: 11
A	0,293331	21
T	0,00119808	50
T	0,000199681	64
A	0,00279553	127
A	0,0597045	224
G	0,000199681	234
T	0,000599042	270
A	0,128994	284
C	0,000399361	316
T	0,000199681	349
G	0,00778754	362
A	0,000199681	409

minor allele	Minor allele frequency	Start on SEQ ID NO: 11
G	0,000199681	425
A	0,000199681	448
T	0,000199681	473
C	0,000199681	491
C	0,327676	564
T	0,0203674	606
-	0,389577	837
-	0,00139776	1317
T	0,000599042	1331
T	0,000199681	1475
T	0,000399361	1483
C	0,01877	1673
A	0,000199681	1682
T	0,00339457	1726
GGTCCTGGCCGGCGCCCGC	0,258586	1736
G	0,000599042	1760
C	0,000199681	1777
G	0,000399361	1780
T	0,000199681	1852
T	0,000199681	1861
T	0,000199681	1889
C	0,000399361	1923
G	0,000399361	1962
T	0,0147764	1987
G	0,000998403	1996
T	0,000399361	2036

Modulation of expression

The term “modulation of expression” as used herein is to be understood as an overall term for a nucleic acid molecules ability to alter the amount of PAPD5 or PAPD7 when compared to the amount of PAPD5 or PAPD7 before administration of the nucleic acid molecule. Alternatively

5 modulation of expression may be determined by reference to a control experiment. It is generally understood that the control is an individual or target cell treated with a saline composition or an individual or target cell treated with a non-targeting or nucleic acid molecule (mock). It may however also be an individual treated with the standard of care.

One type of modulation is a nucleic acid molecules ability to inhibit, down-regulate, reduce,

10 suppress, remove, stop, block, prevent, lessen, lower, avoid or terminate expression of PAPD5 or PAPD7, e.g. by degradation of mRNA or blockage of transcription.

High affinity modified nucleosides

A high affinity modified nucleoside is a modified nucleotide which, when incorporated into the oligonucleotide enhances the affinity of the oligonucleotide for its complementary target, for

15 example as measured by the melting temperature (T^m). A high affinity modified nucleoside of the present invention preferably result in an increase in melting temperature between +0.5 to +12°C, more preferably between +1.5 to +10°C and most preferably between +3 to +8°C per modified nucleoside. Numerous high affinity modified nucleosides are known in the art and

include for example, many 2' substituted nucleosides as well as locked nucleic acids (LNA) (see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213).

Sugar modifications

- 5 The nucleic acid molecule 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 nucleic acid molecules, such as affinity and/or
10 nuclease resistance.

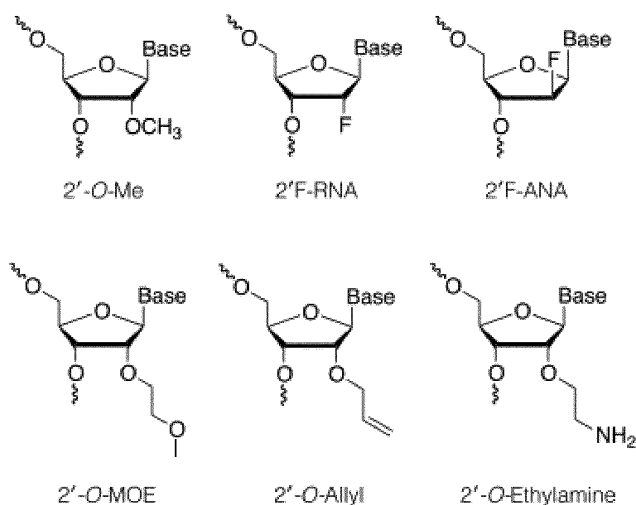
Such modifications include those where the ribose ring structure is modified, *e.g.* by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradicle bridge between the C2 and C4 carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (*e.g.* UNA). Other sugar modified
15 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
20 ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in DNA and RNA nucleosides. Substituents may, for example be introduced at the 2', 3', 4' or 5' positions. Nucleosides with modified sugar moieties also include 2' modified nucleosides, such as 2' substituted nucleosides. Indeed, much focus has been spent on developing 2' substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial
25 properties when incorporated into oligonucleotides, such as enhanced nucleoside resistance and enhanced affinity.

2' modified nucleosides.

A 2' sugar modified nucleoside is a nucleoside which has a substituent other than H or -OH at the 2' position (2' substituted nucleoside) or comprises a 2' linked biradicle, and includes 2'
30 substituted nucleosides and LNA (2' - 4' biradicle bridged) nucleosides. For example, the 2' modified sugar may provide enhanced binding affinity and/or increased nuclease resistance to the oligonucleotide. Examples of 2' substituted modified nucleosides are 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, and 2'-F-ANA nucleoside. For further examples, please see *e.g.* Freier & Altmann; Nucl. Acid
35 Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-

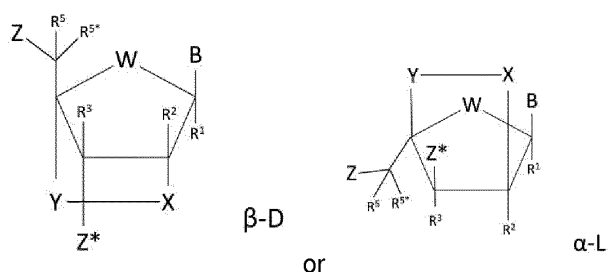
213, and Deleavey and Damha, Chemistry and Biology 2012, 19, 937. Below are illustrations of some 2' substituted modified nucleosides.



5 **Locked Nucleic Acid Nucleosides (LNA).**

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

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



Formula I

Formula II

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

15 Z designates an internucleoside linkage to an adjacent nucleoside, or a 5'-terminal group;
Z* designates an internucleoside linkage to an adjacent nucleoside, or a 3'-terminal group;
X designates a group selected from the list consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-,
-O-, -Si(R^a)₂-, -S-, -SO₂-, -N(R^a)-, and >C=Z

In some embodiments, X is selected from the group consisting of: -O-, -S-, NH-, NR^aR^b-, -CH₂-,
20 CR^aR^b-, -C(=CH₂)-, and -C(=CR^aR^b)-

In some embodiments, X is -O-

Y designates a group selected from the group consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)₂-, -S-, -SO₂-, -N(R^a)-, and >C=Z

In some embodiments, Y is selected from the group consisting of: -CH₂-, -C(R^aR^b)-, -CH₂CH₂-,
5 -C(R^aR^b)-C(R^aR^b)-, -CH₂CH₂CH₂-, -C(R^aR^b)C(R^aR^b)C(R^aR^b)-, -C(R^a)=C(R^b)-, and -C(R^a)=N-

In some embodiments, Y is selected from the group consisting of: -CH₂-, -CHR^a-, -CHCH₃-,
CR^aR^b-

or -X-Y- together designate a bivalent linker group (also referred to as a radicle) together
designate a bivalent linker group consisting of 1, 2, 3 or 4 groups/atoms selected from the group
10 consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)₂-, -S-, -SO₂-, -N(R^a)-, and >C=Z,

In some embodiments, -X-Y- designates a biradicle selected from the groups consisting of: -X-
CH₂-, -X-CR^aR^b-, -X-CHR^a-, -X-C(HCH₃)-, -O-Y-, -O-CH₂-, -S-CH₂-, -NH-CH₂-, -O-CHCH₃-, -CH₂-
O-CH₂-, -O-CH(CH₃CH₃)-, -O-CH₂-CH₂-, OCH₂-CH₂-CH₂-, -O-CH₂OCH₂-, -O-NCH₂-, -C(=CH₂)-
CH₂-, -NR^a-CH₂-, N-O-CH₂-, -S-CR^aR^b- and -S-CHR^a-.

15 In some embodiments -X-Y- designates -O-CH₂- or -O-CH(CH₃)-.

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

and R^a and, when present R^b, each is independently selected from hydrogen, optionally
substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkynyl,
hydroxy, optionally substituted C₁₋₆-alkoxy, C₂₋₆-alkoxyalkyl, C₂₋₆-alkenyloxy, carboxy, C₁₋₆-
20 alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl,
heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-
alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-
aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-
carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphonyl, C₁₋₆-alkylsulphonyloxy, nitro, azido,
25 sulphonyl, C₁₋₆-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted and
where two geminal substituents R^a and R^b together may designate optionally substituted
methylene (=CH₂), wherein for all chiral centers, asymmetric groups may be found in either *R* or
S orientation.

wherein R¹, R², R³, R⁵ and R^{5'} are independently selected from the group consisting of:
30 hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally
substituted C₂₋₆-alkynyl, hydroxy, C₁₋₆-alkoxy, C₂₋₆-alkoxyalkyl, C₂₋₆-alkenyloxy, carboxy, C₁₋₆-
alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl,
heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-
alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-
35 aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-

carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphonyloxy, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene.

- 5 In some embodiments R¹, R², R³, R⁵ and R^{5*} are independently selected from C₁₋₆ alkyl, such as methyl, and hydrogen.

In some embodiments R¹, R², R³, R⁵ and R^{5*} are all hydrogen.

In some embodiments R¹, R², R³, are all hydrogen, and either R⁵ and R^{5*} is also hydrogen and the other of R⁵ and R^{5*} is other than hydrogen, such as C₁₋₆ alkyl such as methyl.

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

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

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

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

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

In some embodiments, the biradicle -X-Y- is -O-CH₂-, W is O, and all of R¹, R², R³, R⁵ and R^{5*} are all hydrogen. Such LNA nucleosides are disclosed in WO99/014226, WO00/66604, WO98/039352 and WO2004/046160 which are all hereby incorporated by reference, and include what are commonly known as **beta-D-oxy LNA** and **alpha-L-oxy LNA** nucleosides.

- 20 In some embodiments, the biradicle -X-Y- is -S-CH₂-, W is O, and all of R¹, R², R³, R⁵ and R^{5*} are all hydrogen. Such **thio LNA** nucleosides are disclosed in WO99/014226 and WO2004/046160 which are hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -NH-CH₂-, W is O, and all of R¹, R², R³, R⁵ and R^{5*} are all hydrogen. Such **amino LNA** nucleosides are disclosed in WO99/014226 and

- 25 WO2004/046160 which are hereby incorporated by reference.

In some embodiments, the biradicle -X-Y- is -O-CH₂-CH₂- or -O-CH₂-CH₂-CH₂-, W is O, and all of R¹, R², R³, R⁵ and R^{5*} are all hydrogen. Such LNA nucleosides are disclosed in WO00/047599 and Morita et al, Bioorganic & Med.Chem. Lett. 12 73-76, which are hereby incorporated by reference, and include what are commonly known as 2'-O-4'C-ethylene bridged nucleic acids (ENA).

30

In some embodiments, the biradicle -X-Y- is -O-CH₂-, W is O, and all of R¹, R², R³, and one of R⁵ and R^{5*} are hydrogen, and the other of R⁵ and R^{5*} is other than hydrogen such as C₁₋₆ alkyl, such as methyl. Such **5' substituted LNA** nucleosides are disclosed in WO2007/134181 which is hereby incorporated by reference.

In some embodiments, the biradicle $-X-Y-$ is $-O-CR^aR^b-$, wherein one or both of R^a and R^b are other than hydrogen, such as methyl, W is O, and all of R^1 , R^2 , R^3 , and one of R^5 and R^{5*} are hydrogen, and the other of R^5 and R^{5*} is other than hydrogen such as C_{1-6} alkyl, such as methyl. Such **bis modified LNA nucleosides** are disclosed in WO2010/077578 which is hereby incorporated by reference.

In some embodiments, the biradicle $-X-Y-$ designate the bivalent linker group $-O-CH(CH_2OCH_3)-$ (2' O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem. Vol 75(5) pp. 1569-81). In some embodiments, the biradicle $-X-Y-$ designate the bivalent linker group $-O-CH(CH_2CH_3)-$ (2' O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem. Vol 75(5) pp. 1569-81). In some embodiments, the biradicle $-X-Y-$ is $-O-CHR^a-$, W is O, and all of R^1 , R^2 , R^3 , R^5 and R^{5*} are all hydrogen. Such **6' substituted LNA nucleosides** are disclosed in WO10036698 and WO07090071 which are both hereby incorporated by reference.

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

In some embodiments, the biradicle $-X-Y-$ designate the bivalent linker group $-O-CH(CH_3)-$. – in either the R- or S- configuration. In some embodiments, the biradicle $-X-Y-$ together designate the bivalent linker group $-O-CH_2-O-CH_2-$ (Seth at al., 2010, J. Org. Chem). In some embodiments, the biradicle $-X-Y-$ is $-O-CH(CH_3)-$, W is O, and all of R^1 , R^2 , R^3 , R^5 and R^{5*} are all hydrogen. Such **6' methyl LNA nucleosides** are also known as **cET nucleosides** in the art, and may be either (S)cET or (R)cET stereoisomers, as disclosed in WO07090071 (beta-D) and WO2010/036698 (alpha-L) which are both hereby incorporated by reference).

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

In some embodiments, the biradicle $-X-Y-$ is $-S-CHR^a-$, W is O, and all of R^1 , R^2 , R^3 , R^5 and R^{5*} are all hydrogen. Such **6' substituted thio LNA nucleosides** are disclosed in WO11156202 which is hereby incorporated by reference. In some 6' substituted thio LNA embodiments R^a is methyl.

In some embodiments, the biradicle $-X-Y-$ is $-C(=CH_2)-C(R^aR^b)-$, such as $-C(=CH_2)-CH_2-$, or $-C(=CH_2)-CH(CH_3)-$ W is O, and all of R^1 , R^2 , R^3 , R^5 and R^{5*} are all hydrogen. Such **vinyl carbo LNA nucleosides** are disclosed in WO08154401 and WO09067647 which are both hereby incorporated by reference.

In some embodiments the biradicle $-X-Y-$ is $-N(-OR^a)-$, W is O, and all of R^1 , R^2 , R^3 , R^5 and R^{5*} are all hydrogen. In some embodiments R^a is C_{1-6} alkyl such as methyl. Such LNA nucleosides

are also known as N substituted LNAs and are disclosed in WO2008/150729 which is hereby incorporated by reference. In some embodiments, the biradicle -X-Y- together designate the bivalent linker group -O-NR^a-CH₃- (Seth et al., 2010, J. Org. Chem). In some embodiments the biradicle -X-Y- is -N(R^a)-, W is O, and all of R¹, R², R³, R⁵ and R^{5'} are all hydrogen. In some
5 embodiments R^a is C₁₋₆ alkyl such as methyl.

In some embodiments, one or both of R⁵ and R^{5'} is hydrogen and, when substituted the other of R⁵ and R^{5'} is C₁₋₆ alkyl such as methyl. In such an embodiment, R¹, R², R³, may all be hydrogen, and the biradicle -X-Y- may be selected from -O-CH₂- or -O-C(HCR^a)-, such as -O-C(HCH₃)-.

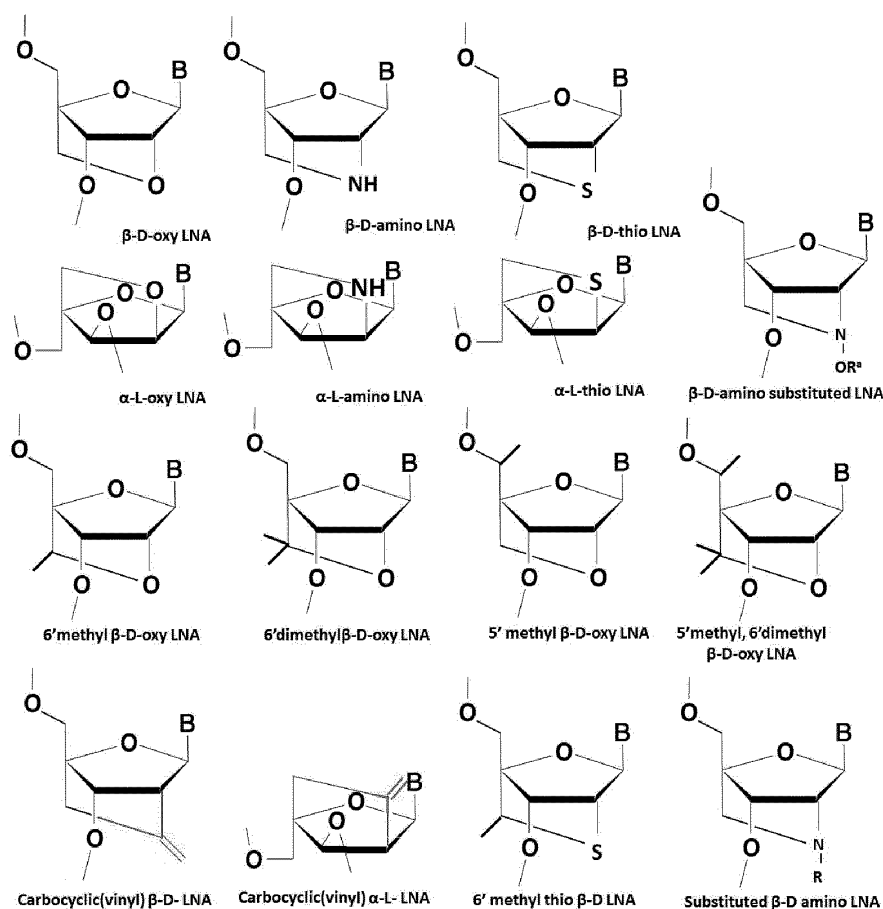
In some embodiments, the biradicle is -CR^aR^b-O-CR^aR^b-, such as CH₂-O-CH₂-, W is O and all
10 of R¹, R², R³, R⁵ and R^{5'} are all hydrogen. In some embodiments R^a is C₁₋₆ alkyl such as methyl. Such LNA nucleosides are also known as conformationally restricted nucleotides (CRNs) and are disclosed in WO2013036868 which is hereby incorporated by reference.

In some embodiments, the biradicle is -O-CR^aR^b-O-CR^aR^b-, such as O-CH₂-O-CH₂-, W is O and all of R¹, R², R³, R⁵ and R^{5'} are all hydrogen. In some embodiments R^a is C₁₋₆ alkyl such as
15 methyl. Such LNA nucleosides are also known as COC nucleotides and are disclosed in Mitsuoka et al., Nucleic Acids Research 2009 37(4), 1225-1238, which is hereby incorporated by reference.

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

20 Certain examples of LNA nucleosides are presented in Scheme 1.

Scheme 1



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

5 ***Nuclease mediated degradation***

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

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

RNase H Activity and Recruitment

The RNase H activity of an antisense oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule. WO01/23613 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH.

- 5 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
- 10 monomers in the oligonucleotide, and using the methodology provided by Example 91 - 95 of WO01/23613 (hereby incorporated by reference).

Gapmer

- The term gapmer as used herein refers to an antisense oligonucleotide which comprises a region of RNase H recruiting oligonucleotides (gap) which is flanked 5' and 3' by regions which
- 15 comprise one or more affinity enhancing modified nucleosides (flanks or wings). Various gapmer designs are described herein. Headmers and tailmers are oligonucleotides capable of recruiting RNase H where one of the flanks is missing, i.e. only one of the ends of the oligonucleotide comprises affinity enhancing modified nucleosides. For headmers the 3' flank is missing (i.e. the 5' flank comprises affinity enhancing modified nucleosides) and for tailmers the
- 20 5' flank is missing (i.e. the 3' flank comprises affinity enhancing modified nucleosides).

LNA Gapmer

The term LNA gapmer is a gapmer oligonucleotide wherein at least one of the affinity enhancing modified nucleosides is an LNA nucleoside.

Mixed Wing Gapmer

- 25 The term mixed wing gapmer or mixed flank gapmer refers to a LNA gapmer wherein at least one of the flank regions comprise at least one LNA nucleoside and at least one non-LNA modified nucleoside, such as at least one 2' substituted modified nucleoside, such as, for example, 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA and 2'-F-ANA nucleoside(s). In some embodiments the mixed wing
- 30 gapmer has one flank which comprises only LNA nucleosides (e.g. 5' or 3') and the other flank (3' or 5' respectively) comprises 2' substituted modified nucleoside(s) and optionally LNA nucleosides.

Conjugate

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

Conjugation of the oligonucleotide of the invention to one or more non-nucleotide moieties may improve the pharmacology of the oligonucleotide, e.g. by affecting the activity, cellular distribution, cellular uptake or stability of the oligonucleotide. In some embodiments the conjugate moiety modify or enhance the pharmacokinetic properties of the oligonucleotide by improving cellular distribution, bioavailability, metabolism, excretion, permeability, and/or cellular uptake of the oligonucleotide. In particular the conjugate may target the oligonucleotide to a specific organ, tissue or cell type and thereby enhance the effectiveness of the oligonucleotide in that organ, tissue or cell type. At the same time the conjugate may serve to reduce activity of the oligonucleotide in non-target cell types, tissues or organs, e.g. off target activity or activity in non-target cell types, tissues or organs. WO 93/07883 and WO2013/033230 provides suitable conjugate moieties, which are hereby incorporated by reference. Further suitable conjugate moieties are those capable of binding to the asialoglycoprotein receptor (ASGPr). In particular tri-valent N-acetylgalactosamine conjugate moieties are suitable for binding to the ASGPr, see for example WO 2014/076196, WO 2014/207232 and WO 2014/179620 (hereby incorporated by reference).

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

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

25 Conjugate 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 to an oligonucleotide (e.g. the termini of region A or C).

In some embodiments of the invention the conjugate or oligonucleotide conjugate of the invention may optionally, comprise a linker region which is positioned between the oligonucleotide and the conjugate moiety. In some embodiments, the linker between the conjugate and oligonucleotide is biocleavable.

Biocleavable linkers comprising or consisting of a physiologically labile bond that is cleavable under conditions normally encountered or analogous to those encountered within a mammalian body. Conditions under which physiologically labile linkers undergo chemical transformation

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

Conjugates may also be linked to the oligonucleotide via non biocleavable linkers, or in some embodiments the conjugate may comprise a non-cleavable linker which is covalently attached to the biocleavable linker. Linkers that are not necessarily biocleavable but primarily serve to covalently connect a conjugate moiety to an oligonucleotide or biocleavable linker. Such linkers may comprise a chain structure or an oligomer of repeating units such as ethylene glycol, amino acid units or amino alkyl groups. 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. Conjugate linker groups may be routinely attached to an oligonucleotide via use of an amino modified oligonucleotide, and an activated ester group on the conjugate group.

Treatment

The terms “treatment”, “treating”, “treats” or the like are used herein to generally mean 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, i.e. arresting its development like the inhibition of increase of HBsAg and/or HBeAg; or (b) ameliorating (i.e. relieving) the disease, i.e. causing regression of the disease, like the repression of HBsAg and/or HBeAg production. Thus, 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.

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
5 mothers are contemplated.

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
10 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.

HBV infection

15 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. Some infected persons have no symptoms during the initial infection and some develop a rapid onset of sickness with vomiting, yellowish skin, tiredness, dark urine and abdominal pain ("Hepatitis B Fact sheet N°204". *who.int*. July
20 2014. Retrieved 4 November 2014). Often these symptoms last a few weeks and can result in death. It may take 30 to 180 days for symptoms to begin. In those who get infected around the time of birth 90% develop a chronic hepatitis B infection while less than 10% of those infected after the age of five do ("Hepatitis B FAQs for the Public - Transmission", U.S. Centers for Disease Control and Prevention (CDC), retrieved 2011-11-29). Most of those with chronic
25 disease have no symptoms; however, cirrhosis and liver cancer may eventually develop (Chang, 2007, *Semin Fetal Neonatal Med*, 12: 160-167). These complications result in the death of 15 to 25% of those with chronic disease ("Hepatitis B Fact sheet N°204". *who.int*. July 2014, retrieved 4 November 2014). Herein, the term "HBV infection" includes the acute and chronic hepatitis B infection. The term "HBV infection" also includes the asymptomatic stage of the
30 initial infection, the symptomatic stages, as well as the asymptomatic chronic stage of the HBV infection.

Enzymatically active fragments

Herein, an enzymatically active fragment of SEQ ID NO: 1 or 2 (i.e. of PAPD5) relates to those polypeptides that comprise a stretch of contiguous amino acid residues of SEQ ID NO: 1 or 2
35 (i.e. of PAPD5) and that retain a biological activity (i.e. functionality) of PAPD5, particularly the poly-A polymerase function. In line with this, herein, an enzymatically active fragment of SEQ ID NO: 3 (i.e. of PAPD7) relates to those polypeptides that comprise a stretch of contiguous amino

acid residues of SEQ ID NO: 3 (i.e. of PAPD7) and that retain a biological activity (i.e. functionality) of PAPD7, particularly the poly-A polymerase function. Examples for enzymatically active fragments of PAPD5 and PAPD7 are the nucleotidyltransferase domain and the Cid1 poly A polymerase.

5 **Polypeptide**

Herein, term "polypeptide" includes all molecules that comprise or consist of amino acid monomers linked by peptide (amide) bonds. Thus, the term "polypeptide" comprises all amino acid sequences, such as peptides, oligopeptides, polypeptides and proteins. The "polypeptide" described herein may be a naturally occurring polypeptide or a non-naturally occurring polypeptide. The non-naturally occurring polypeptide may comprise at least one mutation (e.g. amino acid substitution, amino acid deletion or amino acid addition) as compared to the naturally occurring counterpart. The non-naturally occurring polypeptide may also be cloned in a vector and/or be operable linked to a promoter that is not the natural promoter of said polypeptide. Said promoter may be a constitutively active promoter. The term "amino acid" or
15 "residue" as used herein includes both L- and D-isomers of the naturally occurring amino acids as well as of other amino acids (e.g., non-naturally-occurring amino acids, amino acids which are not encoded by nucleic acid sequences, synthetic amino acids etc.). Examples of naturally-occurring amino acids are alanine (Ala; A), arginine (Arg; R), asparagine (Asn; N), aspartic acid (Asp; D), cysteine (Cys; C), glutamine (Gln; Q), glutamic acid (Glu; E), glycine (Gly; G), histidine
20 (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophane (Trp; W), tyrosine (Tyr; Y), valine (Val; V). Post-translationally modified naturally-occurring amino acids are dehydrobutyrine (Dhb) and labionin (Lab). Examples for non-naturally occurring amino acids are described above. The non-naturally occurring polypeptide may comprise one or more non-
25 amino acid substituents, or heterologous amino acid substituents, compared to the amino acid sequence of a naturally occurring form of the polypeptide, for example a reporter molecule or another ligand, covalently or non-covalently bound to the amino acid sequence.

Compound

Herein, the term "compound" means any nucleic acid molecule, such as RNAi molecules
30 according to the invention or any conjugate comprising such a nucleic acid molecule. For example, herein the compound may be an RNAi molecule against PAPD5 or PAPD7, in particular an antisense oligonucleotide, a siRNA or a shRNA.

Composition

The term "composition" may also be used to describe a nucleic acid molecule compound. A
35 nucleic acid molecule composition has less than 20% impurities, preferably less than 15% or 10% impurities, more preferably less than 9, 8, 7 or 6 % impurities, most preferably less than 5

% impurities. The impurities are typically nucleic acid molecules which are one or two nucleotides shorter (n-1 or n-2) than the primary nucleic acid molecule component.

Inhibitor

The term “inhibitor” is known in the art and relates to a compound/substance or composition capable of fully or partially preventing or reducing the physiologic function (i.e. the activity) of (a) specific protein(s) (e.g. of PAPD5 and/or PAPD7). In the context of the present invention, an “inhibitor” of PAPD5 or PAPD7 is capable of preventing or reducing the activity/function of PAPD5 or PAPD7, respectively, by preventing or reducing the expression of the PAPD5 or PAPD7 gene products. Thus, an inhibitor of PAPD5 or PAPD7 may lead to a decreased expression level of PAPD5 or PAPD7 (e.g. decreased level of PAPD5 or PAPD7 mRNA, or of PAPD5 or PAPD7 protein) which is reflected in a decreased functionality (i.e. activity) of PAPD5 or PAPD7, wherein said function comprises the poly-A polymerase function. An inhibitor of PAPD5 or PAPD7, in the context of the present invention, accordingly, may also encompass transcriptional repressors of PAPD5 or PAPD7 expression that are capable of reducing the level of PAPD5 or PAPD7. Preferred inhibitors are nucleic acid molecules of the invention.

Measuring

Herein, the term “measuring” also means “analyzing” or “determining” (i.e. detecting and/or quantifying). For example, the term “measuring the expression and/or activity of PAPD5 and/or PAPD7” means determining the amount of PAPD5 and/or PAPD7 expression and/or activity, for example, determining the amount of the PAPD5 and/or PAPD7 polypeptide (i.e. protein). Methods for measuring (i.e. determining) the amount and/or activity of PAPD5 and/or PAPD7 protein are known in the art and described herein above. In line with this, the term “measuring whether a test compound inhibits propagation of HBV” means analyzing or determining (i.e. detecting and/or quantifying) whether a test compound or composition inhibits propagation of HBV.

The present invention is further described by reference to the non-limiting figures and examples.

EXAMPLES

The Examples illustrate the invention.

Material and Methods

Oligonucleotide motif sequences and oligonucleotide compounds

Table 3: list of oligonucleotide motif sequences (indicated by SEQ ID NO) targeting the human PAPD5 transcript (SEQ ID NO: 10), designs of these, as well as specific antisense oligonucleotide compounds (indicated by CMP ID NO) designed based on the motif sequence.

SEQ ID NO	Motif Sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 10	dG
12	agggtagatgtgtttaact	2-14-3	AGggtagatgtgtttaACT	12_1	1656	-22

SEQ ID NO	Motif Sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 10	dG
13	cagcctaaacttagtgg	3-12-2	CAGcctaaacttagtGG	13_1	2000	-21
14	aagccctcaatgtaaaacac	2-14-4	AAGccctcaatgtaaaACAC	14_1	2446	-22
15	aatagcaagtagaggagag	3-13-3	AATagcaagtagaggagAG	15_1	3059	-21
16	aaataaggatactggcga	2-12-4	AAataaggatactgGCGA	16_1	4036	-21
17	gaggggaacacataataaaag	4-14-2	GAGGgaacacataataaaAG	17_1	4484	-20
18	gtaatacctctcacattc	2-12-4	GTaatacctctcacATTc	18_1	5928	-21
19	agtaacaccaatctcattg	2-13-4	AGtaacaccaatctcATTG	19_1	6652	-21
20	gtgacagtattcaatgatc	2-13-4	GTgacagtattcaatGATC	20_1	7330	-22
21	cagttccgtatcaccaac	2-13-3	CAGttccgtatcaccAAC	21_1	7702	-22
22	aagtctaactcaaaagccatc	2-15-3	AAGtctaactcaaaagccATC	22_1	8292	-21
23	aggcttccattttattgaa	2-14-3	AGgcttccattttattGAA	23_1	8625	-22
24	ttttagaaaacgaggcta	2-12-4	TTttagaaaacgagGCTA	24_1	9866	-20
25	gtattcttattctgtct	3-12-2	GTAttcttattctgtCT	25_1	10254	-20
26	attattcccacagtaaga	4-12-2	ATTAttcccacagtaaGA	26_1	10881	-22
27	aacaacaaacaggatgggc	3-14-2	AACaacaacaggatggGC	27_1	11370	-21
28	atatccacaatattctgat	4-13-2	ATATccacaatattctgAT	28_1	11790	-20
29	aaagaaataatgtcgtctgg	2-14-4	AAagaaataatgtcgtCTGG	29_1	12413	-20
30	ccagagtaaaccaaatcc	3-11-3	CCAgagtaaaccaaaTCC	30_1	12718	-21
31	attcaacatttttagtcacc	2-15-3	ATtcaacatttttagtcACC	31_1	13555	-21
32	tttgtaattcttttttag	3-14-3	TTTgtaattctttttTAG	32_1	14297	-20
33	caatgaggaaacaagagtca	2-14-4	CAatgaggaaacaagaGTCA	33_1	15137	-22
34	ttcaaaataatgtgggaggt	3-14-3	TTcaaaataatgtgggaGGT	34_1	15695	-22
35	ggatatttgatacggcaaat	2-15-3	GGatatttgatacggcaAAT	35_1	16145	-20
36	ctataagaagcaaaccc	3-11-3	CTAtaagaagcaaaCCC	36_1	16714	-21
37	atataattcacgtttcactt	3-14-3	ATAtaattcacgtttcaCTT	37_1	17097	-21
38	aatgattcacatgaaggtta	3-14-3	AATgattcacatgaaggTTA	38_1	17420	-20
39	gttaggattttgctatg	2-11-4	GTtaggattttgcTATG	39_1	18299	-20
40	gtacaaaatacaaccgtat	3-13-3	GTAcaaaatacaaccgTAT	40_1	18669	-21
41	cacactatttcaagatgcta	3-15-2	CACactatttcaagatgcTA	41_1	19681	-22
42	cacctatacaatggagtatt	3-14-3	CACctatacaatggagtATT	42_1	20352	-22
43	atcatacgtcattagagaac	2-14-4	ATcatacgtcattagaGAAC	43_1	20721	-21
44	cagaacagatactttgcca	2-15-2	CAGaacagatactttgcCA	44_1	21111	-22
45	aagaatgggtgggtaagg	2-14-3	AAGaatgggtgggtaaGGG	45_1	21782	-21
46	agaattggtaactggactg	4-14-2	AGAAttggtaactggacTG	46_1	22378	-22
47	agaattatattggctgg	3-11-3	AGAAttatattggcTGG	47_1	23160	-20
48	cctaaaccagacagaaaaga	2-15-3	CCtaaaccagacagaaaAGA	48_1	23993	-22
49	accaattagagcagaaatc	4-13-2	ACCAattagagcagaaaTC	49_1	24813	-21
50	ttctaaataacagatgggtc	3-14-3	TTctaaataacagatggGTC	50_1	25047	-21
51	tttataatttttccatct	3-14-3	TTTataatttttccaTCT	51_1	26080	-20
52	gcaaatatcagattaacctc	4-14-2	GCAAatcagattaaccTC	52_1	26625	-22
53	aacgggtatggcagaagacaa	3-14-3	AACgggtatggcagaagaCAA	53_1	26973	-22
54	ttcaacctttactgcat	4-11-2	TTCAacctttactgcAT	54_1	27813	-20
55	actgataaagggcatttcaa	2-14-4	ACTgataaagggcattTCAA	55_1	28357	-22
56	cagtaggaatgtggctt	2-12-3	CAGtaggaatgtggCTT	56_1	28718	-21
57	ttttatggcagggtttcac	3-14-2	TTTatggcagggtttcAC	57_1	29327	-21
58	tcactgttaaacctcac	2-11-4	TCactgttaaaccTCAC	58_1	29902	-20
59	caattttctaattcaatggt	4-14-2	CAATTttctaattcaatgGT	59_1	30704	-20
60	aagatataattcaccact	4-13-2	AAGAtataattcaccacCT	60_1	31008	-22
61	gccacataaaggataaagt	2-13-4	GCCacataaaggataAAGT	61_1	31348	-22
62	cccattagaagtaagggtga	2-15-2	CCcattagaagtaagggtGA	62_1	32367	-22
63	atgtaaaataaaactccc	2-13-4	ATgtaaaataaaactTCCC	63_1	32632	-21
64	tgagagcataaaaagtacgga	3-15-2	TGAgagcataaaaagtacgGA	64_1	32945	-22

SEQ ID NO	Motif Sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 10	dG
65	ttcacaacaggtaaaggg	4-12-2	TTCAcaacaggtaaagGG	65_1	33593	-21
66	tgcatccctaagtaacataa	2-14-4	TGcattccctaagtaacATAA	66_1	34801	-21
67	agagaaaagtgtatgagggaa	3-14-3	AGAgaaaagtgtatgaggGAA	67_1	35368	-22
68	atacggatcaccagctaaa	3-14-2	ATAcggatcaccagctaaAA	68_1	36131	-21
69	catgttatgcacagaagat	2-13-4	CAtgttatgcacagaAGAT	69_1	36712	-22
70	cgctgaagaactaagtatta	2-14-4	CGctgaagaactaagtATTA	70_1	37282	-20
71	caaacagatggtggtgata	3-14-2	CAAcagatggtggtgaTA	71_1	37870	-20
72	agtagccattaggatg	3-11-2	AGTagccattaggatG	72_1	38478	-20
73	atacacaggctccataata	2-14-3	ATacacaggctccataATA	73_1	39639	-22
74	gattttgtatagtcacaa	3-15-2	GATtttgtatagtcacAA	74_1	40178	-21
75	gcactataaaaaagggaca	2-14-4	GCactataaaaaaggGACA	75_1	41042	-22
76	agtgcagatcgcct	2-10-3	AGtgcaagatcGCT	76_1	41734	-20
77	ccaaaagaatcaagttcgta	3-15-2	CCAaaagaatcaagttcgTA	77_1	42442	-21
78	cctcagaccaaatttattt	2-13-4	CCtcagaccaaatttATTT	78_1	43203	-22
79	ttaacaagcatctattgta	3-14-3	TTCaacaagcatctattGTA	79_1	43663	-22
80	caaaggtgtgtgactct	3-12-3	CAAaggtgtgtgactTCT	80_1	44220	-21
81	tcataaatcttttccacg	3-14-2	TCAtaaatcttttccaCG	81_1	44756	-20
82	ctgttacggatttaattgtg	2-15-3	CTgttacggatttaattGTG	82_1	45042	-21
83	gctataaaaatagaagcc	3-12-3	GCTataaaaatagaGCC	83_1	46202	-22
84	tccttagcaaaactaaacat	3-13-3	TCCttagcaaaactaaaCAT	84_1	47142	-22
85	agcaaaaggcagggtattcaa	3-15-2	AGCaaaaggcagggtattcAA	85_1	47843	-22
86	gaatccatttacatattcac	3-14-3	GAatccatttacatattCAC	86_1	48267	-21
87	tccagtatccaaaacatac	2-13-4	TCcagtatccaaaacATAC	87_1	49256	-21
88	agcttaagaagaacggtt	4-13-2	AGCTaaagaagaacggTT	88_1	49688	-22
89	cacaacgtgcctacctt	2-13-2	CAcaacgtgcctaccTT	89_1	50508	-22
90	ccagaatccaagaaaatgg	3-14-2	CCAgaatccaagaaaatGG	90_1	50764	-21
91	tcacctgaactaacaagt	2-16-2	TCacctgaactaacaagGT	91_1	51561	-21
92	gtatctttctgtactatt	2-12-4	GTatctttctgtactTATT	92_1	52461	-21
93	gtcatttactaacaacacg	4-13-2	GTCAttactaacaacacCG	93_1	53305	-21
94	tggaaaaggagaaccatt	2-13-4	TGgaaaaggagaaccATT	94_1	53865	-20
95	aatacaactctccgtgat	2-13-4	AAtacaactctccgTGAT	95_1	54638	-22
96	aataccctgacgagctg	2-12-3	AAtaccctgacgagCTG	96_1	54942	-22
97	tcataaacatgatccttgc	3-15-2	TCAtaaaacatgatccttGC	97_1	55741	-22
98	ctaagcagatccatagaa	3-13-3	CTAaagcagatccataGAA	98_1	56277	-21
99	agactataacttttgctaca	3-15-2	AGActataacttttgctaCA	99_1	56942	-22
100	agcaatgacttgaacatagt	2-16-2	AGcaatgacttgaacataGT	100_1	57369	-20
101	ataaaacaagcatacgggc	3-14-2	ATAaaacaagcatacggGC	101_1	58146	-21
102	atgagataccagcagatag	2-15-2	ATgagataccagcagatAG	102_1	58588	-20
103	agaagaatcctgagtaatc	4-14-2	AGAagaatcctgagtaaTC	103_1	59089	-21
104	ccctaaaaagtgcagta	3-12-2	CCctaaaaagtgcagTA	104_1	59461	-21
105	ttaagttagatcacggc	4-11-2	TTAAgttagatcacgGC	105_1	59970	-20
106	gtggatacagaaagcca	2-12-3	GTggatacagaaagCCA	106_1	60738	-22
107	gtatcggcaggagatt	3-10-3	GTAtcggcaggagATT	107_1	61038	-21
108	taactaattgattccattgc	4-14-2	TAACTaattgattccattGC	108_1	61868	-22
109	agaagaacggaaattgcc	3-13-2	AGAagaacggaaattgCC	109_1	62418	-21
110	ataatgattttcctatcc	4-12-2	ATAAtgattttcctatCC	110_1	62822	-20
111	atggttttgggagaagg	2-13-3	ATggttttgggagaAGG	111_1	63000	-22
112	tgctgctgtgaaagaatg	2-14-4	TGctgctgtgaaagaAATG	112_1	63697	-21
113	gtgtccaattttttattat	2-14-3	GTgtccaattttttTAT	113_1	64377	-20
114	gatggaatcaactgtgtagt	2-16-2	GATggaatcaactgtgtgAT	114_1	65307	-21
115	gatggtgacaaattattct	4-13-2	GATGgtgacaaattattCT	115_1	65894	-22
116	tgcttttgggaatcttt	2-11-4	TGcttttgggaatCTTT	116_1	66650	-21

SEQ ID NO	Motif Sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 10	dG
117	gatgtcctacaatgaacacg	2-15-3	GAtgtcctacaatgaacACG	117_1	68024	-22
118	gtacaaggacaaagtaacc	3-13-3	GTAcaggacaaagtaACC	118_1	68732	-22
119	tgaacgcctatctcta	4-11-2	TGAACgcctatctcTA	119_1	69029	-21
120	atactattatgcttatgga	3-15-2	ATActattatgcttatgGA	120_1	69796	-21
121	ttgtaatcaaggcaataagg	3-14-3	TTGtaatcaaggcaataAGG	121_1	70770	-21
122	gaagtccaataacgcaga	4-12-2	GAAGtccaataacgcGA	122_1	71091	-22
123	atatccaatctctatatgtg	3-15-2	ATAtccaatctctatatgTG	123_1	72013	-21
124	gccttacacaagactatatt	2-15-3	GCcttacacaagactatATT	124_1	73444	-22
125	tgctgaatttatgttaac	4-13-2	TGCTgaatttatgttaAC	125_1	73823	-21
126	cacaagatgatgggttaag	2-14-4	CAcaagatgatgggtTAAG	126_1	74559	-22
127	ttagtgtttgggtgc	2-12-2	TTagtgtttgggtGC	127_1	75043	-20
128	agattgttacctactgat	2-14-3	AGattgttacctactGAT	128_1	76110	-21
129	tattacaaatatcaatctcc	3-14-3	TATtacaatatcaatcTCC	129_1	76931	-21
130	taccaaagcatagagtgg	3-14-2	TACcaaagcatagagtGG	130_1	77605	-21
131	aattatcttcccgctac	2-11-4	AAttatcttcccgCTAC	131_1	78652	-22

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

Designs refer to the gapmer design, F-G-F', where each number represents the number of consecutive modified nucleosides, e.g. 2' modified nucleosides (first number=5' flank), followed by the number of DNA nucleosides (second number= gap region), followed by the number of modified nucleosides, e.g. 2'

- 5 modified nucleosides (third number=3' flank), optionally preceded by or followed by further repeated regions of DNA and LNA, which are not necessarily part of the contiguous sequence that is complementary to the target nucleic acid.

Table 4: list of oligonucleotide motif sequences (indicated by SEQ ID NO) targeting the human PAPD7 transcript (SEQ ID NO: 11), designs of these, as well as specific antisense

- 10 oligonucleotide compounds (indicated by CMP ID NO) designed based on the motif sequence.

SEQ ID NO	motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 11	dG
132	gttgggtggaataggca	2-13-2	GTtgggtggaataggCA	132_1	132	-22
133	tcgatttcccggtccaa	2-13-2	TCgatttcccggtccAA	133_1	1962	-21
134	acaacctacacataaattgc	3-15-2	ACAacctacacataaattGC	134_1	2510	-21
135	actataagaactccaaca	2-13-4	ACtataagaactcccAACA	135_1	2668	-21
136	gagaaaaagagtacaagc	4-13-2	GAGAaaaaagagtacaaGC	136_1	2695	-20
137	aactggaggaggagagaagag	4-13-2	AACtggaggaggagagaagAG	137_1	2883	-22
138	tttctaagagcagaggtaca	2-15-3	TTtctaagagcagaggtACA	138_1	3090	-22
139	agaagtaacaagagcct	4-11-2	AGAagtaacaagagcCT	139_1	3463	-20
140	agtatcaaaccagacctc	2-13-3	AGtatcaaaccagacCTC	140_1	3795	-22
141	ccacaaccgaaagactt	4-11-2	CCACaaccgaaagacTT	141_1	4205	-22
142	aatacacactgcattttca	2-13-4	AAtacacactgcattTTCA	142_1	4336	-20
143	ccaggtagatagcacag	2-13-2	CCaggtagatagcacAG	143_1	4686	-21
144	ccatgacaaagtaacaacag	2-14-4	CCatgacaaagtaacaACAG	144_1	4821	-22
145	cagaatttcctttgagtta	2-14-3	CAgaatttcctttgagTTA	145_1	5134	-21
146	ccttcgcaagaagaattga	2-16-2	CCttcgcaagaagaattGA	146_1	5263	-21
147	tcatacatcacgcttct	2-14-2	TCatacatcacgcttCT	147_1	5577	-20
148	tgcgaaaagattggagg	2-11-4	TGcgaaaagattgGAGG	148_1	5945	-21
149	cacaggacgcttacatgaat	2-16-2	CACaggacgcttacatgaAT	149_1	6235	-22
150	gctgtttttttcttaac	3-14-2	GCTgtttttttcttaAC	150_1	6352	-21
151	accataagttagtgttctt	2-14-3	ACcataagttagtgttCTT	151_1	6834	-22

SEQ ID NO	motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 11	dG
152	acacaagcccatagaaacag	2-16-2	ACacaagcccatagaaacAG	152_1	7158	-21
153	cagtagtaaccaccaag	2-11-4	CAGtagtaaccacCAAG	153_1	7447	-22
154	cctgcaaacttttattat	2-14-3	CCtgcaaacttttattTAT	154_1	7708	-21
155	acttagtaatagcagca	2-12-3	ACttagtaatagcaGCA	155_1	8074	-20
156	atgaatactccgaagactt	2-13-4	ATgaatactccgaagACTT	156_1	8249	-21
157	aaagaaaaggatcacaaGCC	3-14-3	AAAgaaaaggatcacaaGCC	157_1	8784	-22
158	agacagaaatcacctaaca	3-14-2	AGAcagaaatcacctaaCA	158_1	8887	-20
159	tagaacagacattattcatc	3-14-3	TAGaacagacattattcATC	159_1	9506	-20
160	agttacacggagcagcac	2-14-2	AGttacacggagcagcAC	160_1	9664	-22
161	cactatacacagaacactat	3-14-3	CACtatacacagaacacTAT	161_1	9770	-22
162	agctgtctaatacatgg	2-12-4	AGctgtctaataacATGG	162_1	10000	-21
163	atgaacctattttatgcttc	3-14-3	ATGaacctattttatgcTTC	163_1	10206	-22
164	accatcattaacctgcgt	2-14-2	ACcatcattaacctgcGT	164_1	10318	-22
165	agtaaagtgccagatgt	2-14-2	AGtaaagtgccagatGT	165_1	10568	-22
166	ttccctatgaaatcctcaa	3-14-2	TTCcctatgaaatcctcAA	166_1	10781	-21
167	cactctcatagaatgcaac	2-14-4	CACtctcatagaatgCAAC	167_1	10917	-22
168	aatgcttaattttctctct	2-14-4	AATgcttaattttctCTCT	168_1	11084	-22
169	ttagagacgatgcctataac	3-14-3	TTAgagacgatgcctatAAC	169_1	11308	-22
170	tgaatagtcccatagatt	4-13-2	TGAatagtcccatagaTT	170_1	11585	-21
171	cagcataattgttttcttt	3-13-3	CAGcataattgtttcTTT	171_1	12330	-21
172	atgtcattatgttttagtt	4-13-2	ATGTcattatgttttagTT	172_1	12634	-21
173	cagcagtatctcttagaa	2-13-3	CAGcagtatctcttaGAA	173_1	12902	-21
174	cggtaagggttcgggtg	2-12-2	CGgtaagggttcggTG	174_1	13126	-21
175	catgaaccacattaggaac	4-13-2	CATGaaccacattaggaAC	175_1	13383	-21
176	cattcaacacacacgacaa	2-13-4	CAttcaacacacacgACAA	176_1	13578	-21
177	aagtatccaagactcaaga	2-13-4	AAgtatccaagactcAAGA	177_1	13889	-20
178	ccacagaaacaccgag	4-10-2	CCACagaaacaccgAG	178_1	14100	-22
179	tggaaaaggggaagggaga	3-14-2	TGGaaaaggggaagggaaGA	179_1	14179	-21
180	agagagtcggaagcctg	2-13-2	AGagagtcggaagccTG	180_1	14432	-22
181	atgggaaaggtaacgagc	3-13-2	ATGggaaaggtaacgaGC	181_1	14616	-22
182	ctatcctacaagtcgga	3-13-2	CTAtcctacaagtcgAA	182_1	15471	-22
183	cattgctttataatccta	4-13-2	CATTgctttataatccTA	183_1	15816	-22
184	cttttaaggacaggagg	4-12-2	CTTTtaaggacaggaGG	184_1	15988	-21
185	gatgaaagataagtgaagcat	2-14-4	GATgaaagataagtgaGCAT	185_1	16395	-22
186	gaagcctgtaataattaagc	2-14-4	GAagcctgtaataattAAGC	186_1	17007	-22
187	caccctagtaaagcaaac	4-12-2	CACCctagtaaagcaaAC	187_1	17151	-22
188	gcaaataagccttttt	3-13-2	GCAaataagcctttTT	188_1	17303	-21
189	acctgacagctaccgac	2-13-2	ACctgacagctaccgAC	189_1	17498	-22
190	aagagtgggttgtaagc	2-12-3	AAgagtgggttgtaAGC	190_1	17963	-20
191	tagtgaataatttgaggtt	2-14-4	TAgtagaataatttgAGTT	191_1	18101	-20
192	tttcagcaccttaaaccc	2-14-2	TTtcagcaccttaaacCC	192_1	18518	-22
193	ttaagggaaggaacacgtca	4-14-2	TTAagggaaggaacacgtCA	193_1	18747	-21
194	gtaggttaagggaacaggaa	3-15-2	GTAaggttaagggaacaggAA	194_1	19007	-22
195	gtgaataaagccaagc	2-12-4	GTgaataaagccaAAGC	195_1	19252	-21
196	tgtttttgtatttagtat	3-13-3	TGTttttgtatttagTAT	196_1	19476	-20
197	gagggttttttagtgaatt	2-14-4	GAggttttttagtgAATT	197_1	19722	-21
198	gaggagctaaacggaca	3-12-2	GAGgagctaaacggCA	198_1	20062	-22
199	gtttagtcttatgttctac	2-16-2	GTttagtcttatgttctAC	199_1	20623	-21
200	caaatactgaatatgcccg	2-14-3	CAaatactgaatatgcCCG	200_1	20726	-22
201	accatttaaatcgccaac	3-12-3	ACCatttaaatcgccAAC	201_1	20926	-21
202	cagtaagagtagcccaacaa	2-16-2	CAGtaagagtagcccaacAA	202_1	21234	-22
203	taacggcaacatcaaatagc	4-14-2	TAACggcaacatcaaataGC	203_1	22017	-22

SEQ ID NO	motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 11	dG
204	actgagcaccaactacac	2-13-3	ACtgagcaccaactaCAC	204_1	22592	-22
205	cctaattttatgtatcacat	2-14-4	CCtaattttatgtatcACAT	205_1	23074	-22
206	ctaggaattataacaaatca	4-14-2	CTAGgaattataacaaatCA	206_1	23462, 23509	-20
207	actccaaagaacatactcac	2-14-4	ACtccaaagaacatacTCAC	207_1	24628	-22
208	taagagaaacaatcacacca	3-14-3	TAAgagaaacaatcacaCCA	208_1	24935	-22
209	tgtcctaaaatatcagcag	2-14-3	TGtcctaaaatatcagCAG	209_1	26022	-21
210	cttttaatgagacagtgc	2-15-2	CTtttaatgagacagtCA	210_1	26212	-20
211	ttgtagcataagatggaaag	4-14-2	TTGTtagcataagatggaaAG	211_1	26500	-21
212	aaactgtagccaataactgt	3-14-3	AAActgtagccaataacTGT	212_1	26945	-21
213	attcatcctaacacaagtag	2-15-3	ATtcatcctaacacaagTAG	213_1	27117	-22
214	cacgaagggaacagctaag	4-13-2	CACGaaagggaacagctaAG	214_1	27264	-21
215	acaacagggaagtagcc	4-10-2	ACAacagggaagtagCC	215_1	27411	-20
216	gctaacactataaggat	3-12-3	GCTaaacactataagGAT	216_1	27505	-21
217	cccgttaagcatttgagaa	2-14-2	CCcgttaagcatttgagAA	217_1	27831	-21
218	agccaatatgcgacagtaac	2-16-2	AGccaatatgcgacagtaAC	218_1	28146	-22
219	taacccaaacaatcagtgct	3-14-3	TAacccaaacaatcagtGTC	219_1	28777	-20
220	aacagggaacaggagtta	3-12-3	AACagggaacaggagTTA	220_1	29195	-20
221	atttatcaacttcacc	2-11-4	ATttatcaacttcCACC	221_1	29906	-22
222	cgtttaagaccaggcac	4-11-2	CGTTtaagaccaggcAC	222_1	30020	-22
223	acaaagggaactcaggaagag	3-15-2	ACaaagggaactcaggaagAG	223_1	30424	-20
224	tcacagacaagcacc	4-11-2	TCACagacaagcaccAA	224_1	31150	-20
225	tacttttaaaacacgtagg	4-14-2	TACTtttaaaacacgtaGG	225_1	31329	-21
226	gcacaatcacaaagaccaa	4-13-2	GCACaatcacaaagaccAA	226_1	31531	-22
227	tcagtaagaacagaggc	2-13-3	TCagtaagaacagaGGC	227_1	31820	-20
228	catattccaccacacaag	2-15-2	CAtattccaccacacaAG	228_1	32222	-21
229	agtaaacactgtcca	2-11-3	AGtaaacactgtCCA	229_1	32601	-21
230	tcctcttggcgatata	2-12-3	TCctcttggcgatATA	230_1	33337	-21
231	cataaataccctgaatac	2-14-3	CAtaaataccctgaaTAC	231_1	33986	-20
232	cgaatttatcacc	4-11-2	CGATtttatcaccCA	232_1	34175	-20
233	acaatcaggttaagtgtgga	2-16-2	ACaatcaggttaagtgtGA	233_1	34771	-21
234	gaagccaaagactacca	3-12-2	GAagccaaagactacCA	234_1	35096	-20
235	tggtagggactgaatttaa	2-15-3	TGgttagggactgaattTAA	235_1	35850	-21
236	cggtagtcaatcacc	2-10-3	CGgtagtcaatcACC	236_1	36584	-20
237	ctatcaaaattattcacct	2-14-4	CTatcaaaattattcACCT	237_1	36886	-22
238	acgaaaatttagcatcctaa	3-14-3	ACGaaaatttagcatccTAA	238_1	37041	-20
239	tggtaaacactgggc	2-11-2	TGgtaaacactggGC	239_1	38059	-19
240	gattgttggtgtcatg	3-11-3	GATgttggtgtcATG	240_1	38173	-21
241	aattataccccacattca	3-14-2	AATataccccacattCA	241_1	38806	-22
242	tgcaattagacacgttacg	2-13-4	TGcaattagacacgtTACG	242_1	39004	-22
243	ggcaaccaattaacta	4-11-2	GGCAaccaattaacTA	243_1	40226	-21
244	cagttttatgctaataca	4-11-2	CAGTtttatgctaataCA	244_1	40272	-20
245	gcaaaggaggagcggaataa	2-16-2	GCaaaggaggagcggaatAA	245_1	40707	-21
246	aaacagaagtaagaaggcc	2-14-4	AAacagaagtaagaagGTCC	246_1	41156	-22
247	tccactatccatagaaa	2-12-4	TCcactatccataGAAA	247_1	41477	-20
248	gctttgacgaacaggaaat	2-14-3	GCTttgacgaacaggAAT	248_1	42282	-21
249	ttccgacccaaagaaaagac	4-14-2	TTCCgacccaaagaaaagAC	249_1	42632	-21
250	gcgacacgatccgttaaa	3-13-2	GCGacacgatccgttaAA	250_1	43104	-22
251	ccccgttttaaaaac	4-9-2	CCCCgttttaaaaAC	251_1	43334	-20

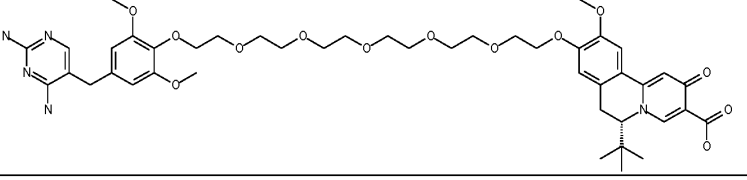
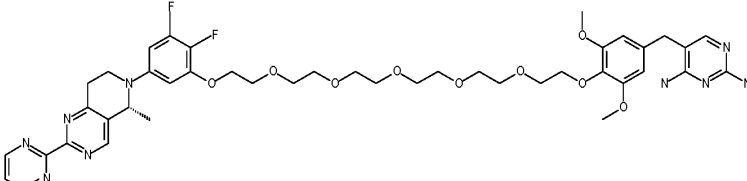
Motif sequences represent the contiguous sequence of nucleobases present in the oligonucleotide.

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

Compound chemistry

- Each one compound from the two chemical series DHQ and THP were synthesized to be suitable for the Y3H screening performed by HYBRIGENICS SERVICES SAS. Both compounds included PEG5 linker and were tagged with a Trimethoprim (TMP) anchor ligand (Table 5).

Table 5: TMP-tagged small molecule compound IDs

	Hybrigenics ID	Structure
DHQ compound -TMP	HBX129653	
THP compound -TMP	HBX129654	

Y3H ULTimate YChemH™ Screen

- The two compounds were provided by Roche to HYBRIGENICS SERVICES SAS and tested for permeability and toxicity. Compounds were then screened against HYBRIGENICS's cDNA Human placenta library (PLA). The screens were carried out according to the optimized cell-to-cell mating protocol developed for Hybrigenics ULTimate Y2H™ using at different compound concentration (Table 6).

Table 6: YChemH screens IDs

	Hybrigenics ID	YChemH Project	YChemH screen Project ID	Probe concentration
DHQ compound	HBX129653	hgx4240	PLA_RP6_hgx4240v1_pB409_A	5μM
THP compound	HBX129654	hgx4241	PLA_RP6_hgx4241v1_pB409_A	10μM

Y3H ULTimate YChemH™ Dependency Assay

- Clones obtained from the screen were picked in 96-well format and clones positive for growth under selective conditions (HIS+) were evaluated in a dependency assay using spot assays. Only clones that were able to grow on selective medium in the presence of the tagged

compound were being picked up, processed (cell lysis, PCR, gene sequencing) and mapped for protein alignment using Blast analysis.

Y3H ULTImate YChemH™ 1-by-1 validation experiment – Prey fragments

In this validation step each one identified fragment prey and one chemical probe (HBX129653, HBX129654) is tested in a 1-by-1 experiment. The plasmids from 3 selected preys from the screening library were extracted from the yeast cells, amplified in *E. coli* and re-transformed into YHGX13 yeast cells. For each interaction, DO1, 1/10, 1/100 and 1/1000 of the diploid yeast culture expressing both hook and prey constructs were spotted on a selective medium without tryptophan, leucine and histidine and supplemented with the chemical probe and FK506. Interactions were tested in duplicate. One plate was used per chemical compound and concentration (DMSO, 5, 10 and 20 μ M of HBX129653, 5, 10 and 20 μ M of HBX129654, 5 μ M of HBX24786 Trimethoprim (TMP) and 5 μ M of HBX129634 (TMP-PEG5-OH)). Plates were incubated at 30°C for 3 days.

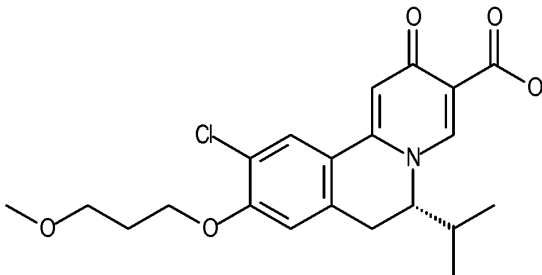
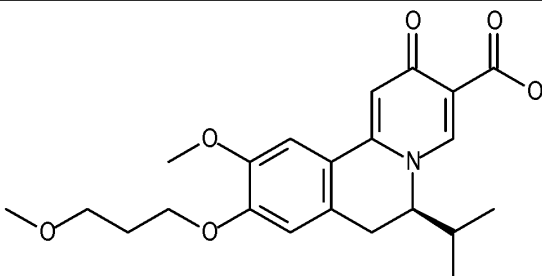
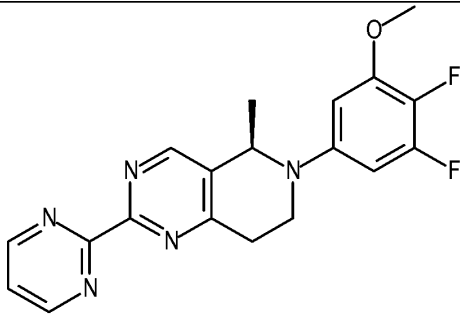
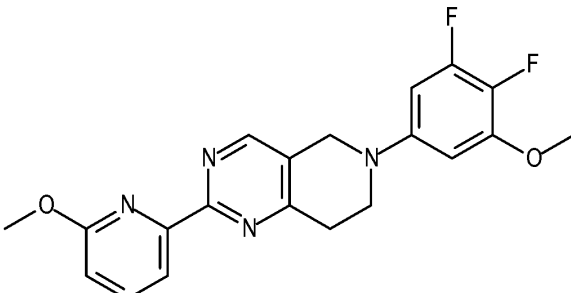
Y3H ULTImate YChemH™ 1-by-1 validation experiment – Full length proteins

The coding sequence of full-length PAPD5var1 (NM_001040284.2) and PAPD7varX1 (XM_005248234.2) were reconstituted from an N-terminal codon-optimized gene fragment (to remove high GC content) and commercially available clones of the C-terminal regions of the proteins and cloned in frame with the Gal4 Activation Domain (AD) into plasmid pP7 (AD-Prey), derived from the original pGADGH (Bartel et al., 1993 in Cellular interactions in development: A practical approach. ed. Hartley, D.A., Oxford University Press, Oxford, pp. 153-179). The constructs were checked by sequencing the entire inserts. For each prey, a mini-mating was carried out between YHGX13 (Y187 ade2-101::loxP-kanMX-loxP, mata α) transformed with the prey plasmids and YPT6AT yeast cells (mata) transformed with the DHFR hook (Dihydrofolate reductase) to produce a diploid yeast culture. For each interaction, DO1, 1/10, 1/100 and 1/1000 of the diploid yeast culture expressing both hook and prey constructs were spotted on a selective medium without tryptophan, leucine and histidine and supplemented with the chemical probe and FK506. Interactions were tested in duplicate. One plate was used per chemical compound and concentration (DMSO, 5, 10 and 20 μ M of HBX129653, 5, 10 and 20 μ M of HBX129654, 5 μ M of HBX24786 Trimethoprim (TMP) and 5 μ M of HBX129634 (TMP-PEG5-OH)). Plates were incubated at 30°C for 3 days.

Y3H ULTImate YChemH™ - Competition with free compound

The competition assay is based on the previously described 1-by-1 validation with a constant concentration for the chemical probe (HBX129653, HBX 129654) and increasing concentrations of the parent compound of the chemical probe (MOL653, MOL654) or its inactive enantiomer (INACT653, INACT654) (Table 7). The competition assays were performed on selective medium at 8 concentrations of the free compound (0, 0.25, 0.5, 1, 2, 5, 10 and 20 μ M) and a consistent concentration for the tagged Y3H-compound (1 μ M).

Table 7: YChemH competition IDs

	Hybrigenics ID	Structure
DHQ compound – active	MOL653	 Formula (III)
DHQ compound – inactive	INACT653	
THP compound – active	MOL654	 Formula (IV)
THP compound – inactive	INACT654	

HepaRG cell culture

- HepaRG cells (Biopredics International, Rennes, France, Cat# HPR101) were cultured at 37°C in a humidified atmosphere with 5% CO₂ in complete HepaRG growth medium consisting of
- William's E Medium (GIBCO), Growth Medium Supplement (Biopredics, Cat# ADD710) and 1% (v/v) GlutaMAX-I (Gibco #32551) and 1x Pen/Strep (Gibco, #15140) for 2 weeks. To initiate differentiation, 0.9% (v/v) DMSO (Sigma-Aldrich, D2650) was added to the growth medium on confluent cells. After one week, medium was replaced by complete differentiation medium

(HepaRG growth medium supplemented with 1.8% (v/v) DMSO) in which cells were maintained for approximately 4 weeks with differentiation medium renewal every 7 days. Differentiated HepaRG cells (dHepaRG), displayed hepatocyte-like cell islands surrounded by monolayer of biliary-like cells. Prior to HBV infection and compound treatment, dHepaRG cells were seeded
 5 into collagen I coated 96-well plates (Gibco, Cat# A11428-03) at 60,000 cells per well in 100 μ L of complete differentiation medium. Cells were allowed to recover their differentiated phenotype in 96-well plates for approximately 1 week after plating prior to HBV infection.

HBV infection of dHepaRG cells

dHepaRG cells were infected with HBV particles at an MOI of 30. The HBV particles were
 10 produced from HBV-producing HepG2.2.15 cells (Sells et al 1987 Proc Natl Acad Sci U S A 84, 1005–1009). dHepaRG culture conditions, differentiation and HBV infection have been described previously (Hantz, 2009, J. Gen. Virol., 2009, 90: 127-135). In brief complete differentiation medium (HepaRG growth medium consisting of William's E Medium (GIBCO), Growth Medium Supplement (Biopredics, Cat# ADD710) and 1% (v/v) GlutaMAX-I (Gibco
 15 #32551) and 1x Pen/Strep (Gibco, #15140), supplemented with 1.8% (v/v) DMSO), containing 4% PEG-8000 and virus stock (20 to 30 GE/cell) was added (120 μ L/well). One day post-infection, the cells were washed three times with phosphate-buffered saline and medium (complete differentiation medium) was replaced every two days during the experiment.

siRNA treatment of HBV-infected HepaRG

20 A pool of four different siRNAs was acquired from GE Dharmacon (ON TARGETplus) (Table 8).

Table 8: Overview siRNAs

ON TARGETplus	siRNA (Cat.No.)	Target Sequence	SEQ ID NO
siPAPD5 (Cat. No. # L-010011-00-0010)	J-010011-05	CAUCAAUGCUUUUAUAUCGA	252
	J-010011-06	GGACGACACUUCAAUUAUU	253
	J-010011-07	GAUAAAGGAUGGUGGUUCA	254
	J-010011-08	GAAUAGACCUGAGCCUUCA	255
siPAPD7 (Cat. No. # L-009807-00-0005)	J-009807-05	GGAGUGACGUUGAUUCAGA	256
	J-009807-06	CGGAGUUCAUCAAGAAUUA	257
	J-009807-07	CGGAGUUCAUCAAGAAUUA	258
	J-009807-08	GCGAAUAGCCACAUGCAAU	259

One day before infection with HBV cells and 4 days after infection cells were treated with the siRNA pool either against PAPD5, PAPD7, both or the non-targeting siRNA as control. The siRNAs (25nM each) were transfected using DharmaFect 4 (GE Dharmacon; Cat. No. T-2004-
 25 01) and OPTI-MEM (Thermo Scientific; Cat.No. 51985034) according to manufacturer's protocol. The experiment was run for 11 days.

HBV antigen measurements

To evaluate the impact on HBV antigen expression and secretion, supernatants were collected on Day 11. HBV HBsAg and HBeAg levels were measured using CLIA ELISA Kits (Autobio

Diagnostic #CL0310-2, #CL0312-2), according to the manufacturer's protocol. Briefly, 25 μ L of supernatant per well were transferred to the respective antibody coated microtiter plate and 25 μ L of enzyme conjugate reagent were added. The plate was incubated for 60min on a shaker at room temperature before the wells were washed five times with washing buffer using an automatic washer. 25 μ L of substrate A and B were added to each well. The plates were incubated on a shaker for 10min at room temperature before luminescence was measured using an Envision luminescence reader (Perkin Elmer).

Cell viability

After the removal of supernatant media from the HBV infected dHepaRG cells, cells were incubated with CellTiterGlo One Solution (Promega) to measure cell viability.

After LNA oligonucleotide treatments of HBV infected dHepaRG cells, cell viability was measured using the Cell Counting kit-8 (Sigma-Aldrich, #96992) according to the manufacturer's protocol. Briefly, Medium was removed from the cells and replaced by 110 μ L of medium containing 9% Cell Counting kit-8. Cells were incubated for 1h at 37°C. The supernatant was transferred into a new 96 wells plate and the absorbance at 450nm was measured using an Envision luminescence reader (Perkin Elmer).

Real-time PCR for intracellular mRNA

For intracellular mRNA isolation, dHepaRG were washed once with PBS (Gibco) and lysed using the MagNA Pure "96 Cellular RNA Large Volume Kit" (Roche #05467535001). The lysates may be stored at at -80°C. For the real-time qPCR reaction an AB7900 HT sequence detection system (Applied Biosystems), the TaqMan® Gene Expression Master Mix (ThermoFisher Scientific) were used. For detection of HBV mRNA HBV core-specific primer (Integrated DNA Technologies) (Table 9) and to measure reduction of PAPD5 and PAPD7, in the presence of siRNA, gene-specific TaqMan® Expression Assay probes (ThermoFisher Scientific; PAPD5 Cat.No. 4331182; PAPD7 Cat.No. 4331182) were used. Samples were normalized using TaqMan® Expression Assay probe against b-Actin (ThermoFisher Scientific; PAPD5 Cat.No. 4331182).

Table 9: HBV core specific TaqMan probes

	Name	Dye	Sequence	SEQ ID NO
HBV core Primer	Forward (F3_HBVcore)		CTG TGC CTT GGG TGG CTT T	265
	Reverse (R3_HBVcore)		AAG GAA AGA AGT CAG AAG GCA AAA	266
	Probe (P3_HBVcore)	FAM-MGB	AGC TCC AAA/ZEN/TTC TTT ATA AGG GTC GAT GTC CAT G	267

HBV DNA extraction and quantification from virus preparation

HBV DNA extraction is performed using the QIAamp UltraSens Virus kit (Qiagen, #53704) according to the manufacturer's protocol with the following optimizations. 30 μ L and 3 μ L of the

virus sample are diluted into 1mL of PBS before adding buffer AC. The first centrifugation step is done for 45min at full speed and 4°C. HBV DNA is quantified in duplicate by qPCR using a QuantStudio 12K Flex (Applied Biosystems), the TaqMan Gene Expression Master Mix (Applied Biosystems, #4369016) and a premix 1:1:0.5 of the primers indicated in Table 9 above and
 5 probe reconstituted at 100µM. The qPCR is performed using the following settings: UDG incubation (2min, 50°C), enzyme activation (10min, 95°C) and qPCR (40 cycles with 15sec, 95°C for denaturation and 1min, 60°C for annealing and extension). Genomes equivalent calculation is based on a standard curve generated from HBV genotype D plasmid dilutions with known concentrations.

10 **Oligonucleotide synthesis**

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

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

Elongation of the oligonucleotide:

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

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

Purification by RP-HPLC:

35 The crude compounds are purified by preparative RP-HPLC on a Phenomenex Jupiter C18 10µ 150x10 mm column. 0.1 M ammonium acetate pH 8 and acetonitrile is used as buffers at a flow

rate of 5 mL/min. The collected fractions are lyophilized to give the purified compound typically as a white solid.

Abbreviations:

- DCI: 4,5-Dicyanoimidazole
- 5 DCM: Dichloromethane
- DMF: Dimethylformamide
- DMT: 4,4'-Dimethoxytrityl
- THF: Tetrahydrofurane
- Bz: Benzoyl
- 10 Ibu: Isobutyryl
- RP-HPLC: Reverse phase high performance liquid chromatography

T_m Assay:

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

Example 1: DHQ and THP binds to PAPD5 and PAPD7

PAPD5/7 were identified in Y3H Ultimate YChemH screen as common interaction partner of DHQ and THP

- Both proteins PAPD5 (variant 1: NP_001035374; variant 2: NP_001035375) and PAPD7
- 25 (XP_005248291) were identified by a numerous number of fragments in the Y3H screen for both compounds (DHQ and THP) as described in the Materials and Method section. The identified proteins were ranked with a confidence score of A (scale A-D) by HYBRIGENICS (Table 10).

Table 10: YChemH screen results for PAPD5/7

	Hybrigenics ID	Protein prey identified	# of fragments	Confidence score
DHQ compound	HBX129653	PAPD5 variant 1	28	A
		PAPD5 variant 2	1	N/A
		PAPD7	12	A
THP compound	HBX129654	PAPD5 variant 1	5	N/A
		PAPD5 variant 2	49	A
		PAPD7	24	A

PAPD5/7 interaction with DHQ and THP could be confirmed using Y3H ULTImate YChemH 1-by-1 validation of identified prey fragments and further with full length proteins

In a first validation step three fragments identified in the first screen were selected for the 1-by-1 validation assay (as described in the Materials and Method section) and tested at three different concentrations (5, 10 and 20 μ M) (Table 11).

Table11: interacting fragment selected for validation assay

Interaction #	Prey fragment ID	Protein Prey
A	PLA_RP6_hgx4240v1_pB409_A-15	PAPD7
B	PLA_RP6_hgx4241v1_pB409_A-112	PAPD5 variant 1
C	PLA_RP6_hgx4240v1_pB409_A-24	PAPD5 variant 2

All three fragments could be validated as specific binders for DHQ and THP already at the lowest tested concentration (Figure 1).

In a second validation step, full length proteins for PAPD5 and PAPD7 were synthesized and used for 1-by-1 validation (as described in the Materials and Method section) with DHQ and THP (Table 12).

Table 12: Reference ID for full length protein prey used in 1-by-1 validation assay

Interaction #	HYBRIGENICS Reference	Protein Prey
A	hgx4386v1_pP7	PAPD5 var1 full length
B	hgx4388v2_pP7	PAPD7 var1 full length

The interaction between these full length proteins and the DHQ and THP compounds were confirmed at the lowest tested concentration and shown to be specific for the chemical probes (Figure 2).

PAPD5/7 interaction with DHQ and THP in Y3H can be competed by both free active compound, but not the inactive enantiomer

After validation of binding of DHQ and THP to protein fragments and full length PAPD5 and PAPD7 the binding was confirmed in a Y3H ULTImate YChenH competition experiment (as described in the Materials and Method section) using either inactive or active free compound (Table 13). A decrease of loss of yeast growth in the presence of the parent active compound, but not in the presence of the inactive enantiomer, means that the parent compound competes with the chemical probe and interacts with the protein target.

For all tested compounds toxicity on non-selective medium at the highest concentration (20 μ M) was tested using CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer's protocol. No toxicity was observed at this concentration for any compound as yeast growth was not affected (data not shown). For both active free parent compounds (DHQ and THP, MOL653 and MOL654, respectively) competition could be observed, with lower concentration needed for competing the binding to the full length protein than for the fragment

interactions (Figure 3+4). Successful cross competition suggests a shared binding side for DHQ and THP to PAPD5/7 or at least binding in close proximity to each other.

Table 13: Reference ID for protein prey used in competition assay

Interaction #	Prey fragment ID	Protein Prey
A	PLA_RP6_hgx4241v1_pB409_A-112	PAPD5 var1 experimental fragment
B	hgx4386v1_pP7	PAPD5 var1 full length
C	PLA_RP6_hgx4240v1_pB409_A-15	PAPD7 varX1 experimental fragment
D	hgx4388v2_pP7	PAPD7 varX1 full length

Example 2: Inhibition of PAPD5 and/or PAPD7 with siRNA results in effective treatment of HBV infection

To correlate the binding of DHQ and THP to PAPD5/7 and the impact of these two proteins on HBV gene expression, we used RNAi technology to reduce these proteins in naturally HBV infected dHepaRG and to monitor the impact of this reduction on viral parameters. For that we used siRNA pools against PAPD5 and PAPD7 (see table 8) in HBV infected dHepaRG cells as described in the Materials and methods section.

Reduction of PAPD5 led to inhibition of viral expression measured by secreted HBsAg and HBeAg as well as intracellular HBV mRNA (measured using CLIA ELISA and real-time PCR as described in the Materials and Methods section). While the reduction of PAPD5 mRNA dramatically reduced HBV gene expression, inhibition of PAPD7 had a modest effect on HBV expression (Figure 7). However, an enhanced synergistic anti-HBV activity was observed when siRNA against PAPD7 and PAPD5 were combined (Figure 7), suggesting a compensative role for PAPD7 in the absence of PAPD5.

Example 3: DHQ and THP effectively reduces HBsAg and HBeAg

The potency of DHQ and THP and their variants against HBV infection were measured in HepG2.2.15 cells using HBsAg and HBeAg as read out.

HepG2.2.15 cells (Sells et al 1987 Proc Natl Acad Sci U S A 84, 1005–1009) were cultured in 96 well plates (15.000 cells/ well in 100 uL) in DMEM+GluTaMax-1 (Gibco Cat. NO. 10569), 1% Pen Strep (Gibco Cat. No.15140), 10% FBS (Clontech Cat.No. 631106), Geneticin 0.25 ug/ml (Invitrogen 10131035). The compounds were tested using three-fold serial dilutions in DMSO with a top concentration of 100 µM and 9 serial dilutions. Each compound was tested in quadruplicate. The cells were incubated for 3 days, supernatants were collected and HBsAg and HBeAg were measured as described in the Materials and Methods section.

The IC₅₀ values of the tested compounds in the reduction of secretion of HBsAg and HBeAg are shown in the following:

HBX129653 (DHQ – TMP): IC₅₀ HBsAg 1.181uM

HBX129654 (THP – TMP): IC₅₀ HBsAg 0.299uM

MOL653 (DHQ – free - active): IC₅₀ HBsAg 0.003uM; IC₅₀ HBeAg 0.007uM

MOL654 (THP – free - active): IC₅₀ HBsAg 0.003uM

INACT653 (DHQ – free - inactive): IC₅₀ HBsAg 3.15uM

5 INACT654 (THP – free - inactive): IC₅₀ HBsAg >25uM

Example 4: Screening for *in vitro* efficacy of antisense oligonucleotides targeting PAPD5

An oligonucleotide screen was done across the PAPD5 mRNA using 16 to 20mer gapmers.

Efficacy testing was performed in an *in vitro* experiment in HeLa cells.

Cell lines

10 HeLa cell line was purchased from European Collection of Authenticated Cell Cultures (ECACC, #93021013) and maintained as recommended by the supplier in a humidified incubator at 37°C with 5% CO₂. For assays, 2,500 cells/well were seeded in a 96 multi well plate in Eagle's Minimum Essential Medium (Sigma, M2279) with 10% fetal bovine serum (FBS), 2mM Glutamin AQ, 1% NEAA, 25µg/ml Gentamicin.

15 Oligonucleotide efficacy

Cells were incubated for 24 hours before addition of oligonucleotides dissolved in PBS. Final concentration of oligonucleotides was 5 and 25 µM, the final culture volume was 100 µl/well. The cells were harvested 3 days after addition of oligonucleotide compounds and RNA was extracted using the PureLink Pro 96 RNA Purification kit (Ambion), according to the manufacturer's instructions. After RNA/LNA duplex denaturation (90 °C, 40 sec) QPCR was done with a one-step protocol (qScript™ XLT One-Step RT-qPCR ToughMix®, Low ROX™ from Quanta Bioscience, #95134-500) in a duplex set up with TaqMan primer assays for the gene of interest PAPD5 (Hs00223727_m1, FAM-MGB, Life Technologies) and a house keeping gene GUSB (Hu_4326320E, VIC-MGB, Life Technologies) following the recommendations of the provider. The relative PAPD5 mRNA expression level is shown in table 14 as % of average control samples (PBS-treated cells). Figure 8 presents the target knockdown achieved with the individual oligonucleotide compounds across the PAPAD5 encoding sequence.

Table 14: *in vitro* efficacy of anti-PAPD5 compounds (single experiment with duplex QPCR). PAPD5 mRNA levels are normalized to GUSB in HeLa cells and shown as % of control (PBS treated cells).

CMP ID NO	Concentration µM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
12_1	25	70,8	9,3	AGgtagatgtgtttaACT	1656
12_1	5	59,3	11,7	AGgtagatgtgtttaACT	1656
13_1	25	43,9	0,2	CAGcctaaacttagtGG	2000
13_1	5	53,2	18,1	CAGcctaaacttagtGG	2000
14_1	25	43,9	8,8	AAGccctcaatgtaaaACAC	2446

CMP ID NO	Concentration μM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
14_1	5	96,6	8,1	AAgccctcaatgtaaaACAC	2446
15_1	25	21,1	3,2	AATagcaagtagaggaGAG	3059
15_1	5	52,9	17,1	AATagcaagtagaggaGAG	3059
16_1	25	28,5	7,3	AAataaggatactgGCGA	4036
16_1	5	68,6	16,4	AAataaggatactgGCGA	4036
17_1	25	50,2	20,4	GAGGgaacacataataaaAG	4484
17_1	5	72,9	29,0	GAGGgaacacataataaaAG	4484
18_1	25	19,7	4,0	GTaatcctctcacATTC	5928
18_1	5	53,8	14,0	GTaatcctctcacATTC	5928
19_1	25	37,0	8,4	AGtaacaccaatctcATTG	6652
19_1	5	58,8	10,7	AGtaacaccaatctcATTG	6652
20_1	25	66,1	5,3	GTgacagtattcaatGATC	7330
20_1	5	58,4	12,5	GTgacagtattcaatGATC	7330
21_1	25	33,2	3,1	CAGttccgtatcaccAAC	7702
21_1	5	45,3	11,6	CAGttccgtatcaccAAC	7702
22_1	25	56,3	6,7	AAgtctaactcaagccATC	8292
22_1	5	70,1	16,8	AAgtctaactcaagccATC	8292
23_1	25	21,7	nd	AGgcttccattttattGAA	8625
23_1	5	44,3	0,5	AGgcttccattttattGAA	8625
24_1	25	71,2	10,2	TTtagaaaaacgagGCTA	9866
24_1	5	63,8	10,6	TTtagaaaaacgagGCTA	9866
25_1	25	13,4	0,1	GTAttcttattcttgCT	10254
25_1	5	27,9	10,6	GTAttcttattcttgCT	10254
26_1	25	12,0	0,9	ATTAttcccacagtaaGA	10881
26_1	5	37,7	1,1	ATTAttcccacagtaaGA	10881
27_1	25	57,5	4,4	AACaacaacaggatggGC	11370
27_1	5	110,0	2,2	AACaacaacaggatggGC	11370
28_1	25	35,2	0,8	ATATccacaatattctgAT	11790
28_1	5	47,7	1,2	ATATccacaatattctgAT	11790
29_1	25	77,8	32,7	AAagaaataatgtcgtCTGG	12413
29_1	5	73,9	2,5	AAagaaataatgtcgtCTGG	12413
30_1	25	23,4	4,0	CCAgagtaaacaaaTCC	12718
30_1	5	48,0	2,0	CCAgagtaaacaaaTCC	12718
31_1	25	57,7	10,6	ATtcaacatttttagtcACC	13555
31_1	5	53,6	4,8	ATtcaacatttttagtcACC	13555
32_1	25	32,8	5,7	TTTggttaattctttttTAG	14297
32_1	5	33,1	5,4	TTTggttaattctttttTAG	14297
33_1	25	58,0	nd	CAatgaggaaacaagaGTCA	15137
33_1	5	80,9	17,9	CAatgaggaaacaagaGTCA	15137
34_1	25	56,3	9,0	TTCaataaatgtgggaGGT	15695
34_1	5	63,6	2,7	TTCaataaatgtgggaGGT	15695
35_1	25	26,4	1,9	GGatatttgatacggcaAAT	16145
35_1	5	39,1	10,1	GGatatttgatacggcaAAT	16145
36_1	25	77,5	12,6	CTAagaagcaaaaCCC	16714
36_1	5	76,6	2,7	CTAagaagcaaaaCCC	16714
37_1	25	45,3	4,5	ATAtaattcacgtttcaCTT	17097
37_1	5	91,9	nd	ATAtaattcacgtttcaCTT	17097
38_1	25	28,9	2,7	AATgattcacatgaaggTTA	17420
38_1	5	38,2	2,2	AATgattcacatgaaggTTA	17420
39_1	25	18,0	4,7	GTtaggattttgcTATG	18299
39_1	5	29,2	4,1	GTtaggattttgcTATG	18299
40_1	25	80,4	2,0	GTAcataatcaaccgTAT	18669

CMP ID NO	Concentration μM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
40_1	5	118,8	10,4	GTAcacaaatcaaccgTAT	18669
41_1	25	32,8	7,7	CACactatttcaagatgcTA	19681
41_1	5	85,6	4,8	CACactatttcaagatgcTA	19681
42_1	25	65,6	20,2	CACctatacaatggagtATT	20352
43_1	25	64,9	12,3	ATcatacgtcattagaGAAC	20721
43_1	5	79,4	25,2	ATcatacgtcattagaGAAC	20721
44_1	25	70,7	2,6	CAgaacagatactttgcCA	21111
44_1	5	84,7	5,5	CAgaacagatactttgcCA	21111
45_1	25	49,8	11,9	AAgaatggttggttaaGGG	21782
45_1	5	79,2	4,3	AAgaatggttggttaaGGG	21782
46_1	25	35,1	0,0	AGAAAttggttaaactggacTG	22378
46_1	5	55,1	7,7	AGAAAttggttaaactggacTG	22378
47_1	25	28,4	3,9	AGAAttatattggcTGG	23160
47_1	5	49,6	2,2	AGAAttatattggcTGG	23160
48_1	25	60,8	13,7	CCtaaaccagacagaaaAGA	23993
48_1	5	69,1	5,0	CCtaaaccagacagaaaAGA	23993
49_1	25	31,9	4,7	ACCAaattagagcagaaaTC	24813
49_1	5	62,2	1,0	ACCAaattagagcagaaaTC	24813
50_1	25	41,3	6,5	TTCTaaataacagatggGTC	25047
50_1	5	64,4	21,7	TTCTaaataacagatggGTC	25047
51_1	25	27,1	5,9	TTTataattttttccaTCT	26080
51_1	5	49,1	7,7	TTTataattttttccaTCT	26080
52_1	25	34,9	6,8	GCAAAtatcagattaaccTC	26625
52_1	5	60,5	3,1	GCAAAtatcagattaaccTC	26625
53_1	25	25,9	0,8	AACggtatggcagaagaCAA	26973
53_1	5	43,6	2,2	AACggtatggcagaagaCAA	26973
54_1	25	5,1	0,1	TTCAacctttactgcAT	27813
54_1	5	25,7	3,1	TTCAacctttactgcAT	27813
55_1	25	40,8	0,9	ACTgataaagggcattTCAA	28357
55_1	5	64,5	1,6	ACTgataaagggcattTCAA	28357
56_1	25	6,4	2,3	CAGtaggaatgtggCTT	28718
56_1	5	19,6	0,7	CAGtaggaatgtggCTT	28718
57_1	25	23,9	0,4	TTTtatggcagggtttcAC	29327
57_1	5	41,2	7,9	TTTtatggcagggtttcAC	29327
58_1	25	19,6	0,7	TCactgttaaaccTCAC	29902
58_1	5	59,0	12,9	TCactgttaaaccTCAC	29902
59_1	25	53,1	4,8	CAATtttctaattcaatgGT	30704
59_1	5	72,4	29,8	CAATtttctaattcaatgGT	30704
60_1	25	42,8	2,0	AAGAtataattcaccctaCT	31008
60_1	5	77,3	13,7	AAGAtataattcaccctaCT	31008
61_1	25	47,4	2,7	GCcacataaaggataAAGT	31348
61_1	5	87,8	12,8	GCcacataaaggataAAGT	31348
62_1	25	49,8	2,7	CCcattagaagtaaggtGA	32367
62_1	5	80,1	11,1	CCcattagaagtaaggtGA	32367
63_1	25	49,4	1,1	ATgtaaattaaaactTCCC	32632
63_1	5	67,2	1,7	ATgtaaattaaaactTCCC	32632
64_1	25	41,7	1,7	TGAgagcataaaagtacgGA	32945
64_1	5	73,1	6,3	TGAgagcataaaagtacgGA	32945
65_1	25	19,4	0,2	TTCaacaacaggtaaagGG	33593
65_1	5	47,7	3,3	TTCaacaacaggtaaagGG	33593
66_1	25	46,6	0,2	TGcattcctaagtaacATAA	34801
66_1	5	52,0	5,3	TGcattcctaagtaacATAA	34801

CMP ID NO	Concentration µM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
67_1	25	34,1	10,4	AGAgaaaagtgatgaggGAA	35368
67_1	5	45,5	2,0	AGAgaaaagtgatgaggGAA	35368
68_1	25	51,7	3,6	ATAcggatcaccagctaAA	36131
68_1	5	74,2	18,8	ATAcggatcaccagctaAA	36131
69_1	25	26,5	3,5	CAtgttatgcacagaAGAT	36712
69_1	5	51,0	1,4	CAtgttatgcacagaAGAT	36712
70_1	25	68,8	11,0	CGctgaagaactaagtATTA	37282
70_1	5	75,2	6,7	CGctgaagaactaagtATTA	37282
71_1	25	36,5	5,1	CAAcacagatggtggtgaTA	37870
71_1	5	66,3	6,4	CAAcacagatggtggtgaTA	37870
72_1	25	62,8	9,4	AGTagccattaggaTG	38478
72_1	5	80,2	3,1	AGTagccattaggaTG	38478
73_1	25	59,9	11,1	ATacacaggctccataATA	39639
73_1	5	70,6	9,5	ATacacaggctccataATA	39639
74_1	25	34,5	9,7	GATttttgtatagtcacAA	40178
74_1	5	43,3	6,9	GATttttgtatagtcacAA	40178
75_1	25	59,8	0,2	GCActataaaaaaggGACA	41042
75_1	5	68,1	2,9	GCActataaaaaaggGACA	41042
76_1	25	50,0	1,8	AGtgcaagtatcGCT	41734
76_1	5	73,2	0,1	AGtgcaagtatcGCT	41734
77_1	25	45,8	3,2	CCAAAagaatcaagttcgTA	42442
77_1	5	75,0	14,2	CCAAAagaatcaagttcgTA	42442
78_1	25	56,1	4,5	CCtcagaccaaattATTT	43203
78_1	5	85,1	1,8	CCtcagaccaaattATTT	43203
79_1	25	27,1	3,4	TTCaacaagcatctattGTA	43663
79_1	5	43,3	1,1	TTCaacaagcatctattGTA	43663
80_1	25	4,4	1,8	CAAagggtgtgtacTCT	44220
80_1	5	19,2	3,4	CAAagggtgtgtacTCT	44220
81_1	25	35,9	11,3	TCAtaaatcttttccaCG	44756
81_1	5	67,6	0,7	TCAtaaatcttttccaCG	44756
82_1	25	36,9	2,1	CTgttacggatttaaatGTG	45042
82_1	5	67,5	10,5	CTgttacggatttaaatGTG	45042
83_1	25	59,1	8,2	GCTataaaaaatagaaGCC	46202
84_1	25	61,3	3,4	TCCTtagcaaaactaaaCAT	47142
84_1	5	92,6	0,3	TCCTtagcaaaactaaaCAT	47142
85_1	25	51,0	1,9	AGCaaaaggcaggatttcAA	47843
85_1	5	92,2	5,5	AGCaaaaggcaggatttcAA	47843
86_1	25	46,6	2,8	GAAtccatttacatattCAC	48267
86_1	5	79,2	0,9	GAAtccatttacatattCAC	48267
87_1	25	49,7	0,0	TCcagtatccaaaacATAC	49256
87_1	5	93,1	2,8	TCcagtatccaaaacATAC	49256
88_1	25	63,4	7,0	AGCTtaaagaagaacggTT	49688
88_1	5	24,2	15,8	AGCTtaaagaagaacggTT	49688
89_1	25	33,3	12,3	CAcaacgtgcctaccTT	50508
89_1	5	71,9	32,7	CAcaacgtgcctaccTT	50508
90_1	25	80,3	18,2	CCAgaatccaagaaaatGG	50764
90_1	5	112,9	36,7	CCAgaatccaagaaaatGG	50764
91_1	25	88,9	10,3	TCacctcgaactaaacaaGT	51561
91_1	5	90,1	5,9	TCacctcgaactaaacaaGT	51561
92_1	5	26,4	7,9	GTatctttctgtacTATT	52461
93_1	25	15,1	7,2	GTCAttctactaacaacaaCG	53305
93_1	5	29,7	15,4	GTCAttctactaacaacaaCG	53305

CMP ID NO	Concentration µM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
94_1	25	48,1	20,2	TGgaaaaggaagaacCATT	53865
94_1	5	42,8	34,7	TGgaaaaggaagaacCATT	53865
95_1	25	59,2	3,6	AAatacaactcttcgTGAT	54638
95_1	5	73,0	9,2	AAatacaactcttcgTGAT	54638
96_1	25	36,8	nd	AAataccctgacgagCTG	54942
96_1	5	75,2	4,1	AAataccctgacgagCTG	54942
97_1	25	40,0	20,5	TCAtaaaacatgatccttGC	55741
97_1	5	96,4	3,0	TCAtaaaacatgatccttGC	55741
98_1	25	55,7	1,0	CTAaagcagatccataGAA	56277
98_1	5	103,3	24,2	CTAaagcagatccataGAA	56277
99_1	25	34,5	16,3	AGActataacttttgctaCA	56942
99_1	5	73,1	6,0	AGActataacttttgctaCA	56942
100_1	25	34,1	nd	AGcaatgactgaacataGT	57369
100_1	5	110,9	22,8	AGcaatgactgaacataGT	57369
101_1	25	87,5	16,7	ATAaaacaagcatacggGC	58146
101_1	5	98,8	15,0	ATAaaacaagcatacggGC	58146
102_1	25	47,2	11,2	ATgagataccagcagatAG	58588
102_1	5	94,0	8,9	ATgagataccagcagatAG	58588
103_1	25	52,0	4,5	AGAAgaaatcctgagtaaTC	59089
103_1	5	72,7	0,6	AGAAgaaatcctgagtaaTC	59089
104_1	25	67,0	9,6	CCCTaaaaagtgcgTA	59461
104_1	5	106,7	9,6	CCCTaaaaagtgcgTA	59461
105_1	25	35,8	4,4	TAAAgtagatcacgGC	59970
105_1	5	54,6	9,3	TAAAgtagatcacgGC	59970
106_1	25	74,8	3,5	GTggatacagaaagCCA	60738
106_1	5	92,6	3,2	GTggatacagaaagCCA	60738
107_1	25	92,2	6,9	GTAtcggcaggagATT	61038
107_1	5	121,5	31,7	GTAtcggcaggagATT	61038
108_1	25	50,2	10,6	TAACtaattgattccattGC	61868
108_1	5	66,4	4,6	TAACtaattgattccattGC	61868
109_1	25	43,3	8,4	AGAagaacggaattgCC	62418
109_1	5	72,9	4,1	AGAagaacggaattgCC	62418
110_1	25	59,3	0,2	ATAAtgattttcctatCC	62822
110_1	5	76,0	3,6	ATAAtgattttcctatCC	62822
111_1	25	28,0	0,4	ATggttttgggagaAGG	63000
111_1	5	11,3	10,2	ATggttttgggagaAGG	63000
112_1	25	97,4	4,2	TGctgctgtgaaaagaAATG	63697
112_1	5	139,8	0,6	TGctgctgtgaaaagaAATG	63697
113_1	25	83,7	7,7	GTgtccaatttttatTAT	64377
113_1	5	86,7	0,4	GTgtccaatttttatTAT	64377
114_1	25	78,7	8,5	GAtggaatcaactgtgaGT	65307
114_1	5	38,6	17,3	GAtggaatcaactgtgaGT	65307
115_1	25	31,4	3,6	GATGgtgacaaattattCT	65894
115_1	5	19,6	4,4	GATGgtgacaaattattCT	65894
116_1	5	21,7	nd	TGcttttgggaatCTTT	66650
117_1	25	40,8	18,6	GAtgtcctacaatgaacACG	68024
117_1	5	39,4	10,3	GAtgtcctacaatgaacACG	68024
118_1	25	74,2	11,3	GTACAaggacaaagtaACC	68732
118_1	5	35,4	7,7	GTACAaggacaaagtaACC	68732
119_1	25	102,8	9,8	TGAAacgcctatctcTA	69029
119_1	5	96,9	2,6	TGAAacgcctatctcTA	69029
120_1	25	56,0	2,4	ATActatttatgcttatgGA	69796

CMP ID NO	Concentration μ M	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
120_1	5	79,7	2,5	ATActatttatgcttatgGA	69796
121_1	25	27,1	0,9	TTGtaatcaaggcaataAGG	70770
121_1	5	56,8	3,8	TTGtaatcaaggcaataAGG	70770
122_1	25	24,4	1,4	GAAGtccaataacgcaGA	71091
122_1	5	63,3	14,8	GAAGtccaataacgcaGA	71091
123_1	25	68,6	11,3	ATAtccaatctctatatgTG	72013
123_1	5	85,3	6,3	ATAtccaatctctatatgTG	72013
124_1	25	83,9	0,8	GCcttacacaagactatATT	73444
124_1	5	77,0	43,8	GCcttacacaagactatATT	73444
125_1	25	93,0	7,4	TGCTgaattttatgttaAC	73823
125_1	5	87,9	4,7	TGCTgaattttatgttaAC	73823
126_1	25	53,9	8,5	CAaagatgatgggttTAAG	74559
126_1	5	77,5	2,5	CAaagatgatgggttTAAG	74559
127_1	25	34,7	10,4	TTagtgggttggtGC	75043
127_1	5	57,6	9,4	TTagtgggttggtGC	75043
128_1	25	71,7	nd	AGattgttacctactGAT	76110
128_1	5	78,9	0,6	AGattgttacctactGAT	76110
129_1	25	79,0	8,9	TATtacaatatcaatcTCC	76931
129_1	5	97,7	nd	TATtacaatatcaatcTCC	76931
130_1	25	61,9	1,5	TACcaaaagcatagagtGG	77605
130_1	5	84,6	1,9	TACcaaaagcatagagtGG	77605
131_1	25	50,3	1,8	AAttatctcccgCTAC	78652
131_1	5	64,9	13,4	AAttatctcccgCTAC	78652

Example 5: Screening for *in vitro* efficacy of antisense oligonucleotides targeting PAPD7

The oligonucleotide screen across the PAPD7 mRNA was conducted essentially as described in Example 4, with the substitution of the TaqMan primer assays for the gene of interest PAPD7 (Hs00173159_m1, FAM-MGB, Life Technologies).

The relative PAPD7 mRNA expression level is shown in table 15 as % of average control samples (PBS-treated cells). Figure 9 presents the target knockdown achieved with the individual oligonucleotide compounds across the PAPAD5 encoding sequence.

Table 15: *in vitro* efficacy of anti-PAPD7 compounds (single experiment with duplex QPCR).

PAPD5 mRNA levels are normalized to GUSB in HeLa cells and shown as % of control (PBS treated cells).

CMP ID NO	Concentration μ M	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
132_1	25	72,8	4,0	GTtgggtggaataggCA	132
132_1	5	79,1	0,3	GTtgggtggaataggCA	132
133_1	25	25,6	1,4	TCgatttccgttccAA	1962
133_1	5	42,7	0,7	TCgatttccgttccAA	1962
134_1	25	65,2	2,3	ACAacctacacataaattGC	2510
134_1	5	93,0	1,1	ACAacctacacataaattGC	2510
135_1	25	66,7	0,6	ACtataagaactcccAACA	2668
135_1	5	58,9	13,0	ACtataagaactcccAACA	2668

CMP ID NO	Concentration μ M	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
136_1	25	76,6	5,0	GAGAAAAagagttacaaGC	2695
136_1	5	76,8	16,6	GAGAAAAagagttacaaGC	2695
137_1	25	53,7	12,7	AACTggagggagagaagAG	2883
137_1	5	87,5	20,1	AACTggagggagagaagAG	2883
138_1	25	76,5	2,2	TTtctaagagcagaggtACA	3090
138_1	5	102,6	44,0	TTtctaagagcagaggtACA	3090
139_1	25	19,6	0,2	AGAAgtaacaagagcCT	3463
139_1	5	44,6	20,4	AGAAgtaacaagagcCT	3463
140_1	25	54,6	3,0	AGtatcaaaccagacCTC	3795
140_1	5	39,9	9,9	AGtatcaaaccagacCTC	3795
141_1	25	32,6	5,1	CCACaaccgaaagacTT	4205
141_1	5	51,2	1,9	CCACaaccgaaagacTT	4205
142_1	25	37,2	3,1	AAtacacactgcattTTCA	4336
142_1	5	53,1	1,7	AAtacacactgcattTTCA	4336
143_1	25	26,2	6,0	CCaggtagatagcacAG	4686
143_1	5	80,5	4,2	CCaggtagatagcacAG	4686
144_1	25	64,2	4,8	CCatgacaaagtaacaACAG	4821
144_1	5	66,4	0,7	CCatgacaaagtaacaACAG	4821
145_1	25	37,4	3,1	CAgaatttcctttgagTTA	5134
145_1	5	44,8	4,7	CAgaatttcctttgagTTA	5134
146_1	25	85,5	4,1	CCttcgcaagaagaattGA	5263
146_1	5	102,4	4,7	CCttcgcaagaagaattGA	5263
147_1	25	23,5	2,3	TCatacatcacgcttCT	5577
147_1	5	55,8	6,5	TCatacatcacgcttCT	5577
148_1	25	30,6	5,8	TGcgaaaagattgGAGG	5945
148_1	5	32,4	5,1	TGcgaaaagattgGAGG	5945
149_1	25	72,9	11,4	CAcaggacgcttacatgaAT	6235
149_1	5	93,9	15,5	CAcaggacgcttacatgaAT	6235
150_1	25	36,1	2,0	GCTgttttttttctaAC	6352
150_1	5	54,3	10,9	GCTgttttttttctaAC	6352
151_1	25	39,5	2,5	ACcataagtgagtgttCTT	6834
151_1	5	120,0	8,9	ACcataagtgagtgttCTT	6834
152_1	25	90,6	9,7	ACacaagcccatagaaacAG	7158
152_1	5	68,2	7,9	ACacaagcccatagaaacAG	7158
153_1	25	3,4	0,2	CAgtagtaaccacCAAG	7447
153_1	5	21,6	4,8	CAgtagtaaccacCAAG	7447
154_1	25	46,6	0,7	CCtgcaaaacttttattTAT	7708
154_1	5	64,7	3,1	CCtgcaaaacttttattTAT	7708
155_1	25	15,7	1,3	ACTtagtaatagcaGCA	8074
155_1	5	37,6	2,1	ACTtagtaatagcaGCA	8074
156_1	25	24,7	0,3	ATgaataactccgaagACTT	8249
156_1	5	59,8	3,1	ATgaataactccgaagACTT	8249
157_1	25	42,7	5,2	AAAgaaaaggatcacaaGCC	8784
157_1	5	60,5	3,2	AAAgaaaaggatcacaaGCC	8784
158_1	25	91,4	19,9	AGAcagaaatcacctaaCA	8887
158_1	5	82,3	4,9	AGAcagaaatcacctaaCA	8887
159_1	25	19,2	2,4	TAGaacagacattattcATC	9506
159_1	5	47,4	10,0	TAGaacagacattattcATC	9506
160_1	25	33,0	5,2	AGttacacggagcagcAC	9664
160_1	5	57,4	3,6	AGttacacggagcagcAC	9664
161_1	25	82,6	22,1	CACtatacacagaacacTAT	9770
161_1	5	85,0	7,0	CACtatacacagaacacTAT	9770

CMP ID NO	Concentration μM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
162_1	25	70,4	2,3	AGctgtctaataacATGG	10000
162_1	5	91,7	16,8	AGctgtctaataacATGG	10000
163_1	25	26,7	0,6	ATGaacctattttatgcTTC	10206
163_1	5	31,9	1,8	ATGaacctattttatgcTTC	10206
164_1	25	36,5	5,5	ACcatcattaacctgcGT	10318
164_1	5	50,0	0,5	ACcatcattaacctgcGT	10318
165_1	25	129,2	5,9	AGtaaagtgcccagatGT	10568
166_1	25	45,8	3,3	TTCcctatgaaatcctcAA	10781
166_1	5	87,1	14,5	TTCcctatgaaatcctcAA	10781
167_1	25	35,6	2,8	CActctcatagaatgCAAC	10917
167_1	5	55,6	9,1	CActctcatagaatgCAAC	10917
168_1	25	19,4	4,9	AAtgcttaattttctCTCT	11084
168_1	5	38,2	1,9	AAtgcttaattttctCTCT	11084
169_1	25	26,8	4,1	TTAgagacgatgcctatAAC	11308
169_1	5	57,6	2,5	TTAgagacgatgcctatAAC	11308
170_1	25	26,1	2,2	TGAAtagttcccatagaTT	11585
170_1	5	54,7	1,8	TGAAtagttcccatagaTT	11585
171_1	25	10,8	0,6	CAGcataattgtttcTTT	12330
171_1	5	33,1	1,3	CAGcataattgtttcTTT	12330
172_1	25	13,8	2,2	ATGTcattatgttttagTT	12634
172_1	5	33,8	3,1	ATGTcattatgttttagTT	12634
173_1	25	22,6	2,5	CAGcagtatctctaGAA	12902
173_1	5	36,8	1,8	CAGcagtatctctaGAA	12902
174_1	25	9,7	0,4	CGgtaagggttcggTG	13126
174_1	5	18,2	0,0	CGgtaagggttcggTG	13126
175_1	25	51,9	3,5	CATGaaccacattaggaAC	13383
175_1	5	67,8	15,0	CATGaaccacattaggaAC	13383
176_1	25	54,6	0,0	CAttcaacacacacgACAA	13578
176_1	5	83,5	0,3	CAttcaacacacacgACAA	13578
177_1	25	38,9	1,1	AAgtatccaagactcAAGA	13889
177_1	5	47,6	3,4	AAgtatccaagactcAAGA	13889
178_1	25	49,8	9,4	CCACagaacacccgAG	14100
178_1	5	74,0	21,3	CCACagaacacccgAG	14100
179_1	25	69,6	0,2	TGGaaaaggaagggaaGA	14179
179_1	5	137,8	15,7	TGGaaaaggaagggaaGA	14179
180_1	25	54,5	0,1	AGagagtccgaagccTG	14432
180_1	5	77,7	18,8	AGagagtccgaagccTG	14432
181_1	25	27,6	5,5	ATGggaaaggtaacgaGC	14616
181_1	5	79,5	nd	ATGggaaaggtaacgaGC	14616
182_1	25	63,3	1,9	CTAtcctacaagtccgAA	15471
182_1	5	106,6	12,5	CTAtcctacaagtccgAA	15471
183_1	25	9,2	0,6	CATTgctttataatccTA	15816
183_1	5	30,6	3,1	CATTgctttataatccTA	15816
184_1	25	9,7	1,0	CTTTtaaggacaggaGG	15988
184_1	5	40,9	0,7	CTTTtaaggacaggaGG	15988
185_1	25	79,7	0,2	GATgaaagataagtgaGCAT	16395
185_1	5	76,9	2,5	GATgaaagataagtgaGCAT	16395
186_1	25	50,5	17,3	GAagcctgtaataattAAGC	17007
186_1	5	54,4	10,9	GAagcctgtaataattAAGC	17007
187_1	25	95,4	6,2	CACCctagtaaagcaaAC	17151
187_1	5	137,3	37,9	CACCctagtaaagcaaAC	17151
188_1	25	15,8	2,5	GCAaatgtaagcctttTT	17303

CMP ID NO	Concentration μM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
188_1	5	25,3	0,4	GCAaatgaagccttTT	17303
189_1	25	45,4	2,3	ACctgacagctaccgAC	17498
189_1	5	46,8	12,3	ACctgacagctaccgAC	17498
190_1	25	9,3	1,5	AAgagtgggttgaAGC	17963
190_1	5	19,1	2,0	AAgagtgggttgaAGC	17963
191_1	25	15,3	2,6	TAgtgaaatatttggAGTT	18101
191_1	5	33,2	4,5	TAgtgaaatatttggAGTT	18101
192_1	25	62,7	7,6	TTtcagcaccttaaacCC	18518
192_1	5	84,9	12,6	TTtcagcaccttaaacCC	18518
193_1	25	86,0	24,0	TTAAgggaaaggaaacgtCA	18747
193_1	5	79,5	6,2	TTAAgggaaaggaaacgtCA	18747
194_1	25	14,4	1,0	GTAgttaaagggaaggAA	19007
194_1	5	37,1	4,6	GTAgttaaagggaaggAA	19007
195_1	25	13,4	0,5	GTgaattaaagccaAAGC	19252
195_1	5	51,9	9,1	GTgaattaaagccaAAGC	19252
196_1	25	80,3	21,3	TGTttttgtatttagTAT	19476
196_1	5	77,5	23,0	TGTttttgtatttagTAT	19476
197_1	25	24,2	1,1	GAggttttttagtgAATT	19722
197_1	5	29,0	2,4	GAggttttttagtgAATT	19722
198_1	25	20,6	1,7	GAGgagctaaacggaCA	20062
198_1	5	33,4	6,4	GAGgagctaaacggaCA	20062
199_1	25	37,7	4,0	GTttagtctatgttctcAC	20623
199_1	5	38,8	5,6	GTttagtctatgttctcAC	20623
200_1	25	23,8	2,1	CAaatactgaatatgcCCG	20726
200_1	5	52,0	10,0	CAaatactgaatatgcCCG	20726
201_1	25	13,6	0,4	ACCatttaaatcgccAAC	20926
201_1	5	54,0	8,8	ACCatttaaatcgccAAC	20926
202_1	25	53,5	2,6	CAGtaagagtagcccaacAA	21234
202_1	5	82,9	0,9	CAGtaagagtagcccaacAA	21234
203_1	25	31,9	8,8	TAAcGgcaacatcaataGC	22017
203_1	5	56,3	4,2	TAAcGgcaacatcaataGC	22017
204_1	25	35,9	5,8	ACtgagcaccaactaCAC	22592
204_1	5	67,9	10,4	ACtgagcaccaactaCAC	22592
205_1	25	36,8	1,7	CCtaattttatgtatcACAT	23074
205_1	5	96,2	8,4	CCtaattttatgtatcACAT	23074
206_1	25	97,7	1,3	CTAGgaattataacaaatCA	23462 23509
207_1	25	88,0	17,6	ACtccaaagaacatacTCAC	24628
207_1	5	97,0	4,8	ACtccaaagaacatacTCAC	24628
208_1	25	77,3	18,8	TAAgagaaacaatcacaCCA	24935
208_1	5	124,2	2,5	TAAgagaaacaatcacaCCA	24935
209_1	25	68,6	6,7	TGtcctaaaaatcagCAG	26022
209_1	5	102,4	4,6	TGtcctaaaaatcagCAG	26022
210_1	25	54,8	0,4	CTtttaatgagacagtCA	26212
210_1	5	73,6	1,8	CTtttaatgagacagtCA	26212
211_1	25	16,7	1,1	TTGTtagcataagatggaaAG	26500
211_1	5	47,0	1,3	TTGTtagcataagatggaaAG	26500
212_1	25	41,0	0,2	AAActgtagccaataacTGT	26945
212_1	5	92,7	9,4	AAActgtagccaataacTGT	26945
213_1	25	68,3	1,6	ATtcacctaacacaagTAG	27117
213_1	5	74,6	6,7	ATtcacctaacacaagTAG	27117
214_1	25	54,5	0,3	CACGaaaggacagctaAG	27264

CMP ID NO	Concentration μM	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
214_1	5	82,0	13,5	CACGaaaggaacagctaAG	27264
215_1	25	32,4	1,7	ACAACaggcaagtaCC	27411
215_1	5	93,5	2,7	ACAACaggcaagtaCC	27411
216_1	25	40,0	2,5	GCTaaacactataagGAT	27505
216_1	5	94,1	0,1	GCTaaacactataagGAT	27505
217_1	25	106,4	4,9	CCcgtaagcatttgagAA	27831
217_1	5	74,6	2,5	CCcgtaagcatttgagAA	27831
218_1	25	47,0	1,4	AGccaatatgcgacagtaAC	28146
218_1	5	78,1	2,2	AGccaatatgcgacagtaAC	28146
219_1	25	42,8	0,3	TAACcaaaaacaatcagtGTC	28777
219_1	5	62,6	1,1	TAACcaaaaacaatcagtGTC	28777
220_1	25	81,1	20,6	AACaggaacaggagTTA	29195
220_1	5	78,4	3,3	AACaggaacaggagTTA	29195
221_1	25	8,6	1,3	ATttatcaacttcCACC	29906
221_1	5	44,4	7,4	ATttatcaacttcCACC	29906
222_1	25	40,0	1,7	CGTTtaagaccaggcAC	30020
222_1	5	79,5	8,0	CGTTtaagaccaggcAC	30020
223_1	25	45,9	6,2	ACAaaggaactcaggaagAG	30424
223_1	5	84,1	9,3	ACAaaggaactcaggaagAG	30424
224_1	25	3,8	0,0	TCACagacaagcaccAA	31150
224_1	5	35,8	0,3	TCACagacaagcaccAA	31150
225_1	25	26,7	1,0	TACTttttaaaacacgtaGG	31329
225_1	5	90,9	3,5	TACTttttaaaacacgtaGG	31329
226_1	25	47,9	0,4	GCACaatcacaagaccAA	31531
226_1	5	65,0	9,4	GCACaatcacaagaccAA	31531
227_1	25	25,4	0,6	TCagtaaagaacagaGGC	31820
227_1	5	64,2	1,5	TCagtaaagaacagaGGC	31820
228_1	25	45,1	2,0	CAtatttccaccacacaAG	32222
228_1	5	66,9	5,1	CAtatttccaccacacaAG	32222
229_1	25	7,8	0,9	AGtaaaccactgtCCA	32601
229_1	5	34,2	2,7	AGtaaaccactgtCCA	32601
230_1	25	23,4	1,2	TCctcttggcgatATA	33337
230_1	5	64,4	3,6	TCctcttggcgatATA	33337
231_1	25	50,2	4,4	CAtaaataccctgaaTAC	33986
231_1	5	96,2	6,9	CAtaaataccctgaaTAC	33986
232_1	25	7,2	0,8	CGATtttatcaccaaCA	34175
232_1	5	20,8	2,3	CGATtttatcaccaaCA	34175
233_1	25	42,1	1,5	ACaatcagggttaagtgtGA	34771
233_1	5	47,4	7,5	ACaatcagggttaagtgtGA	34771
234_1	25	36,5	14,4	GAAgccaagactacCA	35096
234_1	5	67,8	4,2	GAAgccaagactacCA	35096
235_1	25	63,4	6,2	TGgtagggactgaattTAA	35850
235_1	5	80,3	0,4	TGgtagggactgaattTAA	35850
236_1	25	93,2	3,6	CGgtagtcaatcACC	36584
236_1	5	91,2	12,4	CGgtagtcaatcACC	36584
237_1	25	65,0	13,9	CTatcaaaattatttcACCT	36886
237_1	5	71,2	6,8	CTatcaaaattatttcACCT	36886
238_1	25	96,9	0,6	ACGaaaatttagcatccTAA	37041
238_1	5	75,9	13,5	ACGaaaatttagcatccTAA	37041
239_1	25	9,5	1,7	TGgtaaacactggGC	38059
239_1	5	37,9	1,5	TGgtaaacactggGC	38059
240_1	25	52,4	0,7	GATtgttggtgtcATG	38173

CMP ID NO	Concentration μ M	% mRNA of control		Compound (CMP)	Start on SEQ ID NO 10
		Avg	sd		
240_1	5	63,9	3,4	GATgtgtggtgtcATG	38173
241_1	25	38,8	0,7	AATtatacccacatttCA	38806
241_1	5	108,3	7,0	AATtatacccacatttCA	38806
242_1	25	71,1	1,1	TGcaattagacacgtTACG	39004
242_1	5	83,9	3,6	TGcaattagacacgtTACG	39004
243_1	25	42,7	2,6	GGCAaccaattaaacTA	40226
243_1	5	71,3	1,9	GGCAaccaattaaacTA	40226
244_1	25	4,6	0,9	CAGTtttatgctaataCA	40272
244_1	5	10,6	1,7	CAGTtttatgctaataCA	40272
245_1	25	79,2	7,3	GCaaggaggagcggaataAA	40707
245_1	5	81,1	7,2	GCaaggaggagcggaataAA	40707
246_1	25	116,1	0,8	AAacagaagtaagaagGTCC	41156
246_1	5	105,7	10,4	AAacagaagtaagaagGTCC	41156
247_1	25	47,5	5,3	TCcattatccataGAAA	41477
248_1	25	50,6	3,5	GCTtgacgaacaggaAAT	42282
248_1	5	72,3	0,8	GCTtgacgaacaggaAAT	42282
249_1	25	53,7	2,7	TTCCgacccaaaagaaaagAC	42632
249_1	5	115,4	21,3	TTCCgacccaaaagaaaagAC	42632
250_1	25	51,9	0,3	GCGacacgatccgttaAA	43104
250_1	5	50,9	3,1	GCGacacgatccgttaAA	43104
251_1	25	75,9	2,7	CCCCgttttaaaaAC	43334
251_1	5	111,5	3,0	CCCCgttttaaaaAC	43334

Example 6: Effect on HBV infected dHepaRG cells using selected antisense oligonucleotides targeting PAPD5 or PAPD7 delivered to the cells by gymnososis.

A selection of the most efficacious oligonucleotides from Example 4 and 5 were selected to test their effect on HBV propagation parameters in HBV infected dHepaRG cells.

HBV infected dHepaRG cells (described in the Materials and Methods section, HBV infection of dHepaRG cells) were cultured in 96-well plates. One day post HBV infection 20 μ M of oligonucleotide was delivered to the cells using unassisted uptake (gymnososis). A total of 40 oligonucleotides were tested, 20 targeting PAPD5 and 20 targeting PAPD7. The experiments were conducted in triplicate, with PBS controls. The oligonucleotide treatment was repeated at day 4 and 7 including medium replacement (this differs from the every 2 day replacement described in the Materials and Method section).

At day 11 post-infection, supernatants were harvested and HBsAg and HBeAg levels were assessed using the CLIA ELISA assay (see Materials and Methods, HBV antigen measurements). Cell viability was measured as described in the Materials and Method section, Cell viability. 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. HBV mRNA and PAPD5 or PAPD7 mRNA was quantified in technical duplicate by qPCR using a QuantStudio 12K Flex (Applied Biosystems), the TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems, #4392938), Human ACTB endogenous control (Applied

Biosystems, #4310881E). Taqman reagents were used together with the following commercial ThermoFisher Scientific primers (HBV Pa03453406_s1; PAPD5 Hs00900790_m1; and PAPD7 Hs00173159_m1). The mRNA expression was analyzed using the comparative cycle threshold 2- $\Delta\Delta C_t$ method normalized to the reference gene ACTB (ThermoFisher Scientific 4310881E) and to PBS treated cells.

The effects of the oligonucleotide treatment on the PAPD5 or PAPD7 mRNA as well as the effect on the HBV propagation parameter HBsAg are shown in Table 16 and Table 17.

Table 16: *in vitro* effect of PAPD5 targeting compounds on target mRNA and HBsAg (average of 3)

CMP ID NO	% mRNA inhibition		% HBsAg Inhibition		Compound (CMP)	Start on SEQ ID NO 10
	Avg	sd	Avg	sd		
15_1	79,1	8,5	-9,7	5,7	AATagcaagtagaggaGAG	3059
18_1	94,0	1,6	-5,3	6,0	GTaatacctctcacATTC	5928
23_1	95,9	0,8	45,8	3,9	AGgcttccattttattGAA	8625
25_1	98,5	0,3	4,4	7,1	GTAttcttattcttgCT	10254
26_1	97,5	0,4	20,9	7,0	ATTAttcccacagtaaGA	10881
30_1	89,2	3,5	-13,2	1,9	CCAgagtaaacaacTCC	12718
32_1	93,6	1,1	0,8	12,0	TTTggtaattctttttTAG	14297
39_1	72,1	5,6	-19,4	9,9	GTtaggattttgcTATG	18299
54_1	91,0	1,6	5,2	4,9	TTCAacctttactgcAT	27813
56_1	95,6	1,0	5,5	6,6	CAgtaggaatgtggCTT	28718
58_1	82,2	2,6	-18,6	17,5	TCactgttaaaccTCAC	29902
65_1	51,1	6,0	-36,8	15,4	TTCAcaacaggtaagGG	33593
80_1	89,4	1,6	9,4	9,9	CAAaggttgtgtacTCT	44220
88_1	22,6	10,7	-20,1	13,8	AGCTtaaagaagaacggTT	49688
92_1	93,1	1,7	-8,1	6,5	GTatctttctgtacTATT	52461
93_1	67,3	5,5	-14,8	2,5	GTCAttctactaacaacCG	53305
111_1	72,0	4,6	-27,6	8,7	ATggttttgtggagaAGG	63000
115_1	61,7	6,9	25,8	9,4	GATGgtgacaaattattCT	65894
116_1	97,2	0,7	11,2	9,5	TGcttttgggaatCTTT	66650
118_1	44,4	15,0	-24,2	24,1	GTAcaggacaaagtaACC	68732

Table 17: *in vitro* effect of PAPD7 targeting compounds on target mRNA and HBsAg Average of 3).

CMP ID NO	% mRNA inhibition		% HBsAg Inhibition		Compound (CMP)	Start on SEQ ID NO 11
	Avg	sd	Avg	sd		
153_1	87,5	2,1	-32,3	9,5	CAgtagtaaccacCAAG	7447
155_1	83,6	3,0	-35,8	13,5	ACtagtaaatagcaGCA	8074
168_1	96,0	1,0	-45,2	9,5	AAtgcttaatttttctCTCT	11084
171_1	96,3	0,8	-34,4	15,3	CAGcataattgttttctTTT	12330
172_1	96,0	0,8	-45,0	12,5	ATGTcattatgttttagTT	12634
174_1	87,0	1,5	-28,8	4,8	CGgtaagggttcggTG	13126
183_1	96,3	0,7	-31,7	11,6	CATTgctttataatccTA	15816
184_1	85,3	1,9	-46,6	11,2	CTTTttaaggacaggaGG	15988
188_1	89,2	0,8	-34,8	20,7	GCAaatgtaagcctttTT	17303
190_1	92,0	0,9	-31,5	15,7	AAGagtgggttgtaAGC	17963

CMP ID NO	% mRNA inhibition		% HBsAg Inhibition		Compound (CMP)	Start on SEQ ID NO 11
	Avg	sd	Avg	sd		
191_1	93,4	0,4	-36,2	21,8	TAgtgaaaatatttgAGTT	18101
194_1	65,4	4,0	-37,3	17,4	GTAgttaaagggcaaaggAA	19007
195_1	85,9	3,8	-39,2	14,6	GTgaattaaagccaAAGC	19252
197_1	94,0	1,3	-24,9	15,5	GAggttttttagtgAATT	19722
221_1	99,1	0,3	-36,4	4,7	ATttatcaacttcCACC	29906
224_1	97,7	0,6	-22,1	16,0	TCACagacaagcaccAA	31150
229_1	96,6	0,7	60,5	4,6	AGtaaaccactgtCCA	32601
232_1	95,5	1,5	-48,3	31,0	CGATtttatcaccaaCA	34175
239_1	93,1	1,3	-31,6	3,7	TGgtaaacactggGC	38059
244_1	98,7	0,3	-26,9	8,5	CAGTtttatgctaataCA	40272

From these data it can be seen that the inhibition of the PAPD5 mRNA (table 16) and PAPD7 mRNA (table 17) was very efficient for most of the oligonucleotide compounds.

The observed effect of PAPD5 and PAPD7 mRNA reduction on HBsAg levels is however less pronounced even in cells treated for 11 days after un-assisted delivery of oligonucleotide. Here only PAPD5 targeting CMP ID NO: 23_1, 26_1 and 115_1 showed clear HBsAg inhibition and of the PAPD7 targeting compounds only CPM ID NO: 229_1 showed clear HBsAg inhibition. Without being bound by theory this could be due to a slow onset of the effect caused by the target knock down on the HBsAg inhibition in the present assay and thus, for some compounds, HBsAg inhibition would not be seen unless assayed at a later time point.

10 **Example 7: Effect on HBV infected dHepaRG cells using a combined preparation of a PAPD5 and a PAPD7 targeting antisense oligonucleotide delivered to the cells by gymnosis.**

In Example 2 it was observed that mixing a pool of PAPD7 targeting siRNA's with a pool of PAPD5 targeting siRNA's resulted in a synergistic anti-HBV activity. The present example sets out to investigate whether a similar synergy can be observed when combining two individual single stranded antisense oligonucleotides, where one targets PAPD5 and one targets PAPD7.

The experiment was conducted as described in Example 6 with the change that instead of adding individual oligonucleotides, a combination of two oligonucleotides were added to the cells, such that 20 μ M of one oligonucleotide targeting PAPD5 was added together with 20 μ M of a second oligonucleotide targeting PAPD7. Only HBsAg was measured for the combinations.

The combination of oligonucleotides can be seen in table 18, including the results on the HBV propagation parameters, HBsAg inhibition.

Table 18: in vitro effect combinations of PAPD5 and PAPD7 targeting compounds on HBsAg (average of 3). The HBsAg inhibition results from table 16 and 17 on the individual compounds are also included in this table for ease of comparing the individual treatment with the combination treatment.

CMP ID NO combination	% HBsAg inhibition combination		% HBsAg inhibition PAPD5		% HBsAg inhibition PAPD7	
	Avg	sd	Avg	sd	Avg	sd
15_1+184_1	-14,9	13,0	-9,7	5,7	-46,6	11,2
15_1+221_1	-8,1	2,9	-9,7	5,7	-36,4	4,7
18_1+184_1	-8,0	1,8	-5,3	6,0	-46,6	11,2
18_1+221_1	22,0	10,8	-5,3	6,0	-36,4	4,7
23_1+172_1	48,4	6,3	45,8	3,9	-45,0	12,5
23_1+188_1	47,2	14,3	45,8	3,9	-34,8	20,7
25_1+174_1	31,3	7,8	4,4	7,1	-28,8	4,8
25_1+183_1	58,5	9,9	4,4	7,1	-31,7	11,6
26_1+174_1	8,4	2,6	20,9	7,0	-28,8	4,8
26_1+183_1	38,5	11,2	20,9	7,0	-31,7	11,6
30_1+172_1	-19,1	11,2	-13,2	1,9	-45,0	12,5
30_1+188_1	-8,6	5,0	-13,2	1,9	-34,8	20,7
32_1+155_1	21,0	1,2	0,8	12,0	-35,8	13,5
32_1+195_1	8,3	4,7	0,8	12,0	-39,2	14,6
39_1+224_1	-10,7	10,0	-19,4	9,9	-22,1	16,0
39_1+229_1	48,3	4,8	-19,4	9,9	60,5	4,6
54_1+190_1	8,3	9,1	5,2	4,9	-31,5	15,7
54_1+232_1	29,4	2,4	5,2	4,9	-48,3	31,0
56_1+153_1	45,0	9,5	5,5	6,6	-32,3	9,5
56_1+244_1	60,5	5,9	5,5	6,6	-26,9	8,5
58_1+171_1	-8,0	17,8	-18,6	17,5	-34,4	15,3
58_1+239_1	-14,2	13,0	-18,6	17,5	-31,6	3,7
65_1+171_1	-21,2	4,5	-36,8	15,4	-34,4	15,3
65_1+239_1	-26,4	2,3	-36,8	15,4	-31,6	3,7
80_1+153_1	53,6	5,1	9,4	9,9	-32,3	9,5
80_1+244_1	65,4	7,3	9,4	9,9	-26,9	8,5
88_1+168_1	-16,4	7,9	-20,1	13,8	-45,2	9,5
88_1+197_1	-9,7	9,0	-20,1	13,8	-24,9	15,5
92_1+190_1	34,6	7,2	-8,1	6,5	-31,5	15,7
92_1+232_1	23,4	11,6	-8,1	6,5	-48,3	31,0
93_1+224_1	12,8	5,6	-14,8	2,5	-22,1	16,0
93_1+229_1	27,7	10,3	-14,8	2,5	60,5	4,6
111_1+191_1	-20,0	7,0	-27,6	8,7	-36,2	21,8
111_1+194_1	-22,2	5,6	-27,6	8,7	-37,3	17,4
115_1+191_1	4,8	5,4	25,8	9,4	-36,2	21,8
115_1+194_1	-6,7	13,3	25,8	9,4	-37,3	17,4
116_1+155_1	39,2	2,2	11,2	9,5	-35,8	13,5
116_1+195_1	22,9	9,5	11,2	9,5	-39,2	14,6
118_1+168_1	-9,8	11,5	-24,2	24,1	-45,2	9,5

CMP ID NO combination	% HBsAg inhibition combination		% HBsAg inhibition PAPD5		% HBsAg inhibition PAPD7	
	Avg	sd	Avg	sd	Avg	sd
118_1+197_1	4,6	10,5	-24,2	24,1	-24,9	15,5

- The results are also summarized in figure 10. Of the 40 combinations tested above 18 resulted in an apparent synergistic effect on the HBsAg inhibition. 7 of these synergistic combinations are with oligonucleotides that individually did not produce any effect on HBsAg inhibition in the current assay. From this experiment it can be concluded that the combination of PAPD5 and a PAPD7 targeting oligonucleotides have a high likelihood of producing a synergistic effect on HBsAg inhibition.

Example 8: Repeat of selected combinations from Example 7

The experiment in example 7 was repeated with the oligonucleotide combinations indicated in table 19.

- Table 19: in vitro effect combinations of PAPD5 and PAPD7 targeting compounds on HBsAg (average of 3). The HBsAg inhibition results from table 16 and 17 on the individual compounds are also included in this table for ease of comparing the individual treatment with the combination treatment.

CMP ID NO combination	% HBsAg inhibition combination		% HBsAg inhibition PAPD5		% HBsAg inhibition PAPD7	
	Avg	sd	Avg	sd	Avg	sd
23_1+172_1	57,4	13,2	45,8	3,9	-45,0	12,5
23_1+188_1	63,6	8,0	45,8	3,9	-34,8	20,7
25_1+174_1	36,2	14,5	4,4	7,1	-28,8	4,8
25_1+183_1	80,3	1,3	4,4	7,1	-31,7	11,6
39_1+229_1	33,9	18,5	-19,4	9,9	60,5	4,6
54_1+232_1	56,0	8,5	5,2	4,9	-48,3	31,0
56_1+153_1	55,4	6,5	5,5	6,6	-32,3	9,5
80_1+153_1	53,2	9,3	9,4	9,9	-32,3	9,5
80_1+244_1	74,3	5,1	9,4	9,9	-26,9	8,5
92_1+190_1	27,8	9,9	-8,1	6,5	-31,5	15,7
116_1+155_1	31,6	1,1	11,2	9,5	-35,8	13,5

From these data it can be seen that the synergistic effect observed in example 7 is repeatable.

- Example 9: Effect on HBV infected dHepaRG cells using selected antisense oligonucleotides targeting PAPD5 or PAPD7 delivered to the cells by transfection**

In the following experiment it was investigated whether similar synergistic results could be achieved using transfection of the oligonucleotides into HBV infected dHepaRG cells instead of unassisted delivery.

- Twelve PAPD5 targeting oligonucleotides (table 20) and thirteen PAPD7 targeting oligonucleotides (table 21) were tested individually using the transfection assay described here.

Oligonucleotides were transfected in a 96-well plate format at a final concentration of 500nM per well in differentiated HepaRG cells one day post-infection with HBV (described in the Materials and Methods section, HBV infection of dHepaRG cells). Prior to transfection, medium was replaced with 100uL penicillin/streptomycin free complete differentiation medium. For single oligonucleotide treatment, oligonucleotide was diluted in Opti-MEM+Glutamax-I reduced serum medium (Gibco, #51985) and incubated at a ratio 1:1 with Lipofectamine RNAiMax (Invitrogen, #56532) for 5 minutes at room temperature according to the manufacturer's instructions. From this LNA-transfection reagent mixture, 20 µl was then added on top of the cells. After 3 days, medium was replaced with a complete differentiation medium. At day 5 post-transfection, HBsAg was measured as described in the materials and method section, PAPD5 and PAPD7 mRNA was measured as described in Example 6.

In addition some of the PAPD5 and PAPD7 oligonucleotides were tested in combination (table 22). The oligonucleotides were co-transfected into the HBV infected HepaRG cells using the same protocol as single treatment with 500 nM of each oligonucleotide in the final concentration.

Table 20: *in vitro* effect of PAPD5 targeting compounds on target mRNA and HBsAg.

CMP ID NO	% mRNA inhibition		% HBsAg Inhibition		Compound (CMP)	Start on SEQ ID NO 10
	Avg	sd	Avg	sd		
23_1	70,3	4,0	89,2	8,7	AGgcttccattttattGAA	8625
25_1	81,0	2,9	3,9	12,6	GTAttcttattcttgCT	10254
26_1	85,6	1,8	52,7	14,0	ATTAttcccacagtaaGA	10881
39_1	69,3	5,8	14,3	10,0	GTtaggattttgcTATG	18299
54_1	75,6	3,6	31,6	16,5	TTCAacctttactgcAT	27813
56_1	90,7	1,4	67,4	5,3	CAGtaggaatgtggCTT	28718
58_1	39,3	11,1	-4,1	14,6	TCactgttaaaccTCAC	29902
65_1	81,1	4,8	32,3	34,5	TTCAcaacaggtaaagGG	33593
80_1	90,9	1,7	33,2	8,7	CAAaggtgtgtgtacTCT	44220
92_1	80,5	1,9	15,3	19,1	GTatctttctgtacTATT	52461
93_1	72,2	4,3	37,3	12,5	GTCAttctactaacaacCG	53305
116_1	81,6	4,8	10,8	10,3	TGcttttgggaatCTTT	66650

Table 21: *in vitro* effect of PAPD7 targeting compounds on target mRNA and HBsAg

CMP ID NO	% mRNA inhibition		% HBsAg Inhibition		Compound (CMP)	Start on SEQ ID NO 11
	Avg	sd	Avg	sd		
153_1	67,0	8,3	-17,8	9,4	CAGtagtaaccacCAAG	7447
155_1	86,3	4,2	0,1	31,9	ACTtagtaatagcaGCA	8074
171_1	66,8	13,2	-5,8	6,6	CAGcataattgttttCTT	12330
172_1	94,5	1,2	12,1	6,8	ATGTcattatgttttagTT	12634
174_1	58,6	7,3	-6,8	21,9	CGgtaaggggttcggTG	13126
183_1	74,1	5,0	6,7	23,6	CATTgcttttataatccTA	15816
188_1	72,8	4,7	26,9	4,6	GCAaatgtaagcctttTT	17303
190_1	75,2	4,9	87,7	1,0	AAGagtgggtgtgaAGC	17963
224_1	72,6	4,4	4,3	24,1	TCACagacaagcaccAA	31150

CMP ID NO	% mRNA inhibition		% HBsAg Inhibition		Compound (CMP)	Start on SEQ ID NO 11
	Avg	sd	Avg	sd		
229_1	48,0	6,4	27,8	24,9	AGtaaaccactgtCCA	32601
232_1	64,0	4,0	-15,1	38,3	CGATtttatcaccaaCA	34175
239_1	91,9	3,7	88,0	7,0	TGgtaaacactggGC	38059
244_1	70,0	3,6	-12,9	11,3	CAGTtttatgctaataCA	40272

Table 22 *in vitro* effect of combinations of PAPD5 and PAPD7 targeting compounds. The HBsAg inhibition results from table 20 and 21 on the individual compounds are also included in this table for ease of comparing the individual treatment with the combination treatment

CMP ID NO combination	% HBsAg inhibition combination		CMP ID NO PAPD5	% HBsAg inhibition		CMP ID NO PAPD7	% HBsAg inhibition	
	Avg	sd		Avg	sd		Avg	sd
23_1+172_1	44,1	6,9	23_1	89,2	8,7	172_1	12,1	6,8
23_1+188_1	76,6	12,0	23_1	89,2	8,7	188_1	26,9	4,6
25_1+174_1	33,0	22,1	25_1	3,9	12,6	174_1	-6,8	21,9
25_1+183_1	75,3	7,8	25_1	3,9	12,6	183_1	6,7	23,6
39_1+229_1	77,4	13,3	39_1	14,3	10,0	229_1	27,8	24,9
54_1+232_1	79,9	6,5	54_1	31,6	16,5	232_1	-15,1	38,3
56_1+153_1	95,6	1,2	56_1	67,4	5,3	153_1	-17,8	9,4
56_1+244_1	89,2	4,8	56_1	67,4	5,3	244_1	-12,9	11,3
80_1+153_1	85,5	2,3	80_1	33,2	8,7	153_1	-17,8	9,4
80_1+244_1	82,8	6,7	80_1	33,2	8,7	244_1	-12,9	11,3
92_1+190_1	90,5	1,9	92_1	15,3	19,1	190_1	87,7	1,0
116_1+155_1	87,5	2,5	116_1	10,8	10,3	155_1	0,1	31,9

- 5 From these data it can be seen that when using a transfection assay 6 out of the 12 oligonucleotides targeting PAPD5 have a clear effect on HBsAg inhibition, which is considerably more than observed with the gymnotic assay in example 6. Also the number of PAPD7 targeting compounds that have an effect on the HBsAg inhibition has been increased using the transfection assay such that 4 out of 12 compounds have a clear effect on HBsAg inhibition.
- 10 The data from the combinations are summarized in table 22 and figure 11. From these data it can be seen that 9 of the 12 tested combinations produce an apparent synergistic effect on the HBsAg inhibition.

CLAIMS

1. A composition comprising a nucleic acid molecule for use in the treatment and/or prevention of Hepatitis B virus infection, wherein said nucleic acid molecule inhibits expression and/or activity of PAP associated domain containing 5 (PAPD5).
2. The composition for the use of claim 1, wherein said composition is a combined preparation comprising:
 - a. a nucleic acid molecule which inhibits expression and/or activity PAPD5; and
 - b. a nucleic acid molecule which inhibits expression and/or activity of PAP associated domain containing 7 (PAPD7).
3. The composition for the use of claim 1 or 2, wherein the nucleic acid molecules independently are selected from the group consisting of:
 - a. a single stranded antisense oligonucleotide;
 - b. a siRNA molecule;
 - c. a shRNA molecule; and
 - d. a genome editing machinery, comprising:
 - i. a site-specific DNA nuclease or a polynucleotide encoding a site-specific DNA nuclease; and
 - ii. a guide RNA or a polynucleotide encoding a guide RNA.
4. The composition for the use of claim 2 or 3, wherein the nucleic acid molecules are selected from:
 - a. a single stranded antisense oligonucleotide comprising a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 80% complementarity to PAPD5 target nucleic acid and which capable of reducing expression of PAPD5; and
 - b. a single stranded antisense oligonucleotide comprising a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 80% complementarity to a PAPD7 target nucleic acid and which is capable of reducing expression of PAPD7.
5. The composition for the use of any one of claims 1 to 4, wherein the composition reduces secretion of HBsAg, HBeAg and/or inhibits production of intracellular HBV mRNA or HBV DNA.
6. The composition for the use of any one of claims 1 to 5, wherein the composition inhibits development of chronic HBV infection and/or reduces the infectiousness of a HBV infected person.
7. A method for identifying a compound or composition that prevents, ameliorates and/or inhibits a hepatitis B virus (HBV) infection, comprising:
 - a. contacting a test compound or composition with a cell expressing PAPD5 and/or PAPD7;

- b. measuring the expression and/or activity of PAPD5 and/or PAPD7 in the presence and absence of said test compound or composition; and
 - c. identifying a compound or composition that reduces the expression and/or activity of PAPD5 and/or PAPD7 as a compound that prevents, ameliorates and/or inhibits a HBV infection.
8. The method of claim 7, wherein the test compound is a library of nucleic acid molecules and step c) identifies nucleic acid molecules that reduce PAPD5 or PAPD7 mRNA expression by at least 60%.
 9. The method of claim 7, wherein the test composition is a combined preparation of a nucleic acid molecule capable of reducing PAPD5 and a nucleic acid molecule capable of reducing PAPD7.
 10. The method of any one of claims 7 to 9, wherein the compound that inhibits propagation of HBV inhibits secretion of HBV surface antigen (HBsAg), and/or inhibits secretion of HBV envelope antigen (HBeAg), and/or inhibits production of intracellular HBV mRNA.
 11. A single stranded antisense oligonucleotide which comprises or consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 80% complementarity to a PAPD5 target nucleic acid and the antisense oligonucleotide is capable of reducing expression of PAPD5.
 12. A nucleic acid molecule which comprises or consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 80% complementarity to a PAPD7 target nucleic acid and the nucleic acid molecule is capable of reducing expression of PAPD7.
 13. The nucleic acid molecule of claim 12, wherein the nucleic acid molecule is a single stranded antisense oligonucleotide.
 14. The antisense oligonucleotide or nucleic acid molecule of any one of claims 11 to 13, comprising one or more 2' sugar modified nucleoside(s).
 15. The antisense oligonucleotide or nucleic acid molecule of claim 14, wherein the one or more 2' sugar modified nucleoside is independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA and LNA nucleosides.
 16. The antisense oligonucleotide or nucleic acid molecule of claim 14 or 15, wherein the one or more 2' sugar modified nucleoside is a LNA nucleoside.

17. The antisense oligonucleotide or nucleic acid molecule of any one of claims 11 to 16, wherein the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.
18. The antisense oligonucleotide of any one of claims 11 or 13 to 17, wherein the oligonucleotide is a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise 1 - 7 2' sugar modified nucleosides and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH.
19. A conjugate comprising the antisense oligonucleotide or nucleic acid molecule according to any one of claims 11 to 18, and at least one conjugate moiety covalently attached to said oligonucleotide.
20. A combined preparation comprising
 - a. a nucleic acid molecule which inhibits expression and/or activity of PAPD5; and
 - b. a nucleic acid molecule which inhibits expression and/or activity of PAPD7.
21. The combined preparation of claim 20, wherein the nucleic acid molecule is selected from any one of the claims 11 to 18 or the conjugate of claim 19.
22. A pharmaceutical composition comprising the antisense oligonucleotide or nucleic acid molecule according to any one of claims 11 to 18, or the conjugate of claim 19, or the combined preparation of claim 20 or 21..