

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:

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

5 **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

10 **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.

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

15 **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-
20 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

The present disclosure 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
20 molecule inhibits expression and/or activity of PAPD5. In particular a composition 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.

In one aspect, the present disclosure relates to a use of a composition comprising a nucleic acid
25 molecule which directly inhibits expression and/or activity of PAP associated domain containing 5 (PAPD5) in the manufacture of a medicament for the treatment of Hepatitis B virus infection.

The present disclosure further relates to nucleic acid molecules that inhibit expression and/or activity of PAPD7. In particular single stranded antisense, siRNA and shRNA molecules.

A further aspect of the present disclosure relates to a nucleic acid molecule which consists of a
30 contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is 100% complementary to a PAPD7 target nucleic acid and the nucleic acid molecule is capable of reducing expression of PAPD7.

The present disclosure further relates 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.

- 5 A further aspect of the present disclosure relates to a single stranded antisense oligonucleotide which consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is 100% complementary to a PAPD5 target nucleic acid and the antisense oligonucleotide is capable of reducing expression of PAPD5.

- 10 The present disclosure further relates to conjugates of nucleic acid molecules of the disclosure, 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 disclosure.

A further aspect of the present disclosure relates to a conjugate comprising the antisense oligonucleotide or nucleic acid molecule of the disclosure, and at least one conjugate moiety covalently attached to said oligonucleotide.

- 15 A further aspect of the present disclosure 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;

wherein the nucleic acid molecule is selected from the disclosure or the conjugate of the disclosure.

- 20 A further aspect of the present disclosure relates to a pharmaceutical composition comprising the antisense oligonucleotide or nucleic acid molecule of the disclosure, or the conjugate of the disclosure, or the combined preparation of the disclosure.

The present disclosure further relates to 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.

A further aspect of the disclosure is a method for identifying a compound or composition that ameliorates and/or directly 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 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
5 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 240 LNA modified antisense oligonucleotides targeting either PAPD5 or PAPD7 mRNA have
10 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.

CLAIMS

1. A use of a composition comprising a nucleic acid molecule which directly inhibits expression and/or activity of PAP associated domain containing 5 (PAPD5) in the manufacture of a medicament for the treatment of Hepatitis B virus infection.
2. The use of claim 1, wherein said composition is a combined preparation comprising:
 - a. a nucleic acid molecule which directly inhibits expression and/or activity PAPD5; and
 - b. a nucleic acid molecule which directly inhibits expression and/or activity of PAP associated domain containing 7 (PAPD7).
3. 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 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 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 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 ameliorates and/or directly 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 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 consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is 100% complementarity to a PAPD5 target nucleic acid and the antisense oligonucleotide is capable of reducing expression of PAPD5.
12. A nucleic acid molecule which consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length wherein the contiguous nucleotide sequence is 100% 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;
- wherein the nucleic acid molecule is selected from any one of the claims 11 to 18 or the conjugate of claim 19.
21. 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..