and there is an urgent need to develop new therapies for the HBV complete cure that eliminates cccDNA.

OBJECTIVE OF THE INVENTION

The present invention shows that there is a correlation between the inhibition of FUBP1 and reduction of cccDNA in an HBV infected cell, which is relevant in the treatment of HBV infected individuals. An objective of the present invention is to identify FUBP1 inhibitors which reduce cccDNA in an HBV infected cell. Such FUBP1 inhibitors can be used in the treatment of HBV infection.

The present invention further identifies novel nucleic acid molecules, which are capable of inhibiting the expression of FUBP1 *in vivo* and *in vitro*.

BRIEF DESCRIPTION OF THE FIGURES

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

Figure 2: Structural formula of the trivalent GalNAc cluster (GN2). GN2 is useful as conjugation moiety in the present invention. The wavy line illustrates the site of conjugation of the cluster to e.g. a C6 amino linker or directly to the oligonucleotide.

Figure 3: Southern blot from HBV infected PPH cells treated either with control (ctr) or the pool of FUBP1 targeting siRNA’s (Pool) or HBV siRNA (HBV).

SUMMARY ~~OF THE INVENTION~~

~~One aspect of the~~The present ~~invention~~disclosure relates to a FUBP1 inhibitor for use in the treatment and/or prevention of Hepatitis B virus infection. In one aspect, the present disclosure relates to use of a FUBP1 inhibitor in the manufacture of a medicament for the treatment and/or prevention of Hepatitis B virus (HBV) infection. In particular, a FUBP1 inhibitor capable of

reducing cccDNA and/or pre-genomic RNA (pgRNA) is useful. In the event that the FUBP1 inhibitor is a small molecule it is advantageous if it interacts with the DNA binding domain of FUBP1 protein, and prevents or reduces FUBP1 binding to cccDNA. The FUBP1 inhibitor can also be a nucleic acid molecule of 12 to 60 nucleotides in length which is capable of reducing FUBP1 mRNA.

~~A further aspect of the~~The present ~~invention~~disclosure relates to nucleic acid molecules that inhibit expression and/or activity of FUBP1. In particular, a nucleic acid molecule of 12 to 60 nucleotides in length

which comprises or consists of a contiguous nucleotide sequence of 12 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 95% complementary to a mammalian FUBP1 target nucleic acid. Such nucleic acid molecules can be selected from single stranded antisense, siRNA and chemically produced shRNA molecules (not relying on cell based expression from plasmids or viruses). In another aspect, the present disclosure relates to a nucleic acid molecule of 12 to 60 nucleotides in length which comprises or consists of a contiguous nucleotide sequence of 12 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 95% complementarity to a mammalian FUBP1 target nucleic acid, wherein the contiguous nucleotide sequence is complementary to a target sequence selected from the group consisting of position 14200-14218, 14413-14431, 14966-14984 and 30344-30362 on SEQ ID NO: 1.

~~A further aspect of the~~The present ~~invention~~disclosure relates to single stranded antisense oligonucleotides or siRNA that inhibit expression and/or activity of FUBP1. In particular, modified antisense oligonucleotides or modified siRNA comprising one or more 2' sugar modified nucleoside and one or more phosphorthioate linkage that reduce FUBP1 mRNA are of advantageous.

~~Further aspects of the invention are~~The present disclosure relates to conjugates of nucleic acid molecules of the ~~invention~~disclosure and pharmaceutical compositions comprising the molecules of the ~~invention~~disclosure. In particular conjugates targeting the liver are of interest, such as GalNAc clusters. Thus, in another aspect, the present disclosure relates to a conjugate compound comprising a nucleic acid molecule of 12 to 60 nucleotides in length which comprises or consists of a contiguous nucleotide sequence of 12 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 95% complementarity to a mammalian FUBP1 target nucleic acid, and at least one liver targeting conjugate moiety covalently attached to an oligonucleotide component of the nucleic acid molecule.

In another aspect, the present disclosure relates to a pharmaceutical composition comprising one or more nucleic acid molecule(s) as described herein, or one or more of the conjugate compound(s) as described herein, or acceptable salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant.

In another aspect, the present disclosure relates to an *in vitro* method for modulating FUBP1 expression in a target cell which is expressing FUBP1, said method comprising administering the nucleic acid molecule as described herein, or the conjugate compound as described herein or the pharmaceutical composition as described herein in an effective amount to said cell.

In another aspect, the present disclosure relates to the nucleic acid molecule as described herein, or the conjugate compound as described herein or the pharmaceutical composition as described herein for use as a medicament.

Definitions

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 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. Therapeutic nucleic acid molecules are commonly made in the laboratory by solid-phase chemical synthesis followed by purification and isolation. shRNA’s are however often delivered to cells using lentiviral vectors (see for example Soan and Yang 2010 N Am J Med Sci 2(12): 598) which are then transcribed to produce the single stranded RNA that will form a stem loop (hairpin) RNA structure that is capable of interacting with the RNA interference machinery (including the RNA-induced silencing complex (RISC)). When referring to a sequence of the nucleic acid molecule, reference is made to the sequence 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. In some embodiments the nucleic acid molecule of the invention is not a shRNA transcribed from a vector upon entry into the target cell. 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 12 to 60 nucleotides in length, such as from 13 to 50, such as from 14 to 40, such as from 15 to 30, such as from 16 to 22, such as from 16 to 18 or 15 to 17 contiguous nucleotides in length.

CLAIMS

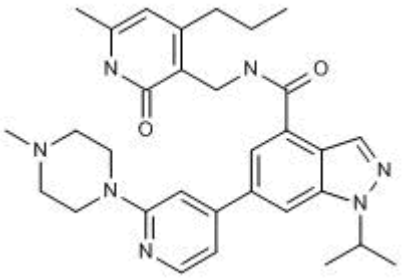
1. ~~A~~ Use of a FUBP1 inhibitor ~~for use~~ in the manufacture of a medicament for the treatment and/or prevention of Hepatitis B virus (HBV) infection.

2. The ~~FUBP1 inhibitor for the~~ use of claim 1, wherein the HBV infection is a chronic infection.

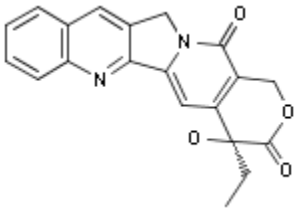
3. The ~~FUBP1 inhibitor for the~~ use of claim 1 or 2, wherein the FUBP1 inhibitor is capable of reducing cccDNA and/or pgRNA in an infected cell.

4. The ~~FUBP1 inhibitor for the~~ use of any one of claims 1 to 3, wherein the inhibitor prevents or reduces the FUBP1/FUSE interaction.

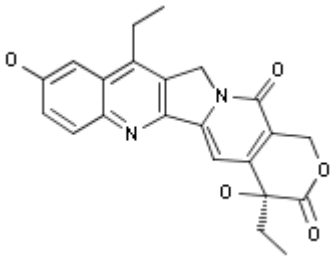
5. The ~~FUBP1 inhibitor for the~~ use of any one of claims 1 to 4, wherein the inhibitor is selected from the compounds of Formula VIII, IX or X



VIII



IX



X

6. The ~~FUBP1 inhibitor for the~~ use of any one of claims 1 to 3, wherein said inhibitor is a nucleic acid molecule of 12 to 60 nucleotides in length comprising or consisting of a contiguous nucleotide sequence of 12 to 30 nucleotides in length which is at least 95% complementary to a mammalian FUBP1 target nucleic acid and capable of reducing FUBP1 mRNA.

7. The ~~nucleic acid molecule for the~~ use of claim 6, wherein the nucleic acid molecule is selected from
   a. a single stranded antisense oligonucleotide;
   b. a siRNA molecule; or
   c. a shRNA molecule.

8. The ~~nucleic acid molecule for the~~ use of claim 6 or 7, wherein the mammalian FUBP1 target nucleic acid is selected from SEQ ID NO: 1 to 8

9. The ~~nucleic acid molecule for the~~ use of any one of claims 6 to 8, wherein the contiguous nucleotide sequence is at least 98% complementarity to the target nucleic acid of SEQ ID NO: 1 and SEQ ID NO: 5.

10. The ~~FUBP1 inhibitor for the~~ use of any one of claims 1 to 9, wherein the cccDNA in an HBV infected cell is reduced by at least 60% when compared to a control and/or wherein the FUBP1 mRNA is reduced by at least 60% when compared to a control.

~~11. The nucleic acid molecule for the use of any one of claims 6 to 9, wherein the FUBP1 mRNA is reduced by at least 60% when compared to a control.~~

~~12.~~11. A nucleic acid molecule of 12 to 60 nucleotides in length which comprises or consists of a contiguous nucleotide sequence of 12 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 95% complementarity to a mammalian FUBP1 target nucleic acid, wherein the contiguous nucleotide sequence is complementary to a target sequence selected from the group consisting of position 14200-14218, 14413-14431, 14966-14984 and 30344-30362 on SEQ ID NO: 1.

~~13. The nucleic acid molecule of claim 12, wherein the nucleic acid molecule is a siRNA or a single stranded antisense oligonucleotide.~~

~~14. The nucleic acid molecule of claim 12 or 13, wherein the nucleic acid molecule comprises one or more 2' sugar modified nucleosides and one or more phosphorthioate linkages.~~

~~15. The nucleic acid molecule of any one of claims 12 to 14, wherein contiguous nucleotide sequence is complementary to a target sequence selected from the group consisting of position14200-14218, 14413-14431, 14966-14984 and 30344-30362 on SEQ ID NO: 1.~~

~~16~~12. A conjugate compound comprising a nucleic acid molecule of 12 to 60 nucleotides in length which comprises or consists of a contiguous nucleotide sequence of 12 to 30 nucleotides in length wherein the contiguous nucleotide sequence is at least 95% complementarity to a mammalian FUBP1 target nucleic acid, ~~the nucleic acid molecule of any one of claims 12 to 15~~ and at least one liver targeting conjugate moiety covalently attached to an oligonucleotide component of the nucleic acid molecule.

13. The conjugate compound of claim 12, wherein the liver targeting conjugate moiety is selected from moieties comprising cholesterol or other lipids or a conjugate moiety capable of binding to the asialoglycoprotein receptor (ASGPR), and/or wherein the candidate compound comprises a physiologically labile linker composed of 2 to 5 linked nucleosides

comprising at least two consecutive phosphodiester linkages, wherein the physiologically labile linker covalently bound at the 5' or 3' terminal of the oligonucleotide component.

~~17~~14. The conjugate compound of claim ~~16~~13, wherein the liver targeting conjugate moiety is selected from one of the trivalent GalNAc moieties in figure 1.

15. The nucleic acid molecule of claim 11, or the conjugate compound of any one of claims 12 to 14, wherein the nucleic acid molecule is a siRNA or a single stranded antisense oligonucleotide, and/or wherein the nucleic acid molecule comprises one or more 2' sugar modified nucleosides and one or more phosphorothioate linkages.

~~18. The conjugate compound of claim 16 or 17 comprising a physiologically labile linker composed of 2 to 5 linked nucleosides comprising at least two consecutive phosphodiester linkages, wherein the physiologically labile linker covalently bound at the 5' or 3' terminal of the oligonucleotide component.~~

~~19~~16. A pharmaceutical composition comprising one or more nucleic acid molecule(s) of ~~any one of claims 12 to 15~~claim 11 or 15, or one or more of the conjugate compound(s) of any one of claims ~~16 to 18~~12 to 15, or acceptable salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant.

~~20~~17. An ~~*in vivo* or~~ *in vitro* method for modulating FUBP1 expression in a target cell which is expressing FUBP1, said method comprising administering the nucleic acid molecule of ~~any one of claims 12 to 15~~claim 11 or 15, or the conjugate compound of any one of claims ~~16 to 18~~12 to 15 or the pharmaceutical composition of claim ~~19~~ 16 in an effective amount to said cell.

~~21~~18. The nucleic acid molecule of ~~any one of claims 12 to 15~~claim 11 or 15, or the conjugate compound of any one of claims ~~16 to18~~12 to 15 or the pharmaceutical composition of claim ~~19~~ 16 for use as a medicament.