strand invasion events and thereby promotes non-crossover repair by meiotic synthesis dependent strand annealing (SDSA) as well as disassembly of D loop recombination intermediates. In additional RTEL1 disassembles T loops and prevents telomere fragility by counteracting telomeric G4-DNA structures, which together ensure the dynamics and stability of the telomere.

RTEL1 has been identified in a siRNA screen as a stabilizer of HPV episomes: (Edwards et al 2013 PLoS One Vol 8, e75406). siRNA targeting RTEL1 has likewise been used to identify interactants with RTEL1 in Hoyeraal-Hreidarsson syndrome (Schertzer et al 2015 Nucleic Acid Res Vol 43 p. 1834). In addition, RTEL1 was identified as a HIV host dependency factor from a siRNA screen for essential host proteins to provide targets for inhibition HIV infection (WO 2007/094818).

To our knowledge RTEL1 has never been identified as a cccDNA dependency factor in the context of cccDNA stability and maintenance, nor have molecules inhibiting RTEL1 ever been suggested as cccDNA destabilizers for the treatment of HBV infection.

OBJECTIVE OF THE INVENTION

The present invention shows that there is a correlation between the inhibition of RTEL1 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 RTEL1 inhibitors which reduce cccDNA in an HBV infected cell. Such RTEL1 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 RTEL1 *in vitro and in vivo*.

SUMMARY

The present disclosure relates to oligonucleotides targeting a nucleic acid capable of modulating the expression of RTEL1 and to treat or prevent diseases related to the functioning of the RTEL1.

Accordingly, the disclosure provides a RTEL1 inhibitor for use in the treatment and/or prevention of Hepatitis B virus (HBV) infection. In a first aspect, the disclosure provides use of a RTEL1 inhibitor in the manufacture of a medicament for the treatment and/or prevention of Hepatitis B virus (HBV) infection. In particular, a RTEL1 inhibitor capable of reducing cccDNA and/or pre-genomic RNA (pgRNA) is useful. Such an inhibitor is advantageously selected from a nucleic acid molecule of 12 to 60 nucleotides in length, which is capable of reducing RTEL1 mRNA, such as a single stranded antisense oligonucleotide, a siRNA or a shRNA complementary to mammalian RTEL1.

The disclosure also relates to an oligonucleotide of 12-60 nucleotides, such as 12-30 nucleotides, comprising a contiguous nucleotides sequence of at least 10 nucleotides, in particular of 16 to 20 nucleotides, which is complementary to a mammalian RTEL1. Such an

oligonucleotide is capable of inhibiting the expression of RTEL1. In another aspect, the disclosure relates to a single stranded antisense oligonucleotide of 12-30 nucleotides in length comprising a contiguous nucleotides sequence of at least 10 nucleotides which is complementary to a mammalian RTEL1, in particular a human RTEL1, wherein the oligonucleotide is capable of inhibiting the expression of RTEL1. The oligonucleotide can be a single stranded antisense oligonucleotide or a shRNA nucleic acid molecule.

The antisense oligonucleotide can have a gapmer design. Preferably, the antisense oligonucleotide is capable of inhibiting the expression of RTEL1 by cleavage of the target nucleic acid. The cleavage is preferably achieved via nuclease recruitment.

In another aspect, the disclosure relates to an oligonucleotide of the disclosure, wherein the contiguous nucleotide sequence is 100% complementary to a target sequence selected from SEQ ID NO: 3-21.

In another aspect, the disclosure relates to a conjugate comprising an oligonucleotide of the disclosure and at least one conjugate moiety covalently attached to said oligonucleotide.

In another aspect, the disclosure relates to a pharmaceutically acceptable salt of an oligonucleotide of the disclosure, or of a conjugate of the disclosure.

The disclosure provides pharmaceutical compositions comprising the antisense oligonucleotide of the invention and a pharmaceutically excipient. In another aspect, the disclosure relates to a pharmaceutical composition comprising an oligonucleotide of the disclosure, or of a conjugate of the disclosure or a pharmaceutically acceptable salt of the disclosure and a pharmaceutically acceptable excipient.

In a further aspect, the disclosure provides methods for *in vivo* or *in vitro* method for modulation of RTEL1 expression in a target cell which is expressing RTEL1, by administering an antisense oligonucleotide or composition of the invention in an effective amount to said cell. In another aspect, the disclosure relates to an *in vitro* method for modulating RTEL1 expression in a target cell which is expressing RTEL1, said method comprising administering an oligonucleotide of the disclosure, or of a conjugate of the disclosure, a pharmaceutically acceptable salt of the disclosure, or a pharmaceutical composition of the disclosure in an effective amount to said cell.

In a further aspect the disclosure provides methods for treating or preventing a disease, disorder or dysfunction associated with *in vivo* activity of RTEL1 comprising administering a therapeutically or prophylactically effective amount of the antisense oligonucleotide of the invention to a subject suffering from or susceptible to the disease, disorder or dysfunction.

In another aspect, the disclosure relates to an antisense oligonucleotide of the disclosure, or of a conjugate of the disclosure, a pharmaceutically acceptable salt of the disclosure, or a pharmaceutical composition of the disclosure for use in medicine.

In another aspect, the disclosure relates to use of an oligonucleotide of the disclosure, or of a conjugate of the disclosure, a pharmaceutically acceptable salt of the disclosure, or a pharmaceutical composition of the disclosure, in the preparation of a medicament for the treatment or prevention of Hepatitis B Virus HBV.

Further aspects of the disclosure are conjugates of nucleic acid molecules of the disclosure and pharmaceutical compositions comprising the molecules of the disclosure. In particular conjugates targeting the liver are of interest, such as GalNAc clusters.

BRIEF DESCRIPTION OF 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.

DEFINITIONS

HBV infection

The term “hepatitis B virus infection” or “HBV infection” is commonly known in the art and refers to an infectious disease that is caused by the hepatitis B virus (HBV) and affects the liver. A HBV infection can be an acute or a chronic infection. Chronic hepatitis B virus (CHB) infection is a global disease burden affecting 248 million individuals worldwide. Approximately 686,000

CLAIMS

1. Use of a RTEL1 inhibitor in the manufacture of a medicament for the treatment and/or prevention of Hepatitis B virus (HBV) infection.
2. The use according to claim 1, wherein the HBV infection is a chronic infection.
3. The use according to claim 1 or 2, wherein the RTEL1 inhibitor is capable of reducing cccDNA in an infected cell.
4. The use according to any one of claims 1 to 3, wherein said inhibitor is an oligonucleotide of 12 to 60 nucleotides in length comprising a contiguous nucleotide sequence of at least 10 nucleotides in length which is at least 95% complementary to a mammalian RTEL1 target nucleic acid, in particular a human RTEL1 nucleic acid, and is capable of reducing RTEL1 mRNA.
5. The use according to any one of claims 1 to 4 selected from a single stranded antisense oligonucleotide, siRNA or a shRNA molecule.
6. The use according to any one of claim 1 to 5, wherein the mammalian RTEL1 target nucleic acid is selected from SEQ ID NO: 1 or 2.
7. The use according to any one of claims 4 to 6, wherein the contiguous nucleotide sequence is at least 98% complementarity to the target nucleic acid of SEQ ID NO: 1 and SEQ ID NO: 2.
8. The use according to any one of claims 3 to 7, wherein the cccDNA in an HBV infected cell is reduced by at least 60% when compared to a control.
9. The use according to any one of claims 4 to 7, wherein the RTEL1 mRNA is reduced by at least 60% when compared to a control.
10. A single stranded antisense oligonucleotide of 12-30 nucleotides in length comprising a contiguous nucleotides sequence of at least 10 nucleotides which is complementary to a mammalian RTEL1, in particular a human RTEL1, wherein the oligonucleotide is capable of inhibiting the expression of RTEL1.
11. The antisense oligonucleotide according to claim 10, wherein the contiguous nucleotide sequence is at least 90% complementary, such as fully complementary, to SEQ ID NO: 1.
12. The antisense oligonucleotide according to claim 10 or 11 comprising a contiguous nucleotide sequence of 12 to 25, in particular 15 to 21 nucleotides in length.
13. An oligonucleotide according to any one of claims 11 to 12, wherein the contiguous nucleotide sequence is 100% complementary to a target sequence selected from SEQ ID NO: 3-21.

14. The oligonucleotide according to any one of claims 10 to 13, wherein the oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 22-237.

15. The antisense oligonucleotide according to any one of claims 10 to 14, comprising one or more 2’ sugar modified nucleoside.

16. The antisense oligonucleotide according to claim 15, 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.

17. The antisense oligonucleotide according to any one of claims 15 or 16, wherein the one or more 2’ sugar modified nucleoside is a LNA nucleoside.

18. The antisense oligonucleotide according to any one of claims 10 to 17, where the oligonucleotide comprises at least one phosphorothioate internucleoside linkage.

19. The antisense oligonucleotide according to claim 18, wherein all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

20. The antisense oligonucleotide according to any one of claims 10 to 19, wherein the oligonucleotide is capable of recruiting RNase H.

21. The antisense oligonucleotide according to any one of claims 10 to 20, wherein the antisense oligonucleotide, or contiguous nucleotide sequence thereof, consists or comprises a gapmer of formula 5’-F-G-F’-3’, where region F and F’ independently comprise 1 - 4 2’ sugar modified nucleosides and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH, such as a region comprising between 6 and 18 DNA nucleosides.

22. A conjugate comprising an oligonucleotide according to any one of claims 10 to 21 and at least one conjugate moiety covalently attached to said oligonucleotide.

23. The conjugate compound of claim 22, wherein the conjugate moiety is selected from one of the trivalent GalNAc moieties in figure 1.

24. The conjugate compound of claim 22 or 23 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.

25. A pharmaceutically acceptable salt of an oligonucleotide according to any one of claims 10 to 21, or of a conjugate according to claim 22 to 24.

26. A pharmaceutical composition comprising an oligonucleotide according to any one of claims 10 to 21, or of a conjugate according to claim 22 to 24 or a pharmaceutically acceptable salt according to claim 25 and a pharmaceutically acceptable excipient.

27. An *in vitro* method for modulating RTEL1 expression in a target cell which is expressing RTEL1, said method comprising administering an oligonucleotide according to any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26 in an effective amount to said cell.

28. An antisense oligonucleotide according any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26 for use in medicine.

29. The use of an oligonucleotide according any one of claims 10 to 21, or of a conjugate according to claim 22 to 24, a pharmaceutically acceptable salt according to claim 25, or a pharmaceutical composition according to claim 26, in the preparation of a medicament for the treatment or prevention of Hepatitis B Virus HBV.

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