METHODS FOR TREATING HEPATITIS B INFECTION

FIELD OF THE INVENTION

[0001] The present application relates to oligonucleotides and uses thereof, particularly uses relating to the treatment of hepatitis B infection.

BACKGROUND OF THE INVENTION

[0002] Chronic Hepatitis B Virus (HBV) infection is a significant cause of worldwide morbidity and mortality. Current HBV therapies, such as nucleoside analogs require lifelong therapy to reduce plasma viremia, and they are generally ineffective in the long term. Functional cure of chronic HBV has traditionally been a best treatment outcome. RNA interference (RNAi) technology offers the potential for pharmacologically intervention.

[0003] The outer envelope proteins are collectively known as hepatitis B surface antigen (HBsAg). HBsAg consists of three related polypeptides called S, M, and L encoded by overlapping open reading frames (ORF). The smallest envelope protein is S with 226 amino acids, called the S-ORF. M and L are produced from upstream translation initiation sites and add 55 and 108 amino acids, repectively, to S. HBV S, M, and L glycoproteins are found in the viral envelope of intact, infectious HBV virions, named Dane particles, and all three are produced and secreted in a vast excess that forms non-infectious subviral spherical and filamentous particles (both referred to as decoy particles) found in the blood of chronic HBV patients. The abundance of HBsAg on the surface of decoy particles is believed to inhibit humoral immunity and spontaneous clearance in patients with chronic HBV infection (CHB).

BRIEF SUMMARY

[0003a] In one aspect, the present disclosure refers to an oligonucleotide comprising a sense strand forming a duplex region with an antisense strand, wherein:

the sense strand consists of a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8) and comprising 2′-fluoro modified nucleotides at positions 3, 8-10, 12, 13, and 17, 2′-O-methyl modified nucleotides at positions 1, 2, 4-7, 11, 14-16, 18-26, and 31-36, and a phosphorothioate linkage between the nucleotides at positions 1 and 2, wherein each of the nucleotides of the -GAAA- sequence on the sense strand is conjugated to a monovalent GalNac moiety; and

the antisense strand consists of a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 5.1) and comprising 2′-fluoro modified nucleotides at positions 2, 3, 5, 7, 8, 10, 12, 14, 16, and 19, 2′-O-methyl modified nucleotides at positions 1, 4, 6, 9, 11, 13, 15, 17, 18, and 20-22, and phosphorothioate linkages between nucleotides at positions 1 and 2, between nucleotides at positions 2 and 3, between nucleotides at positions 3 and 4, between nucleotides at positions 20 and 21, and between nucleotides at positions 21 and 22, wherein the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a methoxy phosphonate (MOP).

[0003b] In another aspect, the present disclosure refers to a composition comprising the oligonucleotide disclosed herein.

[0003c] In yet another aspect, the present disclosure refers to an *in vitro* method of reducing expression of hepatitis B virus (HBV) surface antigen (HBsAg) in a cell, the method comprising delivering to the cell the oligonucleotide disclosed herein.

[0003d] In a further aspect, the present disclosure refers to a use of the oligonucleotide disclosed herein, or the composition disclosed herein, in the manufacture of a medicament for treating HBV infection in a subject.

[0004] Examples of the present disclosure relate to improved oligonucleotide-based compositions and related methods for treating HBV infection in a subject. In some examples, the present disclosure relates to the development of potent oligonucleotides that produce durable knockdown of HBV surface antigen (HBsAg) expression. The oligonucleotide induce RNA interference (RNAi)-mediated recognition and destruction of mRNA which encodes all forms of HBsAg in hepatocytes. This includes protein translated from viral RNAs transcribed from both cccDNA and HBV DNA which has been integrated into the host genome. Oligonucleotides are provided herein designed to target an expansive set of HBsAg transcripts encoded by HBV genomes across all known genotypes. In some examples, it has been

found that oligonucleotides disclosed herein that target HBsAg transcripts in hepatocytes can produce a stable reduction in HBsAg expression with high specificity that persists for an extended period of time (e.g., greater than 7 weeks to several months) following administration to a subject (See, e.g., Examples 1 and 3). In some examples, it has been discovered that targeting of HBsAg expression using oligonucleotides disclosed herein results in reduction of pre-genomic RNA (pgRNA) and other viral life cycle intermediates. It has also been found that certain RNAi oligonucleotides disclosed herein can knockdown HBsAg mRNA transcripts that originate from either cccDNA or integrated virus. In further examples, it has been discovered that targeting of HBsAg expression using oligonucleotides disclosed herein reduces expression of all HBV proteins (with the exception of HBx), namely HBcAg, HBeAg, and HBV Polymerase, resulting in cytosolic retention of HBV core protein. Furthermore, in some examples, clearance of circulating HBsAg resulting from the mRNA knockdown using methods provided herein is clinically advantageous because it enables breaking of HBV immune tolerance caused by high levels of circulating HBsAg in CHB patients. Reactivation of immune system activity against HBV infection is believed to be a cornerstone of achieving functional cure, defined as permanent seroclearance of HBsAg.

[0005] Previous work has indicated that the use of a combination of RNAi agents targeting multiple different HBV genes (namely, S, C, P, and X genes), or in some cases targeting X gene transcripts alone, achieves effective inhibition of HBV replication and gene expression. However, results provided herein demonstrate that the use of RNAi oligonucleotides targeting HBsAg transcripts alone also achieves effective inhibition of HBV replication and gene expression, which provides a new therapeutic approach to treating HBV infections. In addition to the direct effect of silencing viral RNAs, HSB(s)-219 precursors prevent nuclear localization of HBV Core Antigen (HBcAg). Importantly, targeting of HBV-X or both genes simultaneously does not prevent HBcAg nuclear localization. Preclinical data strongly suggest that the inhibition of nuclear core localization caused by S-targeting RNAi therapy results in significantly improved duration of HBsAg suppression. Notably, lack of nuclear localization of HBcAg in patients has been shown to correlate to favorable responses to antiviral therapy.

[0006] Some examples of the present disclosure provide oligonucleotides for reducing expression of hepatitis B virus surface antigen (HBsAg) mRNA, the oligonucleotide comprising an antisense strand of 19 to 30 nucleotides in length, wherein the antisense strand comprises a region of complementarity to a sequence of HBsAg mRNA as set forth in ACAANAAUCCUCACAAUA (SEQ ID NO: 1).

[0007] In some examples, the oligonucleotide further comprises a sense strand of 19 to 50 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand. In some examples, sense strand comprises a region of complementarity to a sequence as set forth in UUNUUGUGAGGAUUN (SEQ ID NO: 2). In some examples, the sense strand comprises a region of complementarity to a sequence as set forth in 5′-UUAUUGUGAGGAUUNUUGUC (SEQ ID NO: 3).

[0008] In some examples, the antisense strand comprises a sequence as set forth in UUAUUGUGAGGAUUNUUGUCGG (SEQ ID NO: 4). In some examples, the antisense strand consists of a sequence as set forth in UUAUUGUGAGGAUUCUUGUCGG (SEQ ID NO: 5). In some examples, the antisense strand consists of a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 5.1).

[0009] In some examples, the sense strand comprises a sequence as set forth in ACAANAAUCCUCACAAUAA (SEQ ID NO: 6). In some examples, the sense strand comprises a sequence as set forth in GACAANAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 7). In some examples, the sense strand consists of a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8). In some examples, the sense strand consists of a sequence as set forth in GACAAGAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8.1).

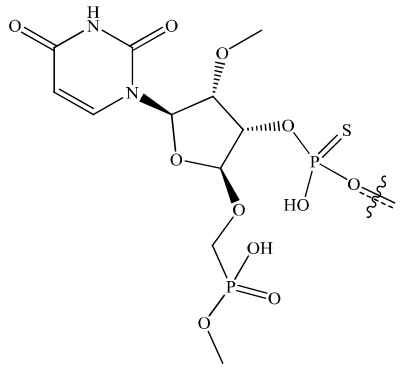
[00010] Other examples of the present disclosure provide oligonucleotides for reducing expression of hepatitis B virus surface antigen (HBsAg) mRNA, the oligonucleotide comprising a sense strand forming a duplex region with an antisense strand, wherein the sense strand comprises a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8), wherein the antisense strand comprises a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG(SEQ ID NO: 5.1), wherein each of the antisense strand and the sense strand comprises one or more 2′-fluoro and 2′-O-methyl modified nucleotides and at least one phosphorothioate linkage, wherein the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog, and wherein the sense strand is conjugated to one or more N-acetylgalactosamine (GalNAc) moiety.

[00011] Further provided herein are oligonucleotides for reducing expression of hepatitis B virus surface antigen (HBsAg) mRNA, the oligonucleotide comprising a sense strand forming a duplex region with an antisense strand, wherein: the sense strand comprises a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ

ID NO: 8) and comprising 2′-fluoro modified nucleotides at positions 3, 8-10, 12, 13, and 17, 2′-O-methyl modified nucleotides at positions 1, 2, 4-7, 11, 14-16, 18-26, and 31-36, and at least one phosphorothioate internucleotide linkage, wherein the sense strand is conjugated to one or more N-acetylgalactosamine (GalNAc) moiety; and the antisense strand comprises a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 5.1) and comprising 2′-fluoro modified nucleotides at positions 2, 3, 5, 7, 8, 10, 12, 14, 16, and 19, 2′-O-methyl modified nucleotides at positions 1, 4, 6, 9, 11, 13, 15, 17, 18, and 20-22, and at least three phosphorothioate internucleotide linkages, wherein the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog.the sense strand comprises a phosphorothioate linkage between the nucleotides at postions 1 and 2.

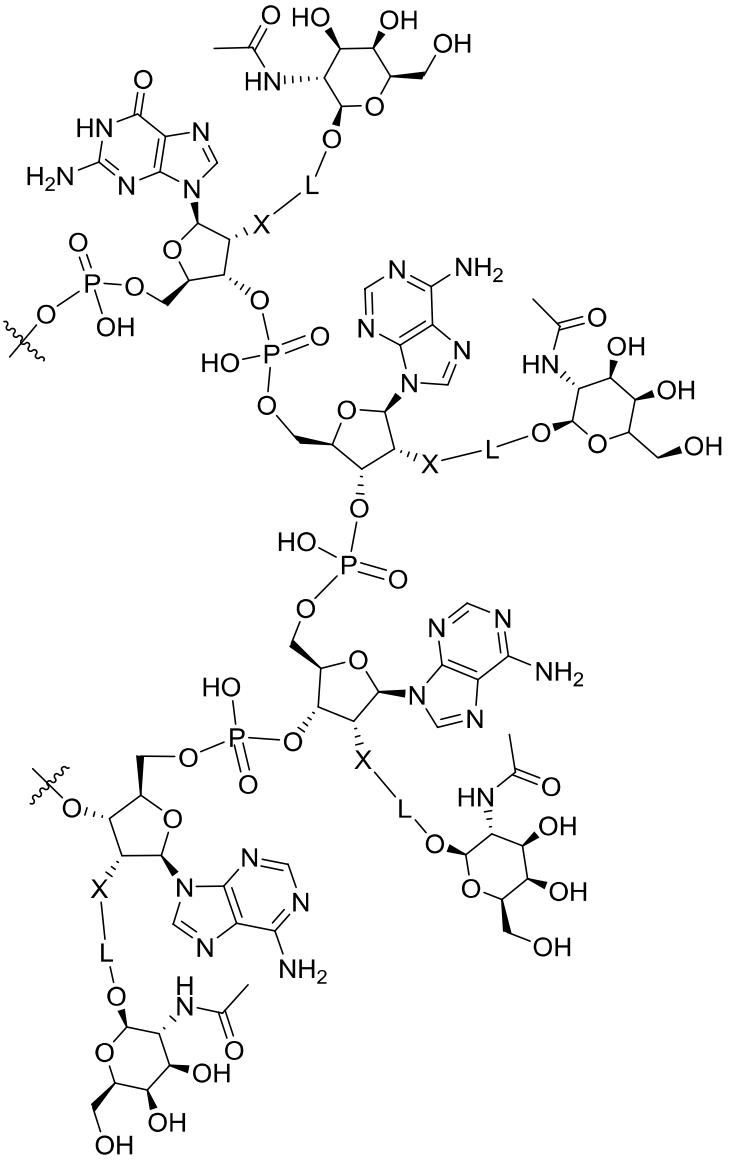
[00012] In some examples, the antisense strand comprises five phosphorothioate linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 20 and 21, and 21 and 22.

[00013] In some examples, the 5’-nucleotide of the antisense strand has the following structure:



[00014] In some examples, one or more of the nucleotides of the –GAAA– sequence on the sense strand is conjugated to a monovalent GalNac moiety.

[00015] In some examples each of the nucleotides of the –GAAA– sequence on the sense strand is conjugated to a monovalent GalNac moiety. In some examples, the –GAAA– motif comprises the structure:



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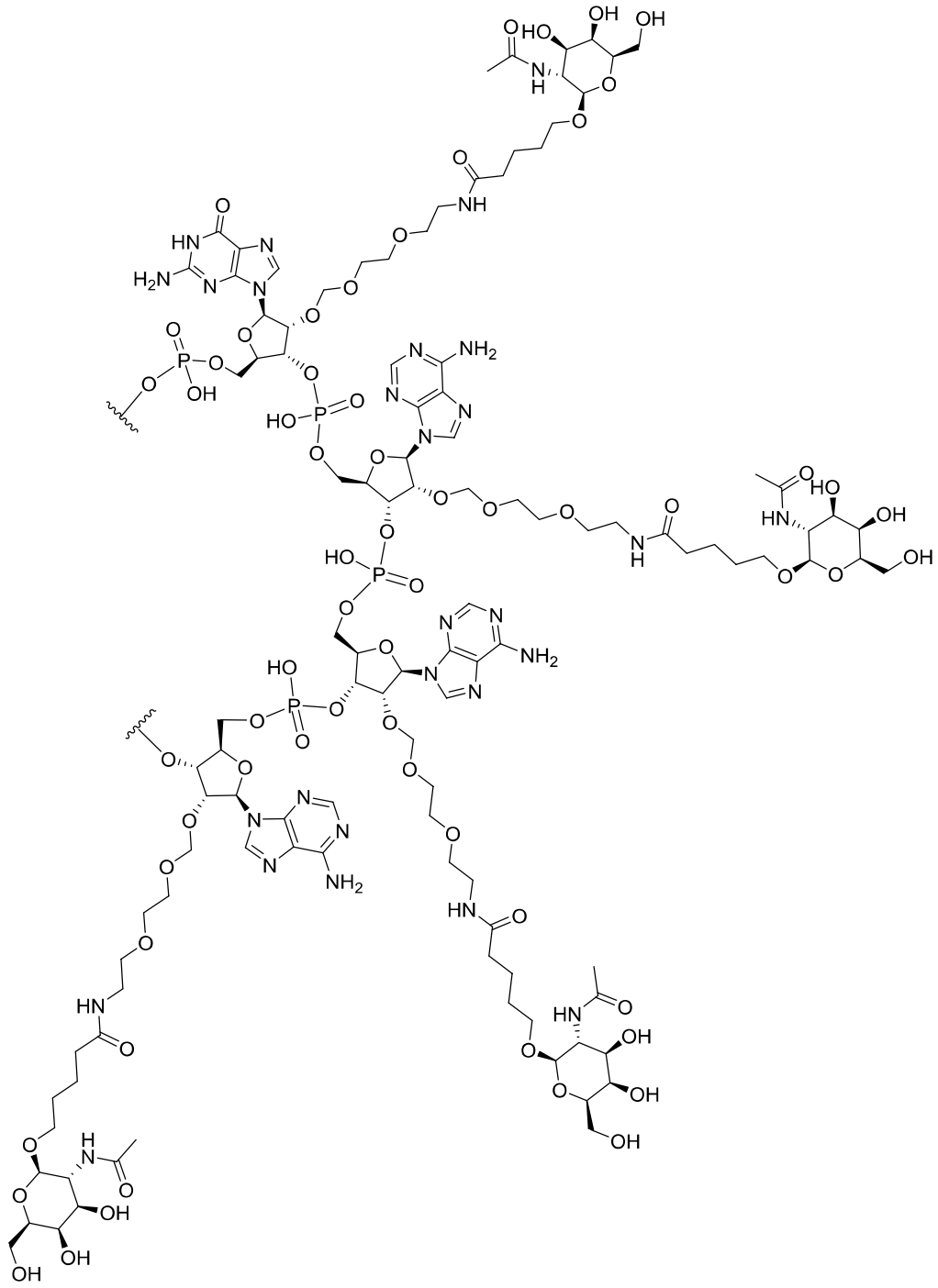
wherein:

L represents a bond, click chemistry handle, or a linker of 1 to 20, inclusive, consecutive, covalently bonded atoms in length, selected from the group consisting of substituted and unsubstituted alkylene, substituted and unsubstituted alkenylene, substituted and unsubstituted alkynylene, substituted and unsubstituted heteroalkylene, substituted and unsubstituted heteroalkenylene, substituted and unsubstituted heteroalkynylene, and combinations thereof; and

X is a O, S, or N.

[00016] In some examples, L is an acetal linker. In some examples, X is O.

[00017] In some examples, the –GAAA– sequence comprises the structure:



[00018] In some examples, the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of up to 6 nucleotides in length. In some examples, L is a tetraloop. In some examples, L forms a loop between S_1 and S_2 of 4 nucleotides in length. In some examples, L comprises a sequence set forth as GAAA. In some examples, up to 4 nucleotides of L of the stem-loop are each conjugated to a separate GalNAc.

[00019] In some examples, the oligonucleotide comprises at least one modified nucleotide. In some examples, the modified nucleotide comprises a 2′-modification. In

some examples, the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid. In some examples, all of the nucleotides of the oligonucleotide are modified nucleotides. In some examples, the oligonucleotide comprises at least one modified internucleotide linkage. In some examples, the at least one modified internucleotide linkage is a phosphorothioate linkage.the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog.

[00020] In some examples, at least one nucleotide of the oligonucleotide is conjugated to a targeting ligand. In some examples, the targeting ligand is a N-acetylgalactosamine (GalNAc) moiety.

[00021] Further provided herein are compositions comprising an oligonucleotide of any one of the preceding claims and an counterion, and compositions comprising an oligonucleotide of any one of the preceding claims and a pharmaceutically acceptable carrier.

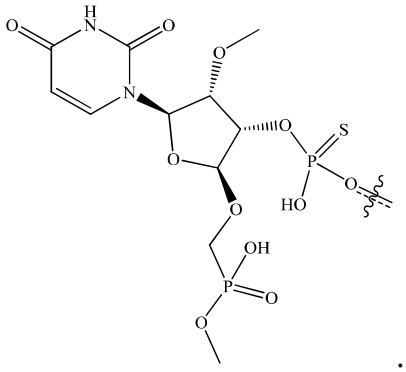
[00022] Other examples of the present disclosure provide methods of reducing expression of hepatitis B virus (HBV) surface antigen in a cell, the method comprising delivering to the cell an oligonucleotide described herein. In some examples, the cell is a hepatocyte. In some examples, the cell is *in vivo*. In some examples, the cell is *in vitro*.

[00023] Other examples of the present disclosure provide methods of treating a hepatitis B virus (HBV) infection in a subject, the method comprising administering to the subject an oligonucleotide of or a composition described herein.

[00024] Methods of treating HBV infection in a subject are also provided, the method comprising administering to the subject an RNAi oligonucleotide that selectively targets HBsAg mRNA, wherein the RNAi oligonucleotide is administered in the absence of treatment with an RNAi oligonucleotide targeting a non-surface antigen encoding HBV mRNA transcript. Methods of treating HBV infection in a subject are also provided, the method comprising administering to the subject an RNAi oligonucleotide that selectively targets HBsAg mRNA, wherein the subject is not administered an RNAi oligonucleotide that selectively targets HBxAg mRNA transcript. In some examples, the method further comprises administering to the subject an effective amount of Entecavir.

[00025] Other examples of the present disclosure provide oligonucleotides for reducing expression of hepatitis B virus surface antigen (HBsAg) mRNA, the oligonucleotide comprising a sense strand forming a duplex region with an antisense strand, wherein:

the sense strand comprises a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8) and



[00026] Composition comprising the oligonucleotide is also provided. In some examples, the composition further comprises Na+ counterions.

[00027] Also provided are methods of reducing expression of hepatitis B virus (HBV) surface antigen in a cell, the method comprising delivering to the oligonucleotide or the composition. In some examples, the cell is a hepatocyte. In some examples, the cell is *in vivo*. In some examples, the cell is *in vitro*.

[00028] Methods of treating a hepatitis B virus (HBV) infection in a subject are provided, the method comprising administering to the subject the oligonucleotide or the composition. In some examples, the method further comprises administering to the subject an effective amount of Entecavir.

BRIEF DESCRIPTION OF THE DRAWINGS

[00029] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments, and together with the written description, serve to provide non-limiting examples of certain aspects of the compositions and methods disclosed herein.

[00030] FIG. 1 shows an example of an RNAi target site on a schematic representation of the organization of the HBV genome.

[00031] FIG. 2 shows single dose evaluation of an oligonucleotide for reducing HBsAg expression in HDI-mice.

[00032] FIG. 3 is a graphical representation of plasma HBsAg levels over time during a specified dosing regimen with an HBsAg-targeting oligonucleotide. As shown in this example, the oligonucleotide demonstrated preclinical potency and maintained decreased levels well beyond the dosing period.

[00033] FIG. 4 shows graphs depicting the results of HBsAg mapping in HeLa cells using a reporter assay. An unmodified siRNA targeting position 254 of the HBV genome was

CLAIMS

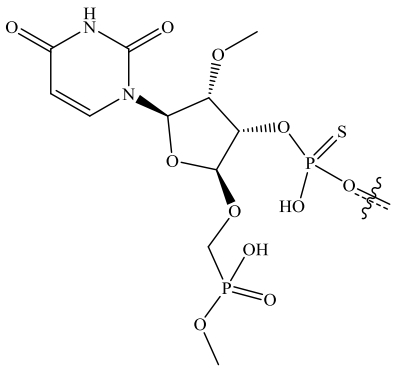
What is claimed is:

1. An oligonucleotide comprising a sense strand forming a duplex region with an antisense strand, wherein:

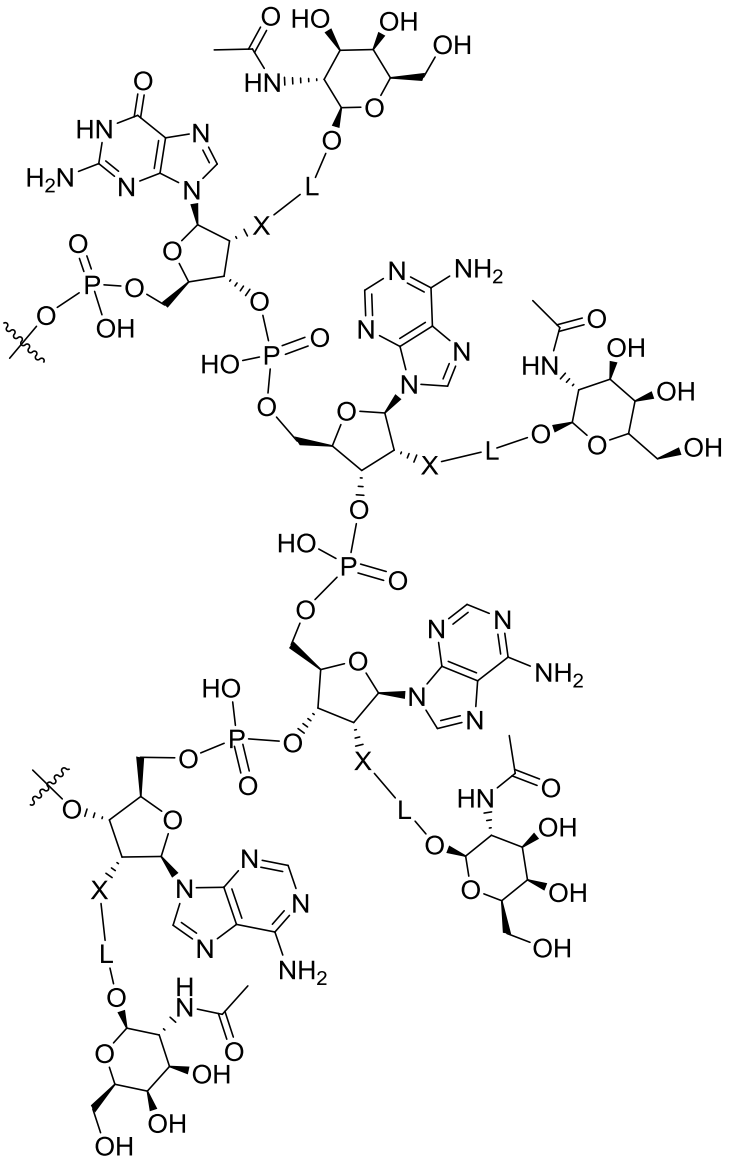
the sense strand consists of a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8) and comprising 2′-fluoro modified nucleotides at positions 3, 8-10, 12, 13, and 17, 2′-O-methyl modified nucleotides at positions 1, 2, 4-7, 11, 14-16, 18-26, and 31-36, and a phosphorothioate linkage between the nucleotides at positions 1 and 2, wherein each of the nucleotides of the -GAAA- sequence on the sense strand is conjugated to a monovalent GalNac moiety; and

the antisense strand consists of a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 5.1) and comprising 2′-fluoro modified nucleotides at positions 2, 3, 5, 7, 8, 10, 12, 14, 16, and 19, 2′-O-methyl modified nucleotides at positions 1, 4, 6, 9, 11, 13, 15, 17, 18, and 20-22, and phosphorothioate linkages between nucleotides at positions 1 and 2, between nucleotides at positions 2 and 3, between nucleotides at positions 3 and 4, between nucleotides at positions 20 and 21, and between nucleotides at positions 21 and 22, wherein the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a methoxy phosphonate (MOP).

2. The oligonucleotide of claim 1, wherein the 5’-nucleotide of the antisense strand has the following structure:



3. The oligonucleotide of claim 1 or 2, wherein the –GAAA– motif comprises the structure:



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wherein:

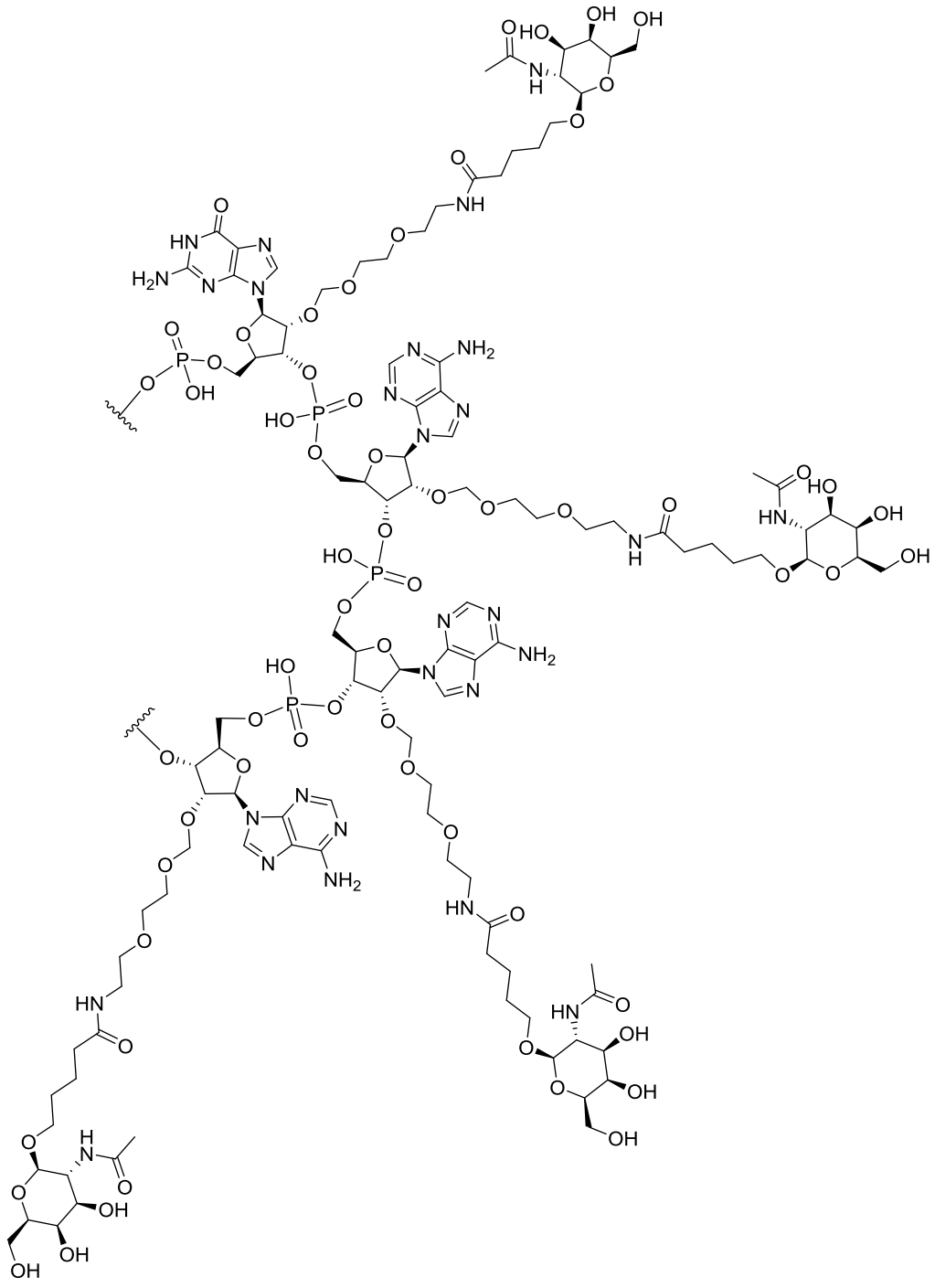
L represents a bond, click chemistry handle, or a linker of 1 to 20, inclusive, consecutive, covalently bonded atoms in length, selected from the group consisting of substituted and unsubstituted alkylene, substituted and unsubstituted alkenylene, substituted and unsubstituted alkynylene, substituted and unsubstituted heteroalkylene, substituted and unsubstituted heteroalkenylene, substituted and unsubstituted heteroalkynylene, and combinations thereof; and

X is a O, S, or N.

4. The oligonucleotide of claim 3, wherein L is an acetal linker.

5. The oligonucleotide of claim 3 or 4, wherein X is O.

6. The oligonucleotide of any one of claims 1 to 5, wherein the –GAAA– sequence comprises the structure:



7. A composition comprising the oligonucleotide of any one of claims 1 to 6.

8. The composition of claim 7 comprising a counterion.

9. The composition of claim 7 or claim 8 comprising a pharmaceutically acceptable carrier.

10. The composition of any one of claims 7 to 9 comprising an excipient.

11. The composition of any one of claims 7 to 10 comprising phosphate-buffered saline solution.

12. An *in vitro* method of reducing expression of hepatitis B virus (HBV) surface antigen (HBsAg) in a cell, the method comprising delivering to the cell the oligonucleotide of any one of claims 1 to 6.

13. The method of claim 12, wherein the cell is a hepatocyte.

14. The method of claim 12 or 13, wherein the oligonucleotide is delivered in the form of a transgene that is engineered to express the oligonucleotide in the cell.

15. Use of the oligonucleotide according to any one of claims 1 to 6, or the composition according to any one of claims 7 to 11, in the manufacture of a medicament for treating HBV infection in a subject.

16. The use of claim 15, wherein the oligonucleotide or composition is to be administered subcutaneously to the subject.

17. The use of claim 15, wherein the oligonucleotide or composition is to be administered intravenously to the subject.

18. The use of claim 17, wherein the medicament comprises a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is a solvent or dispersion medium containing water.