

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, respectively, 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: 9) 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: 6) 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

[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: 6).

[0009] In some examples, the sense strand comprises a sequence as set forth in ACAANAAUCCUCACAAUAA (SEQ ID NO: 7). In some examples, the sense strand comprises a sequence as set forth in GACAANAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8). In some examples, the sense strand consists of a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 9). In some examples, the sense strand consists of a sequence as set forth in GACAAGAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 10).

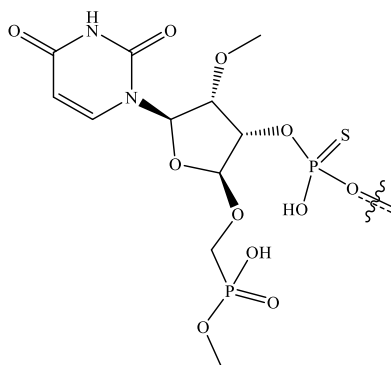
[00010] Other examples of the present disclosure provide oligonucleotides for reducing expression of hepatitis B virus surface antigen (HBsAg) mRNA, the oligonucleotide comprising a sense strand forming a duplex region with an antisense strand, wherein the sense strand comprises a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 9), wherein the antisense strand comprises a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 6), 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: 9) 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: 6) 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 positions 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:

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*.

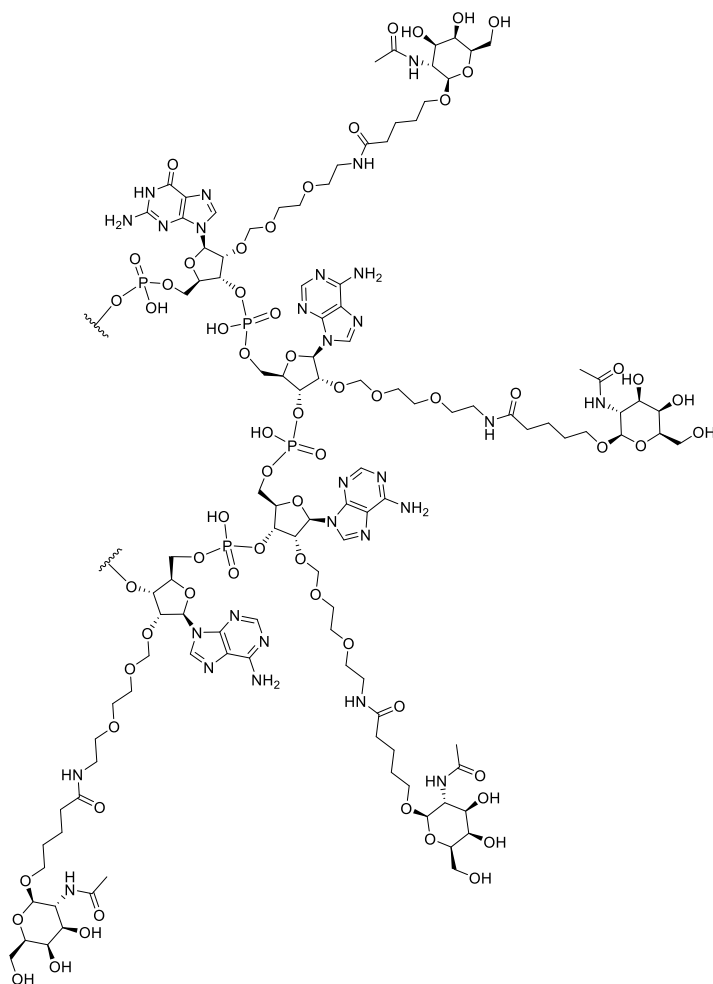
[00023] Other examples of the present disclosure provide methods of treating a hepatitis B virus (HBV) infection in a subject, the method comprising administering to the subject an oligonucleotide of or a composition described herein.

[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
GACAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 9) 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 one phosphorothioate internucleotide 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, wherein the –GAAA– sequence comprises the structure:



the antisense strand comprises a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 6) 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 five phosphorothioate internucleotide linkages between nucleotides 1 and 2, 2 and 3, 3 and 4, 20 and 21, and 21 and 22, wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand has the following structure:

embodiments, the antisense strand comprises, or consists of, a sequence set forth as: UUAUUGUGAGGAUUCUUGUCGG (SEQ ID NO: 5). In some embodiments, the antisense strand comprises, or consists of, a sequence set forth as:

UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 6). In some embodiments, the sense strand comprises, or consists of, a sequence set forth as: ACAANAAUCCUCACAAUAA (SEQ ID NO: 7). In some embodiments, the sense strand comprises, or consists of, a sequence set forth as: GACAANAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8).

In some embodiments, the sense strand comprises, or consists of, a sequence set forth as: GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 9). In some embodiments, the sense strand comprises, or consists of, a sequence set forth as: GACAAGAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 10).

[00089] In some embodiments, an oligonucleotide for reducing expression of HBsAg mRNA comprises a sense strand forming a duplex region with an antisense strand, where the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 7-10, and the antisense strand comprises a sequence as set forth in any one of SEQ ID NOs: 4-6. In some embodiments, the sense strand comprises 2'-fluoro and 2'-O-methyl modified nucleotides and at least one phosphorothioate internucleotide linkage. In some embodiments, the sense strand is conjugated to a N-acetylgalactosamine (GalNAc) moiety. In some embodiments, the antisense strand comprises 2'-fluoro and 2'-O-methyl modified nucleotides and at least one phosphorothioate internucleotide linkage. In some embodiments, the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog. In some embodiments, each of the antisense strand and the sense strand comprises 2'-fluoro and 2'-O-methyl modified nucleotides and at least one phosphorothioate internucleotide linkage, where the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog, and the sense strand is conjugated to a N-acetylgalactosamine (GalNAc) moiety.

[00090] In some embodiments, a sense strand comprising a sequence as set forth in any one of SEQ ID NOs: 8-10 comprises 2'-fluoro modified nucleotides at positions 3, 8-10, 12, 13, and 17. In some embodiments, the sense strand comprises 2'-O-methyl modified nucleotides at positions 1, 2, 4-7, 11, 14-16, 18-26, and 31-36. In some embodiments, the sense strand comprises one phosphorothioate internucleotide linkage. In some embodiments, the sense strand comprises a phosphorothioate internucleotide linkage between nucleotides at positions 1 and 2. In some embodiments, the sense strand is conjugated to a N-acetylgalactosamine (GalNAc) moiety.

[00091] In some embodiments, an antisense strand comprising a sequence as set forth

in any one of SEQ ID NOs: 4-6 comprises 2'-fluoro modified nucleotides at positions 2, 3, 5, 7, 8, 10, 12, 14, 16, and 19. In some embodiments, the antisense strand comprises 2'-O-methyl modified nucleotides at positions 1, 4, 6, 9, 11, 13, 15, 17, 18, and 20-22. In some embodiments, the antisense strand comprises three phosphorothioate internucleotide linkages. In some embodiments, the antisense strand comprises phosphorothioate internucleotide 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. In some embodiments, the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog.

ii. Double-Stranded Oligonucleotides

[00092] There are a variety of structures of oligonucleotides that are useful for targeting HBsAg mRNA expression in the methods of the present disclosure, including RNAi, antisense, miRNA, etc. Any of the structures described herein or elsewhere may be used as a framework to incorporate or target a sequence described herein. Double-stranded oligonucleotides for targeting HBV antigen expression (e.g., via the RNAi pathway) generally have a sense strand and an antisense strand that form a duplex with one another. In some embodiments, the sense and antisense strands are not covalently linked. However, in some embodiments, the sense and antisense strands are covalently linked.

[00093] In some embodiments, double-stranded oligonucleotides for reducing the expression of HBsAg mRNA expression engage RNA interference (RNAi). For example, RNAi oligonucleotides have been developed with each strand having sizes of 19-25 nucleotides with at least one 3' overhang of 1 to 5 nucleotides (see, *e.g.*, U.S. Patent No. 8,372,968). Longer oligonucleotides have also been developed that are processed by Dicer to generate active RNAi products (see, *e.g.*, U.S. Patent No. 8,883,996). Further work produced extended double-stranded oligonucleotides where at least one end of at least one strand is extended beyond a duplex targeting region, including structures where one of the strands includes a thermodynamically-stabilizing tetraloop structure (see, *e.g.*, U.S. Patent Nos. 8,513,207 and 8,927,705, as well as WO2010033225, which are incorporated by reference herein for their disclosure of these oligonucleotides). Such structures may include single-stranded extensions (on one or both sides of the molecule) as well as double-stranded extensions.

[00094] In some embodiments, oligonucleotides provided herein are cleavable by Dicer enzymes. Such oligonucleotides may have an overhang (*e.g.*, of 1, 2, or 3 nucleotides in

internally segmented interfering RNA (sisiRNA; see, *e.g.*, Bramsen *et al.*, Nucleic Acids Res. 2007 Sep; 35(17): 5886–5897). Each of the foregoing references is incorporated by reference in its entirety for the related disclosures therein. Further non-limiting examples of an oligonucleotide structures that may be used in some embodiments to reduce or inhibit the expression of HBsAg are microRNA (miRNA), short hairpin RNA (shRNA), and short siRNA (see, *e.g.*, Hamilton *et al.*, Embo J., 2002, 21(17): 4671-4679; see also U.S. Application No. 20090099115).

a. Antisense Strands

[00097] In some embodiments, an antisense strand of an oligonucleotide may be referred to as a “guide strand.” For example, if an antisense strand can engage with RNA-induced silencing complex (RISC) and bind to an Argonaut protein, or engage with or bind to one or more similar factors, and direct silencing of a target gene, it may be referred to as a guide strand. In some embodiments, a sense strand complementary with a guide strand may be referred to as a “passenger strand.”

[00098] In some embodiments, an oligonucleotide provided herein comprises an antisense strand that is up to 50 nucleotides in length (*e.g.*, up to 30, up to 27, up to 25, up to 21, or up to 19 nucleotides in length). In some embodiments, an oligonucleotide provided herein comprises an antisense strand that is at least 12 nucleotides in length (*e.g.*, at least 12, at least 15, at least 19, at least 21, at least 25, or at least 27 nucleotides in length). In some embodiments, an antisense strand of an oligonucleotide disclosed herein is in the range of 12 to 50 or 12 to 30 (*e.g.*, 12 to 30, 11 to 27, 11 to 25, 15 to 21, 15 to 27, 17 to 21, 17 to 25, 19 to 27, or 19 to 30) nucleotides in length. In some embodiments, an antisense strand of any one of the oligonucleotides disclosed herein is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length.

[00099] In some embodiments, the antisense strand comprises a region of complementarity to a sequence as set forth in (shown 5' to 3'): AATCCTCACA (SEQ ID NO: 11). In some embodiments, the antisense strand comprises a sequence as set forth in (shown 5' to 3'): UGUGAGGAUU (SEQ ID NO: 12). In some embodiments, the antisense strand comprises a sequence as set forth in (shown 5' to 3'): TGTGAGGATT (SEQ ID NO: 13).

[000100] In some embodiments, an oligonucleotide for reducing expression of HBsAg mRNA can comprise an antisense strand having a region of complementarity to a sequence as set forth in SEQ ID NO: 11, and one or two non-complementary nucleotides at its 3' terminus.

In some embodiments, the antisense strand comprises the nucleotide sequence set forth in any one of SEQ ID NOs: 4-6.

[000101] In some embodiments, an oligonucleotide for reducing expression of HBsAg mRNA can comprise an antisense strand that has a region of complementarity to a sequence as set forth in SEQ ID NO: 11, where the antisense strand does not have a sequence as set forth in any one of the following (shown 5' to 3'): TATTGTGAGGATTCTTGTCA (SEQ ID NO: 14); CGGTATTGTGAGGATTCTTG (SEQ ID NO: 15); TGTGAGGATTCTTGTCAACA (SEQ ID NO: 16); UAUUGUGAGGAUUUUUGUCA (SEQ ID NO: 17); UGCGGUAUUGUGAGGAUUCTT (SEQ ID NO: 18); ACAGCATTGTGAGGATTCTTGTC (SEQ ID NO: 19); UAUUGUGAGGAUUUUUGUCAACA (SEQ ID NO: 20); AUUGUGAGGAUUUUUGUCAACAA (SEQ ID NO: 21); and UUGUGAGGAUUUUUGUCAACAAG (SEQ ID NO: 22). In some embodiments, the antisense strand differs from the nucleotide sequence set forth in SEQ ID NOs: 4, 5, or 6 by no more than three nucleotides.

b. Sense Strands

[000102] In some embodiments, a double-stranded oligonucleotide may have a sense strand of up to 40 nucleotides in length (*e.g.*, up to 40, up to 35, up to 30, up to 27, up to 25, up to 21, up to 19, up to 17, or up to 12 nucleotides in length). In some embodiments, an oligonucleotide may have a sense strand of at least 12 nucleotides in length (*e.g.*, at least 12, at least 15, at least 19, at least 21, at least 25, at least 27, at least 30, at least 35, or at least 38 nucleotides in length). In some embodiments, an oligonucleotide may have a sense strand in a range of 12 to 50 (*e.g.*, 12 to 40, 12 to 36, 12 to 32, 12 to 28, 15 to 40, 15 to 36, 15 to 32, 15 to 28, 17 to 21, 17 to 25, 19 to 27, 19 to 30, 20 to 40, 22 to 40, 25 to 40, or 32 to 40) nucleotides in length. In some embodiments, an oligonucleotide may have a sense strand of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleotides in length. In some embodiments, a sense strand of an oligonucleotide is longer than 27 nucleotides (*e.g.*, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotides). In some embodiments, a sense strand of an oligonucleotide is longer than 25 nucleotides (*e.g.*, 26, 27, 28, 29 or 30 nucleotides).

[000103] In some embodiments, a sense strand comprises a stem-loop at its 3'-end. In some embodiments, a sense strand comprises a stem-loop at its 5'-end. In some embodiments, a strand comprising a stem loop is in the range of 2 to 66 nucleotides long (*e.g.*, 2 to 66, 10 to 52, 14 to 40, 2 to 30, 4 to 26, 8 to 22, 12 to 18, 10 to 22, 14 to 26, or 14 to 30 nucleotides).

[000163] As a non-limiting set of examples, the oligonucleotides of the instant disclosure would typically be administered quarterly (once every three months), bi-monthly (once every two months), monthly, or weekly. For example, the oligonucleotides may be administered every one, two, or three weeks. The oligonucleotides may be administered daily.

[000164] In some embodiments, the subject to be treated is a human or non-human primate or other mammalian subject. Other exemplary subjects include domesticated animals such as dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and animals such as mice, rats, guinea pigs, and hamsters.

EXAMPLES

Example 1. Development of potent oligonucleotide inhibitors of HBsAg expression

[000165] HBV surface antigen was identified as a target for RNAi-based therapy to treat HBV infection. As depicted in the HBV genome organization shown in FIG. 1, HBsAg is encoded by three RNA molecules transcribed from a single ORF. Oligonucleotides were designed for purposes of silencing one or more RNA transcripts that contribute to HBsAg assembly (example RNAi target site indicated by “X” in FIG. 1). An HBsAg-targeting oligonucleotide, HBV-254, was designed and evaluated *in vitro* and *in vivo*. HBV-254 was selected and designed based on an ability to directly target mRNA transcripts for four HBV RNA species. The HBV-254 duplex oligonucleotide used in the experiments included a sense strand of a sequence as set forth in (shown 5' to 3'):

GUGGUGGACUUCUCUCAAUAGCAGCCGAAAGGCUGC (SEQ ID NO: 23); and an antisense strand of a sequence as set forth in (shown 5' to 3'):

UAUUGAGAGAAGUCCACCACGG (SEQ ID NO: 24).

[000166] A single dose evaluation of oligonucleotide HBV-254 in HDI-mice was conducted, demonstrating the ability to subcutaneously target HBsAg viral transcript (FIG. 2). As shown, HBV-254 systematically reduced HBsAg levels in mice with increasing dosage. Preclinical potency was further evaluated in mice following a QW×3 dosing regimen in which HBV-254 was subcutaneously administered at 3 mg/kg (FIG. 3). The administration points are indicated by arrows in the figure. HBsAg levels were monitored in both oligonucleotide treated and untreated control mice for a period spanning 147 days. Diminished HBsAg levels persisted in treated mice throughout the entirety of the study, with expression levels (relative to control) appearing to settle at a reduced baseline at approximately two months following the first administration.

CLAIMS

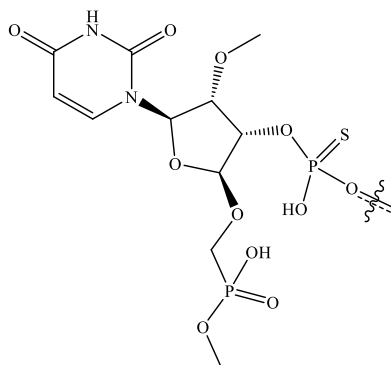
What is claimed is:

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

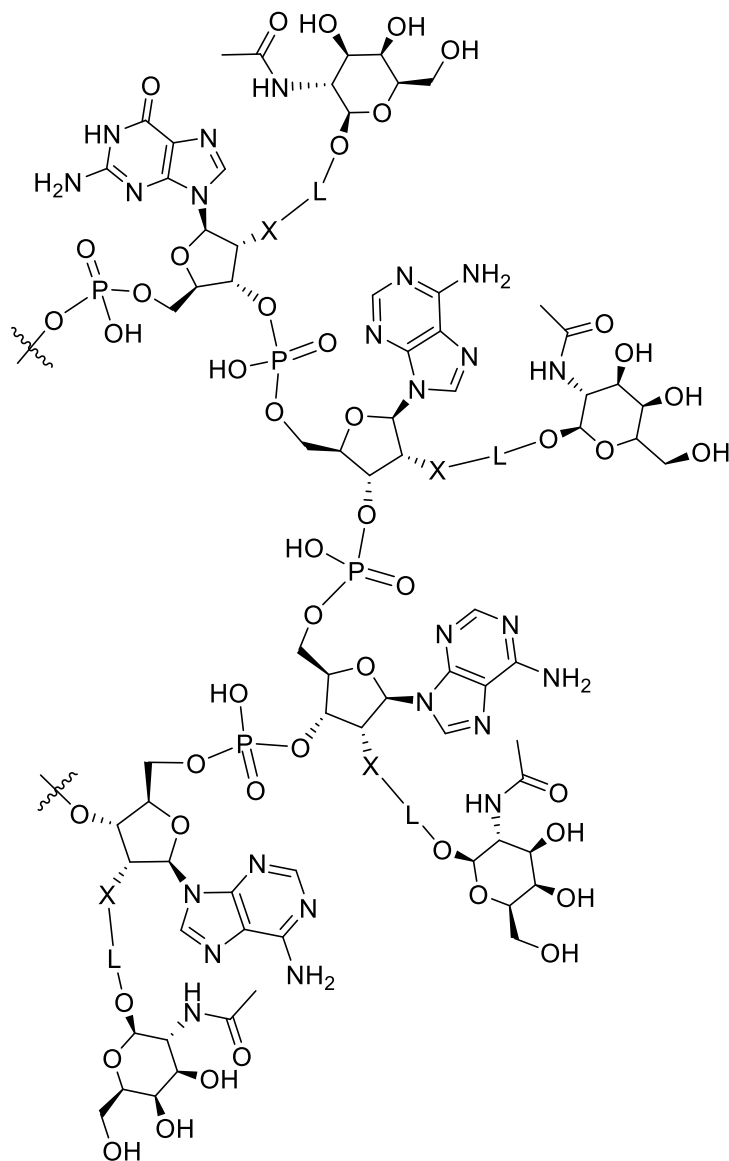
the sense strand consists of a sequence as set forth in GACAAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 9) and comprising 2'-fluoro modified nucleotides at positions 3, 8-10, 12, 13, and 17, 2'-O-methyl modified nucleotides at positions 1, 2, 4-7, 11, 14-16, 18-26, and 31-36, and a phosphorothioate 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: 6) 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:

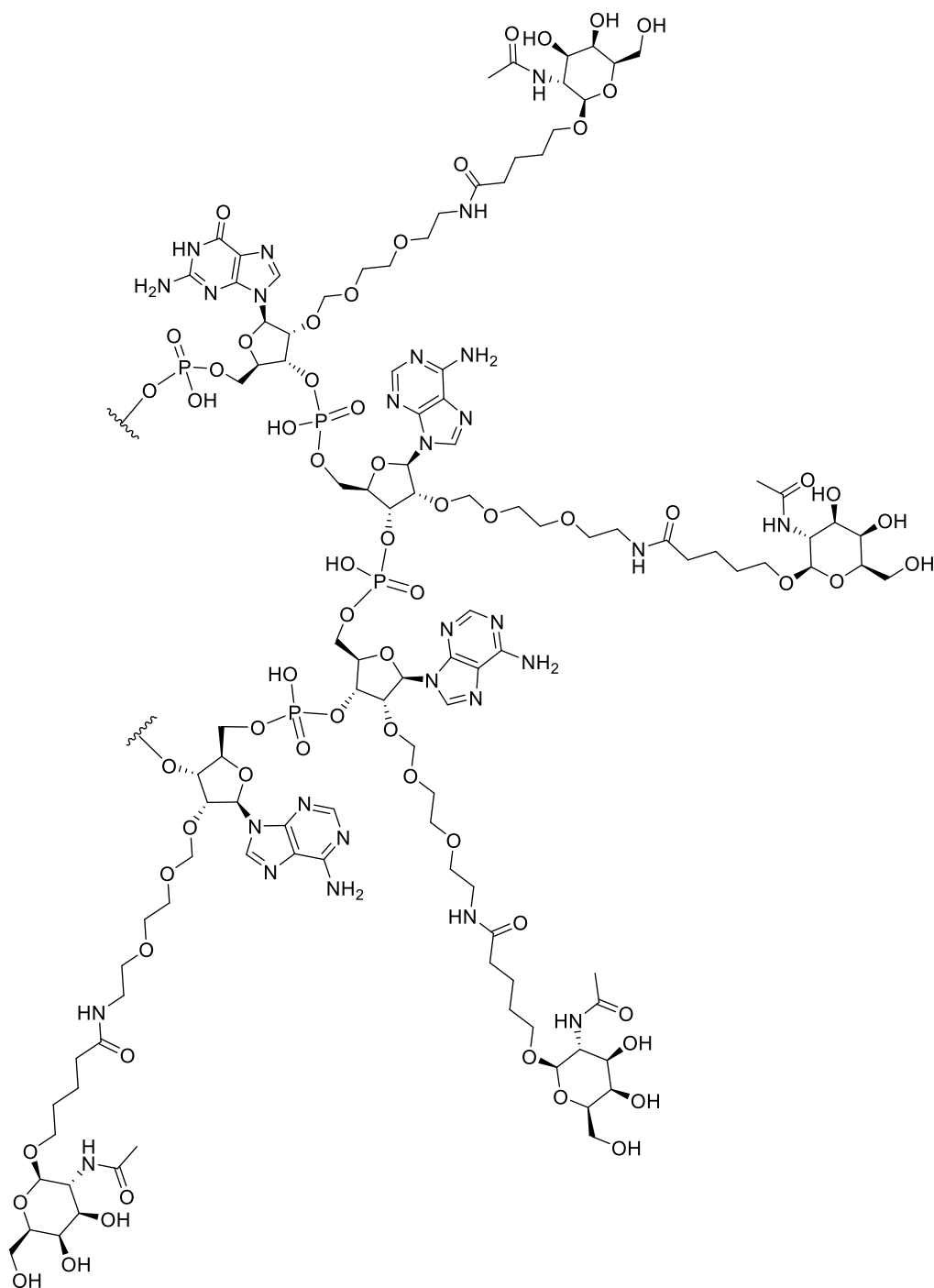


wherein:

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 or S.

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. 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.
15. The use of claim 14, wherein the oligonucleotide or composition is to be administered subcutaneously to the subject.
16. The use of claim 14, wherein the oligonucleotide or composition is to be administered intravenously to the subject.
17. The use of claim 16, wherein the medicament comprises a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is a solvent or dispersion medium containing water.