

CLAIMS

What is claimed is:

1. An oligonucleotide 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).
2. The oligonucleotide of claim 1, further comprising a sense strand of 19 to 50 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand.
3. The oligonucleotide of claim 2, wherein the sense strand comprises a region of complementarity to a sequence as set forth in UUNUUGUGAGGAUUN (SEQ ID NO: 2).
4. The oligonucleotide of claim 2 or 3, wherein the sense strand comprises a region of complementarity to a sequence as set forth in 5'-UUAUUGUGAGGAUUNUUGUC (SEQ ID NO: 3).
5. The oligonucleotide of claim 3, wherein the antisense strand comprises a sequence as set forth in UUAUUGUGAGGAUUNUUGUCGG (SEQ ID NO: 4).
6. The oligonucleotide of claim 3, wherein the antisense strand consists of a sequence as set forth in UUAUUGUGAGGAUUCUUGUCGG (SEQ ID NO: 5).
7. The oligonucleotide of claim 3, wherein the antisense strand consists of a sequence as set forth in UUAUUGUGAGGAUUUUUGUCGG (SEQ ID NO: 5.1).
8. The oligonucleotide of any one of claims 2 to 6, wherein the sense strand comprises a sequence as set forth in ACAANAAUCCUCACAAUAA (SEQ ID NO: 6).
9. The oligonucleotide of any one of claims 2 to 8, wherein the sense strand comprises a sequence as set forth in GACAANAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 7).
10. The oligonucleotide of any one of claims 2 to 8, wherein the sense strand consists of a sequence as set forth in GACAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8).

11. The oligonucleotide of any one of claims 2 to 8, wherein the sense strand consists of a sequence as set forth in GACAAGAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8.1).

12. An oligonucleotide 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: 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.

13. An oligonucleotide 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:

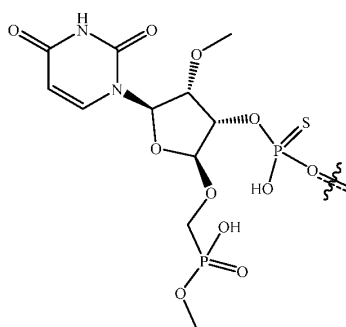
the sense strand comprises a sequence as set forth in GACAAAAUCCUCACAAUAAGCAGCCGAAAGGCUGC (SEQ ID NO: 8) and comprising 2'-fluoro modified nucleotides at positions 3, 8-10, 12, 13, and 17, 2'-O-methyl modified nucleotides at positions 1, 2, 4-7, 11, 14-16, 18-26, and 31-36, and at least one phosphorothioate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

14. The oligonucleotide of claim 13, wherein the sense strand comprises a phosphorothioate linkage between the nucleotides at positions 1 and 2.

15. The oligonucleotide of claim 13 or 14, wherein 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.

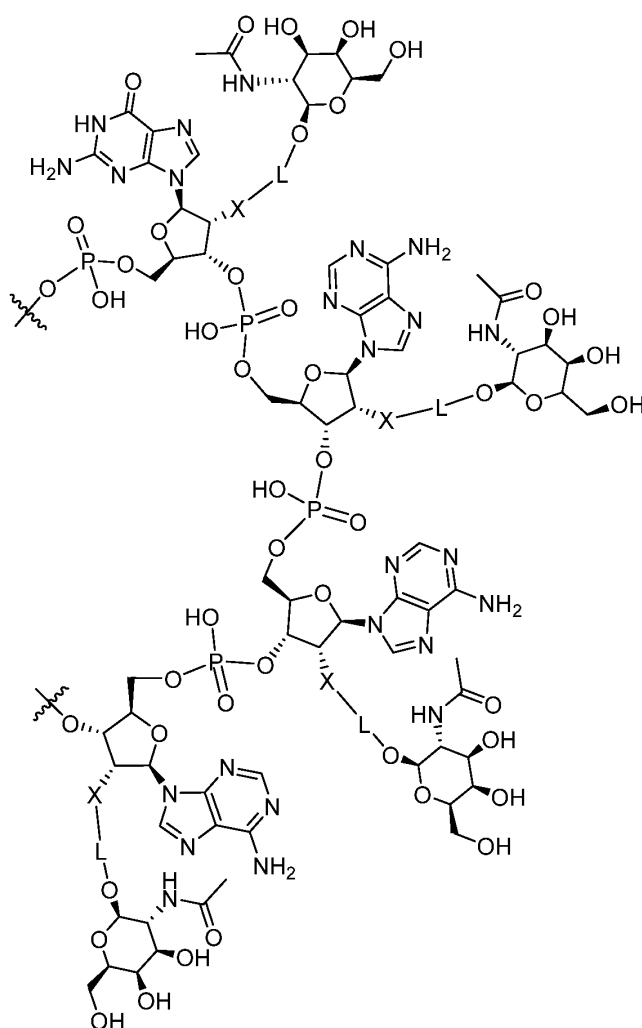
16. The oligonucleotide of any of claims 13 to 14, wherein the 5'-nucleotide of the antisense strand has the following structure:



17. The oligonucleotide of any of claims 13 to 14, wherein one or more of the nucleotides of the –GAAA– sequence on the sense strand is conjugated to a monovalent GalNac moiety.

18. The oligonucleotide of claim 17, wherein each of the nucleotides of the –GAAA– sequence on the sense strand is conjugated to a monovalent GalNac moiety.

19. The oligonucleotide of claim 18, wherein the –GAAA– motif comprises the structure:



wherein:

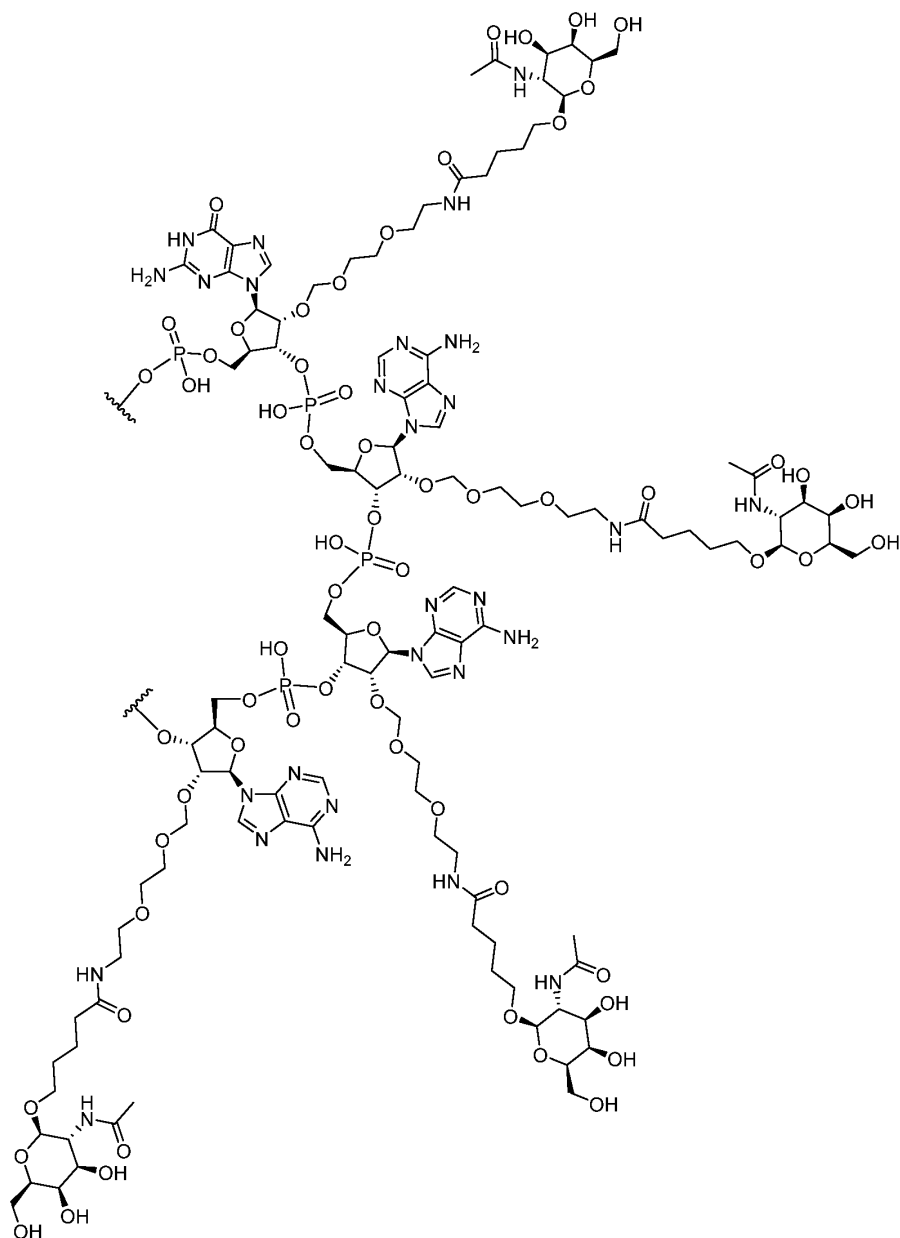
L represents a bond, click chemistry handle, or a linker of 1 to 20, inclusive, consecutive, covalently bonded atoms in length, selected from the group consisting of substituted and unsubstituted alkylene, substituted and unsubstituted alkenylene, substituted and unsubstituted alkynylene, substituted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

X is a O, S, or N.

20. The oligonucleotide of claim 19, wherein L is an acetal linker.

21. The oligonucleotide of claim 19 or claim 20, wherein X is O.

22. The oligonucleotide of claim 16, wherein the –GAAA– sequence comprises the structure:

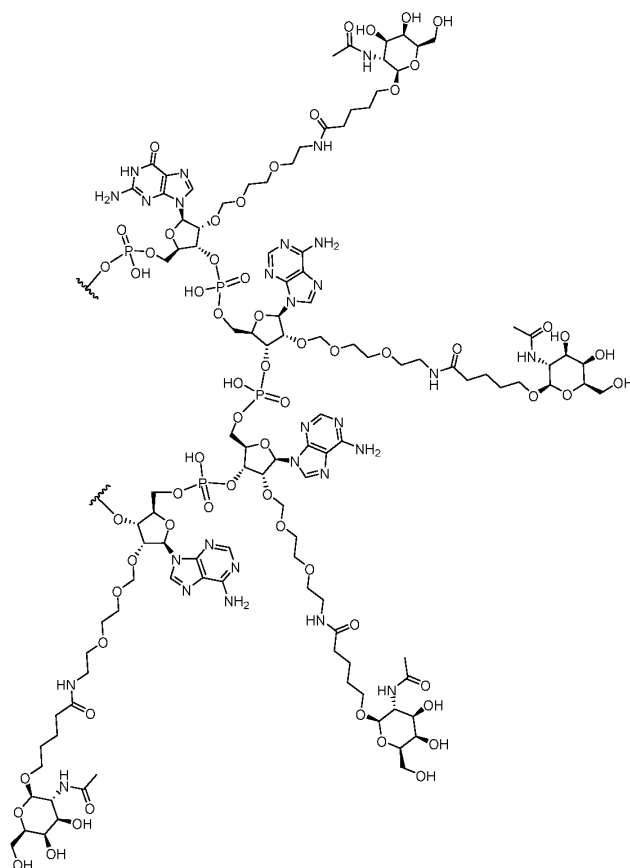


23. The oligonucleotide of claim 2, wherein the sense strand comprises at its 3'-end a stem-loop set forth as: S₁-L-S₂, wherein S₁ is complementary to S₂, and wherein L forms a loop between S₁ and S₂ of up to 6 nucleotides in length.

24. The oligonucleotide of claim 23, wherein L is a tetraloop.
25. The oligonucleotide of claim 23 or 24, wherein L forms a loop between S₁ and S₂ of 4 nucleotides in length.
26. The oligonucleotide of any one of claims 23 to 25, wherein L comprises a sequence set forth as GAAA.
27. The oligonucleotide of claims 23 to 26, wherein up to 4 nucleotides of L of the stem-loop are each conjugated to a separate GalNAc.
28. The oligonucleotide of any one of claims 1 to 10, wherein the oligonucleotide comprises at least one modified nucleotide.
29. The oligonucleotide of claim 28, wherein the modified nucleotide comprises a 2'-modification.
30. The oligonucleotide of claim 29, wherein 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.
31. The oligonucleotide of any one of claims 1 to 10, wherein all of the nucleotides of the oligonucleotide are modified nucleotides.
32. The oligonucleotide of any one of claims 1 to 10, wherein the oligonucleotide comprises at least one modified internucleotide linkage.
33. The oligonucleotide of claim 32, wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.
34. The oligonucleotide of any one of claims 1 to 10, wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog.
35. The oligonucleotide of claims 1 to 10, wherein at least one nucleotide of the oligonucleotide is conjugated to a targeting ligand.
36. The oligonucleotide of claim 35, wherein the targeting ligand is a N-acetylgalactosamine (GalNAc) moiety.

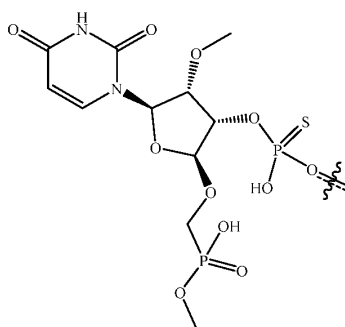
37. A composition comprising an oligonucleotide of any one of the preceding claims and an counterion.
38. A composition comprising an oligonucleotide of any one of the preceding claims and a pharmaceutically acceptable carrier.
39. A method of reducing expression of hepatitis B virus (HBV) surface antigen in a cell, the method comprising delivering to the cell an oligonucleotide of any one of claims 1 to 36.
40. The method of claim 39, wherein the cell is a hepatocyte.
41. The method of claim 30 to 40, wherein the cell is *in vivo*.
42. The method of claim 39 or 40, wherein the cell is *in vitro*.
43. A method of treating a hepatitis B virus (HBV) infection in a subject, the method comprising administering to the subject an oligonucleotide of any one of claims 1 to 36 or a composition of claim 37 or 38.
44. A method of treating HBV infection in a subject, 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.
45. A method of treating HBV infection in a subject, 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.
46. The method of any one of claims 39-45, further comprising administering to the subject an effective amount of Entecavir.
47. An oligonucleotide 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: 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 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:



; 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 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:



48. A composition comprising the oligonucleotide of claim 47.
49. The composition of claim 48, further comprising Na⁺ counterions.
50. A method of reducing expression of hepatitis B virus (HBV) surface antigen in a cell, the method comprising delivering to the cell an oligonucleotide of claim 47, or the composition of claim 48 or claim 49.
51. The method of claim 50, wherein the cell is a hepatocyte.
52. The method of claim 50 or claim 51, wherein the cell is *in vivo*.
53. The method of claim 50 or claim 51, wherein the cell is *in vitro*.
54. A method of treating a hepatitis B virus (HBV) infection in a subject, the method comprising administering to the subject an oligonucleotide of claim 47 or a composition of claim 48 or 49.
55. The method of any one of claims 50-54, further comprising administering to the subject an effective amount of Entecavir.