for long term storage making them less desirable for use in countries where HBV infection is most prevalent.

Treatment of chronic HBV is further complicated by the ability of HBV to evade or suppress the immune response resulting in persistence of the infection. The HBV proteins have immune-inhibitory properties, with hepatitis B s antigen (HBsAg) comprising the overwhelming majority of HBV protein in the circulation of HBV infected subjects. Additionally, while the removal (*via* seroconversion) of hepatitis B e antigen (HBeAg) or reductions in serum viremia are not correlated with the development of sustained control of HBV infection off treatment, the removal of serum HBsAg from the blood (and seroconversion) in HBV infection is a well-recognized prognostic indicator of antiviral response to treatment which will lead to control of HBV infection off treatment. However, this only occurs in a small fraction of patients receiving immunotherapy. Therefore, it is possible, although rare, for patients to mount a sufficiently robust immune response to suppress or clear an HBV infection resulting in at least a functional cure of the disease.

## SUMMARY

The disclosure provides RNAi agents and HBV vaccines for use in treatment of hepatitis B virus (HBV) infection and methods for the treatment of a subject having a hepatitis B virus (HBV) infection, *e.g.,* a chronic HBV infection, which includes a combination therapy or treatment regimen including an RNAi agent targeting at least one HBV transcript and a therapeutic vaccination.

In one aspect, the present disclosure refers to the use of an RNAi agent, a protein-based HBV vaccine, and a nucleic acid-based HBV vaccine in the manufacture of a medicament for treating a hepatitis B virus (HBV) infection, wherein: a) the RNAi agent inhibits expression of at least three HBV transcripts, and comprises a sense strand and an antisense strand forming a double stranded region; b) the protein-based HBV vaccine comprises a first HBV core antigen (HBcAg) polypeptide, or immunogenic fragment thereof, and a first HBV surface antigen (HBsAg) polypeptide, or immunogenic fragment thereof; c) the nucleic acid-based HBV vaccine comprising an expression vector construct encoding a second HBcAg polypeptide, or immunogenic fragment thereof, and/or a second HBsAg polypeptide, or immunogenic fragment thereof; d) the second HBcAg polypeptide, or immunogenic fragment thereof, and/or the second HBsAg polypeptide, or immunogenic fragment thereof, shares at least one epitope with at least one of the first HBcAg polypeptide, or immunogenic fragment thereof, and/or the first HBsAg polypeptide, or immunogenic fragment thereof; and (e) the RNAi agent, the protein-based HBV vaccine, and the nucleic acid-based HBV vaccine are to be sequentially administered to a subject having an HBV infection.

In another aspect, the present disclosure refers to a kit for treating a subject having an HBV infection, the kit comprising: a) an RNAi agent that inhibits expression of at least three HBV transcripts, wherein the RNAi agent comprises a sense strand and an antisense strand forming a double stranded region; b) a protein-based vaccine comprising a first HBV core antigen (HBcAg) polypeptide, or immunogenic fragment thereof, and a first HBV surface antigen (HBsAg)

polypeptide, or immunogenic fragment thereof; and c) a nucleic acid-based vaccine comprising an expression vector construct encoding a second HBcAg polypeptide, or immunogenic fragment thereof, and/or a second HBsAg polypeptide, or immunogenic fragment thereof, wherein the second HBcAg polypeptide, or immunogenic fragment thereof, and/or the second HBsAg polypeptide, or immunogenic fragment thereof, shares at least one epitope with at least one of the first HBcAg polypeptide, or immunogenic fragment thereof, and/or the first HBsAg polypeptide, or immunogenic fragment thereof; and d) instructions for use according to the uses as described herein.

Accordingly, in one example, the present disclosure provides RNAi agents and HBV vaccines for use in treatment of hepatitis B virus (HBV) infection and methods for treating a subject having a hepatitis B virus (HBV) infection, *e.g.,* a chronic HBV infection. The methods include a regimen which includes administering, *e.g.,* sequentially administering, to the subject having the HBV infection, an RNAi agent that targets at least three HBV transcripts, wherein the RNAi agent comprises a sense strand and an antisense strand; a protein-based vaccine comprising an HBV core antigen (HBcAg) and an HBV surface antigen (HBsAg); and a nucleic acid-based vaccine comprising an expression construct encoding an HBcAg or an HBsAg, wherein the construct encodes a protein that shares an epitope with the protein-based vaccine, thereby treating the subject.

In another example, the present disclosure provides a regimen for treating a subject having a hepatitis B virus (HBV) infection, *e.g.,* a chronic HBV infection. The regimen includes the use of an RNAi agent that targets at least three HBV transcripts, wherein the RNAi agent comprises a sense strand and an antisense strand; a protein-based vaccine comprising an HBV core antigen (HBcAg) and an HBV surface antigen (HBsAg); and a nucleic acid-based vaccine comprising an expression construct encoding an HBcAg or an HBsAg, wherein the construct encodes a protein that shares an epitope with the protein-based vaccine.

In one example, the HBcAg protein, or immunogenic fragment thereof, shares an epitope with the HBV core antigen (HBcAg) polypeptide, or immunogenic fragment thereof, present in the protein-based vaccine and/or the HBsAg protein, or immunogenic fragment thereof, shares an epitope with the HBV surface antigen (HBsAg) polypeptide, or immunogenic fragment thereof, present in the protein-based vaccine.

In certain examples, the RNAi agent comprises at least one modified nucleotide.

In certain examples, the nucleic acid-based vaccine comprises an expression construct encoding an HBcAg and an HBsAg.

In certain examples, the RNAi agent targeting HBV is administered to the subject at least two times.

In certain examples, the RNAi agent targeting HBV administered to the subject decreases HBsAg in the serum of the subject by at least 0.5 log 10 IU/ml. In certain examples, the subject has at least a 0.5 log10 IU/ml decrease in HBsAg in serum prior to administration of the first dose of the protein based vaccine. In certain examples, the subject has at least a 1 log10 IU/ml decrease in HBsAg in serum prior to administration of the first dose of the protein based vaccine. In certain

examples, the subject has an HBsAg of 2 log 10 IU/ml or less in serum prior to administration of the vaccine.

In certain examples, the RNAi agent is administered to the subject no more than once per week. In certain examples, the RNAi agent is administered to the subject no more than once every four weeks.

In certain examples, the RNAi ageint is administered to the subject at a dose of 0.01 mg/kg to 10 mg/kg; or 0.5 mg/kg to 50 mg/kg; or 10 mg/kg to 30 mg/kg. In certain examples, the RNAi agent is administered to the subject at a dose selected from 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, and 30 mg/kg. In some examples, a dose of the RNAi agent is administered to the subject about once per week; about one every two weeks; about once every three weeks; about once every four weeks; or a dose of the RNAi agent is administered to the subject not more than once per week; or a dose of the RNAi agent is administered to the subject no more than once every four weeks.

In certain examples, the protein-based vaccine comprises an amino acid sequence of at least one determinant of HBsAg and at least one determinant of HBcAg.

In certain examples, the nucleic acid-based vaccine comprising an expression vector construct encoding an HBcAg or an HBsAg encodes an amino acid sequence comprising at least one determinant of HBsAg or at least one determinant of HBcAg

In certain examples, the determinant of HBsAg comprises a sequence at least 90% identical to amino acids 124 to 147 of SEQ ID NO: 22. In certain examples, the determinant of HBsAg comprises a sequence at least 90% identical to amino acids 99 to 168 of SEQ ID NO: 23.

In certain examples, the determinant of HBcAg comprises a sequence comprising amino acid 80 of SEQ ID NO: 24. In certain examples, the sequence comprising amino acid 80 of SEQ ID NO: 24 comprises an amino acid sequence at lest 90% identical to at least amino acids 70 to 90 of SEQ ID NO: 24. In certain examples, the determinant of HBcAg comprises a sequence comprising amino acid 138 of SEQ ID NO: 24. In certain examples, the sequence comprising amino acid 80 of SEQ ID NO: 14 comprises an amino acid sequence at lest 90% identical to at least amino acids 128 to 143 of SEQ ID NO: 24. In certain examples, the determinant of HBcAg comprises an amino acid sequence at least 90% identical to at least 40, 50, 60, 70, 80, 90, or 100 contiguous amino acids of SEQ ID NO: 24. In certain examples, the determinant of HBcAg comprises a sequence at least 90% identical to amino acids 18 to 143 of SEQ ID NO: 24.

In certain examples, the protein-based vaccine administered to the subject comprises a dose of 0.1 μg to 1.0 mg of the HBcAg and a dose of 0.1 μg to 1.0 mg of HBsAg. In some examples, a dose of the protein-based vaccine is administered to the subject about once per week; about one every two weeks; about once every three weeks; about once every four weeks; or a dose of the protein-based vaccine is administered to the subject no more than once per week; or a dose of the protein-based vaccine is administered to the subject no more than once every four weeks.

In certain examples, the HBcAg protein and the HBsAg protein are present in a single formulation. In certain examples, the HBcAg protein and the HBsAg protein are not present in a

single formulation. In certain examples, the protein-based vaccine comprises an adjuvant. In some examples, the adjuvant stimulates a balanced Th1/Th2 response. In certain examples, the adjuvant is selected from monophosphoryl lipid A (MPL), poly(I:C), polyICLC adjuvant, CpG DNA, polyICLC adjuvant ,a STING agonist, c-di-AMP, c-di-GMP, c-di-CMP; short, blunt-ended 5′-triphosphate dsRNA (3pRNA) Rig-Iligand, poly[di(sodiumcarboxylatoethylphenoxy)phosphazene] (PCEP)), alum, virosomes, cytokines, IL-12, AS02, AS03, AS04, MF59, ISCOMATRIX®, IC31®, or Rig-I ligand. In certain examples, the adjuvant is selected from polyI:C adjuvant, a CpG adjuvant, a STING agonist, or a PCEP adjuvant.

In certain examples, the protein based vaccine is administered to the subject at least two times. In certain examples, the protein-based vaccine is administered to the subject no more than once every two weeks. In certain examples, the protein-based vaccine is administered to the subject no sooner than the day on which the final dose of the RNAi agent has been administered to the subject. In certain examples, the protein-based vaccine is administered to the subject on the same day on which the final dose of the RNAi agent has been administered to the subject. In certain examples, the protein based vaccine is administered to the subject no later than one month after the final dose of the RNAi agent has been administered to the subject. In certain examples, the protein based vaccine is administered to the subject no later than two months after the final dose of the RNAi agent has been administered to the subject. In certain examples, the protein based vaccine is administered to the subject no later than three months after the final dose of the RNAi agent has been administered to the subject.

In certain examples the methods of the disclosure further comprise determining the serum HBsAg level after administration of at least one dose of the RNAi agent and prior to administration of the protein based vaccine. That is, the serum HBsAg level is determined in the subject after administration of at least one dose of the RNAi agent and prior to administration of the protein based vaccine.

In certain examples the methods of the disclosure further comprise determining the serum HBeAg level after administration of at least one dose of the RNAi agent and prior to administration of the protein based vaccine. That is, the serum HBeAg level is determined in the subject after administration of at least one dose of the RNAi agent and prior to administration of the protein based vaccine.

In certain examples the methods of the disclosure further comprise determining the serum HBsAg level and the HBeAg level after administration of at least one dose of the RNAi agent and prior to administration of the protein based vaccine. That is, the serum HBsAg level and the serum HBeAg level are determined in the subject after administration of at least one dose of the RNAi agent and prior to administration of the protein based vaccine.

In certain examples, the nucleic acid-based vaccine comprises at least one expression vector construct encoding both an HBcAg and an HBsAg. In certain examples, the expression construct

promotes expression of HBcAg and HBsAg from a single promoter. In other examples, the expression construct promotes expression of HBcAg and HBsAg from separate promoters.

In certain examples, at least one promoter is selected from a respiratory syncytial virus (RSV) promoter, a cytomegalovirus (CMV) promoter, a PH5 promoter, and an H1 promoter. In certain examples, the expression construct comprises a viral vector. In certain examples, the viral vector is selected from adenovirus vector; retrovirus vector, lentiviral vector, moloney murine leukemia virus vector, adeno- associated virus vector; herpes simplex virus vector; SV 40 vector; polyoma virus vector; papilloma virus vector; picornavirus vector; pox virus vector, orthopox virus vector, vaccinia virus vector, modified vaccinia virus Ankara (MVA) vector, avipox vector, canary pox vector, fowl pox vector, adenovirus vector, and Epstein Barr virus vector. In certain examples, the viral vector is an MVA vector.

In certain emboidments, the nucleic acid-based vaccine administered to the subject comprises a tissue-culture infectious dose ($TCID_{50}$) of $10^6$ to $10^{10}$ $TCID_{50}$; or $10^6$ to $10^9$ $TCID_{50}$; or $10^6$ to $10^8$ $TCID_{50}$

In certain examples, the nucleic acid-based vector is administered to the subject no sooner than two weeks after administration of the final dose of the protein-based vaccine is administered to the subject.

In certain examples, the methods further comprise determining the serum HBsAg level after administration of at least one dose of the RNAi agent and prior to administration of the nucleic acid-based vaccine. That is, the serum HBsAg level is determined in the subject after administration of at least one dose of the RNAi agent and prior to administration of the nucleic acid-based vaccine. In certain examples, the nucleic acid-based vaccine is administered to the subject after a further decrease of at least 0.5 log 10 of serum HBsAg after at least one dose of the protein-based vaccine is administered to the subject. In certain examples of the regimen, a single dose of the nucleic-acid based vaccine is administered to the subject.

In certain examples, the methods further comprise determining the serum HBeAg level after administration of at least one dose of the RNAi agent and prior to administration of the nucleic acid-based vaccine. That is, the serum HBeAg level is determined in the subject after administration of at least one dose of the RNAi agent and prior to administration of the nucleic acid-based vaccine. In certain examples, the nucleic acid-based vaccine is administered to the subject after a further decrease of at least 0.5 log 10 of serum HBeAg after at least one dose of the protein-based vaccine is administered to the subject. In certain examples of the regimen, a single dose of the nucleic-acid based vaccine is administered to the subject.

In certain examples, the methods further comprise determining the serum HBsAg level and the HBeAg level after administration of at least one dose of the RNAi agent and prior to administration of the nucleic acid-based vaccine. That is, the serum HBsAg level and the serum HBeAg level are determined in the subject after administration of at least one dose of the RNAi agent and prior to administration of the nucleic acid-based vaccine. In certain examples, the nucleic acid-

based vaccine is administered to the subject after a further decrease of at least 0.5 log 10 of serum HBsAg and serum HBeAg after at least one dose of the protein-based vaccine is administered to the subject. In certain examples of the regimen, a single dose of the nucleic-acid based vaccine is administered to the subject.

In certain examples, the methods further comprise administering a nucleot(s)ide analog to the subject at least prior to administration of the iRNA targeted to HBV. In certain examples, the nucleot(s)ide analog is administered throughout the entire regimen. In certain examples, the nucleot(s)ide analog is selected from Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide (TAF), Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, AGX-1009, emtricitabine, clevudine, ritonavir, dipivoxil, lobucavir, famvir, FTC, N-Acetyl-Cysteine (NAC), PC1323, theradigm-HBV, thymosin-alpha, and ganciclovir, besifovir (ANA-380/LB-80380), and tenofovir-exaliades (TLX/CMX157).

In certain examples, the subject has serum HBsAg below 3000 IU/ml prior to administration of the RNAi agent. In certain examples, the subject has serum HBsAg below 4000 IU/ml prior to administration of the RNAi agent. In certain examples, subject has serum HBsAg below 5000 IU/ml prior to administration of the RNAi agent.

In certain examples, the subject has a reduction of HBsAg level of at least 2 $\log_{10}$ scales after administration of the RNAi agent and prior to administration of the first dose of a protein-based vaccine. In certain examples, the subject has a reduction of HBeAg level of at least 1 $\log_{10}$ scale after administration of the RNAi agent and prior to administration of the first dose of a protein-based vaccine. In certain examples, the subject has a reduction of HBsAg level of at least 2 $\log_{10}$ scales and a reduction of HBeAg level of at least 1 $\log_{10}$ scale after administration of the RNAi agent and prior to administration of the first dose of a protein-based vaccine.

In certain examples, the subject has a reduction of HBsAg level to 500 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine. In certain examples, the subject has a reduction of HBsAg level to 200 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine. In certain examples, the subject has a reduction of HBsAg level to 100 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine.

In certain examples, the subject has a reduction of HBeAg level to 500 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine. In certain examples, the subject has a reduction of HBeAg level to 200 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine. In certain examples, the subject has a reduction of HBeAg level to 100 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine.

In certain examples, the subject has a reduction of HBsAg level and HBeAg level to 500 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine. In certain examples, the subject has a reduction of HBsAg level and HBeAg level to 200 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine. In certain examples, the subject has a reduction of HBsAg level and HBeAg level to 100 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein based vaccine.

In certain examples, the level of serum HBsAg and HBeAg in the subject are decreased to below the level of detection using a clinical assay for at least three months after the end of the dose of the nucleic acid-based vaccine. In certain examples, the level of serum HBsAg and HBeAg in the subject are decreased to below the level of detection using a clinical assay for at least six months after the end of the dose of the nucleic acid-based vaccine.

In certain examples, serum HBsAg in the subject is decreased to below the level of detection using a clinical assay for at least six months after the end of the dose of the nucleic acid-based vaccine.

In certain examples, the methods further comprise administration of an immune stimulator to the subject. In certain examples, the immune stimulator is selected from the group pegylated interferon alfa 2a (PEG-IFN-alpha-2a), Interferon alfa-2b, PEG-IFN-alpha-2b, Interferon lambda a recombinant human interleukin-7, and a Toll-like receptor 3, 7, 8 or 9 (TLR3, TLR7, TLR8, TLR9) agonist, a viral entry inhibitor, Myrcludex, an oligonucleotide that inhibits the secretion or release of HBsAg, REP 9AC, a capsid inhibitor, Bay41-4109, NVR-1221, a cccDNA inhibitor, IHVR-25) a viral capsid, an MVA capsid, an immune checkpoint regulator, an CTLA-4 inhibitor, ipilimumab, a PD-1 inhibitor, Nivolumab, Pembrolizumab, BGB-A317 antibody, a PD-L1 inhibitor, atezolizumab, avelumab, durvalumab, and an affimer biotherapeutic.

In certain examples of the regimen, the subject is human.

In certain examples of the regimen, the RNAi agent targets four transcripts of HBV. In certain examples, the RNAi agent is selected from an iRNA in Appendix A. In certain examples, the RNAi agent is selected from any of the agents in any one of Tables 2-11 in Appendix A. In certain examples, the RNAi agent targets at least 15 contiguous nucleotides of nucleotides 206-228, 207-229, 210-232, 212-234, 214-236, 215-237, 216-238, 226-248, 245-267, 250-272, 252-274, 253-275, 254-276, 256-278, 258-280, 263-285, 370-392, 373-395, 375-397, 401-423, 405-427, 410-432, 411-433, 422-444, 424-446, 425-447, 426-448, 731-753, 734-756, 1174-1196, 1250-1272, 1255-1277, 1256-1278, 1545-1567, 1547-1569, 1551-1571, 1577-1597, 1579-1597, 1580-1598, 1806-1825, 1812-1831, 1814-1836, 1829-1851, 1831-1853, 1857-1879, 1864-1886, 2259-2281, 2298-2320, or 2828-2850 of SEQ ID NO: 1 (NC_003977.1). In certain examples, the RNAi agent targets at least 15 contiguous nucleotides of nucleotides 1579-1597 or 1812-1831 of SEQ ID NO: 1 (NC_003977.1). In certain examples, the RNAi agent targets nucleotides 1579-1597 or 1812-1831 of SEQ ID NO: 1 (NC_003977.1).

In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of at least 15 contiguous nucleotides of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 25) or AGGUGAAAAAGUUGCAUGGUGUU (SEQ ID NO: 26). In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of at least 19 contiguous nucleotides of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 25) or AGGUGAAAAAGUUGCAUGGUGUU (SEQ ID NO: 26). In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 25) or AGGUGAAAAAGUUGCAUGGUGUU (SEQ ID NO: 26).

In certain examples, the sense strand of the RNAi agent comprises a nucleotide sequence of at least 15 contiguous nucleotides of GUGUGCACUUCGCUUCACA (SEQ ID NO: 27) or CACCAUGCAACUUUUUCACCU (SEQ ID NO: 28). In certain examples, the sense strand of the RNAi agent comprises a nucleotide sequence of at least 19 contiguous nucleotides of GUGUGCACUUCGCUUCACA (SEQ ID NO: 27) or CACCAUGCAACUUUUUCACCU (SEQ ID NO: 28). In certain examples, the sense strand of the RNAi agent comprises a nucleotide sequence of GUGUGCACUUCGCUUCACA (SEQ ID NO: 27) or CACCAUGCAACUUUUUCACCU (SEQ ID NO: 28).

In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of at least 15 contiguous nucleotides of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 25) and the sense strand comprises a nucleotide sequence of at least 15 contiguous nucleotides of GUGUGCACUUCGCUUCACA (SEQ ID NO: 27). In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of at least 19 contiguous nucleotides of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 25) and the sense strand comprises a nucleotide sequence of at least 19 contiguous nucleotides of GUGUGCACUUCGCUUCACA (SEQ ID NO: 27). In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 25) and the sense strand comprises a nucleotide sequence of GUGUGCACUUCGCUUCACA (SEQ ID NO: 27).

In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of at least 15 contiguous nucleotides of AGGUGAAAAAGUUGCAUGGUGUU (SEQ ID NO: 26) and the sense strand of the RNAi agent comprises a nucleotide sequence of at least 15 contiguous nucleotides of CACCAUGCAACUUUUUCACCU (SEQ ID NO: 28). In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of at least 19 contiguous nucleotides of AGGUGAAAAAGUUGCAUGGUGUU (SEQ ID NO: 26) and the sense strand of the RNAi agent comprises a nucleotide sequence of at least 19 contiguous nucleotides of CACCAUGCAACUUUUUCACCU (SEQ ID NO: 28). In certain examples, the antisense strand of the RNAi agent comprises a nucleotide sequence of AGGUGAAAAAGUUGCAUGGUGUU (SEQ ID NO: 26) and the sense strand of the RNAi agent comprises a nucleotide sequence of CACCAUGCAACUUUUUCACCU (SEQ ID NO: 28).

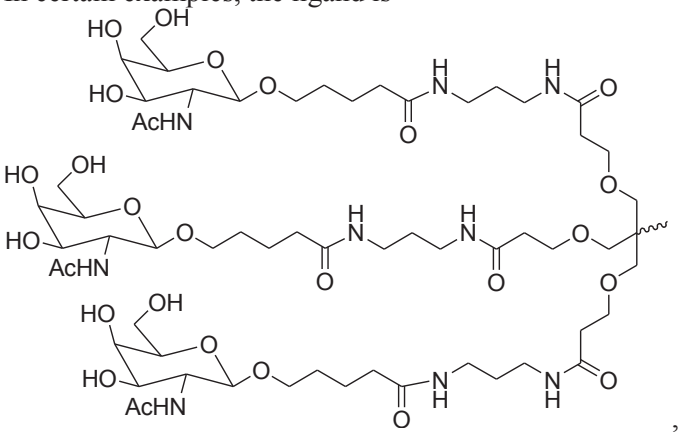
In certain examples of the regimen, substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, wherein said sense strand is conjugated to a ligand attached at the 3'-terminus. In certain examples, the ligand is one or more GalNAc derivatives attached through a monovalent linker, bivalent branched linker, or trivalent branched linker. In certain examples, the at least one of said modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

In certain examples, at least one strand of the RNAi agent comprises a 3' overhang of at least 1 nucleotide. In certain examples, at least one strand if the RNAi agent comprises a 3' overhang of at least 2 nucleotides. In certain examples, the double-stranded region of the RNAi agent is 15-30 nucleotide pairs in length; 17-23 nucleotide pairs in length; 17-25 nucleotide pairs in length; 23-27 nucleotide pairs in length; 19-21 nucleotide pairs in length; 21-23 nucleotide pairs in length.

In certain examples, each strand of the RNAi agent has 15-30 nucleotides.

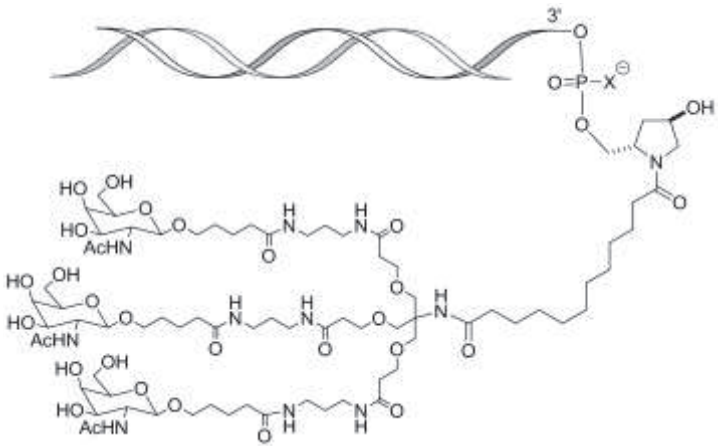
In certain examples, each strand of the RNAi agent has 19-30 nucleotides.

In certain examples, the ligand is



,

and the RNAi agent is optionally conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In certain examples, the sense strand comprises the nucleotide sequence 5'-gsusguGfcAfCfUfucgcuucaca-3' (SEQ ID NO: 29) and the antisense strand comprises the nucleotide sequence 5'-usGfsugaAfgCfGfaaguGfcAfcacsusu-3' (SEQ ID NO: 30); or the sense strand comprises the nucleotide sequence 5'-csasccauGfcAfAfCfuuuuucaccu-3' (SEQ ID NO: 31) and the antisense strand comprises the nucleotide sequence 5'-asGfsgugAfaAfAfaguuGfcAfuggugsusu-3' (SEQ ID NO: 32), wherein a, c, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively; as, cs, gs, and us are 2'-O-methyladenosine-3'- phosphorothioate, 2'-O-methylcytidine-3'- phosphorothioate, 2'-O-methylguanosine-3'- phosphorothioate, and 2'-O-methyluridine-3'-phosphorothioate, respectively; Af, Cf, Gf, and Uf are 2'-fluoroadenosine-3'-phosphate, 2'-fluorocytidine-3'-phosphate, 2'-fluoroguanosine-3'-phosphate, and 2'-fluorouridine-3'-phosphate, respectively; and Gfs is 2'-fluoroguanosine-3'-phosphorothioate. In certain examples, the RNAi agent is AD-66810 or AD-66816.

In certain examples, the protein-based vaccine comprises epitopes present in at least 4, 5, 6, 7, 8, 9, or 10 genotypes of HBV.

In certain examples, the nucleic acid-based vaccine comprises epitopes present in at least 4, 5, 6, 7, 8, 9, or 10 genotypes of HBV.

The present disclosure further provides uses of the RNAi agents and vaccines provided herein for treatment of subjects having a hepatitis B virus infection based on the methods provided herein. In certain example, the RNAi agents and the HBV vaccines are used in the manufacture of medicaments for treatment of a subject with an HBV infection.

The present disclosure further provides kits comprising RNAi agents and vaccines provided herein and instructions providing treatment regimens for their use for treatment of subjects having a hepatitis B virus infection.

The present disclosure is further illustrated by the following detailed description and drawings.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically depicts the structure of the approximately 3.2 kb double-stranded HBV genome. Replication of the HBV genome occurs through an RNA intermediate and produces 4 overlapping viral transcripts of about 3.5 kb, 2.4 kb, 2.1 kb, and 0.7 kb (termination sites indicated by arrows), and the common 3' end encoding seven viral proteins (pre-S1, pre-S2, S, P, X, pre-C, and C) that are translated across three reading frames.

Figures 2A-2E are graphs showing suppression of HBV by siRNA in a transgenic mouse model. The level of (2A) HBsAg, (2B) HBeAg, and (2C) HBV-DNA in serum of HBV1.3-xfs mice (n = 6 per group) after a single subcutaneous 3 mg/kg or 9 mg/kg dose of a control GalNAc-siRNA (an siRNA that does not target HBV), AD-66816, or AD-66810. The level of (2D) total HBV RNA and (2E) 3.5 kb HBV transcripts from liver lysates determined via RT-qPCR and normalized to expression of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Figure 3 is a schematic showing a dosing regimen for an experiment assaying the effect of pretreatment of HBV1.3-xfs mice with the nucleoside inhibitor, Entecavir, an HBV-shRNA, a control GalNAc-siRNA, AD-66816, or AD-66810 prior to administration of a therapeutic vaccine against HBV.

Figures 4A-4C are graphs showing the level of (4A) HBsAg, (4B) HBeAg, and (4C) HBV-DNA in serum of HBV1.3-xfs mice after treatment based on the dosing regimen provided in Figure 3.

Figures 5A-5F are graphs showing T cell immune response in the liver against peptides (5A) HBs(S208), (5B) HBc(C93), and (5C) MVA(B8R) in HBV1.3-xfs mice after treatment based on the dosing regimen provided in Figure 3. Figures 5D-5F show a reanalysis of the same data in Figures 5A-5C performed to accommodate for an insufficient exclusion of dead immune cells and shows T cell immune response in the liver against peptides (5D) HBs(S208), (5E) HBc(C93), and (5F) MVA(B8R).

Figures 6A-6C show the level of HBV RNA and protein levels in liver cells in HBV1.3-xfs mice after treatment based on the dosing regimen provided in Figure 3. Figure 6A shows total HBV RNA. Figure 6B shows HBV 3.5 kb transcript relative to GAPDH as determined by RT-qPCR. Figure 6C shows the number of HBcAg positive cells per $mm^2$ of liver section detected by immunohistochemical staining.

We claim:

1. Use of an RNAi agent, a protein-based HBV vaccine, and a nucleic acid-based HBV vaccine in the manufacture of a medicament for treating a hepatitis B virus (HBV) infection, wherein:

a) the RNAi agent inhibits expression of at least three HBV transcripts, and comprises a sense strand and an antisense strand forming a double stranded region;

b) the protein-based HBV vaccine comprises a first HBV core antigen (HBcAg) polypeptide, or immunogenic fragment thereof, and a first HBV surface antigen (HBsAg) polypeptide, or immunogenic fragment thereof;

c) the nucleic acid-based HBV vaccine comprising an expression vector construct encoding a second HBcAg polypeptide, or immunogenic fragment thereof, and/or a second HBsAg polypeptide, or immunogenic fragment thereof;

d) the second HBcAg polypeptide, or immunogenic fragment thereof, and/or the second HBsAg polypeptide, or immunogenic fragment thereof, shares at least one epitope with at least one of the first HBcAg polypeptide, or immunogenic fragment thereof, and/or the first HBsAg polypeptide, or immunogenic fragment thereof; and

(e) the RNAi agent, the protein-based HBV vaccine, and the nucleic acid-based HBV vaccine are to be sequentially administered to a subject having an HBV infection.

2. The use of claim 1, wherein the nucleic acid-based HBV vaccine comprises an expression vector construct encoding the second HBcAg polypeptide, or immunogenic fragment thereof, and the second HBsAg polypeptide, or immunogenic fragment thereof.

3. The use of any of claims 1-2, wherein (a) the RNAi agent is to be administered to the subject such that the level of HBsAg in the serum of the subject is decreased by at least 0.5 log 10 IU/ml; (b) the level of HBsAg in the serum of the subject is decreased by at least a 0.5 log10 IU/ml, at least a 1 log10 IU/ml, or at least a 2 log 10 IU/ml prior to administration of a first dose of the protein-based HBV vaccine; (c) the level of HBeAg in the serum of the subject is decreased by at least a 1 log 10 IU/ml prior to administration of a first dose of the protein-based HBV vaccine; (d) the level of HBsAg in the serum of the subject is decreased by at least a 2 log 10 IU/ml and the level of HBeAg in the serum of the subject is decreased by at least a 1 log 10 IU/ml prior to administration of a first dose of the protein based vaccine; (e) the subject has a serum HBsAg level of 500 IU/ml or less, 200 IU/ml or less, or 100 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein-based HBV vaccine; (f) the subject has a serum HBeAg level of 500 IU/ml or less, 200 IU/ml or less, or 100 IU/ml or less after administration of the RNAi agent and prior to administration of a first dose of the protein-based HBV vaccine; (g) the level of serum HBsAg in the subject is below 3000 IU/ml, 4000 IU/ml, or 5000 IU/ml prior to administration of the RNAi agent; (h) the level of serum HBsAg in the subject is decreased to below the level of detection using a

clinical assay for at least six months after the end of the dose of the nucleic acid-based HBV vaccine; (i) the level of serum HBeAg in the subject is decreased to below the level of detection using a clinical assay for at least six months after the end of the dose of the nucleic acid-based HBV vaccine; (j) the level of serum HBeAg in the subject is decreased to below the level of detection using a clinical assay for at least six months after the end of the dose of the nucleic acid-based HBV vaccine; or (k) the level of serum HBsAg and HBeAg in the subject are decreased to below the level of detection using a clinical assay for at least six months after the end of the dose of the nucleic acid-based HBV vaccine.

4. The use of any of claims 1-3, wherein (a) at least two doses of the RNAi agent are to be administered to the subject; or (b) a dose of the RNAi agent is to be administered to the subject no more than once per week or no more than once every four weeks.

5. The use of any one of claims 1-4, wherein the RNAi agent is to be administered to the subject at a dose of 0.01 mg/kg to 10 mg/kg; 0.5 mg/kg to 50 mg/kg; 10 mg/kg to 30 mg/kg; 0.5 mg/kg; 1 mg/kg; 1.5 mg/kg; 3 mg/kg; 5 mg/kg; 10 mg/kg; or 30 mg/kg.

6. The use of any of claims 1-5, wherein the first HBcAg polypeptide, or immunogenic fragment thereof, comprises at least one HBcAg determinant and the first HBsAg polypeptide, or immunogenic fragment thereof, comprises at least one HBsAg determinant.

7. The use of any one of claims 1-6, wherein the second HBcAg polypeptide, or immunogenic fragment thereof, comprises at least one HBcAg determinant and the second HBsAg polypeptide, or immunogenic fragment thereof, comprises at least one HBsAg determinant.

8. The use or of claim 6 or 7, wherein the first and/or second HBsAg determinant comprises an amino acid sequence at least 90% identical to amino acids 124 to 147 of SEQ ID NO: 22; or an amino acid sequence at least 90% identical to amino acids 99 to 168 of SEQ ID NO: 23.

9. The use of claim 6 or 7, wherein the first and/or second HBcAg determinant comprises: an amino acid sequence comprising amino acid residue 80 of SEQ ID NO: 24; an amino acid sequence at least 90% identical to at least amino acids 70 to 90 of SEQ ID NO: 24; an amino acid sequence comprising amino acid residue 138 of SEQ ID NO: 24; an amino acid sequence at least 90% identical to at least amino acids 128 to 143 of SEQ ID NO: 24; an amino acid sequence at least 90% identical to at least 40, 50, 60, 70, 80, 90, or 100 contiguous amino acids of SEQ ID NO: 24; or an amino acid sequence at least 90% identical to amino acids 18 to 143 of SEQ ID NO: 24.

10. The use of any one of claims 1-9, wherein the protein-based HBV vaccine is to be administered to the subject at a dose of about 0.1 □g to about 1.0 mg of the first HBcAg polypeptide, or immunogenic fragment thereof, and a dose of about 0.1 □g to about 1.0 mg of the first HBsAg polypeptide, or immunogenic fragment thereof.

11. The use of any of claims 1-10, wherein the protein-based HBV vaccine further comprises an adjuvant.

12. The use of claim 11, wherein the adjuvant is monophosphoryl lipid A (MPL), poly(I:C), polyICLC adjuvant, CpG DNA, polyICLC adjuvant ,a STING agonist, c-di-AMP, c-di-GMP, c-di-CMP; short, blunt-ended 5′-triphosphate dsRNA (3pRNA) Rig-Iligand, poly[di(sodiumcarboxylatoethylphenoxy)phosphazene] (PCEP)), alum, virosomes, cytokines, IL-12, AS02, AS03, AS04, MF59, ISCOMATRIX®, IC31®, or Rig-I ligand.

13. The use of any of claims 1-12, wherein a dose of the protein-based HBV vaccine is to be administered to the subject: at least two times; no more than once every two weeks; no sooner than the day on which a final dose of the RNAi agent has been administered to the subject; on the same day as a final dose of the RNAi agent has been administered to the subject; no later than one month after a final dose of the RNAi agent has been administered to the subject; or no later than three months after a final dose of the RNAi agent has been administered to the subject.

14. The use of any of claims 1-13, wherein the nucleic acid-based HBV vaccine comprises at least one expression vector construct encoding the second HBcAg polypeptide, or immunogenic fragment thereof, and the second HBsAg polypeptide, or immunogenic fragment thereof.

15. The use of claim 14, wherein the at least one expression construct comprises (a) a promoter that promotes expression of the second HBcAg polypeptide, or immunogenic fragment thereof, and the second HBsAg polypeptide, or immunogenic fragment thereof; or (b) a first and second promoter, the first promoter promoting expression of the second HBcAg polypeptide, or immunogenic fragment thereof, and the second promoter promoting expression of the second HBsAg polypeptide, or immunogenic fragment thereof.

16. The use of claim 15 wherein at least one the promoters is selected from the group consisting of a respiratory syncytial virus (RSV) promoter, a cytomegalovirus (CMV) promoter, a PH5 promoter, and an H1 promoter.

17. The use of any of claims 1-16, wherein the expression construct comprises a viral vector.

18. The use of claim 17, wherein the viral vector is selected from the group consisting of an adenovirus vector; a retrovirus vector, a lentiviral vector, a moloney murine leukemia virus vector,an adeno- associated virus vector; a herpes simplex virus vector; a SV 40 vector; a polyoma virus vector; a papilloma virus vector; a picornavirus vector; a pox virus vector, an orthopox virus vector, a vaccinia virus vector, a modified vaccinia virus Ankara (MVA) vector, an avipox vector, a canary pox vector, a fowl pox vector, and an Epstein Barr virus vector.

19. The use of any one of claims 1-18, wherein the nucleic acid-based HBV vaccine is to be administered to the subject at a tissue-culture infectious dose ($TCID_{50}$) of $10^6$ to $10^{10}$ $TCID_{50}$; or $10^6$ to $10^9$ $TCID_{50}$; or $10^6$ to $10^8$ $TCID_{50}$.

20. The use of any of claims 1-19, wherein (a) a dose of the nucleic acid-based HBV vaccine is to be administered to the subject no sooner than two weeks after administration of a final dose of the protein-based vaccine is to be administered to the subject; (b) a first dose of the nucleic acid-based HBV vaccine is to be administered to the subject when the level of HBsAg in the serum of the subject is further decreased to at least 0.5 log 10 IU/ml following administration of at least one dose of the protein-based HBV vaccine; or (c) a single dose of the nucleic acid-based HBV vaccine is to be administered to the subject.

21. The use of any of claims 1-20, further, comprising that a nucleot(s)ide analog is also to be administered to the subject.

22. The use of claim 21, wherein at least one dose of the nucleot(s)ide analog is to be administered to the subject prior to administration of the RNAi agent to the subject; or multiple doses of the nucleot(s)ide analog are to be administered to the subject.

23. The use of claim 21 or 22, wherein the nucleot(s)ide analog is Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide (TAF), Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, AGX-1009, emtricitabine, clevudine, ritonavir, dipivoxil, lobucavir, famvir, FTC, N-Acetyl-Cysteine (NAC), PC1323, theradigm-HBV, thymosin-alpha, and ganciclovir, besifovir (ANA-380/LB-80380), or tenofvir-exaliades (TLX/CMX157).

24. The use of any of claims 1-23, further comprising that an immune stimulator is also to be administered to the subject.

25. The use of claim 24, wherein immune stimulator is pegylated interferon alfa 2a (PEG-IFN-alpha-2a), Interferon alfa-2b, PEG-IFN-alpha-2b, Interferon lambda a recombinant human interleukin-7, and a Toll-like receptor 3, 7, 8 or 9 (TLR3, TLR7, TLR8, TLR9) agonist, a viral entry inhibitor, Myrcludex, an oligonucleotide that inhibits the secretion or release of HBsAg, REP 9AC, a capsid inhibitor, Bay41-4109, NVR-1221, a cccDNA inhibitor, IHVR-25) a viral capsid, an MVA capsid, an immune checkpoint regulator, an CTLA-4 inhibitor, ipilimumab, a PD-1 inhibitor, Nivolumab, Pembrolizumab, BGB-A317 antibody, a PD-L1 inhibitor, atezolizumab, avelumab, durvalumab, or an affimer biotherapeutic.

26. The use of any one of claims 1-25, wherein the subject is human.

27. The use of any of claims 1-26, wherein the RNAi agent inhibits expression of four HBV transcripts or at least three HBV transcripts.

28. The use of any of claims 1-27, wherein the RNAi agent is selected from any one of the iRNA agents in Appendix A.

29. The use of any of claims 1-28, wherein the RNAi agent targets at least 15 contiguous nucleotides of nucleotides 1579-1597, 206-228, 207-229, 210-232, 212-234, 214-236, 215-237, 216-238, 226-248, 245-267, 250-272, 252-274, 253-275, 254-276, 256-278, 258-280, 263-285, 370-392, 373-395, 375-397, 401-423, 405-427, 410-432, 411-433, 422-444, 424-446, 425-447, 426-448, 731-753, 734-756, 1174-1196, 1250-1272, 1255-1277, 1256-1278, 1545-1567, 1547-1569, 1551-1571, 1577-1597, 1580-1598, 1806-1825, 1812-1831, 1814-1836, 1829-1851, 1831-1853, 1857-1879, 1864-1886, 2259-2281, 2298-2320, or 2828-2850 of SEQ ID NO: 1 (NC_003977.1).

30. The use of any of claims 1-29, wherein the RNAi agent targets at least 15 contiguous nucleotides of nucleotides 1579-1597 or 1812-1831 of SEQ ID NO: 1 (NC_003977.1).

31. The use of claim 29, wherein the antisense strand of the RNAi agent comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'-UGUGAAGCGAAGUGCACACUU-3' (SEQ ID NO: 25) or 5'-AGGUGAAAAAGUUGCAUGGUGUU-3' (SEQ ID NO: 26).

32. The use of claim 29, wherein the antisense strand of the RNAi agent comprises the nucleotide sequence of 5'-UGUGAAGCGAAGUGCACACUU-3' (SEQ ID NO: 25) or 5'-AGGUGAAAAAGUUGCAUGGUGUU-3' (SEQ ID NO: 26).

33. The use of any of claims 29-32, wherein the sense strand of the RNAi agent comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'-GUGUGCACUUCGCUUCACA-3' (SEQ ID NO: 27) or 5'-CACCAUGCAACUUUUUCACCU-3' (SEQ ID NO: 28).

34. The use of any of claims 29-32, wherein the sense strand of the RNAi agent comprises the nucleotide sequence of 5'-GUGUGCACUUCGCUUCACA-3' (SEQ ID NO: 27) or 5'-CACCAUGCAACUUUUUCACCU-3' (SEQ ID NO: 28).

35. The use of any of claims 29-30, wherein the antisense strand of the RNAi agent comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'-UGUGAAGCGAAGUGCACACUU-3' (SEQ ID NO: 25) and the sense strand comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'-GUGUGCACUUCGCUUCACA-3' (SEQ ID NO: 27).

36. The use of any of claims 29-30, wherein the antisense strand of the RNAi agent comprises the nucleotide sequence of 5'-UGUGAAGCGAAGUGCACACUU-3' (SEQ ID NO: 25) and the sense strand comprises the nucleotide sequence of 5'-GUGUGCACUUCGCUUCACA-3' (SEQ ID NO: 27).

37. The use of any of claims 29-30, wherein the antisense strand of the RNAi agent comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'-AGGUGAAAAAGUUGCAUGGUGUU-3' (SEQ ID NO: 26) and the sense strand of the RNAi agent comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'-CACCAUGCAACUUUUUCACCU-3' (SEQ ID NO: 28).

38. The use of any of claims 29-30, wherein the antisense strand of the RNAi agent comprises the nucleotide sequence of 5'-AGGUGAAAAAGUUGCAUGGUGUU-3' (SEQ ID NO: 26) and the sense strand of the RNAi agent comprises the nucleotide sequence of 5'-CACCAUGCAACUUUUUCACCU-3' (SEQ ID NO: 28).

39. The use of any of claims 1-38, wherein

(a) the RNAi agent comprises at least one modified nucleotide;

(b) substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, wherein said sense strand is conjugated to a ligand attached at the 3'-terminus;

(c) the RNAi agent comprises a 3' overhang of at least 1 nucleotide or at least 2 nucleotides;

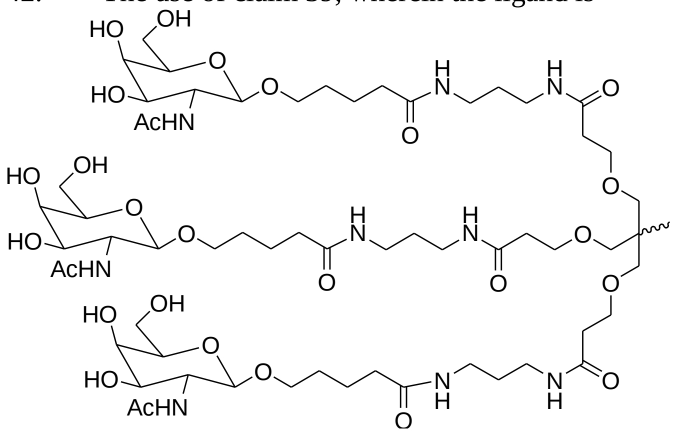
(d) the double-stranded region of the RNAi agent is 15-30, 17-23, 17-25, 23-27, 19-21, or 21-23 nucleotide pairs in length; or

(e) each strand of the RNAi agent has 15-30 or 19-30 nucleotides.

40. The use of claim 39, wherein the ligand is one or more GalNAc derivatives attached through a monovalent linker, bivalent branched linker, or trivalent branched linker.

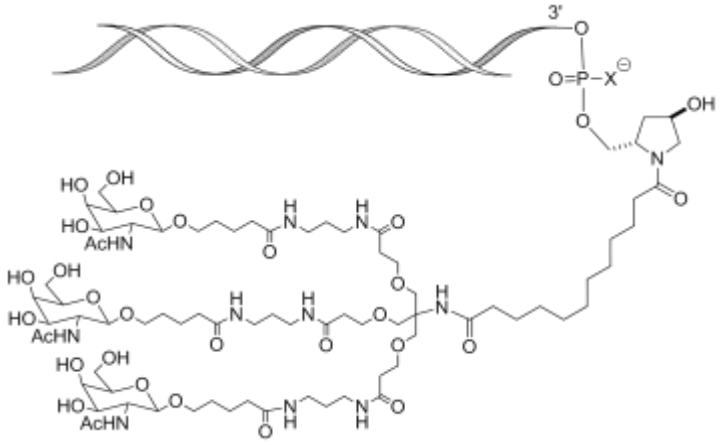
41. The use of claim 40, wherein at least one of said modified nucleotides is a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, or a nucleotide comprising a 5'-phosphate mimic.

42. The use of claim 39, wherein the ligand is




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43. The use of claim 39, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

44. The use of any of claims 1-29 wherein the RNAi agent is AD-66810 or AD-66816.

45. The use of any of claims 1-44, wherein the first or second HBcAg polypeptide, or immunogenic fragment thereof, and/or the first or second HBsAg polypeptide, or immunogenic fragment thereof, comprise at least one determinant present in at least four, at least six, or at least seven genotypes of HBV.

46. A kit for treating a subject having an HBV infection, the kit comprising:

a) an RNAi agent that inhibits expression of at least three HBV transcripts, wherein the RNAi agent comprises a sense strand and an antisense strand forming a double stranded region;

b) a protein-based vaccine comprising a first HBV core antigen (HBcAg) polypeptide, or immunogenic fragment thereof, and a first HBV surface antigen (HBsAg) polypeptide, or immunogenic fragment thereof; and

c) a nucleic acid-based vaccine comprising an expression vector construct encoding a second HBcAg polypeptide, or immunogenic fragment thereof, and/or a second HBsAg polypeptide, or immunogenic fragment thereof,

wherein the second HBcAg polypeptide, or immunogenic fragment thereof, and/or the second HBsAg polypeptide, or immunogenic fragment thereof, shares at least one epitope with at least one of the first HBcAg polypeptide, or immunogenic fragment thereof, and/or the first HBsAg polypeptide, or immunogenic fragment thereof; and

d) instructions for use according to the uses of any one of claims 1-45.