METHODS FOR THE TREATMENT OF SUBJECTS HAVING A HEPATITIS B VIRUS (HBV) INFECTION

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/486,618, filed on April 18, 2017, U.S. Provisional Patent Application No. 62/553,358, filed on September 1, 2017, U.S. Provisional Patent Application No. 62/646,978, filed on March 23, 2018, and U.S. Provisional Patent Application No. 62/655,862, filed on April 11, 2018. The entire contents of each of the foregoing provisional patent applications are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 12, 2018, is named 121301-07220_SL.txt and is 429,841 bytes in size.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) is an enveloped DNA virus that infects the liver that causes hepatocellular necrosis, inflammation, and is a major risk factor for development of cirrhosis and hepatocellular carcinoma (HCC). The World Health Organization (WHO) estimates that there are 240 million chronically HBV infected individuals worldwide largely in low to middle income countries and that about 650,000 people will die annually of complications from HBV infection. Although an effective HBV vaccine is available and efforts to vaccinate infants at birth have been effective in reducing incidence and prevalence of HBV infection, such programs do not have a demonstrable effect on death rates for years (WHO Guidelines for prevention, care and treatment of persons with chronic Hepatitis B Infection, 2015 at apps.who.int/iris/bitstream/10665/154590/1/9789241549059_eng.pdf?ua=1&ua=1).

A number of therapeutic agents have been developed for the treatment of HBV that effectively reduce the disease burden of HBV infection, but they are not typically curative as they do not fully eliminate all replicative forms of the virus including the covalently closed circular DNA (cccDNA) that resides in the hepatocyte nucleus and becomes a template for viral replication and transcription of viral RNAs. Nucleotide and nucleoside analogs, typically considered to be the gold standard for treatment of chronic HBV infection due to their safety and efficacy, are effective in suppressing HBV replication, but they do not eliminate cccDNA, do not prevent expression of viral proteins, must be dosed chronically, and can result in the development of resistance. Further, treatment with nucleot(s)ide inhibitors does not fully mitigate the risk of the development of hepatocellular carcinoma (HCC) which remains significant (about 7% in 5-7 years despite treatment). Interferon-based therapies can result in sero-conversion and cure of about 10-15% of patients allowing discontinuation of treatment, but the agents have severe side effects and must be refrigerated

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 in the manufacture of a first medicament, use of a protein-based HBV vaccine in the manufacture of a second medicament, and use of a nucleic acid-based HBV vaccine in the manufacture of a third medicament, wherein the first, second and third medicaments are to be used for a conmbination therapy 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, and (i) the sense strand comprises the nucleotide sequence of 5'-GUGUGCACUUCGCUUCACA-3' (SEQ ID NO: 27) and the antisense strand comprises the nucleotide sequence of 5'-UGUGAAGCGAAGUGCACACUU-3' (SEQ ID NO: 25), or (ii) the sense strand comprises the nucleotide sequence of 5'-CACCAUGCAACUUUUUCACCU-3' (SEQ ID NO: 28) and the antisense strand comprises the nucleotide sequence of 5'-AGGUGAAAAAGUUGCAUGGUGUU-3' (SEQ ID NO: 26); 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 is to be administered to a subject having an HBV infection prior to the protein-based HBV vaccine that is to be administered to the subject, and and the nucleic acid-based HBV vaccine that is to be administered to the subject.

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).

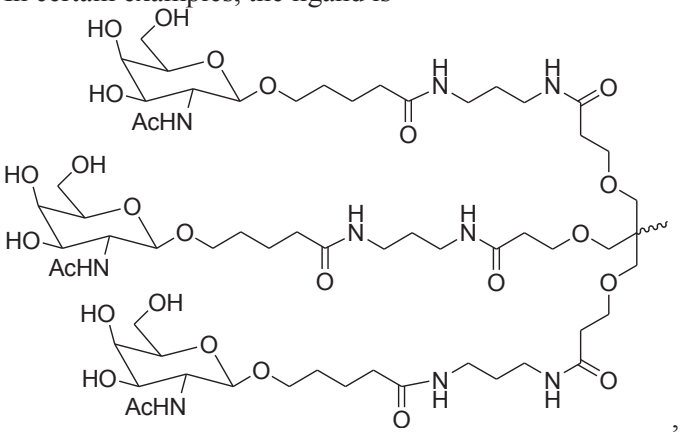
In certain examples of the regimen, substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, wherein said sense strand is conjugated to a ligand attached at the 3'-terminus. In certain examples, the ligand is 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.

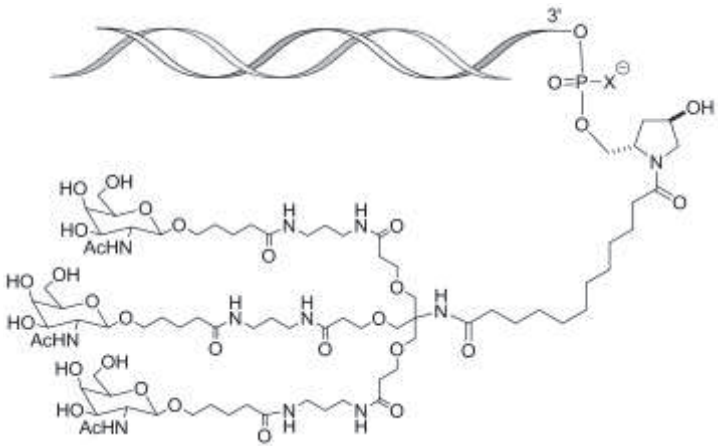
In certain examples, each strand of the RNAi agent has 19-30 nucleotides.

In certain examples, the ligand is



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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.

Figure 7 is a schematic showing a dosing regimen for the experiment assaying the effect of pretreatment of HBV1.3-xfs mice with control siRNA or AD-66816 prior to administration of a therapeutic vaccine against HBV.

Figures 8A-8C are graphs showing the level of (8A) HBsAg, (8B) HBeAg, and (8C) HBV-DNA in serum of HBV1.3-xfs mice after treatment based on the dosing regimen provided in Figure 7.

Figures 9A-9D are graphs showing T cell immune response in the liver against peptides (9A) HBs(S208), (9B) HBc(C93), (9C) HBc(Cpool) and (9D) MVA(B8R) in HBV1.3-xfs mice after treatment based on the dosing regimen provided in Figure 7.

Figures 10A-10C show the level of HBV RNA and protein levels in liver cells of HBV1.3-xfs mice after treatment based on the dosing regimen provided in Figure 7. Figure 10A shows total HBV RNA. Figure 10B shows HBV 3.5 kb transcript relative to GAPDH as determined by RT-qPCR. Figure 10C shows the number of HBcAg positive cells per mm^2 of liver section detected by immunohistochemical staining.

Figures 11A and 11B are graphs showing the level of (11A) HBsAg and (11B) HBeAg in serum of individual HBV1.3-xfs mice at week 7 after the first vaccine dose after the 6 week regimen in the dosing regimen provided in Figure 7.

Figures 11C and 11D are graphs showing (11C) anti-HBs antibody response and (11D) T cell immune response in the liver against HBs(S208) of individual HBV1.3-xfs mice at week 7 after the first vaccine dose after the 6 week regimen in the dosing regimen provided in Figure 7.

Figure 12 is a schematic showing a dosing regimen for the experiment assaying the effect of pretreatment of mice infected with an AAV vector encoding HBV with control siRNA or AD-66816 prior to administration of a therapeutic vaccine against HBV.

Figures 13A-13G are based on the dosing regimen provided in Figure 12. Figures 13A and 13B are graphs showing the level of (13A) HBsAg and (13B) HBeAg in serum of HBV-AAV mice (inset 13C is an exploded portion of the later time points in graph 13B). Figure 13D shows the serum HBV DNA level at week 22. Figures 13D and 13E show the number of copies per liver cell of (13D) total HBV DNA and (13E) AAV-DNA at week 22. Figures 13F and 13G show the relative expression in liver of (13F) HBV 3.5 RNA relative to GAPDH RNA and (13G) total HBV RNA relative to GAPDH RNA.

Figures 14A-14C are graphs showing (14A) anti-HBs antibody response throughout the course of the experiment and (14B) anti-HBe antibody response at day 116 of the experiment based on the dosing regimen provided in Figure 12. Figure 14C is an exploded portion of the later time points in graph 14B.

Figures 15A and 15B show (15A) ALT levels and (15B) body weights of the animals throughout the experiment based on the dosing regimen provided in Figure 12.

A formal sequence listing is provided herewith that forms part of the specification.

An Appendix A providing exemplary target sequences for siRNA targeting and iRNA agents is provided herewith and forms part of the specification.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides treatment regimens and methods of use of iRNA agents which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of one or more HBV genes (open reading frames/ transcripts) and hepatitis B vaccines to stimulate an immune response against one or more HBV proteins in the treatment of HBV infection. The treatment regimens and methods preferably provide a functional cure within a defined period of time.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an HBV gene as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from inhibition or reduction of the expression of an HBV gene.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The term “about” is used herein to mean within the typical ranges of tolerances in the art. For example, “about” can be understood as within 2 standard deviations from the mean. In certain embodiments, “about” means +/-10%. In certain embodiments, “about” means +/-5%. When about is present before a series of numbers or a range, it is understood that “about” can modify each of the numbers in the series or range.

The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term “at least”, and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, “at least 18 nucleotides of a 21 nucleotide nucleic acid molecule” means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a

series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range. As used herein, ranges include both the upper and lower limit.

In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

Various embodiments of the invention can be combined as determined appropriate by one of skill in the art.

As used herein, "Hepatitis B virus," used interchangeably with the term "HBV" refers to the well-known non-cytopathic, liver-tropic DNA virus belonging to the Hepadnaviridae family.

The HBV genome is partially double-stranded, circular DNA with overlapping reading frames (see, *e.g.*, Figure 1).

There are four transcripts (that may be referred to herein as "genes" or "open reading frames") based on size, encoded by the HBV genome. These contain open reading frames called C, X, P, and S. The core protein is coded for by gene C (HBcAg). Hepatitis B e antigen (HBeAg) is produced by proteolytic processing of the pre-core (pre-C) protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigens (HBsAg). The HBsAg gene is one long open reading frame which contains three in frame "start" (ATG) codons resulting in polypeptides of three different sizes called large, middle, and small S antigens, pre-S1 + pre-S2 + S, pre-S2 + S, or S. Surface antigens in addition to decorating the envelope of HBV, are also part of subviral particles, which are produced at large excess as compared to virion particles, and play a role in immune tolerance and in sequestering anti-HBsAg antibodies, thereby allowing for infectious particles to escape immune detection. The function of the non-structural protein coded for by gene X is not fully understood, but it plays a role in transcriptional transactivation and replication and is associated with the development of liver cancer. Exemplary protein sequences are provided in the attached sequence listing (see, *e.g.,* SEQ ID NOs:1, 3, 16, and 20).

HBV is one of the few DNA viruses that utilize reverse transcriptase in the replication process which involves multiple stages including entry, uncoating, and transport of the virus genome to the nucleus. Initially, replication of the HBV genome involves the generation of an RNA intermediate that is then reverse transcribed to produce the DNA viral genome.

Upon infection of a cell with HBV, the viral genomic relaxed circular DNA (rcDNA) is transported into the cell nucleus and converted into episomal covalently closed circular DNA (cccDNA), which serves as the transcription template for the viral mRNAs. After transcription and nuclear export, cytoplasmic viral pregenomic RNA (pgRNA) is assembled with HBV polymerase and

capsid proteins to form the nucleocapsid, inside which polymerase-catalyzed reverse transcription yields minus-strand DNA, which is subsequently copied into plus-strand DNA to form the progeny rcDNA genome. The mature nucleocapsids are then either packaged with viral envelope proteins to egress as virion particles or shuttled to the nucleus to amplify the cccDNA reservoir through the intracellular cccDNA amplification pathway. cccDNA is an essential component of the HBV replication cycle and is responsible for the establishment of infection and viral persistence.

HBV infection results in the production of two different particles: 1) the infectious HBV virus itself (or Dane particle) which includes a viral capsid assembled from the HBcAg and is covered by an envelope consisting of a lipid membrane with HBV surface antigens, and 2) subviral particles (or SVPs) which contain the small and medium forms of the hepatitis B surface antigen HBsAg which are non-infectious. For each viral particle produced, over 10,000 SVPs are released into the blood. As such, SVPs (and the HBsAg protein they carry) represent the overwhelming majority of viral protein in the blood. HBV infected cells also secrete a soluble proteolytic product of the pre-core protein called the HBV e-antigen (HBeAg).

Eight genotypes of HBV, designated A to H, have been determined, and two additional genotypes I and J have been proposed, each having a distinct geographical distribution. The virus is non-cytopathic, with virus-specific cellular immunity being the main determinant for the outcome of exposure to HBV- acute infection with resolution of liver diseases with 6 months, or chronic HBV infection that is frequently associated with progressive liver injury.

The term "HBV" includes any of the genotypes of HBV (A to J). The complete coding sequence of the reference sequence of the HBV genome may be found in for example, GenBank Accession Nos. GI:21326584 (SEQ ID NO:1) and GI:3582357 (SEQ ID NO:3). Antisense sequences are provided in SEQ ID NO: 2 and 4, respectively. Amino acid sequences for the C, X, P, and S proteins can be found, for example at NCBI Accession numbers YP_009173857.1 (C protein); YP_009173867.1 and BAA32912.1 (X protein); YP_009173866.1 and BAA32913.1 (P protein); and YP_009173869.1, YP_009173870.1, YP_009173871.1, and BAA32914.1 (S protein) (SEQ ID NOs: 5-13) . Protein and DNA sequences from HBV genotype D, strain ayw are provided in SEQ ID NOs.: 14-17. Protein and DNA sequences from HBV genotype A, strain adw are provided in SEQ ID NOs.: 18-21.

Additional examples of HBV mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM. The International Repository for Hepatitis B Virus Strain Data can be accessed at http://www.hpa-bioinformatics.org.uk/HepSEQ/main.php.

The term "HBV," as used herein, also refers to naturally occurring DNA sequence variations of the HBV genome, *i.e.*, genotypes A-J and variants thereof.

As used herein, "epitope" also referred to as "an antigenic determinant," or "determinant," is understood as the part of a protein antigen that is recognized by the immune system, specifically by antibodies, B cells, and/or T cells. Epitopes include conformational epitopes and linear epitopes. Proteins share an epitope when they share an amino acid sequence of sufficient length or size and

antigenicity to be recognized by an antibody, B cell, and/or T cell. T cell epitopes presented by MHC class I molecules are typically peptides about 8 to 11 amino acids in length, whereas MHC class II molecules present longer peptides about 13 to 17 amino acids in length. Conformational epitopes are typically discontinuous and span a longer amino acid sequence.

As used herein, the term "nucelot(s)ide analog" or "reverse transcriptase inhibitor" is an inhibitor of DNA replication that is structurally similar to a nucleotide or nucleoside and specifically inhibits replication of the HBV cccDNA and does not significantly inhibit the replication of the host (*e.g.*, human) DNA. Such inhibitors include 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, ganciclovir, besifovir (ANA-380/LB-80380), and tenofvir-exaliades (TLX/CMX157). In certain embodiments, the nucelot(s)ide analog is Entecavir (ETV). Nucleot(s)ide analogs are commercially available from a number of sources and are used in the methods provided herein according to their label indication (*e.g.*, typically orally administered at a specific dose) or as determined by a skilled practitioner in the treatment of HBV.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an HBV transcript, including mRNA that is a product of RNA processing of a primary transcription product. In one embodment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an HBV transcript. In one embodiment, the target sequence is within the protein coding region of an HBV transcript.

The target sequence may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing

properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

As used herein, an "RNAi agent", an "iRNA agent", an "siRNA agent", and the like, is a double stranded RNA that preferably target regions in the HBV genome that are conserved across all serotypes of HBV and are preferably designed to inhibit all steps of the HBV life cycle, *e.g.*, replication, assembly, secretion of virus, and secretion of viral antigens, by inhibiting expression of more than one HBV transcript. In particular, since transcription of the HBV genome results in polycistronic, overlapping RNAs, an RNAi agent for use in the invention targeting a single HBV transcript preferably results in significant inhibition of expression of most or all HBV transcripts. All HBV transcripts are at least partly overlapping and share the same polyadenylation signal. Therefore, all viral transcripts have an identical 3´end and, thus, an RNAi agent of the invention targeting the X gene will target all viral transcripts and should result in inhibition of not only X gene expression but also the expression of all other viral transcripts, including pregenomic RNA (pgRNA). Furthermore, the RNAi agents of the invention have been designed to inhibit HBV viral replication by targeting pgRNA, HBV structural genes, polymerase, and the HBV X gene. In addition, they have been designed to mediate the silencing of SVP and other viral protiens that play a role in immune tolerance, thereby permitting a subject's immune system to detect and respond to the presence of viral antigens such that an immune response to control and to clear an HBV infection is mounted. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites and/or the specific modifications in these RNAi agents confer to the RNAi agents of the invention improved efficacy, stability, safety, potency, and durability. Such agents are provided, for example, in PCT Publication Nos. WO 2016/077321, WO 2012/024170, WO 2017/027350, and WO 2013/003520, the entire contents of each of which is incorporated herein by reference. Exemplary target sites for RNAi agents and exemplary RNAi agents are provided in Appendix A, filed herewith, which forms a part of the specification. The term RNAi agents further includes shRNAs, *e.g.*, adeno-associated virus (AAV) 8 vectors for delivery of an shRNA in an artificial mi(cro)RNA under a liver-specific promoter; optionally co-delivered a decoy ("TuD") directed against the shRNA sense strand to curb off-target gene regulation are provided in Michler *et al.*, 2016 (*EMBO Mol. Med.*, 8:1082-1098, incorporated herein by reference).

The majority of nucleotides of each strand of an iRNA agent may be ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an

"RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides.

As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, and/or modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.*, a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

As used herein, a "therapeutic HBV vaccine," and the like, can be a peptide vaccine, a DNA vaccine including a vector-based vaccine, or a cell-based vaccine that induces an immune response, preferably an effector T cell induced response, against one or more HBV proteins. Preferably the vaccine is a multi-epitope vaccine that is cross-reactive with multiple HBV serotypes, preferably all HBV serotypes. A number of therapeutic HBV vaccines are known in the art and are at various stages of pre-clinical and clinical development. Protein based vaccines include hepatitis B surface antigen (HBsAg) and core antigen (HBcAg) vaccines (*e.g.*, Li *et al.*, 2015, Vaccine. 33:4247-4254, incorporated herein by reference). Exemplary DNA vaccines include HB-110 (Genexine, Kim *et al.*, 2008. Exp Mol Med. 40: 669–676.), pDPSC18 (PowderMed), INO-1800 (Inovio Pharmaceuticals), HB02 VAC-AND (ANRS), and CVI-HBV-002 (CHA Vaccine Institute Co., Ltd.). Exemplary protein based vaccines include Theravax/DV-601 (Dynavax Technologies Corp.), εPA-44 (Chongqing Jiachen Biotechnology Ltd.), and ABX 203 (ABIVAX S.A.). Exemplary cell based vaccines include HPDCs-T immune therapy (Sun Yat-Sen University). Combination vaccines and products are also known and include HepTcell™ (FP-02.2 vaccine (peptide) + IC31® Adjuvant (a combination peptide-oligonucleotide adjuvant),(see U.S. Patent Publication Nos. 2013/0330382, 2012/0276138, and 2015/0216967, the entire contents of each of which is incorporated herein by reference)); GS-4774 (Gilead, a fusion protein S. core X vaccine + Tarmogen T cell immune stimulator), pSG2.HBs/MVA.HBs (protein prime/viral vector boost, Oxxon Therapeutics), and a protein-prime/modified vaccinia virus Ankara vector-boost (HBsAg and HBsAg protein + HBcAg and HBsAg in MVA expression vector, Backes *et al.*, 2016, Vaccine. 34:923-32, and WO2017121791, both of which are incorporated herein by reference).

As used herein, the term "adjuvant" is understood to be an agent that promotes (*e.g.*, enhances, accelerates, or prolongs) an immune response to an antigen with which it is administered to elicit long-term protective immunity. No substantial immune response is directed at the adjuvant itself. Adjuvants include, but are not limited to, pathogen components, particulate adjuvants, and combination adjuvants (see, *e.g.*, www.niaid.nih.gov/research/vaccine-adjuvants-types). Pathogen components (*e.g.*, monophosphoryl lipid A (MPL), poly(I:C), polyICLC adjuvant, CpG DNA, c-di-AMP, c-di-GMP, c-di-CMP; short, blunt-ended 5′-triphosphate dsRNA (3pRNA) RIG-1 ligand, and emulsions such as poly[di(sodiumcarboxylatoethylphenoxy)phosphazene] (PCEP)) can help trigger

early non-specific, or innate, immune responses to vaccines by targeting various receptors inside or on the surface of innate immune cells. The innate immune system influences adaptive immune responses, which provide long-lasting protection against the pathogen that the vaccine targets. Particulate adjuvants (*e.g.*, alum, virosomes, cytokines, *e.g.*, IL-12) form very small particles that can stimulate the immune system and also may enhance delivery of antigen to immune cells. Combination adjuvants (*e.g.*, AS02, AS03, and AS04 (all GSK); MF59 (Novartis); ISCOMATRIX® (CSL Limited); and IC31® (Altimmune) elicit multiple protective immune responses. Adjuvants that have a modest effect when used alone may induce a more potent immune response when used together.

In preferred embodiments of the invention, adjuvants for use in the invention promote a humoral as well as a cellular immune response. For this, a balanced Th1/Th2 helper T cell response is desired to support neutralizing antibody responses as well as effector cytotoxic T cell responses. Preferably the adjuvant provides a balanced Th1/Th2 response. In certain embodiments, the adjuvant is one or more of a polyI:C adjuvant, a polyICLC adjuvant, a CpG adjuvant, a STING agonist (a c-di-AMP adjuvant, a c-di-GMP adjuvant, or a c-di-CMP adjuvant), an ISCOMATRIX® adjuvant, a PCEP adjuvant, and a Rig-I-ligand adjuvant. In certain embodiments, the adjuvant is a polyI:C adjuvant, a CpG adjuvant, a STING agonist, or a PCEP adjuvant. In certain embodiments, the adjuvant is not alum.

As used herein, an "immune stimulator" is an agent that stimulates an immune response that may or may not be administered independently of an antigen. Immune stimulators include, but are not limited to, 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 (*e.g.*, Myrcludex), an oligonucleotide that inhibits the secretion or release of HBsAg (*e.g.*, REP 9AC), a capsid inhibitor (*e.g.*, Bay41-4109 and NVR-1221), a cccDNA inhibitor (*e.g.*, IHVR-25). In certain embodiments, an immune stimulator can include a viral capsid, optionally an empty viral capsid, *e.g.*, MVA capsid.

Immune stimulators can also include immune checkpoint regulators. Immune checkpoint regulators can be stimulatory or inhibitory. As used herein, immune checkpoint regulators potentiate an immune response. Immune checkpoint regulators include, but are not limited to, CTLA-4 inhibitors, such as ipilimumab, PD-1 inhibitors, such as Nivolumab, Pembrolizumab, and the BGB-A317 antibody. PD-L1 inhibitors include atezolizumab, avelumab, and durvalumab, in addition to an affimer biotherapeutic.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (*e.g.*, a mouse model or other animal model that can be infected with HBV). In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder, or condition that would benefit from reduction in HBV gene expression or replication. In certain embodiments, the subject has a chronic hepatitis B virus (HBV) infection. In certain embodiments, the subject has both a chronic hepatitis B virus (HBV) infection and a hepatitis D virus (HDV) infection.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation of one or more signs or symptoms in a subject with HBV infection including, but not limited to, the presence of serum HBV DNA or liver HBV ccc DNA, the presence of serum or liver HBV antigen, *e.g.*, HBsAg or HBeAg. Diagnostic criteria for HBV infection are well known in the art. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment, or lower risk of HCC development.

In certain embodiments, an HBV-associated disease is chronic hepatitis B (CHB). In certain embodiments, subjects have been infected with HBV for at least five years. In certain embodiments, subjects have been infected with HBV for at least ten years. In certain embodiments, subjects became infected with HBV at birth. Subjects having chronic hepatitis B disease are immune tolerant, have an active chronic infection accompanied by necroinflammatory liver disease, have increased hepatocyte turn-over in the absence of detectable necroinflammation, or have an inactive chronic infection without any evidence of active disease, and they are also asymptomatic. Subjects having chronic hepatitis B disease are HBsAg positive and have either high viremia ($\geq 10^4$ HBV-DNA copies / ml blood) or low viremia ($<10^3$ HBV-DNA copies / ml blood). Patients with chronic active hepatitis, especially during the high replicative state, may have symptoms similar to those of acute hepatitis. The persistence of HBV infection in CHB subjects is the result of ccc HBV DNA persistence. In certain embodiments, a subject having CHB is HBeAg positive. In other embodiments, a subject having CHB is HBeAg negative.

In preferred embodiments, treatment of HBV infection results in a "functional cure" of hepatitis B. As used herein, the term "functional cure" is understood to be clearance of circulating HBsAg and is preferably accompanied by conversion to a status in which HBsAg antibodies become undetectable using a clinically relevant assay. For example, undetectable antibodies can include a signal lower than 10 mIU/ml as measured by Chemiluminescent Microparticle Immunoassay (CMIA) or any other immunoassay, and may be called anti-HBs seroconversion. Functional cure does not require clearance of all replicative forms of HBV (*e.g.*, cccDNA from the liver). Anti-HBs seroconversion occurs spontaneously in about 0.2-1% of chronically infected patients per year. However, even after anti-HBs seroconversion, low level persistence of HBV is observed for decades indicating that a functional rather than a complete cure occurs. Without being bound to mechanism, it is proposed that the immune system is able to keep HBV in check. A functional cure permits discontinuation of any treatment for the HBV infection. However, it is understood that a "functional cure" for HBV infection may not be sufficient to prevent or treat diseases or conditions that result from HBV infection, *e.g.*, liver fibrosis, HCC, cirrhosis.

The term "lower" in the context of the level of HBV gene expression or HBV replication in a subject, or a disease marker or symptom, refers to a statistically significant decrease in such level. The decrease can be, for example, at least 70%, 75%, 80%, 85%, 90%, 95%, or more. In monitoring of HBV infection, a log10 scale is typically used to describe the level of antigenemia (*e.g.*, HBsAg level in serum) or viremia (HBV DNA level in serum). It is understood that a 1 log10 decrease is a

90% decrease (10% remaining), a 2 log10 decrease is a 99% decrease (1% remaining), *etc*. In certain embodiments, a disease marker is lowered to below the level of detection.

In certain embodiments, the expression of a disease marker is normalized, *i.e.*, decreased to a level accepted as within the range of normal for an individual without such disorder, *e.g.*, the level of a disease marker, such as, ALT or AST, is decreased to a level accepted as within the range of normal for an individual without such disorder. When the disease associated level is elevated from the normal level, the change is calculated from the upper level of normal (ULN). When the disease associated level is decreased from the normal level, the change is calculated from the lower level of normal (LLN). The lowering is the percent difference in the change between the subject value and the normal value. For example, a normal AST level can be reported as 10 to 40 units per liter. If, prior to treatment, a subject has an AST level of 200 units per liter (*i.e.*, 5 times the ULN, 160 units per liter above the upper level of normal) and, after treatment, the subject has an AST level of 120 units per liter (*i.e.*, 3 times the ULN, 80 units per liter above the upper level of normal), the elevated AST would be lowered towards normal by 50% (80/160).

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition. Preferably inhibiting includes a statistically significant or clinically significant inhibition.

The phrase "inhibiting expression of an HBV gene" is intended to refer to knockdown of any HBV transcript (*e.g.*, 3.5 kb, 2.4 kb, 2.1 kb, or 0.7 kb transcript) encoding one or more HBV viral proteins (such as, *e.g.*, preS1/2-S, preS, S, P, X, preC, and C), as well as variants or mutants of an HBV gene.

"Inhibiting expression of an HBV gene" includes any significant level of inhibition of an HBV gene or transcript, *e.g.*, at least partial suppression of the expression of an HBV gene S, P, X, or C, or any combination thereof, *e.g.*, S, P, and C. The expression of the HBV gene may be assessed based on the level, or the change in the level, of any variable associated with HBV gene expression, *e.g.*, an HBV mRNA level, an HBV protein level, and/or an HBV cccDNA level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject, *e.g.*, levels may be monitored in serum.

In some embodiments of the methods of the invention, expression of an HBV gene is inhibited by at least 70%, 75%, 80%, 85%, 90%, 95%, or to below the level of detection of the assay. In preferred embodiments, the inhibition of expression of an HBV gene results in a clinically relevant inhibition of the level of gene expression, *e.g.*, sufficiently inhibited to permit an effective immune response to a vaccine against an HBV protein.

Inhibition of the expression of an HBV gene may be manifested by a reduction of the amount of RNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which an HBV gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an RNAi agent of the invention, or by administering an RNAi agent of the invention to a subject in which the cells are or were present) such that the expression of

an HBV gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by the rtPCR method provided in Example 2 of PCT Publication No. WO 2016/077321 (the entire contents of which are incorporated herein by reference), with *in vitro* assays being performed in an appropriately matched cell line with the duplex at a 10 nM concentration, and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(\text{RNA in control cells}) - (\text{RNA in treated cells})}{\text{RNA in control cells}} \bullet 100\%$$

Alternatively, inhibition of the expression of an HBV gene may be assessed in terms of a reduction of a parameter that is functionally linked to HBV gene expression, *e.g.,* as described herein. HBV gene silencing may be determined in any cell expressing an HBV gene, either constitutively or by genomic engineering, and by any assay known in the art.

Inhibition of the expression of an HBV protein may be manifested by a reduction in the level of an HBV protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells or in serum.

A control cell or group of cells that may be used to assess the inhibition of the expression of an HBV gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent. In alternative embodiments, the level may be compared to an appropriate control sample, *e.g.*, a known population control sample.

The level of HBV RNA that is expressed by a cell or group of cells, or the level of circulating HBV RNA, may be determined using any method known in the art for assessing mRNA expression, preferably using the rtPCR method provided in Example 2 of PCT Publication No. WO 2016/077321,or Example 1 provided herein. In one embodiment, the level of expression of an HBV gene (*e.g.*, total HBV RNA, an HBV transcript, *e.g.*, HBV 3.5 kb transcript) in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* RNA of the HBV gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton *et al.*, *Nuc. Acids Res.* 12:7035), northern blotting, *in situ* hybridization, and microarray analysis. Circulating HBV mRNA may be detected using methods the described in PCT Publication No. WO 2012/177906, the entire contents of which are hereby incorporated herein by reference.

In one embodiment, the level of expression of an HBV gene is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific HBV gene. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated RNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses, and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to an HBV mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of an HBV mRNA.

An alternative method for determining the level of expression of an HBV gene in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of, for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, US Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, US Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of an HBV gene is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System), *e.g.*, using the method provided herein.

The expression levels of an HBV RNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads, or fibers (or any solid support comprising bound nucleic acids). See U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195, and 5,445,934, the entire contents of each of which are incorporated herein by reference. The determination of HBV expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of RNA expression is assessed using real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

The level of HBV protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipiting reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in a symptom of an HBV infection. Symptoms may be assessed using any method known in the art.

As used herein, the term "Hepatitis B virus-associated disease" or "HBV-associated disease," is a disease or disorder that is caused by, or associated with HBV infection or replication. The term "HBV-associated disease" includes a disease, disorder, or condition that would benefit from reduction in HBV gene expression or replication. Non-limiting examples of HBV-associated diseases include, for example, hepatitis D virus infection,; hepatitis delta; chronic hepatitis B; liver fibrosis; end-stage liver disease; cryoglobulinemia; hepatocellular carcinoma.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject or prepared therefrom, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum, plasma, immune cells, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes, resident liver immune cells). In preferred embodiments, a "sample derived from a subject" refers to blood drawn from the subject or plasma, serum, or selected cell pools derived therefrom (*e.g.*, populations of immune cells). In further embodiments, a "sample derived from a subject" refers to liver tissue (or subcomponents thereof) obtained from the subject.

As used herein, "coding sequence" is understood to refer to a DNA sequence that encodes for a specific amino acid sequence. In certain embodiments, the DNA sequence can be reverse transcribed from an RNA sequence. In certain embodiments, an iRNA, *e.g.*, an shRNA, targets a coding sequence.

The terms, "suitable regulatory sequences," and the like, are used herein is to refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" as used herein refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter

sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. A promoter may be selected to promote expression of a coding sequence in a particular cell type or at different stages of development, or in response to different environmental conditions. In certain embodiments, the promoter is a promoter that is active in liver, *e.g.*, a liver-specific promoter. Many promoter sequences are known in the art and selection of an appropriate promoter sequence for a specific context is within the ability of those of skill in the art.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (*i.e.*, the coding sequence is under the transcriptional control of the promoter).

The term "expression" as used herein refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA, or an RNAi agent (*e.g.*, an shRNA) derived from the nucleic acid fragment of the subject technology. "Over-expression" refers to the production of a gene product in transgenic or recombinant organisms that exceeds levels of production in normal or non-transformed organisms.

"Expression vector" or "expression construct," as used herein, refers to a nucleic acid in which a coding sequence is operably linked to a promoter sequence to permit expression of the coding sequence under the control of the promoter. Expression vectors include, but are not limited to, viral vectors or plasmid vectors. Methods for delivery of expression vectors into cells are known in the art.

II. Treatment Methods of the Invention

The present invention provides treatment regimens and methods for the sequential use of an agent to reduce the expression of an HBV gene, *e.g.*, iRNA agents which effect the RNA-induced silencing complex (RISC)-mediated cleavage of one or more HBV transcripts, and hepatitis B vaccines to stimulate an immune response against one or more HBV proteins in the treatment of HBV infection. The treatment regimens and methods preferably provide a functional cure of HBV within a defined period of time.

The treatment regimens and methods provided herein include the ordered administration of therapeutic agents to provide treatment, and preferably a functional cure, for HBV infection. The agents used in the methods are known in the art. However, the agents alone fail to consistently and durably decrease HBV disease burden, *e.g.*, reducing HBsAg levels to below 2 log10, preferably 1 log10 IU/ml or to below the level of detection, in most subjects. Significantly reducing HBV disease burden, *e.g.*, reducing HBsAg levels to below 2 log10, preferably 1 log10 IU/ml or to below the level of detection, will provide the opportunity of discontinuation of administration of therapeutic agents and provide a functional cure for HBV in a substantial number of subjects. Without being bound by mechanism, it is proposed that the treatment regimens and methods provided herein, including administration of an iRNA agent targeted to HBV, substantially reduces HBV antigens and nucleic

acid for a sufficient magnitude and duration in a subject to allow an effective immune response induced by administration of multiple doses of a therapeutic vaccine. The regimens and methods, provided herein, consistently provide a substantial reduction of disease burden and a functional cure in a significant number of subjects, preferably at least 30%, 40%, 50%, 60%, or 70% of subjects.

A transgenic mouse model of HBV infection, HBV1.3 xfs was used to assess the combination therapy provided herein. Primary studies demonstrated the efficacy of two different chemically modified GalNAc- iRNA agents targeted to HBV (AD-66816 and AD-66810) to inhibit the level of HBsAg and HBeAg proteins, and HBV DNA in serum for at least 21 days with a single subcutaneous dose at 3 mg/kg or 9 mg/kg, with similar efficacy. No significant knockdown was observed with a non-HBV iRNA control (see Figure 2). Based on this result, the lower dose of 3 mg/kg was selected for combination therapy studies.

In the first combination therapy trial (see Figure 3), mice were pretreated with one of six treatment regimens (n= 6 per group):

(1) No pretreatment;

(2) Entecavir at 1 μg/ml in water throughout the course of the study beginning on the first day of Week 0;

(3) A 3 mg/kg dose on the first day of Weeks 0, 4, 8, and 12 of the control iRNA agent.

(4) A single dose on the first day of Week 0 with an expression vector encoding an shRNA targeted to HBV (HBV-shRNA) (Michler *et al.*, 2016); or

(5-6) A 3 mg/kg dose on the first day of Weeks 0, 4, 8, and 12 of AD-66816 or AD-66810 (generically, HBV-siRNA).

On the first day of Weeks 12 and 14, a mixture of recombinantly expressed yeast HBsAg (15 μg) and *E. coli* expressed HBcAg (15 μg) adjuvanted with 31.9 μg synthetic phosphorothioated CpGODN 1668 (CpG) and 25 μg poly[di(sodiumcarboxylatoethyl-phenoxy)phosphazene] (PCEP) was subcutaneously administered to all mice as a protein prime vaccination (Backes, 2016).

On the first day of week 16, a mixture of modified vaccinia virus Ankara expressing HBsAg or HBcAg (5×10^7 particles of each virus) was subcutaneously administered to all mice as a boost vaccination (Backes, 2016).

Blood samples were obtained on the first days of Week 0, 2, 4, 8, 12, 16, and 17 and were assayed for levels of HBsAg, HBeAg, and HBV DNA. Results observed for HBsAg and HBeAg levels mice in groups 1, 2, and 3 (mock, Entecavir, control iRNA agent) were similar (Figure 4A and 4B). The HBV-shRNA or HBV-siRNAs alone caused a significant decrease in HBsAg, HBeAg, and HBV DNA in serum. The three dose prime-boost vaccination scheme resulted in a further decrease in HBsAg in all groups, and reduced the level of HBsAg in at least some animals in the HBV-shRNA and HBV-siRNA groups to below the level of detection. However, vaccine treatment did not decrease HBeAg levels in any of the groups. HBV DNA levels were decreased to about the lower limit of quantitation with Entecavir alone so no effect of the three dose prime-boost vaccine could be detected (Figure 4C). Mock treatment and treatment with the HBV-shRNA, the HBV-siRNAs, and control

siRNA all decreased HBV DNA levels, and the level of HBV-DNA was further decreased by the prime-boost vaccine in all groups. These data demonstrate that RNAi is superior to nucleot(s)ide analog therapy in reducing viral antigens. Also, the combination of RNAi and subsequent vaccination have a greater effect on HBsAg and HBV DNA levels than either agent alone.

On the final day of the experiment (the first day of week 17), mice were sacrificed and their livers were harvested. Intrahepatic CD8+ T cell responses were assessed for response to HBsAg, HBcAg, and the MVA virus particle using the method provided in Backes, 2016. Mice treated with the HBV-shRNA or the HBV-siRNAs were able to generate a CD8+ immune response against the HBsAg and HBcAg (Figure 5A and 5B). No significant immune response against the HBV antigens was observed in the mock, Entecavir, or control siRNA groups. However, a similar immune response against the MVA virus was observed in all animals independent of pretreatment or viral antigen levels (Figure 5C) showing that vaccination had worked equally well in all animals, demonstrating the presence of a competent immune system. The data shown in Figure 5A-C were reanalyzed to accomodated for an insufficient exclusion of dead immune cells during the first analysis. The data obtained from the second analysis (shown in Figure 5 D-F) again shows that only the mice pretreated with the HBV-shRNA or the HBV-siRNAs were able to generate HBV-specific CD8+ T cell responses after therapeutic vaccination, but that MVA-specific responses were not influenced by the pretreatment. The reduced variances in the second analysis are attributed to the more rigorous exclussion of dead cells. These data demonstrate that RNAi treatment, in contrast to the current standard of care treatment with a nucleoside analog, can restore HBV-specific T cell immunity and enable the induction of HBV-specific CD8 T cell responses after therapeutic vaccination.

Serum was also assessed for antibody immune response to HBV antigens. Although the vaccine was able to induce a T-cell immune response only in animals which had received HBV-siRNA or HBV-shRNA pretreatment, antibody responses against HBsAg and HBcAg were similar across all groups regardless of pretreatment at the time points evaluated until week 17. No HBeAg antibody responses could be detected. These data demonstrate that high HBV antigen loads preferentially inhibit HBV-specific T cell, not B cell responses.

Livers were also assessed for the presence of HBV transcripts by RT-qPCR and normalized to the liver GAPDH transcript. Mice treated with the HBV-shRNA or the HBV-siRNAs demonstrated a significant decrease in HBV transcript levels (Figure 6A and 6B). No significant difference was observed between the mock and Entecavir or control siRNA groups. Liver sections were analysed by immunohistochemical stainings for expression of core antigen to assess the antiviral effect in the liver (Figure 6C). In animals that were not pretreated before vaccination, there were, on average, 83 hepatocytes per mm^2 with cytoplasmic expression of HBc. This number was not significantly changed in animals that were pretreated with Entecavir or the control siRNA. However, the number of cytoplamic HBcAg positive cells was significantly reduced in AAV-shRNA or HBV siRNA pretreated animals. These data demonstrate that a combinatorial RNAi/vaccination therapy suppresses not only antigens in the serum, but also viral antigen expression in the liver.

Having demonstrated that suppression of expression of HBV antigens using shRNA or siRNA is effective at potentiating an immune response to an HBV vaccine regimen, a study was designed to determine if the duration of HBV antigen suppression had an effect on the potentiation an HBV immune response. Using the HBV 1.3xfs mouse model, mice were treated for eight, six, or three weeks with HBV-siRNA AD-66816, or the control siRNA for 8 weeks, subcutaneously administered at 3 mg/kg/dose, followed by administration of the prime-boost vaccine regimen as set forth above, except 10 μg c-di-AMP was used as an adjuvant rather than PCEP + CPG (n= 6 per 8 and 3 group, n = 6 for 6 week group) (see Figure 7).

A significant decrease in the levels of each of HBsAg, HBeAg, and HBV DNA was observed after the first administration of AD-66816 (Figures 8A-8C). A further significant decrease in HBsAg was observed after treatment with the vaccine boost with the greatest decrease observed in the 8 week pretreatment group to below the level of detection of the assay, representing a greater than 5 log10 decrease in HBsAg level in all treated animals. Immunization caused only slight further reductions (<0.5 log10) of HBV DNA and no further reduction in HBeAg levels. These data demonstrate that efficacy of therapeutic vaccination correlates with duration of antigen suppression before start of vaccination. Reconstitution of HBV-specific CD8 T cell responses takes several weeks, with a 6 or preferably 8 week pretreatment resulting in more HBsAg knockdown than a 3 week pretreatment.

Similarly, T-cell responses against HBsAg and HBcAg in liver corresponded with the duration of HBV antigen knockdown, with longer duration of HBV antigen suppression resulting in greater T-cell responses (Figure 9A-9C). Similar responses to MVA virus antigens were observed across all groups independent of pretreatment (Figure 9D). Antibody responses were similar across all groups.

Livers were also assessed for the presence of HBV transcripts by RT-qPCR and normalized to the liver GAPDH transcript. Longer pretreatment durations trended to higher levels of HBV RNA knockdown (Figure 10A and 10B).

Liver sections were also analysed by immunohistochemical staining for cytoplasmic expression of HBcAg to assess the antiviral effect of the treatment in the liver (Figure 10C). In animals pretreated with the control siRNA before vaccination, there were, on average, 172 hepatocytes per mm^2 which showed cytoplasmic expression of HBc. With longer duration of HBV suppression before vaccination, there was a gradual decrease of cytoplasmic HBcAg positive hepatocytes with an average of only 12 cytoplasmic HBcAg positive hepatocytes per mm^2 in the 8 week pretreatment group. These data demonstrate that the increasing HBV-specific CD8+ T cell response observed with longer antigen suppression before vaccination led to decreased HBV antigen expression in the liver. To assess the durability of response, blood samples were collected from mice (n = 6) pretreated with the AD-66816 HBV-siRNA for six weeks at 2 and 3 weeks after administration of the boost vaccination. In three of the six mice, HBsAg levels continued to dropped to below the level of detection of the assay (Figure 11A and 11B). Specifically, the three animals which had the highest HBsAg and HBeAg levels at start of vaccination (2, 4, and 5) did show a

decline of antigen titers after vaccination, but rebounded with antigen titers towards the end of the experiment. In contrast, the three animals with the lowest antigen titers at start of vaccination (1, 3, and 6) showed a further decline of HBsAg to below the detection limit. These data demonstrate that a functional cure is possible using the treatment regimens provided herein. These data also suggest that the antigen levels at start of vaccination can affect the response to therapeutic vaccination. Finally, without being bound by mechanism, these data suggest that the decline of antigen levels after therapeutic vaccination is mediated, at least in part, by CD8+ T cells. These data demonstrate that knockdown of circulating HBV antigens (*e.g.*, HBsAg, HBcAg), but not inhibition of HBV DNA replication alone, potentiates immune response to HBV therapeutic vaccine, *e.g.*, a prime boost therapeutic vaccination regimen. That is, an immune response can be potentiated by pretreatment with an siRNA, but not with nucleot(s)ide inhibitors alone as the immune response is a CD8+ T-cell based immune response.

The magnitude and duration of knockdown required depends on, for example, the level of disease burden in the subject. The higher the level of circulating antigen, the greater the magnitude and duration of HBV antigen knockdown required to potentiate an immune response. Knockdown of HBsAg in serum should be at least 0.5 log 10 (IU/ml), preferably 1log 10 (IU/ml), 1.5 log 10 (IU/ml), 2 log 10 (IU/ml), or more from the level in the subject prior to treatment with the therapeutic vaccine. In certain embodiments, the level of serum HBsAg is no more than 2.5 log 10 (IU/ml), 2 log 10 (IU/ml), 1.5 log 10 (IU/ml) prior to vaccine administration.

Further, as demonstrated herein, a longer duration of HBV antigen knockdown trended towards a more robust immune response. Therefore, knockdown of serum HBsAg to a sufficiently low level for a duration of at least four weeks, six weeks, or eight weeks is preferred prior to administration of the vaccine.

A second series of experiments were designed to study the combination siRNA/vaccination treatment regimen in a mouse model of aquired HBV infection using an adeno-associated virus infection system. For these studies, wildtype C57/Bl6 mice (9 weeks of age) were injected i.v. with $2x10^{10}$ genome equivalents of Adeno-Associated-Virus Serotype 8 (AAV8) carrying a 1.2-fold overlength HBV genome of genotype D (AAV-HBV1.2) (see, *e.g.,* Yang, *et al.* (2014) *Cell and Mol Immunol* 11:71). Starting 4 weeks after AAV-transduction (on day -28), animals were treated with three doses of either a control siRNA, or HBV siRNA (HBV AD-66816 or AD-66810) on days 0, 29, and 57, and subsequently half of the animals in each group were treated with a vaccine regimen consisting of prime protein vaccination with HBsAg, HbcAg, and 10 µg c-di-AMP at days 57 and 70, and boosted with MVA-HBs and MVA-HBc at day 84. The treatment regimen is shown in Figure 12.

Following AAV-HBV1.2 transduction, HBsAg and HBeAg values rose to levels comparable to the levels seen in HBV transgenic mice (HBVxfs) (see, *e.g.*, Figures 13A and 13B). Mice treated with the HBV siRNAs showed mean reductions of HBsAg levels of about 2 $\log_{10}$ scales and of HBeAg levels greater than 1 $\log_{10}$ scale such that HBsAg and HBeAg levels dropped to less than about 500 IU/per ml, whereas the control siRNA had no effect on antigen levels. Animals treated with

one of the HBV siRNAs that did not receive the vaccine regimen showed slowly rebounding antigen levels after the last application of the siRNA. Antigenemia returned to baseline levels after 18 weeks. The combination treatment with the HBV-siRNA and vaccine regimen resulted in a durable response with a decrease in HBsAg and HBeAg to below the limit of detection through the duration of the experiment. A decrease in HBsAg and HBeAg levels was observed prior to the administration of the MVA boost, suggesting that the protein vaccination may be sufficient to affect a cure. Both serum and liver HBV DNA and RNA were significantly decreased after combination treatment with the HBV-siRNA and vaccine regimen (Figures 13D-13H). This demonstrates, that RNAi-mediated suppression can strongly reduce antigen expression, but that treatment with the vaccine regimen extends the durability of response.

The vaccine regimen alone in animals that received the control siRNA produced a transient decline of antigen levels which rebounded towards the end of the experiment. In contrast, all animals that received an HBV-siRNA and vaccination, HBsAg and HBeAg levels decreaased to below the detection limit after start of vaccination. Antigen levels remained largely undetectable at all through the last time point measured at least 22 weeks after the last siRNA application (Figures 13A and 13B). The durable loss of antigenemia in HBV siRNA pretreated animals, in contrast to the antigen rebound in control siRNA pretreated animals, further demonstrates that immune control was only achieved in animals which had lowered antigen titers before vaccination.

Coinciding with the loss of antigenemia, animals treated with HBV siRNA plus the vaccine regimen developed high titers of anti-HBs antibodies and resulted in anti-HBs and anti-HBe seroconversion in all vaccinated animals (Figure 14). siRNA-pretreated animals developed 10-fold higher and more constant anti-HBs titers and were able to completely and persistently clear serum HBsAg and HBeAg. Interestingly, 3/12 mice vaccinated after HBV siRNA treatment showed a transient drop in anti-HBe levels between week 15 and 22 resulting in a low-level relapse of HBeAg (Figure 13C) that was again controlled. Although anti-HBs antibodies could also be measured in animals that received the control siRNA plus the vaccine regimen, the levels were lower. Further, only animals that received HBV siRNA plus the vaccine regimen developed anti-HBe antibodies. Taken together, functional cure was not achieved by the siRNA treatement regimen or the therapeutic vaccination regimen alone. However, the loss of antigenemia, as well as development of anti-HBs and anti-HBe antibodies, demonstrates that the combination HBV siRNA plus vaccine regimen can achieve a functional cure.

Throughout the experiment, ALT and body weight of the animals were monitored. The loss of antigenemia concided with slight increases of ALT activity seen in treatment groups which had received HBV siRNA in conjunction with the vaccination regimen (Figure 15A). These groups showed significant but mild increases (both $p>0.05$ or smaller by repeated measure two-way ANOVA; only comparing time points after start of vaccination) as compared to all other treatment groups that did not receive the combination HBVsiRNA-vaccine regimen. Without being bound by

mechanism, it is suggested that the CD8+ T cells induced by the combinatorial HBV siRNA-vaccine regimen killed HBV-expressing hepatocytes resulting in elevated ALT.

The body weight of the animals was measured throughout the experiment to assess tolerability of the treatments. There was steady increases throughout the experiment independent of siRNA treatment. Animals that were vaccinated showed a slight and transient decrease (approximately 5%) of body weight after vaccination, but rebounded to normal levels within nine days, and subsequently gained weight comparable to the control groups (Figure 15B). Taken together, both, ALT activity and body weight data demonstrate that all examined treatments, including siRNA only, vaccine only, as well as the combinatorial siRNA/vaccine regimen are well tolerated.

The number and timing of doses of siRNA to knockdown HBsAg level in serum depends, for example, on the specific agent used. Due to the duration and potency of the exemplary GalNAc siRNAs used in the methods herein and provided, for example, in Appendix A and in PCT Publication No. WO 2016/077321 (the entire contents of which are incorporated herein by reference), a single dose of siRNA may be sufficient to provide the level and duration of knockdown required prior to administration of the therapeutic vaccine. As shown in Figure 4, a single dose of an AAV vector encoded shRNA was sufficient to provide durable knockdown of HBV antigens and HBV DNA. Those of skill in the art are able to monitor HBV disease status, *e.g.*, by measuring HBsAg levels in blood, to determine the timing and level of siRNA and vaccine administration appropriate for a specific subject.

A number of therapeutic HBV vaccines are known in the art and discussed herein. In preferred embodiments, a prime-boost vaccination protocol, such as the protocol that is used herein, is preferred. However, the HBV antigen knockdown method provided herein can be used in combination with other therapeutic HBV vaccines known in the art, including those found to be insufficient when administered alone. Vaccinations include at least two doses of an antigen in protein or nucleic acid form. In certain embodiments, the vaccination includes three doses of a protein-based vaccine. In preferred embodiments, the methods include heterologous vaccine administration, *i.e.*, at least one protein-based vaccine dose and at least one nucleic-acid based vaccine dose. Exemplary embodiments of vaccines and dosing regimens are provided, for example, in PCT Publication No. WO 2017/121791, the entire contents of which are incorporated herein by reference.

The methods provided herein include the use of a nucleic acid-based vaccine comprising an expression vector construct encoding an HBcAg or an HBsAg, wherein the construct encodes a protein that shares an epitope with the protein-based vaccine. Therefore, it is clearly understood that neither the nucleic acid-based vaccine nor the protein-based vaccine are required to provide the full length protein. The nucleic acid-based vaccine and the protein-based vaccine are required to provide at least one shared epitope that is present in HBcAg or HBsAg, and does not require that the full length protein be provided. As noted above, epitopes may be relatively short, MHC class I molecules that are typically peptides about 8 to 11 amino acids in length, whereas MHC class II molecules present longer peptides about 13 to 17 amino acids in length, with conformational epitopes being

longer. However, it is understood that the use of protein antigens and coding sequences for protein antigens that encode multiple epitopes is preferred. Further, it is understood that the antigens present in the protein-based vaccine and encoded by the nucleic-acid based vaccine may or may not be identical. It is also obvious that antigens should also be selected for their immunogenicity. Such antigens are well known in the art.

In certain embodiments, the order of administration of the protein-based vaccine and the nucleic acid-based vaccine are reversed as compared to the order exemplified in the methods provided herein. That is, the nucleic acid-based vaccine is administered first and the protein-based vaccine is administered second. In certain embodiments, a total of three doses of vaccine are administered, two doses of the nucleic acid-based vaccine followed by a single dose of the protein-based vaccine. In alternative embodiments, a single dose of the nucleic acid-based vaccine is followed by two doses of a protein based vaccine. In other embodiments, one dose of each vaccine is administered.

In preferred embodiments, the prime-boost vaccination method includes the use of an adjuvant with protein antigens. Appropriate adjuvants for use in the methods promote a cell-based response to the antigens. Adjuvants preferably provide a balanced Th1/Th2 response.

The siRNA + vaccine methods provided herein can be used in combination with administration of nucleot(s)ide inhibitors which are the standard of care for treatment of HBV. In certain embodiments, subjects are treated with nucleot(s)ide inhibitors prior to treatment with the siRNA + vaccine treatment regimen. In certain embodiments, subjects are treated throughout the siRNA + vaccine treatment regimen with nucleot(s)ide inhibitors. In certain embodiments, the nucleot(s)ide inhibitor is dosed to reduce pre-existing inflammation associated with HBV infection prior to administration of the nucleic acid therapeutic targeted to HBV (*e.g.*, siRNA, shRNA, antisense oligonucleotide).

In certain embodiments, subjects may be pretreated, or concurrently treated, with other agents used for the treatment of HBV. Such agents include, but are not limited to an immune stimulator (*e.g.*, pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and aToll-like receptor 7 (TLR7) agonist), a viral entry inhibitor (*e.g.*, Myrcludex), an oligonucleotide that inhibits the secretion or release of HbsAg (*e.g.*, REP 9AC), a capsid inhibitor (*e.g.*, Bay41-4109 and NVR-1221), a cccDNA inhibitor (*e.g.*, IHVR-25), a Rig-I ligand, or an immune checkpoint regulator. In certain embodiments, the immune stimulator is a Rig-I ligand or an immune checkpoint regulator. A functional cure includes a sustained period of at least 3 months, preferably 6 months of HBsAg below 50 IU/ml, or a detectable antibody response to HBsAg. In preferred embodiments, a functional cure includes both a sustained period of at least 3 months, preferably 6 months of HBsAg below 50 IU/ml and a detectable antibody response to HBsAg.

A. RNAi Agents For Use in the Methods of the Invention

The present invention includes the use of iRNAs, which inhibit the expression of at least one HBV transcript, and preferably three or four HBV transcripts (open reading frames, sometimes

referred to herein as genes). Due to the highly condensed structure of the HBV genome, it is possible to design single iRNAs that will inhibit the expression of three or four HBV transcripts (see Figure 1). For the sake of simplicity, the text herein refers to "an HBV transcript" or "the HBV transcript." It is understood that preferred embodiments include inhibition of more than one HBV transcript (or open reading frame), preferably at least three HBV transcripts (or open reading frames). Further, it is understood that there are eight HBV genotypes, and two proposed additional genotypes, that may further include mutations from published sequences. Therefore, certain iRNA agents may inhibit different numbers of genes based on the specific genotype and subject infected with HBV.

In some embodiments, the iRNA agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression or decreasing the level of an HBV transcript in a cell in a subject with an HBV infection. The dsRNA includes an antisense strand having a region of complementarity, which is complementary to at least a part of an mRNA formed in the expression of an HBV transcript. The region of complementarity is about 30 nucleotides or less in length (*e.g.,* about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the HBV gene, the iRNA selectively inhibits the expression of at least one, preferably three or four HBV genes. In preferred embodiments, inhibition of expression is determined by the qPCR method in an appropriate cell line as provided in the examples. For *in vitro* assessment of activity, percent inhibition is determined using the methods provided in Example 2 of PCT Publication No.WO 2016/077321 at a single dose at a 10 nM duplex final concentration. For *in vivo* studies, the level after treatment can be compared to, for example, an appropriate historical control or a pooled population sample control to determine the level of reduction, *e.g.*, when a baseline value is not available for the subject. An appropriate control must be carefully selected by one of skill in the art.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of an HBV gene. As multiple HBV genotypes are known, iRNA agents are preferably designed to inhibit expression of HBV genes across as many genotypes as possible. It is understood that an siRNA that is perfectly complementary to one or more HBV genotypes will not be perfectly complementary to all genotypes. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 and 30 base pairs in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-

26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence is 15 and 30 nucleotides in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, the dsRNA is about 15 to 20 nucleotides in length, or about 25 to 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target HBV gene expression is not generated in the target cell by cleavage of a larger dsRNA.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.*, about 15-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28,

15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides.

In some embodiments, a dsRNA agent of the invention comprises a tetraloop. As used herein, "tetraloop" in the context of a dsRNA refers to a loop (a single stranded region) consisting of four nucleotides that forms a stable secondary structure that contributes to the stability of adjacent Watson-Crick hybridized nucleotides. Without being limited to theory, a tetraloop may stabilize an adjacent Watson-Crick base pair by stacking interactions. In addition, interactions among the four nucleotides in a tetraloop include, but are not limited to, non-Watson-Crick base pairing, stacking interactions, hydrogen bonding, and contact interactions (Cheong *et al.*, Nature 1990 Aug 16;346(6285):680-2; Heus and Pardi, Science 1991 Jul 12;253(5016): 191-4). A tetraloop confers an increase in the melting temperature (Tm) of an adjacent duplex that is higher than expected from a simple model loop sequence consisting of four random bases. For example, a tetraloop can confer a melting temperature of at least 55°C in l0mM $NaHPO_4$ to a hairpin comprising a duplex of at least 2 base pairs in length. A tetraloop may contain ribonucleotides, deoxyribonucleotides, modified nucleotides, and combinations thereof. Examples of RNA tetraloops include the UNCG family of tetraloops (*e.g.*, UUCG), the GNRA family of tetraloops (*e.g.*, GAAA), and the CUUG tetraloop. (Woese *et al.*, Proc Natl Acad Sci U S A. 1990 Nov;87(21):8467-71 ; Antao *et al.*, Nucleic Acids Res. 1991 Nov l l;19(21):5901-5). Examples of DNA tetraloops include the d(GNNA) family of tetraloops (*e.g.*, d(GTTA), the d(GNRA)) family of tetraloops, the d(GNAB) family of tetraloops, the d(CNNG) family of tetraloops, the d(TNCG) family of tetraloops (*e.g.*, d(TTCG)). (Nakano *et al.* Biochemistry, 41 (48), 14281 -14292, 2002; Shinji *et al.* Nippon Kagakkai Koen Yokoshu vol.78th; no.2; page.731 (2000).)

In certain embodiments of the invention, tetraloop- and modified nucleotide- containing dsNAs are contemplated as described, *e.g.*, as described in U.S. Patent Publication No. 2011/0288147, the entire contents of which are incorporated by reference herein. In certain such embodiments, a dsNA of the invention possesses a first strand and a second strand, where the first strand and the

second strand form a duplex region of 19-25 nucleotides in length, wherein the first strand comprises a 3' region that extends beyond the first strand-second strand duplex region and comprises a tetraloop, and the dsNA comprises a discontinuity between the 3' terminus of the first strand and the 5' terminus of the second strand.

Optionally, the discontinuity is positioned at a projected Dicer cleavage site of the tetraloop-containing double stranded nucleic acid (dsNA). It is contemplated that, as for any of the other dupexed oligonucleotides of the invention, tetraloop-containing duplexes of the invention can possess any range of modifications disclosed herein or otherwise known in the art, including, *e.g.*, 2'-O-methyl, 2'- fluoro, inverted base, GalNAc moieties, *etc*. Typically, every nucleotide on both strands of the tetraloop-containing dsNA is chemically modified if the tetraloop-containing dsNA is going to be delivered without using lipid nanoparticles or some other delivery method that protects the dsNA from degradation during the delivery process. However, in certain embodiments, one or more nucleotides are not modified.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.,* 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA. In certain embodiments, longer, extended overhangs are possible.

A dsRNA can be synthesized by standard methods known in the art. iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. In certain embodiments, the iRNA compound is produced from an expression vector delivered into a cell.

In one aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences

provided in any one of the Tables in Appendix A, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of the Tables in Appendix A.

In some embodiments, the sense strand is selected from the group of sequences provided in any one of the Tables in Appendix A, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of the Tables in Appendix A. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of an HBV gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of the Tables in Appendix A and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of the Tables in Appendix A. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It is understood that, although some of the sequences in the Tables in Appendix A are described as modified or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in the Tables in Appendix A that is un-modified, unconjugated, or modified or conjugated differently than described therein. Additional target sites are provided, for example, in PCT Publication Nos. WO 2016/077321, WO 2012/024170, WO 2017/027350, and WO 2013/003520; and in Michler, 2016, the entire contents of wach of which are incorporated herein by reference.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of the Tables in Appendix A, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of any one of the Tables in Appendix A minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of any one of the Tables in Appendix A and differing in their ability to inhibit the expression of an HBV gene by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence.

An iRNA as described herein can contain one or more mismatches to the target sequence, or to one or more HBV target sequences due, *e.g.*, to sequence variations among the HBV genotypes. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA

contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of an HBV gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of an HBV gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of an HBV gene is important, especially if the particular region of complementarity in an HBV gene is known to have polymorphic sequence variation within various genotypes and the population.

i. Modified iRNAs For Use in the Methods of the Invention

In some embodiments, the RNA of the iRNA for use in the invention *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications or conjugations known in the art and described herein, *e.g.*, when produced from an expression vector. In other embodiments, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA of the invention are modified. iRNAs of the invention in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl

phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative US patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, US Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative US patents that teach the preparation of the above oligonucleosides include, but are not limited to, US Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

In other embodiments, suitable RNA mimetics are contemplated for use in iRNAs, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative US patents that teach the preparation of PNA compounds include, but are not limited to, US Patent Nos. 5,539,082; 5,714,331;

and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular –CH_2–NH–CH_2-, --CH_2–N(CH_3)–O–CH_2–[known as a methylene (methylimino) or MMI backbone], --CH_2–O–N(CH_3)–CH_2--, --CH_2–N(CH_3)–N(CH_3)–CH_2–and –N(CH_3)–CH_2–CH_2–[wherein the native phosphodiester backbone is represented as –O–P–O–CH_2--] of the above-referenced US Patent No. 5,489,677, and the amide backbones of the above-referenced US Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced US Patent No. 5,034,506.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include O[(CH_2)$_n$O]$_m$$CH_3$, O($CH_2$)$_nOCH_3$, O($CH_2$)$_nNH_2$, O($CH_2$)$_nCH_3$, O($CH_2$)$_nONH_2$, and O($CH_2$)$_n$ON[($CH_2$)$_nCH_3$)]$_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O–$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a O(CH_2)$_2$ON(CH_3)$_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O–CH_2–O–CH_2–N(CH_2)$_2$.

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative US patents that teach the preparation of such modified sugar structures include, but are not limited to, US Patent Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873;

5,670,633; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

The RNA of an iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxy-thymine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in US Patent No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative US patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted US Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

The RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has

been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

In some embodiments, the iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series,* 52, 133-134 (2008) and Fluiter *et al.*, *Mol. Biosyst.,* 2009, 10, 1039 hereby incorporated by reference).

The RNA of an iRNA can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A"bicyclic nucleoside"("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *Et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4′ to 2′ bridge. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH2)—O-2′ (LNA); 4′-(CH2)—S-2′; 4′-(CH2)2—O-2′ (ENA); 4′-CH(CH3)—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-CH(CH2OCH3)—O-2′ (and analogs thereof; see, *e.g.*, US Patent No. 7,399,845); 4′-C(CH3)(CH3)—O-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4′-CH2—N(OCH3)-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4′-CH2—O—N(CH3)-2′ (see, *e.g.*,US20040171570); 4′-CH2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, US Patent No. 7,427,672); 4′-CH2—C(H)(CH3)-2′ (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH2—C(=CH2)-2′ (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative US patents and US patent publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: US Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133;7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 20080039618; and US 20090012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH3)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2'and C4' carbons of ribose or the C3 and -C5′ carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US 20130190383 and WO 2013036868, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"- phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in WO 2011005861.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of an RNAi agent. Suitable phosphate mimics are disclosed in, for example US20120157511, the entire contents of which are incorporated herein by reference.

In certain specific embodiments, the RNAi agent for use in the methods of the invention is an agent selected from the group of agents listed in any one of the Tables in Appendix A. These agents may further comprise a ligand.

ii. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA. Such moieties include but are not limited to lipid moieties such as a

cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. *Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In one embodiment, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid, or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, monovalent or multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucoseamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic.

In certain embodiments, ligands include monovalent or multivalent galactose. In certain embodiments, ligands include cholesterol.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, monovalent or multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc*. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

1. Lipid Conjugates

In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In a preferred embodiment, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

2. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 33). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 34) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO: 35) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 36) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such

as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

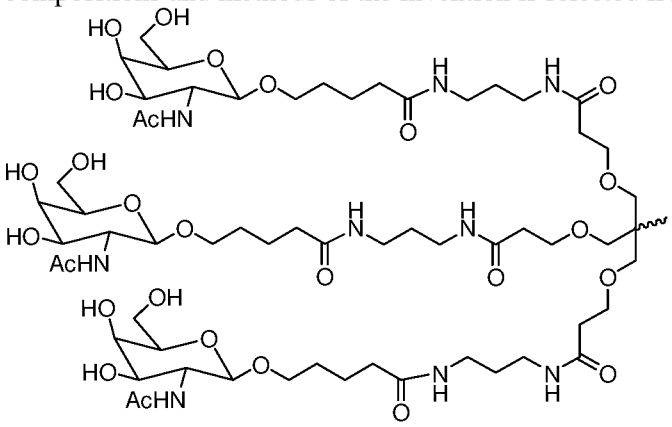
An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.,* glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

A "cell permeation peptide" is capable of permeating a cell, *e.g.,* a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, Nucl. Acids Res. 31:2717-2724, 2003).

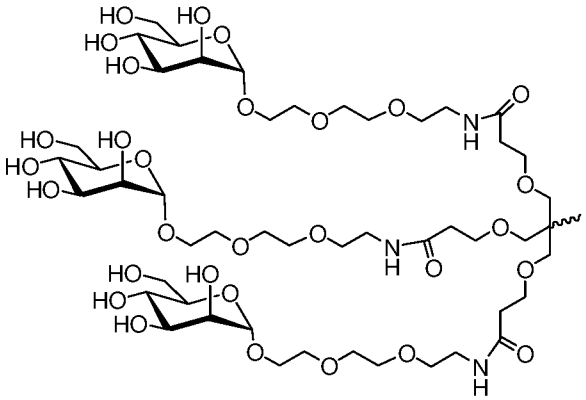
3. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.,* C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.,* C5, C6, C7, or C8).

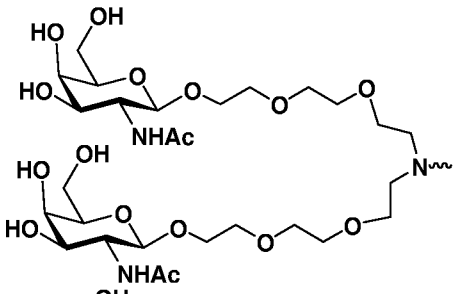
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



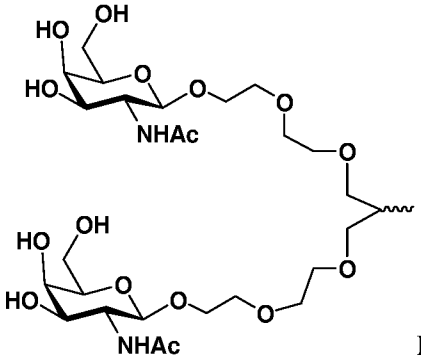
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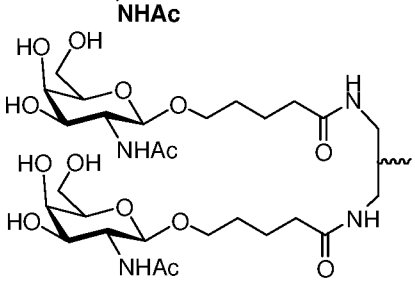
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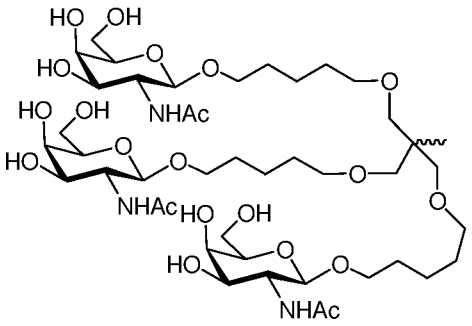
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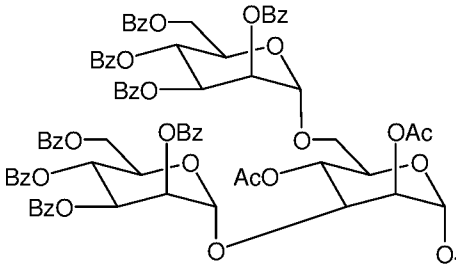
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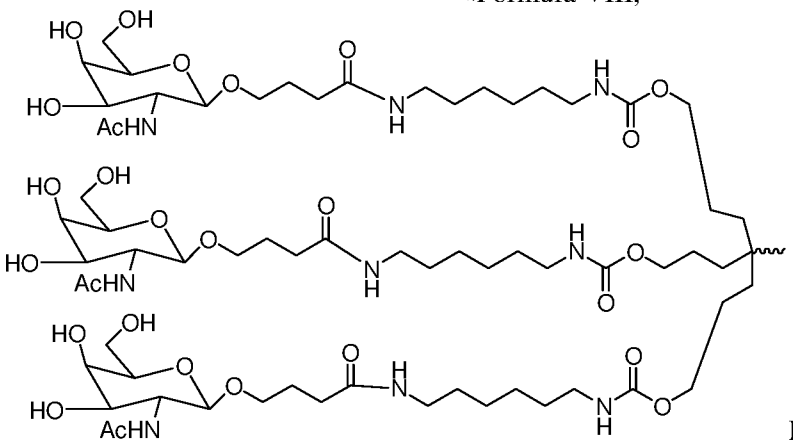
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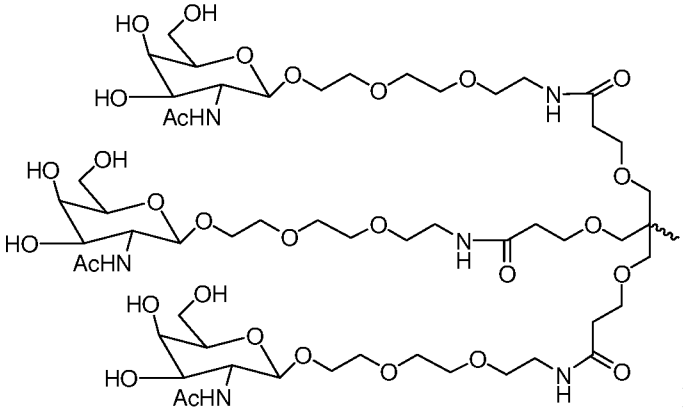
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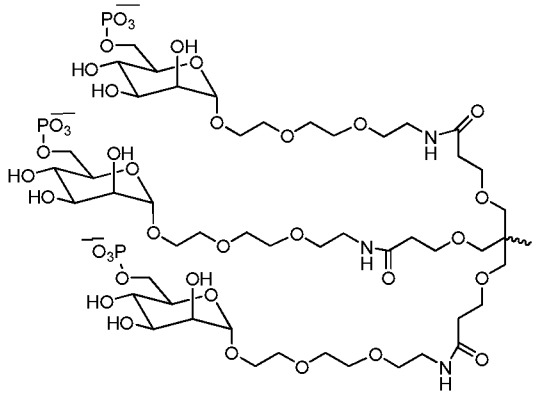
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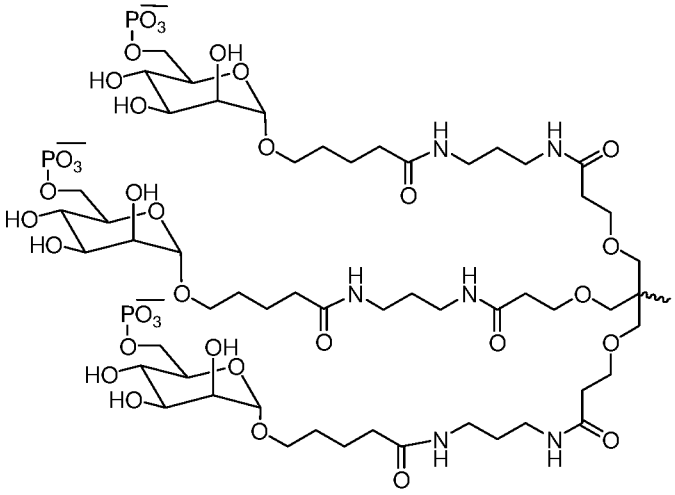
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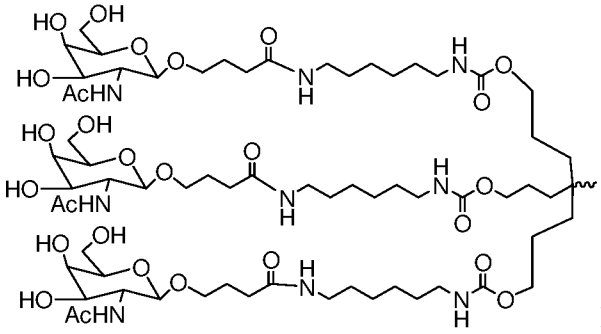
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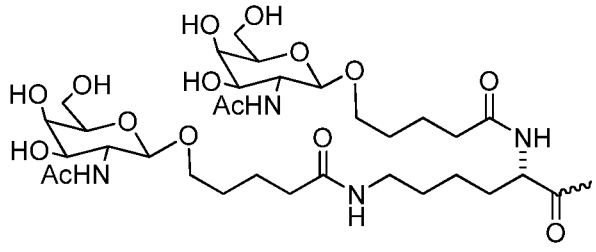
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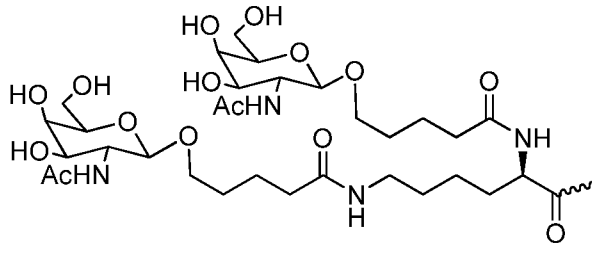
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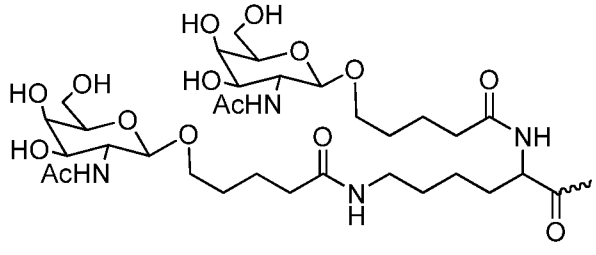
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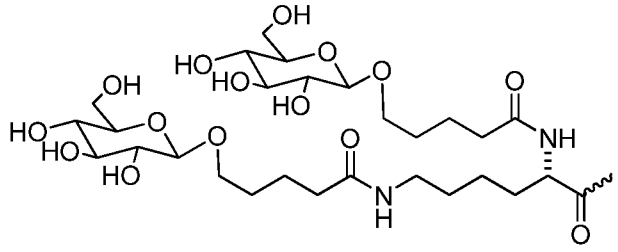
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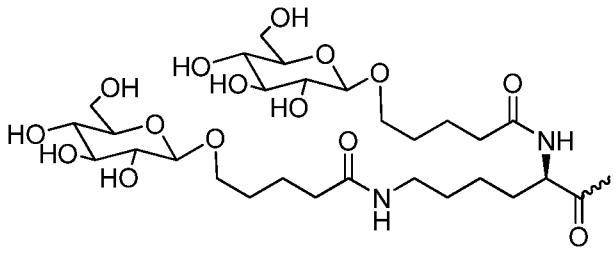
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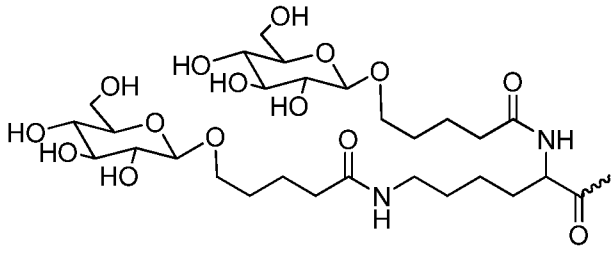
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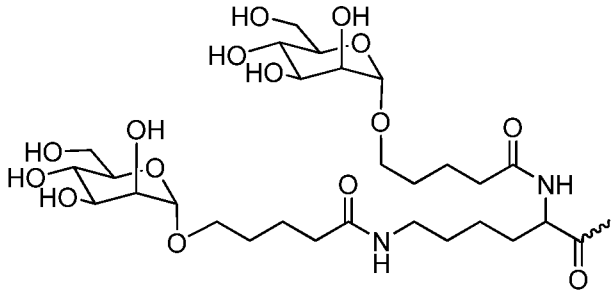
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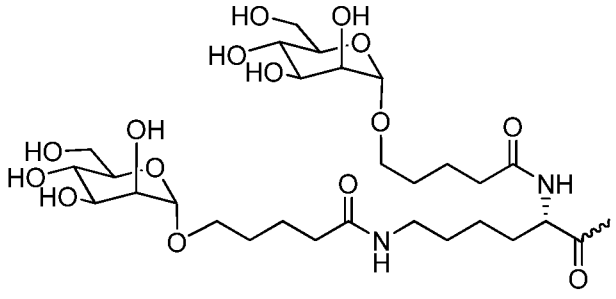
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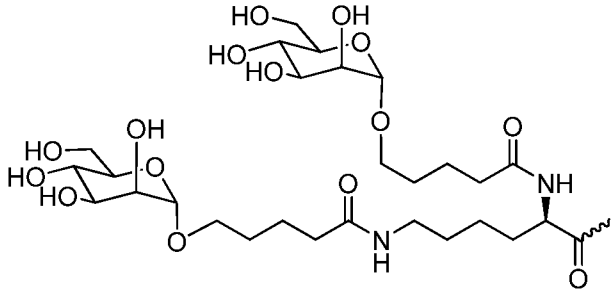
Formula XIX,



Formula XX,

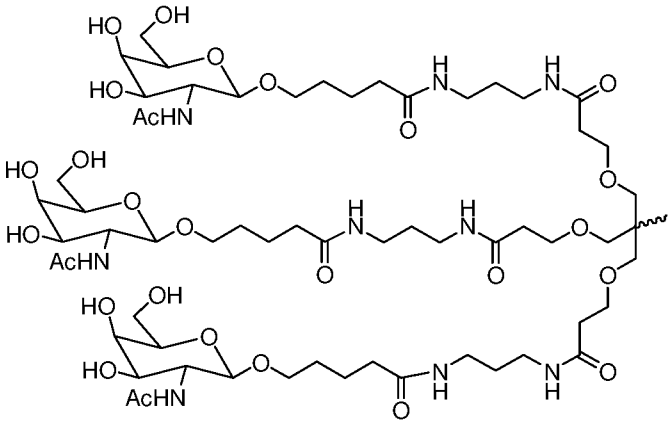


Formula XXI,



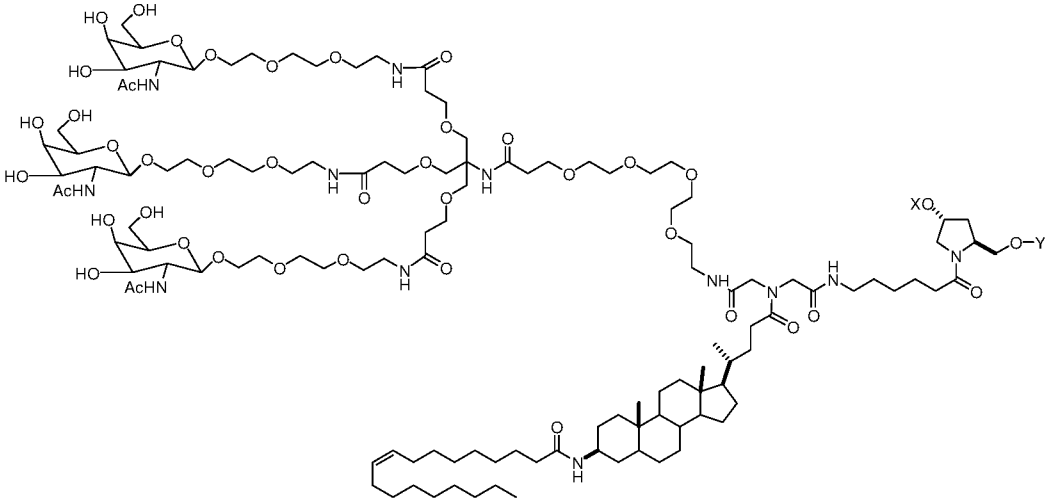
Formula XXII.

In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as



Formula II.

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (*e.g.,* 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

Additional carbohydrate conjugates suitable for use in the present invention include those described in WO 2014179620 and WO 2014179627, the entire contents of each of which are incorporated herein by reference.

4. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include:

redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

a. Redox cleavable linking groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA

moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

b. Phosphate-based cleavable linking groups

In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

c. Acid cleavable linking groups

In another embodiment, a cleavable linker comprises an acid cleavable linking group. An *a*cid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

d. Ester-based linking groups

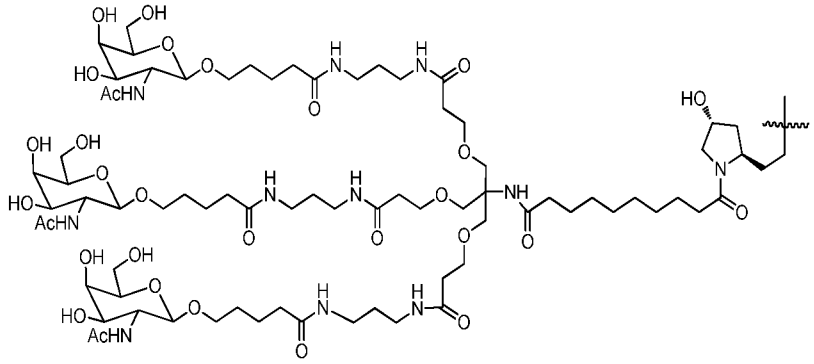
In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells.

Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated using methods analogous to those described above.

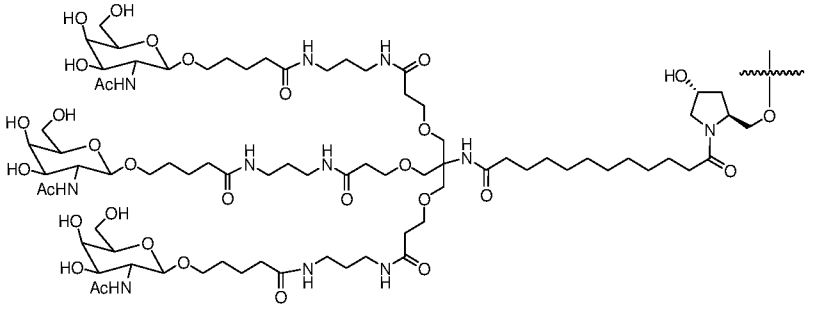
e. Peptide-based cleaving groups

In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

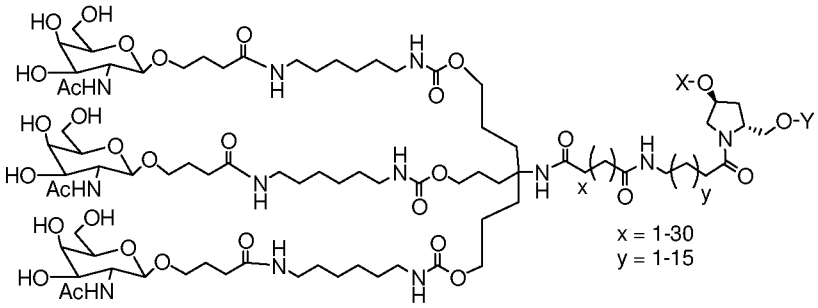
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



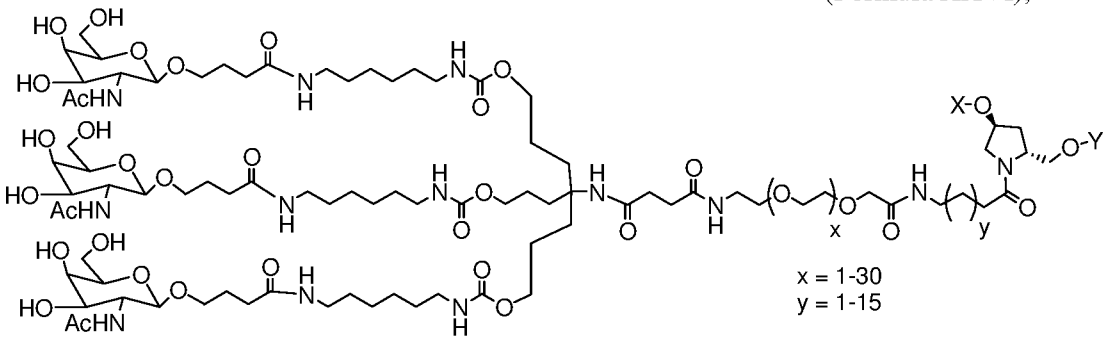
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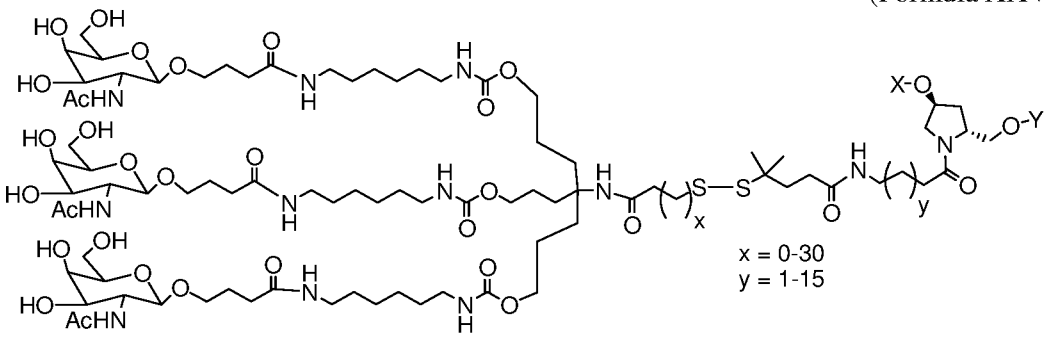
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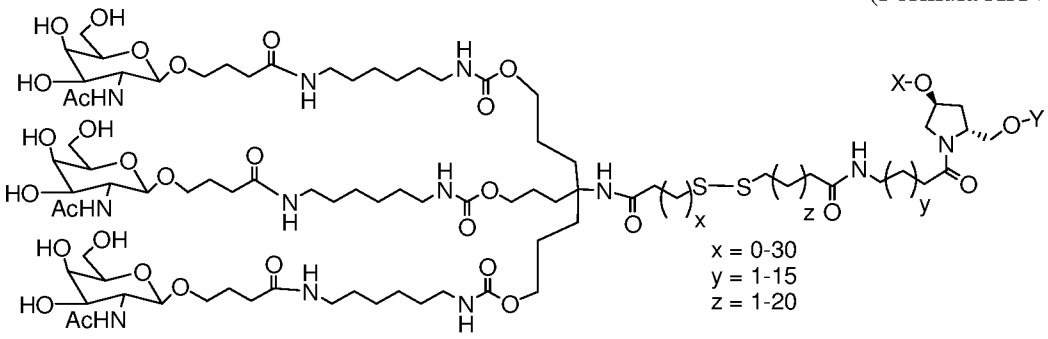
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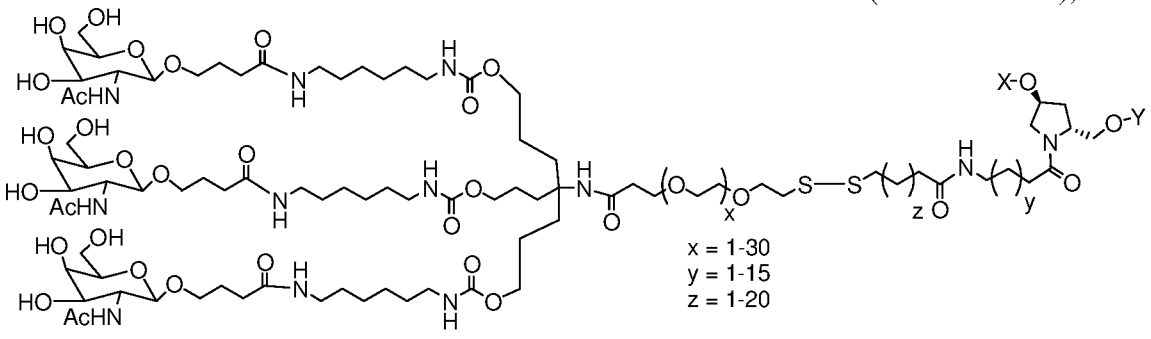
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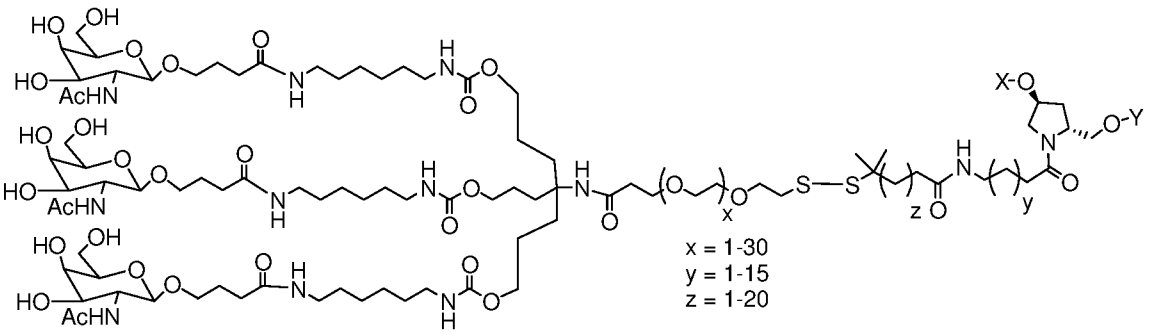
(Formula XXVIII),



(Formula XXIX),



(Formula XXX), and



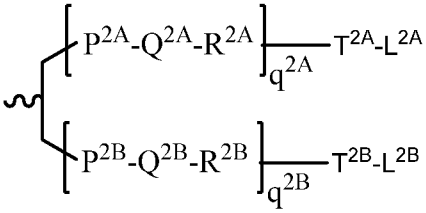
(Formula XXXI),

when one of X or Y is an oligonucleotide, the other is a hydrogen.

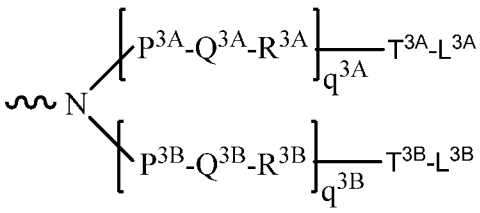
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII) – (XXXV):

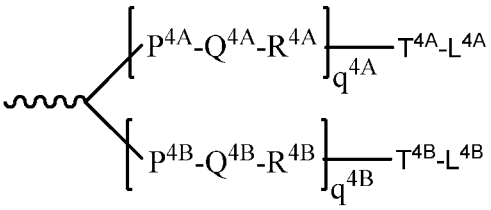
Formula XXXII



Formula XXXIII

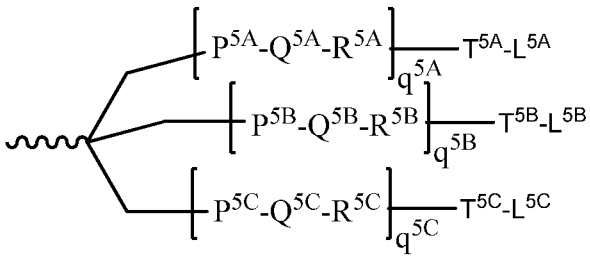


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Formula XXXIV



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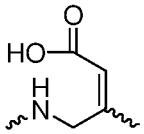
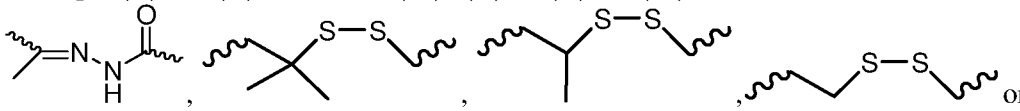
Formula XXXV

wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

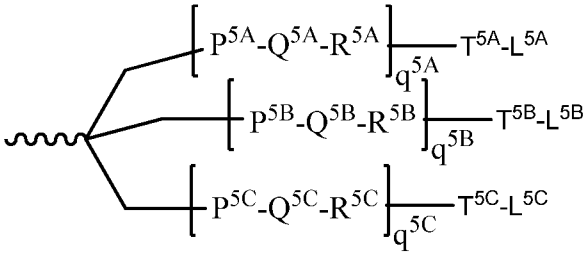
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO, CH=N-O, , , or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; andR^a is H or amino acid side chain.Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XXXV):

Formula XXXV



,

wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative US patents that teach the preparation of RNA conjugates include, but are not limited to, US Patent Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds or "chimeras," in the context of this invention, are iRNA compounds, preferably dsRNAs, which contain two or more chemically distinct regions, each made

up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNa:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNa strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al., Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of an RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

iii. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.,* a cell within a subject, such as a human subject infected with HBV can be achieved in a number of different ways. Delivery may also

be performed directly by administering a composition comprising an iRNA, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO9402595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., *et al* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN., *et al* (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN., *et al* (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., *et al* (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in US Patent No. 7,427,605, which is herein incorporated by reference in its entirety. In certain embodiments, the iRNA agents can be administered with amphipathic peptides to facilitate pH-

dependent endosomal escape (see, *e.g.*, Bartz *et al.*, 2011. Biochem. J. 435:475-87, incorporated herein by reference)

1. Vector encoded iRNAs for use in the Invention

iRNA targeting an HBV gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, WO 00/22113, WO 00/22114, and US Patent No. 6,054,299). Exemplary expression vectors for expression of shRNA targeted to HBV are provided in Michler *et al.*, 2016 which discloses adeno-associated virus (AAV) 8 vectors for delivery included (i) embedded the shRNA in an artificial mi(cro)RNA under a liver-specific promoter; (ii) co-expressed Argonaute-2, a rate limiting cellular factor whose saturation with excess RNAi triggers can be toxic; or (iii) co-delivered a decoy ("TuD") directed against the shRNA sense strand to curb off-target gene regulation. The plasmids expressing shRNAs shHBV4 to 7 that were used in the cell culture studies were cloned by direct insertion of the respective shRNA-encoding oligonucleotides into a self-complementary AAV vector plasmid previously reported by Grimm et al, 2006 (Nature 441: 537 – 541), containing an H1 promoter followed by two BbsI sites for oligonucleotide insertion as well as an RSV promoter. Such constructs can also be used for the expression of vaccine antigens if appropriately sized for the expression vector.

Expression can be transient (on the order of days to weeks) or sustained (weeks to months, or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

The individual strand or strands of an iRNA can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA expressing vectors can be systemic, such as by subcutaneous, intravenous, or intramuscular administration. Such vectors can also be used for expression of viral antigens from nucleic acid-based vaccines.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno-associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors including modified vaccinia virus Ankara vector or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA or HBV antigen will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are further described below.

Such constructs and vectors can also be used for the expression of vaccine antigens if appropriately sized for the expression vector. Such limitations are well understood by those of skill in the art.

B. Therapeutic HBV Vaccines For Use in the Mehods of the Invention

Therapeutic HBV vaccines for use in the regimens and methods of the invention can be a peptide vaccine, a DNA vaccine including a vector-based vaccine, or cell-based vaccine that induces an immune response, preferably an effector T cell induced response, against one or more HBV proteins. Preferably the vaccine is a multi-epitope vaccine that is cross-reactive with multiple HBV serotypes, preferably all HBV serotypes.

A therapeutic vaccine is designed to activate the patient's immune system to recognize and control or eliminate an already established pathogen infection. This is clearly distinct from a prophylactic vaccination which is designed to promote rapid antibody-mediated neutralization of an invading pathogen. Control and elimination of persistent viruses such as hepatitis, herpes, or papilloma viruses requires multi-specific and poly-functional effector T cell responses. These T cell responses are ideally directed against continuously expressed viral antigens to keep the pathogen in check. Therapeutic vaccines are under development for a number of chronic infections. Hepatitis B virus infection is a candidate for treatment by therapeutic vaccination since a spontaneous, immune-mediated recovery of chronic hepatitis B and an elimination of the virus has been observed in very rare cases.

Robust T cell responses seem to be essential to achieve HBV cure. While HBV-specific CD4+ and CD8+ T cell responses are readily detectable in patients resolving HBV infection, HBV-specific T cells are scarce and functionally impaired in chronic hepatitis B most likely due to high amounts of circulating viral HBeAg and HBsAg. T cells eliminate HBV infected cells by their cytotoxic activity but also control HBV gene expression and replication in a non-cytolytic fashion.

To overcome immune tolerance in chronic hepatitis B different approaches have been investigated in preclinical models using DNA or peptide vaccines, or vector- or cell-based vaccines to induce an effector T cell response. Multi-epitope therapeutic vaccine candidates that cover sufficient different HBV genotypes and most frequent HLA types have been developed. Although proper peptide presentation was demonstrated, immunogenicity was limited and the approach was not translated into the clinics. A non-exhaustive list is provided in the table below.

Therapeutic HBV Vaccine Tables

Vaccine Name	**Proteins/ coding sequences**	**Vaccine type/ Composition**	**Results available**	**Sponsor**	**Development Stage**	**Clinical Trial Reference No.**	**References (the entire contents of each of which are incorporated herein by reference)**
HB-110	HBsAg,, HBcAg, human IL-12	3 Plasmid based DNA vaccine	Study completed, results not reported	Genexine	Phase I	NCT01813487 NCT01641536 NCT00513968	Kim *et al.*, 2008. Exp Mol Med. 40: 669–676
HB-100	pGX10 S +pGX10 S1/S2/X +pGX10 core +pGX10 Pol +pGX10 hIL-12N222L	5 Plasmid based DNA vaccine – 4 antigens + human IL-12					Yang *et al.*, 2006. Gene Therapy. 13:1110-1117
ppdpSC18		DNA vaccine adjuvanted with particle mediated epidermal delivery	Study completed, results not reported	PowderMed	Phase I	NCT00277576	
INO-1800	HbsAg, HBcAg	DNA plasmid	Recruiting	Inovio Pharmaceuticals	Phase I	NCT02431312	

HB02 VAC-ADN	HB preS/S	pCMV-S2.S DNA vaccine; CMV promoter, plasmid vector	Well tolerated; No change in relapse rate in HBV treated patients or decrease in virological breakthrough	ANRS	Phase I/II	NCT00536627	Mancini – Bourgine *et al.*, 2006. Vaccine 24:4482-4489
CVI-HBV-002	HbsAg	DNA+ L-pampo	Recruiting	CHA Vaccine Institute Co., Ltd.	Phase I/II	NCT02693652	
Theravax (DV-601)	HBsAg, HBcAg	Protein + adjuvant	Well tolerated; anti-viral response observed in all patients	Dynavax Technologies Corp.	Phase Ib	NCT01023230	
HepTcell™		IC31® adjuvanted peptide	Recruiting	Altimmune, Inc.	Phase I	NCT02496897	US 20130330382 US 20120276138 US 20150216967
HBsAg/HBcAg	HBsAg HBcAg	protein			Research		Li *et al.*, 2015, Vaccine. 33:4247-4254
GS-4774	Fusion protein HBsAg, HBcAg, HBxAg	Protein + Tamogen T cell stimulator	Phase II naïve group ongoing; no significant viral decrease in treatment-	Gilead	Phase II	NCT01943799 NCT01779505 NCT02174276	Gaggar *et al.*, 2014. Vaccine. 32:4925-4931.

			experience patients				
εPA-44		Multi-peptide vaccine	Some seroconversion observed in all groups, no statistical analysis	Chongqing Jaichen Biotechnology, Ltd.	Phase II	NCT00869778 NCT02862106	
ABX 203	HBsAg, HBcAg	HBsAg, HBcAg	Ongoing	ABIVAX S.A.	Phase II/III	NCT02249988	
pSG2.HBs/ MVA.HBs		Protein prime-viral vector boost	Well tolerated, but did not control HBV infection	Oxxon Therapeutics	Phase IIa	ISRCTN 67270384	Cavenaugh *et al.*, 2011. PLoS ONE 6: e14626.
	HBsAg, HBcAg	Adjuvanted protein prime-viral vector boost			Research		Backes, 2016, Vaccine; WO2017121791

Existing prophylactic vaccines have been used to restore HBV-specific immunity in chronically infected patients, but have failed to provide a functional cure. These subviral particle-based vaccines were able to reduce HBV replication in animal models of chronic hepadnaviral infection, but have not been successful in patients with chronic hepatitis B. An antigen-antibody (HBsAg-HBIG) immunogenic complex therapeutic vaccine candidate with alum as adjuvant first showed promising results in a double-blind, placebo controlled, phase IIb clinical trial, but results of a phase III clinical trial including 450 patients were disappointing. This is most likely due to the fact that subviral particle vaccines with alum-based adjuvants are designed as prophylactic vaccines and preferentially induce antibodies but not cytotoxic T cell responses that would be required for therapeutic efficacy.

Alternatively, DNA vaccines encoding HBV envelope proteins were designed to induce HBV-specific T cells but also had limited success. Since DNA-based vaccines hardly induce antibody responses, they failed to achieve HBeAg or HBsAg seroconversion. An alternative DNA prime, poxvirus-boost vaccine encompassing the HBV preS/S region encoding for the HBV envelope proteins showed promising results in chimpanzees, but also failed in a clinical phase IIa trial neither inducing sustained T cell responses nor reducing viremia in chronic HBV carriers. They may have failed to induce T cell help or broad enough, multi-specific immune responses.

Any vaccines known in the art can be used in the methods and regimens provided herein. Preferred embodiments include the prime-boost vaccination scheme with protein antigens administered twice and a nucleic acid vaccine administered once as provided in the Examples and provided in PCT Publication No. WO 2017/121791 (the entire contents of which are incorporated herein by reference). As discussed above, the sequence of the protein antigen in the vaccine and the amino acid sequence encoded by nucleic acid-based vaccine need not be identical. They simply must share at least one epitope, preferably multiple epitopes, so that the sequential administration of the vaccines has the desired prime-boost effect. Further, as discussed herein, in preferred embodiments, the treatment regimen provided herein would provide a treatment regimen effective across multiple, if not all, HBV serotypes. Therefore, it is understood that the antigen delivered to the subject may not have antigen sequences identical to the antigens expressed by the HBV virus that infected the subject.

It is known in the art that some portions of HBV antigens are the main targets of antibodies generated during the initial immune response to infection with HBV known as determinants. For example the HBsAg includes the "a" determinant epitope that is located at amino acids 124 to 147 within the major hydrophilic region (MHR, amino acids 100 to 169) of the 226 amino acid S gene (SEQ ID NO: 8 or 22). This "a" determinant is one of the main targets of anti-HBs antibodies during the course of the initial immune response in acute hepatitis B. In certain embodiments, an immunogenic fragment of HBsAg comprises compared to the full length protein at least amino acids 99 to 168 corresponding to the amino acid positons of the small envelope protein (SEQ ID NO: 23) (see, *e.g.*, Lada *et al.*, J. Virol.(2006) 80:2968-2975, the entire contents of which are incorporated herein by reference).

Similarly, determinants have been identified in HBcAg (see, *e.g.*, Salfeld *et al.*, J. Virol. (1989) 63:798-808, the entire contents of which are incorporated herein by reference). The full-length core protein is 183 amino acids in length and consists of an assembly domain (amino acids 1 to 149) and a nucleic acid-binding domain (amino acids 150 to 183). Three distinct major determinants have been characterized. The single conformational determinant responsible for HBc antigenicity in the assembled core (HBc) and a linear HBe-related determinant (HBel) were both mapped to an overlapping hydrophilic sequence around amino acid 80; a second HBe determinant (HBe2) was assigned to a location in the vicinity of amino acid 138 but found to require for its antigenicity the intramolecular participation of the extended sequence between amino acids 10 and 140. Typically, such an immunogenic fragment comprises, compared to the full length core protein, at least amino acids 18 to 143 corresponding to the sequence positions set forth in SEQ ID NO: 24. Analogous sequences can be identified in SEQ ID NO: 10.

In preferred embodiments, the vaccines include an amino acid sequence or encode an amino acid sequence that includes at least one determinant of HBsAg or HBcAg. Specifically, in certain embodiments, a vaccine targeted to HBsAg includes at least the amino acid sequence of amino acids 127 to 147 of HBsAg, *e.g.*, includes at least amino acids 99 to 168 of the amino acid sequence of the small envelope protein (SEQ ID NO: 23). In certain embodiments, a vaccine targeted to a hydrophilic sequence at least around amino acid 80 of HBcAg or an amino acid sequence at least around amino acid 138, *e.g.*, at least a 40 amino acid portion, at least a 50 amino acid portion, at least a 60 amino acid portion, at least a 70 amino acid portion, at least an 80 amino acid portion, at least a 90 amino acid portion, or at least a 100 amino acid portion of SEQ ID NO: 43 including amino acid 80 or amino acid 138, or a coding sequence therefor. In preferred embodiments, the antigen amino sequence of the antigen targeted to HBcAg includes at least 20 amino acids N-terminal and C-terminal to amino acid 80 or 138 of SEQ ID NO: 24. In certain embodiments, a vaccine targeted to HBcAg includes at least the amino acid sequence of amino acids 10 to 140 or 18 to 143 of HBsAg.

In certain embodiments, the vaccine may comprise the entire amino acid sequence or encode the entire amino acid sequence of any one or more of SEQ ID NO: 22, 23, or 24.

It is understood that there are multiple serotypes of HBV with different nucleic acid sequences that encode different amino acid sequences. Therefore, it is understood that the amino acid sequence of a protein-based vaccine or the amino acid sequence encoded by a nucleic acid-based vaccine may not be 100% identical to the sequences provided in the SEQ ID NOs. In certain embodiments of the invention, the the amino acid sequence of a protein-based vaccine or the amino acid sequence encoded by a nucleic acid-based vaccine is at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, or at least 98% identical to the portion of SEQ ID NO: 22, 23, or 24. Additional exemplary HBsAg and HBcAg amino acid sequenences are provided in the sequence listing. Alignment methods to identify appropriate sequences corresponding to the HBsAg and HBcAg determinants in the sequences indicated above are known in the art.

The vaccine preferably comprises MVA viruses in a concentration range of 10^4 to 10^9 tissue-culture infectious dose $(TCID)_{50}$/ml, preferably in a concentration range of 10^5 to $5x10^8$ $TCID_{50}$/ml, more preferably in a concentration range of 10^6 to 10^8 $TCID_{50}$/ml, and most preferably in a concentration range of 10^7 to 10^8 $TCID_{50}$/ml.

A preferred vaccination dose for humans comprises 10^6 to 10^9 $TCID_{50}$, most preferably a dose of 10^6 $TCID_{50}$ or 10^7 $TCID_{50}$ or 10^8 $TCID_{50}$.

The preferred methods of the invention include administration of both a protein-based vaccine and a nucleic acid-based vaccine. However, other methods include administration of only protein antigens. Less preferred embodiments include administration of only nucleic acids encoding antigens.

i. Adjuvants

As used herein "adjuvant" is understood as an agent that promotes (*e.g.*, enhances, accelerates, or prolongs) an immune response to an antigen with which it is administered to elicit long-term protective immunity. No substantial immune response is directed at the adjuvant itself. Adjuvants include, but are not limited to, pathogen components, particulate adjuvants, and combination adjuvants (see, *e.g.*, www.niaid.nih.gov/research/vaccine-adjuvants-types). Pathogen components (*e.g.*, monophosphoryl lipid A (MPL), poly(I:C), CpG DNA, emulsions such as poly[di(sodiumcarboxylatoethylphenoxy)phosphazene] (PCEP)) can help trigger early non-specific, or innate, immune responses to vaccines by targeting various receptors inside or on the surface of innate immune cells. The innate immune system influences adaptive immune responses, which provide long-lasting protection against the pathogen that the vaccine targets. Particulate adjuvants (*e.g.*, alum, virosomes, cytokines, *e.g.*, IL-12) form very small particles that can stimulate the immune system and also may enhance delivery of antigen to immune cells. Combination adjuvants (*e.g.*, AS02, AS03 and AS04 (GSK); MF59 (Novartis); and IC31® (Altimmune) elicit multiple protective immune responses. Adjuvants that have a modest effect when used alone may induce a more potent immune response when used together. In certain embodiments, preferred adjuvants include c-di-AMP, c-di-GMP, c-di-CMP, PolyICLC, CpG, ISCOMATRIX®, AS02, AS03, AS04, or a RIG-I ligand such as 5' 3P-RNA. In certain embodiments, a viral capsid, with or without a nucleic acid expressing an HBV antigen can be used as an adjuvant. For example, a vaccine that preferentially stimulates T cells such as an MVA-only or a DNA prime, MVA boost or an adenovirus vector prime-MVA boost can be used in the methods of the invention.

In preferred embodiments of the invention, adjuvants for use in the invention promote a humoral and a cellular immune response as discussed above. In certain embodiments, adjuvants provide a balanced Th1/Th2 response.

ii. Non-adjuvant immune stimulators and additional agents

Methods of the invention can further include administration of additional agents used in the treatment of HBV or to stimulate an immune response. Such agents can include an immune

stimulator (*e.g.*, pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and aToll-like receptor 3, 7, 8, or 9 (TLR3, TLR7, TLR8, or TLR9) agonist), a viral entry inhibitor (*e.g.*, Myrcludex), an oligonucleotide that inhibits the secretion or release of HBsAg (*e.g.*, REP 9AC), a capsid inhibitor (*e.g.*, Bay41-4109 and NVR-1221), a cccDNA inhibitor (*e.g.*, IHVR-25), a Rig-I-ligand, a STING agonist, an antibody based immune therapy against HBV (mono-, bi-, or trispecific antibody against HBV), or an immune checkpoint regulator. Such agents are known in the art.

C. Nucleotide and Nucleoside Analogs For Use in the Methods of the Invention

Nucleotide and nucleoside analogs are considered to be the standard of care for HBV infection as they are generally considered safe and inexpensive. However, nucleotide and nucleoside analogs cannot cure HBV infection, may cause the development of resistance, and must be taken indefinitely. Nucleotide analog and nucleoside analogs include, but are not limited to, Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide, Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, AGX-1009, emtricitabine, clevudine, ritonavir, dipivoxil, lobucavir, famvir, FTC, N-Acetyl-Cysteine (NAC), PC1323, theradigm-HBV, thymosin-alpha, ganciclovir, Besifovir (ANA-380/LB-80380), and Tenofvir-Exalidex (TXL/CMX157) . In certain embodiments, the nucelot(s)ide analog is Entecavir (ETV) or Tenofovir or a derivative thereof. In certain embodiments, the nucleot(s)ide analog is not Lamivudine. Nucleot(s)ide analogs are commercially available from a number of sources and are used in the methods provided herein according to their label indication (*e.g.*, typically orally administered at a specific dose) or as determined by a skilled practitioner in the treatment of HBV.

III. Antisense Oligonucleotides Targeting HBV

The present invention includes the use of iRNAs which promote cleavage of at least one HBV transcript, and preferably three or four HBV transcripts. Antisense oligonucleotides can similarly be used to promote cleavage of at least one HBV transcript, preferably three or four HBV transcripts, in the methods of the invention provided here. Exemplary antisense oligonucleotides targeted to HBV are provided, for example, in U.S. Patent Publication Nos. 2013/0035366, 2012/0207709, and 2004/0127446, the contents of each of which is incorporated by reference herein in its entirety.

It is understood by those of skill in the art that conjugates, linkers, and formulations for the delivery of siRNAs as provided above can be used for the formulation and delivery of antisense oligonucleotide therapeutic agents to subjects.

IV. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs or vaccines for use in the invention. It is understood that approved therapeutic agents are formulated and administered by the route indicated on their package instructions.

In some embodiments, provided herein are pharmaceutical compositions containing an iRNA or a vaccine, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA or vaccine are useful for treating subject with an HBV infection. Such pharmaceutical compositions are formulated based on the mode of delivery, *e.g.*, for systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery.

The pharmaceutical RNAi compositions for use in the invention may be administered in dosages sufficient to significantly reduce the level of at least one HBV transcript. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every other week or once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (*i.e.*, about once every three months). In preferred embodiments, the RNAi agent is administered subcutaneously.

Formulation and dosing of the vaccine will depend on the nature of the vaccine administered. In a protein prime-expression vector boost vaccination strategy, the protein-based priming vaccines are administered about 2, 3, or 4 weeks apart with the expression vector vaccine boost being administered about 2, 3, or 4 weeks after the second protein-based vaccine dose. In certain embodiments, it is about two weeks between the first and second doses of the protein-based vaccine. In certain embodiments, it is about two weeks between the second dose of the protein based vaccine and the DNA expression vector vaccine boost. In certain embodiments, the prime and boost vaccinations are administered by routes independently selected from intramuscularly, intradermally, or subcutaneously.

The pharmaceutical nucleic acid-based vaccines for use in the invention may be administered in dosages sufficient to promote an immune response, as either a prime agent or a boost agent. The amount of nucleic acid-based vaccine to be administered will depend, for example, on the design of the vaccine. As the regimens provided herein can include the use of existing nucleic acid-based vaccines, knowledge regarding appropriate dosages based on therapeutic efficacy and safety should be based on the specific agent used.

The pharmaceutical protein-based vaccines for use in the invention may be administered in dosages sufficient to promote an immune response, as either a prime or a boost agent. The amount of protein-based vaccine to be administered will depend, for example, on the adjuvant used. Protein-based vaccines can be dosed, for example, at about 5-100 mg/kg/dose, about 10-50 mg/kg/dose, or about 20-40 mg/kg/dose. As the regimens provided herein can include the use of existing protein-based vaccines, knowledge regarding appropriate dosages based on therapeutic efficacy and safety should be based on the specific agent used.

After an initial treatment regimen, the treatments can be administered on a less frequent basis. In preferred embodiments, the treatment regimens and methods provided herein result in a functional cure allowing for discontinuation of treatment after completion of the regimen or after diagnostic criteria indicate a functional cure, *e.g.*, decreased HBsAg levels preferably to below the level of detection of the methods provided herein and a detectable immune response to HBsAg.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual therapeutic agent used in the methods and regimens invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein and known in the art.

A. iRNA Formulations Comprising Membranous Molecular Assemblies

An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the iRNA composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate iRNA. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the iRNA to particular cell types.

A liposome containing an iRNA agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The iRNA agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the iRNA agent and condense around the iRNA agent to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of the iRNA agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). pH can also adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, *e.g.*, WO 9637194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al., Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987; US Patent No. 4,897,355; US Patent No. 5,171,678; Bangham, *et al. M. Mol. Biol.* 23:238, 1965; Olson, *et al. Biochim. Biophys. Acta* 557:9, 1979; Szoka, *et al. Proc. Natl. Acad. Sci. USA* 75: 4194, 1978; Mayhew, e*t al. Biochim. Biophys. Acta* 775:169, 1984; Kim, *et al. Biochim. Biophys. Acta* 728:339, 1983; and Fukunaga, *et al. Endocrinol.* 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.,* Mayer, *et al. Biochim. Biophys. Acta* 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, *et al. Biochim. Biophys. Acta* 775:169, 1984). These methods are readily adapted to packaging iRNA agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.*, *Biochem. Biophys. Res. Commun.*, 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap nucleic acids rather than complex with it. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, *Journal of Controlled Release*, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC and egg PC. Another type is formed from mixtures of phospholipid or phosphatidylcholine or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include US Patent Nos. 5,283,185 and 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, *J. Biol. Chem.* 269:2550, 1994; Nabel, *Proc. Natl. Acad. Sci.USA* 90:11307, 1993; Nabel, *Human Gene Ther.* 3:649, 1992; Gershon, *Biochem.* 32:7143, 1993; and Strauss *EMBO J.* 11:417, 1992.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu *et al. S.T.P.Pharma. Sci.*, 1994, 4(6) 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, *FEBS Letters*, 1987, 223, 42; Wu *et al.*, *Cancer Research*, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. USA*, 1988, 85, 6949). US Patent No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. US Patent No. 5,543,152 discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 9713499 (Lim *et al*).

Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages *in vivo* and can be used to deliver iRNA agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated iRNA agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and

Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of iRNA agent (see, *e.g.*, Felgner, P. L. *et al.*, *Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987 and US Patent No. 4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, *e.g.*, US Patent No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Commun.* 179:280, 1991). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. *et al.*, *Biochim. Biophys. Acta* 1065:8, 1991). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration. Liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at

the desired target, and the ability to administer iRNA agent into the skin. In some implementations, liposomes are used for delivering iRNA agent to epidermal cells and also to enhance the penetration of iRNA agent into dermal tissues, *e.g.*, into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner *et al., Journal of Drug Targeting,* 1992, vol. 2,405-410 and du Plessis *et al., Antiviral Research*, 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682-690, 1988; Itani, T. *et al. Gene* 56:267-276. 1987; Nicolau, C. *et al. Meth. Enz.* 149:157-176, 1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, L., P*roc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

Liposomes that include iRNA can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transferosomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include iRNA agent can be delivered, for example, subcutaneously by infection in order to deliver iRNA agent to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transferosomes can be self-optimizing (adaptive to the shape of pores, *e.g.*, in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present invention are described in WO 2008042973.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in “Pharmaceutical Dosage Forms”, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and

sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The iRNA for use in the methods of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

B. *Lipid particles*

iRNAs, *e.g.,* dsRNAs of in the invention may be fully encapsulated in a lipid formulation, *e.g.,* a LNP, or other nucleic acid-lipid particle. Expression vectors or RNAs containing coding sequences for viral antigens under the control of an appropriate promoter can be formulated in lipid particles for delivery.

As used herein, the term "LNP" refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in WO 0003683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, US Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; and 6,815,432; US20100324120 and WO 9640964.

In some embodiments, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3- dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3- dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (Dlin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDaP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPz), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle. In other embodiments, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles.

In some embodiments, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

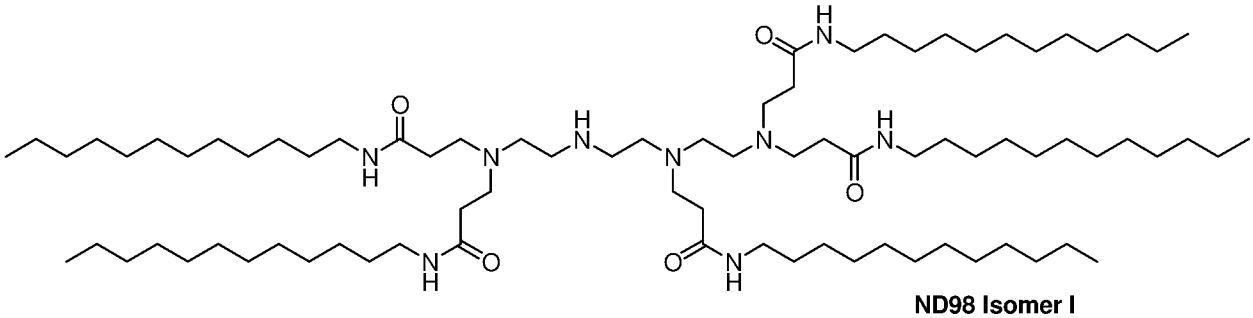
The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-

dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG- distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see US20090023673, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*i.e.,* LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous dsRNA (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

LNP01 formulations are described, *e.g.*, in WO 2008042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are described in Table 1.

Table 1

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
SNALP-1	l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5

	hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO 2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described in PCT Publication No. WO 2010/088537, the entire contents of which are hereby incorporated herein by reference.

MC3 comprising formulations are described, *e.g.*, in U.S. Publication No. 2010/0324120, the entire contents of which are hereby incorporated by reference.

ALNY-100 comprising formulations are described in PCT Publication No. WO 2010/054406, the entire contents of which are hereby incorporated herein by reference.

C12-200 comprising formulations are described in PCT Publication No. WO 2010/129709, the entire contents of which are hereby incorporated herein by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids or esters or salts thereof, bile acids or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan,

poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in US Patent Nos. 6,887,906 and 6,747,014, and US 20030027780, each of which is incorporated herein by reference.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

V. Uses of the Methods of the Invention

The invention sets forth various methods and treatment regimens. It is understood that the methods can be provided as uses of the RNAi agents and vaccines provided herein. That is, the invention provides

a) an RNAi agent that targets at least three HBV transcripts, wherein the RNAi agent comprises a sense strand and an antisense strand;

b) a protein-based vaccine comprising an HBV core antigen (HBcAg) and an HBV surface antigen (HBsAg); and

c) a nucleic acid-based vaccine comprising an expression vector 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

for use in methods of treating a subject having a hepatitis B infection.

The uses include all of the variations and exemplary RNAi agents, protein-based vaccines, and nucleic acid-based vaccines provided herein.

VI. Kits for Practicing the Methods of the Invention

The invention sets forth various methods, treatment regimensm and uses of agents for the treatment of a subject having a hepatitis B infection. It is understood that agents for practicing the

methods of the invention can be prepared based in the disclosure provided herein. Such a kit would include,

a) an RNAi agent that targets at least three HBV transcripts, wherein the RNAi agent comprises a sense strand and an antisense strand;

b) a protein-based vaccine comprising an HBV core antigen (HBcAg) and an HBV surface antigen (HBsAg);

c) a nucleic acid-based vaccine comprising an expression vector 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; and

d) instructions for use in methods of treating a subject having a hepatitis B infection.

The uses include all of the variations and exemplary RNAi agents, protein-based vaccines, and nucleic acid-based vaccines provided herein. The components of the kit may be provided together, *e.g.*, in a box. In certain embodiments, the components of the invention may be provided separately, *e.g.*, due to different storage requirements, but be provided for use together, *e.g.*, based on package instructions for use.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures, Appendix A, and the Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

Example 1. Materials and methods

Exemplary iRNAs

Exemplary iRNA target sites and unmodified and modified siRNA sequences are provided in the tables in Appendix A.

The chemically modified HBV-siRNA duplexes used in the experiments below have the following sequences:

Unmodified sequences:

DuplexID	Sense Sequence Unmodified (5' to 3')	SEQ ID:	Antisense Sequence Umodified (5' to 3')	SEQ ID:
AD-66810	GUGUGCACUUCGCUUCACA	27	UGUGAAGCGAAGUGCACACUU	25
AD-66816	CACCAUGCAACUUUUUCACCU	28	AGGUGAAAAAGUUGCAUGGUGUU	26

Modified sequences:

DuplexID	Sense Sequence Modified (5' to 3')	SEQ ID:	Antisense Sequence Modified (5' to 3')	SEQ ID:
AD-66810	gsusguGfcAfCfUfucgcuucacaL96	37	usGfsugaAfgCfGfaaguGfcAfcacsusu	30
AD-66816	csasccauGfcAfAfCfuuuuucaccuL96	38	asGfsgugAfaAfAfaguuGfcAfuggugsusu	32

Abbreviations for nucleotide monomers in modified nucleic acid sequences are provided in Table 1 of Appendix A.

The target site of AD-66810 is GTGTGCACTTCGCTTCACA (SEQ ID NO: 39) which is nucleotides 1579-1597 of NC_003977.1 (SEQ ID NO: 1).

The target site of AD-66816 is CACCATGCAACTTTTTCACCT (SEQ ID NO: 40) which is nucleotides 1812-1832 of NC_003977.1 (SEQ ID NO: 1).

Cell Culture Evaluation of HBV-siRNA

hNTCP-expressing HepG2 cells were infected (100 multiplicity of infection (MOI)) HBV particles/cell (subtype ayw)) in duplicate. At day 4 after infection, cells were trypsinized and reseeded into multiwell plates and transfected with control or HBV-siRNAs AD-66810 (having the chemical modifications shown in the table above and sense and antisense sequences as set forth in SEQ ID NOs: 37 and 30, respectively) or AD-66816 (having the chemical modifications shown in the table above and sense and antisense sequences as set forth in SEQ ID NOs: 38 and 32, respectively) each delivered at 100 nM, 10 nM, or 1 nM using Lipofectamine® RNAiMax. Supernatant was harvested at days 3, 6, 10, 13, and 17 after reseeding and HBeAg and HBsAg levels were determined relative to non-transfected control. HBsAg and HBeAg levels were determined using a chemiluminescent microparticle immunoassay (CMIA) measured in an Abbott Architect immunoassay analyzer (Abbott Laboratories, Abbott Park, IL, USA).

Mice, siRNA Administration, and Vaccinations

HBV-transgenic mice (StrainHBV1.3xfs (HBV genotype D, subtype ayw)), were derived from in-house breeding under specific pathogen-free conditions following institutional guidelines.

For siRNA administration, mice were subcutaneously administered a 3 mg/kg or 9 mg/kg dose of control siRNA or HBV-siRNA (modified AD-66810 or modified AD-66816); or intravenously administered by tail vein injection 1 x 10^{11} AAV particles for expression of the HBV-shRNA as indicated in the Figures and Examples below.

For protein vaccinations, mice were immunized subcutaneously with recombinant yeast HBsAg and Escherichia coli HBcAg (APP Latvijas Biomedicinas, Riga, Latvia) mixed with 31.91 µg of synthetic phosphorothioated CpG ODN 1668 and 25 µg poly[di(sodiumcarboxylatoethyl-phenoxy)phosphazene] (PCEP) in 50 µl PBS.

Recombinant MVA were generated by homologous recombination and host range selection as described previously (Staib *et al.*, 2003. *Biotechniques*. 34:694–700). The entire HBcAg (genotype D, subtype ayw) and HBsAg open reading frames (genotype A, subtype ayw or adw) were cloned into MVA transfer plasmids pIIIΔHR-PH5 or pIIIΔHR-P7.5, thereby placing the HBV proteins under the control of the early/late Vaccinia virus-specific promoters PH5 (HBcAg ayw /HBsAg ayw/HBsAg adw) or P7.5 (HBsAg ayw). After construction of each virus, gene expression, sequence of inserted DNA, and viral purity were verified. For generation of vaccine preparations, MVA were routinely propagated in CEF, purified by ultracentrifugation through sucrose, reconstituted in 1 mM Tris-HCl pH 9.0 and titrated following standard methodology (Staib *et al.*, 2004. *Methods Mol Biol*. 269:77–100). For MVA vaccination, mice were vaccinated intraperitoneally with 1×10^8 infectious units of respective recombinant MVA in 500 µl PBS.

Specific dosing regimens are provided in the Examples below and in Figures 3, 7, and 12.

Serological analysis

Serum levels of HBsAg and HBeAg were determined in 1:33 dilutions; and anti-HBs levels were determined in a 1:100 dilution using chemiluminescent microparticle immunoassay (CMIA) measured in an Abbott Architect immunoassay analyzer (Abbott Laboratories, Abbott Park, IL, USA). Quantification of serum HBV titers by real-time polymerase chain reaction and determination of HBV DNA levels in serum was performed as described in Untergasser *et al.*, 2006 (*Hepatology*. 43:539–47). The amount of HBV DNA was normalized to DNA level prior to treatment.

Levels of anti-HBc antibodies were determined in 1:20 dilution using the Enzgnost anti-HBc monoclonal test on the BEPIII platform (Both Siemens Healthcare, Eschborn, Germany). If a sample was measured outside the linear range, higher or lower dilutions were used as appropiate.

Quantification of serum HBV titers by real-time polymerase chain reaction and determination of HBV DNA levels in serum was performed as described in Untergasser *et al.*, 2006 (*Hepatology*. 43:539–47). The amount of HBV DNA was normalized to DNA level prior to treatment.

ALT activity was measured at the day of bleeding in a 1:4 dilution in phosphate buffered saline (PBS) using the Reflotron GPT/ALT test (Roche Diagnostics, Mannheim, Germany).

Lymphocyte stimulation assay

Liver-associated lymphocytes (LAL) were isolated as described in Stross *et al.*, 2012 (Hepatology 56:873–83) and stimulated with H2-kb-or H-2Db-restricted peptides (see Backes, 2016) for 12 hours in the presence of 1 mg/ml Brefeldin A (Sigma-Aldrich, Taufkirchen,Germany). Cells were live/dead-stained with ethidium monoazidebromide (Invitrogen®, Karlsruhe, Germany) and blocked with anti-CD16/CD32-Fc-Block (BD Biosciences, Heidelberg, Germany). Surface markers were stained with PB-conjugated anti-CD8-alpha and PE-conjugated anti-CD4 (eBiosciences, Eching, Germany). Intracellular cytokine staining (ICS) was performed with FITC anti-IFN-gamma (XMG1.2), PE-Cy7 anti-TNF-alpha and APC anti-IL-2 (eBiosciences, Ech-ing, Germany) using the

Cytofix/Cytoperm kit (BD Biosciences, Heidelberg, Germany) according to the manufacturer's recommendations. The same data were analyzed twice, the second time with a more rigorous exclusion of dead cells.

Immunohistochemical stainings for HBc-expressing hepatocytes

Livers were harvested, fixed in 4% paraformaldehyde, and paraffin-embedded. The liver was sectioned (2 µm) and sections were stained with rabbit anti-HBcAg as the primary antibody (Diagnostic Biosystems, Pleasanton, CA; #RP 017; 1:50 dilution; retrieval at 100°C for 30 min with EDTA) and a horseradish peroxide coupled secondary antibody. Incubation in Ventana buffer and staining were performed on a NEXES immunohistochemistry robot (Ventana Instruments) using an IVIEW DAB Detection Kit (Ventana Instruments) or on a Bond MAX (Leica Biosystems). For analysis, slides were scanned using a SCN 400 slide scanner and positive cells were counted using the integrated Tissue AI software (both Leica Biosystems).

HBV transcripts from liver lysate

For analysis of HBV RNA from liver lysate, RNA was extracted from 30 mg liver tissue with the RNeasy mini kit (Qiagen) and cDNA was synthesized with the Superscript III kit (Thermo Fisher Scientific). HBV transcripts were amplified with primers specific for only the 3.5 kb transcripts (forward primer 5'- GAGTGTGGATTCGCACTCC-3' (SEQ ID NO: 41); reverese primer 5'- GAGGCGAGGGAGTTCTTCT-3' (SEQ ID NO: 42)), or with primers binding to the common 3´ end of all HBV transcripts (forward primer 5'- TCACCAGCACCATGCAAC-3' (SEQ ID NO: 43); reverse primer 5'- AAGCCACCCAAGGCACAG-3' (SEQ ID NO: 44)) (Denaturation: 95°C 5 min; Amplification: 95°C 3s, 60°C 30s (40 cycles)) (Yan *et al.*, 2012). Results were normalised to murine GAPDH expression (forward primer 5'- ACCAACTGCTTAGCCC-3' (SEQ ID NO: 45); reverse primer 5'- CCACGACGGACACATT-3' (SEQ ID NO: 46)) (Denaturation: 95°C 5 min; Amplification: 95°C 15s, 60C° 10s, 72°C 25s (45 cycles)). All PCR reactions were performed on a LightCycler 480 (Roche Diagnostics).

AAV-HBV mouse model

For the AAV mouse model, wildtype C57/Bl6 mice (9 weeks of age; 6 animals per treatment group) were injected i.v. with $2x10^{10}$ genome equivalents (geq) of Adeno-Associated-Virus Serotype 8 (AAV8) carrying a 1.2-fold overlength HBV genome of genotype D (AAV-HBV1.2) (day -28) (see, *e.g.*, Yang, *et al.* (2014) *Cell and Mol Immunol* 11:71) . Starting 4 weeks after AAV-transduction (day 0), animals were treated with 3 injections (3 mg/kg bw, n=12 per group) of either a control siRNA, or HBV siRNA (AD-66816 or AD-66810) (days 0, 29, and 57). Each siRNA treatment group was divided into two groups (n = 6 per group) to be not treated or treated with the vaccine regimen consisting of recombinant HBsAg, HBcAg (15 µg of each) and 10 µg c-di-AMP given at day 57 and

70 and boosted with MVA-HBs and MVA-HBc ($5x10^7$ geq of each) at day 84. A schematic showing the treatment regimen is provided in Figure 12.

Example 2 – Evaluation of HBV-siRNA in HepG2-NTCP Cell Culture and Dose Finding Experiments in HBVxfs Mice

Anti-HBV siRNAs were evaluated for efficient knockdown of HBV antigens and DNA in an *in vitro* infection model using HBV-infected HepG2-NTCP cells treated with 1 nM, 10 nM, or 100 nM of one of modified AD-66810 or modified AD-66816 HBV-siRNA, or a control siRNA as provided above. Supernatants were collected at days 3, 6, 10, 13, and 17 days after siRNA treatment and assayed for HBeAg and HBsAg levels as compared to untransfected control. Both HBV-siRNAs were demonstrated to effectively knockdown expression of both HBeAg and HBsAg with the highest levels of knockdown observed at days 13 and 17. No significant knockdown was observed with the control siRNA. These data demonstrate that the AD-66810 or AD-66816 HBV-siRNAs are effective at knocking down expression of HBV antigens in a sustained manner in an *in vitro* system of HBV infection.

The HBV and control siRNAs were then tested in the HBVxfs transgenic model of chronic hepatitis B. The HBVxfs mice include an integrated HBV genome that is expressed under the control of a liver-specific promoter. At about 10 weeks of age, mice were administered a 3 or 9 mg/kg dose of one of AD-66810 or AD-66816 HBV-siRNA or a control siRNA (n = 6 per group). Blood samples were collected at days 6, 13, and 21 after siRNA treatment and serum was prepared. HBeAg, HBsAg, and HBV DNA levels in serum (Figures 2A-2C) were determined as provided above. Further, RNA was isolated from liver and total HBV RNA and HBV 3.5 kB transcript levels were detected using the method of Yan, 2012 and normalized to GAPDH expression (Figures 2D and 2E).

Both of the HBV-siRNAs at both doses were demonstrated to effectively knockdown expression of HBsAg and HBeAg and to decrease HBV DNA levels in serum as compared to control levels (see Figures 2A-2C). Futher, total liver HBV RNA relative to GAPDH DNA and 3.5 kb HBV RNA relative to GAPDH RNA levels were strongly decreased by both doses of the HBV siRNAs as compared to control levels (Figures 2D and 2E). Based on these results, the 3 mg/kg dose was selected for use in further experiments.

Example 3 – Comparison of Reducing Antigen Load with siRNA to Nucleot(s)ide Analogs Prior to Therapeutic Vaccine Administration in an HBV Transgenic Mouse Model

Having demonstrated that siRNA targeted to HBV can effectively knockdown expression of HBsAg and HBeAg and decrease HBV DNA levels in serum in the HBVxfs mouse model, the effect of treatment of mice with the nucleoside analog Entecavir or HBV-siRNA prior to therapeutic vaccine administration using a prime-boost regimen was tested. The treatment scheme is shown in Figure 3.

Mice were pretreated with one of six treatment regimens prior to vaccination using a prime-boost regimen (n= 6 per group):

(1) No pretreatment;

(2) Entecavir at 1 μg/ml in water throughout the course of the study beginning on the first day of Week 0 (expected dose of about 4 mg/day based on calculations provided in Lütgehetmann *et al.*, 2011, *Gastroenterology*.140:2074-83);

(3) A 3 mg/kg dose on the first day of Weeks 0, 4, 8, and 12 of the control iRNA agent.

(4) A single dose on the first day of Week 0 with an expression vector encoding an shRNA targeted to HBV (HBV-shRNA) (Michler *et al.*, 2016); or

(5-6) A 3 mg/kg dose on the first day of Weeks 0, 4, 8, and 12 of modified AD-66816 or modified AD-86610 (generically HBV-siRNA).

On the first day of Weeks 12 and 14, a mixture of recombinantly expressed yeast HBsAg (15 μg) and *E. coli* expressed HBcAg (15 μg) adjuvanted with 31.9 μg synthetic phosphorothioated CpGODN 1668 (CpG) and 25 μg poly[di(sodiumcarboxylatoethyl-phenoxy)phosphazene] (PCEP) was subcutaneously administered to all mice as a protein-prime vaccination (Backes, 2016).

On the first day of week 16, a mixture of modified vaccinia viruses Ankara expressing HBsAg or HBcAg (5×10^7 particles of each virus) was subcutaneously administered to all mice as a boost vaccination (Backes, 2016).

Blood samples were obtained on the first days of Week 0, 2, 4, 8, 12, 16, and 17 and serum samples prepared therefrom were assayed for levels of HBsAg, HBeAg, and HBV DNA. Results are shown in Figure 4.

HBsAg and HBeAg levels mice in groups 1, 2, and 3 (mock, Entecavir, control iRNA agent) were similar. The HBV-shRNA or HBV-siRNAs (AD-66816 or AD-86610) alone caused a significant decrease in HBsAg, HBeAg, and HBV DNA in serum (Figures 4A-4C). The three dose prime-boost vaccination scheme resulted in a further decrease in HBsAg in all groups, and reduced the level of HBsAg in at least some animals in the HBV-shRNA and HBV-siRNA groups to below the level of detection. However, vaccine treatment did not decrease HBeAg levels in any of the groups. Without being bound by mechanism, it is proposed that the decrease in HBsAg, but not HBeAg, results from the immune response induced by the vaccine against the s antigen, but not the e antigen, which is produced by proteolytic processing of the C protein (see Figure 5 discussed below).

HBV DNA levels were decreased to about the lower limit of quantitation with Entecavir alone so no effect of the three dose prime-boost vaccine could be detected (Figure 4C). Mock treatment and treatment with the HBV-shRNA, the HBV-siRNAs, and control siRNA all decreased HBV DNA levels and the level of HBV-DNA was further decreased by the prime-boost vaccine in all groups. It is unclear why the mock treatment and control siRNA decreased HBV DNA levels in this experiment. No decrease in HBV DNA was observed in response to treatment with control siRNA in other experiments (see, *e.g.*, Figures 2C and 8C). These data demonstrate that RNAi is superior to nucleot(s)ide analog therapy in reducing viral antigens. Also, RNAi and subsequent vaccination have a combined effect on HBsAg and HBV DNA levels greater than either agent alone.

On the final day of the experiment (first day of Week 17), mice were sacrificed and their livers harvested. Liver associated lymphocytes were isolated from liver and after peptide stimulation CD8+ T cell responses measured via intracellular cytokine staining. Specifically, intrahepatic CD8+ T cell responses were assessed for response to HBsAg, HBcAg, and the MVA virus particle using the method provided in Backes, 2016. The data were analyzed twice using two different thresholds for the exclusion of dead immune cells as the exclusion of dead immune cells in the first analysis provided in Figures 5A-5C was determined to be insufficient. The second analysis is presented in Figures 5D-5F. The second analysis confirmed the conclusions from the first analysis.

Mice pretreated with the HBV-shRNA or the HBV-siRNAs before vaccination were able to generate a CD8+ T cell immune response against the HBsAg and HBcAg (Figures 5A, 5B, 5D, and 5E) indicating that cytotoxic T cells able to clear HBV infection are induced when HBV antigen levels are suppressed prior to vaccination. No significant CD8+ T cell immune response against the HBV antigens was observed in the mock, Entecavir, or control siRNA groups. A significant and similar CD8+ T cell immune response against the MVA virus in all animals, independent of pretreatment or viral antigen levels, demonstrated that vaccination had worked equally well in all animals and was not influenced by HBV antigen levels, thereby demonstrating the presence of a competent immune system (Figures 5C and 5F). No significant differences in antibody production were observed between mock treated animals and any of the other groups indicating that high HBV antigen levels may induce T cell tolerance.

These data demonstrate that RNAi treatment, in contrast to the current standard of care treatment with a nucleoside analog, can restore HBV-specific T cell immunity in the liver and enable the induction of HBV-specific CD8+ T cell responses after therapeutic vaccination. A robust CD8+ effector T cell response has been associated with viral clearance and functional cure (see, *e.g.*, Thimme *et al.*, 2003. *J. Virol.* 77:68-76, and Backes *et al.*, 2016. *Vaccine*. 34:923-932).

RNA was also isolated from liver and total HBV RNA and HBV 3.5 kB transcript levels were detected using the method of Yan, 2012 and normalized to GAPDH expression. Treatment of mice with HBV-siRNA or HBV-shRNA prior to vaccine administration resulted in a significant decrease in HBV total RNA and HBV 3.5 kb transcript as compared to the mock treated control (Figures 6A and 6B). No significant change in HBV total RNA and HBV 3.5 kb transcript was observed in the mice treated with Entecavir or the control siRNA prior to vaccination as compared to the mock treated control. These data demonstrate that both HBV siRNAs and the shRNA, in contrast to the control and Entecavir groups, successfully led to a decrease in HBV transcript levels.

Further, expression of HBV antigens in the liver was analyzed by immunohistochemical staining for HBc of liver sections and counting of HBc positive cells per mm^2 (Figure 6C). Only groups of animals pre-treated with HBV siRNA or shRNA, but not Entecavir or the control siRNA, showed reduced HBc expression following vaccination.

Throughout the experiment, body weight and ALT levels were monitored. No significant differences were observed in any of the treatment groups as compared to mock treated control.

Example 4 – Evaluation of the Effect of Duration of HBV Antigen Knockdown on Response to Immunization in an HBV Transgenic Mouse Model

Having demonstrated that suppression of expression of HBV antigens using shRNA or siRNA is effective at potentiating an immune response to an HBV vaccine regimen, a study was designed to determine if the length of time of HBV antigen suppression had an effect on potentiation of an HBV immune response. A treatment scheme is shown in Figure 7.

Using the HBV1.3xfs mouse model, mice were treated for eight, six, or three weeks with HBV-siRNA AD-66816 (modified) or the control siRNA for 8 weeks, administered subcutaneously at 3 mg/kg/dose. siRNA administration was followed by administration of the prime-boost vaccine regimen as set forth above with the exception that c-di-AMP was used as an adjuvant with protein administration rather than PCEP + CPG (n= 6 per group). Therefore, mice in the 8 week group (n = 6) received three doses of siRNA with the third dose being administered on the first day of vaccine administration. The mice in the 6 week group (n = 12) received two doses of siRNA with the second dose being administered two weeks prior to the first day of vaccine administration. Finally, the mice in the 3 week group (n = 6) received one dose of siRNA with the dose being administered three weeks prior to the first day of vaccine administration.

Blood samples were collected on the first day of -8 weeks, -6 weeks, -4 weeks, -2 weeks, 0 weeks, 2 weeks, 4 weeks, and 6 weeks, before (negative numbers) and after the first dose of vaccine administration on the first day of Week 0. Serum was prepared and HBsAg, HBeAg, and HBV DNA levels were assessed as provided above.

A significant decrease in each HBsAg, HBeAg, and HBV DNA was observed after the first administration of AD-66816 (Figures 8A-8C). A further significant decrease in HBsAg was observed after treatment with the vaccine boost, with the greatest decrease observed in the 8 week pretreatment group to below the level of detection of the assay, representing a greater than 5 log10 decrease in HBsAg level in all treated animals. Immunization caused only slight further reductions (<0.5 log10) of HBV DNA which had been significantly decreased by the siRNA treatment. No further decrease in HBeAg levels was observed in response to the vaccination regimen.

These data demonstrate that efficacy of therapeutic vaccination correlates with duration of antigen suppression before start of vaccination. Reconstitution of HBV-specific CD8+ T cell responses takes several weeks, with a 6 or preferably 8 week pretreatment rather than a 3 week pretreatment.

On the final day of the experiment, on the first day of Week 6 after the start of immunization, mice were sacrificed and their livers harvested for six mice from each group. Liver associated lymphocytes were isolated and a lymphocyte stimulation assay was performed as provided above. Specifically, intrahepatic CD8+ T cell responses were assessed for response to HBsAg, HBcAg, and the MVA virus particle using the method provided in Backes, 2016. T-cell responses against HBsAg and HBcAg in liver corresponded with the duration of HBV antigen knockdown, with a trend of

higher levels of immune response observed with longer duration of HBV antigen resulting in greater T-cell response (see Figures 9A-9C). Similar responses to MVA virus antigens were observed across all groups, independent of pretreatment, showing that vaccination had worked equally well in all animals and was not influenced by HBV antigen levels demonstrating the presence of a competent immune system (Figure 9D). No significant differences in antibody production were observed between control siRNA treated animals and any of the other groups. This demonstrates, that reconstitution of HBV-specific CD8+ T cell responses does not occur immediately, with stronger responses seen after therapeutic vaccination if animals had lowered HBV antigen titers for at least 6 or 8 weeks compared to only 3 weeks. It further confirms the previous finding that, in contrast to T cell responses, B cell immunity does not seem to be significant influenced by HBV antigen titers.

RNA was also isolated from liver and total HBV RNA and HBV 3.5 kB transcript levels were detected using the method of Yan, 2012 and normalized to GAPDH expression. Treatment of mice with HBV-siRNA AD-66816 prior to vaccine administration resulted in a significant decrease in HBV total RNA and HBV 3.5 kb transcript in liver lysates as compared to the control siRNA treated control (Figures 10A and 10B). Further, HBV antigen expression in the liver was analyzed by immunohistochemical staning and counting of HBc positive cells per mm^2 (Figure 10C). Correlating the observed increase in HBV-specific CD8 responses with increased duration of siRNA pretreatment, decreased numbers of HBc expressing cells where observed in the liver. These results demonstrate that the CD8+ T cell responses did prevent antigen expression in the liver..

To assess the durability of response, blood samples were collected from mice pretreated with the AD-66816 HBV-siRNA using the six week treatment regimen at 2 and 3 weeks after administration of the boost vaccination (Figures 11A-11D). In three of the six mice, HBsAg levels continued to drop to below the level of detection of the assay (Figure 11A). No similar decrease in HBeAg levels were observed during the course of the experiment (Figure 11B). These data show that the maximum effect by the siRNA-vaccination combinatorial therapy provided herein is later than 1 week after the MVA vaccination, which was chosen as termination time point to best asses CD8+ T cell responses. Anti-HBs antibody response (Figure 11C) and T cell immune response in the liver against HBs(S208) (Figure 11D) at week 7 after the first vaccine dose after the 6 week regimen in the dosing regimen. The antibody response varied among animals.

These data demonstrate that a functional cure is possible using the treatment regimens provided herein. Further, these data suggest that a lower HBsAg and HBeAg burden can result in a greater level of immune clearance of HB antigens and potentiate an immune response, at least within the short time course of the experiment.

Throughout the experiment, body weight and ALT levels were monitored. No significant differences were observed in any of the treatment groups as compared to mock treated control.

Example 5 – Evaluation of the Effect of Duration of HBV Antigen Knockdown on Response to Immunization in an AAV-HBV Mouse Model

Having demonstrated the efficacy of the siRNA-vaccine combination treatment regimen in a transgenic mouse model, an AAV-HBV infection mouse model was used to study the efficacy of the treatment regimen in acquired infection model (see, *e.g.,* Yang, *et al.* (2014) *Cell Mol Immunol* 11:71). This mouse model exhibits sustained HBV viremia after infection with a recombinant adeno-associated virus (AAV) carrying a replicable HBV genome.

There are a number of differences between the HBV-transgenic and AAV-HBV mouse models. The HBV transgenic mice can express HBV antigens essentially from birth, whereas the AAV-HBV model allows for the introduction of the HBV genome at a later time in the life of the mice. This may have an effect on immune tolerance. Further, the HBV transgenic mice carry the transgene in every cell of the body, providing the possibility of "leaky" extrahepatic expression. Although the AAV8 serotype could infect cells outside of the liver, it has a strong liver tropism. Moreover, it is not possible to clear an HBV infection in a transgenic mouse. When a transgenic HBV expressing liver cell is killed, it is replaced by a new HBV expressing cell. In the infection model, if the infected cells are killed, the newly dividing cells are not infected at the time of cell division.

Nine week old C57/Bl6 mice were infected with AAV-HBV (- 28 days). Mice were then treated with one of control siRNA, or one of two HBV-siRNAs, modified AD-66816 or modified AD-66810 at 3 mg/kg administered subcutaneously on days 0, 29, and 57 (*i.e.*, 0 weeks, 4 weeks, 8 weeks) (n=12 per group). Each siRNA treatment group was divided into two groups (n=6 per group). One group was treated with the HBV vaccine protocol (protein prime on days 57 and 70, and MVA boost on day 84, *i.e.*, weeks 8, 10, and 12, respectively) and one group was not. A schematic showing the dosing regimen is provided in Figure 12. Mice were monitored throughout the experiment for serum HBsAg, HBeAg, anti-HBs antibodies, body weight, and ALT. Anti-HBe antibody levels were also periodically tested.

Figures 13A and 13B show an increase in HBsAg and HBeAg levels comparable to that seen in transgenic mice within two weeks of transduction with the AAV-HBV virus. Mice treated only with the control siRNA replicated HBV for greater than 8 months at levels comparable to chronically infected humans (HBsAg levels around 2,000 IU/ml, HBV viremia 10^6-10^7 IU/ml). HBV siRNAs AD-66816 and AD-66810 reduced HBsAg by 2 and 2.5 $\log_{10}$, respectively, and HBeAg by $\geq$1 $\log_{10}$. The effect persisted for at least 4 weeks after stopping siRNA treatment before antigenemia slowly rebounded to baseline levels after 18 weeks (Figures 13A and13B).

Intrahepatic HBV DNA (Figure 13D) and AAV DNA (Figure 13E) levels were determined at week 22 by qPCR as described above. The relative expression in liver of HBV 3.5 RNA relative to GAPDH RNA (Figure 13F) and total HBV RNA relative to GAPDH RNA (Figure 13G) were determined using rtPCR. Mice treated with the combined siRNA-vaccine protocol demonstrated a significant decrease in total HBV DNA and AAV DNA as compared to untreated control or siRNA or

vaccine treatement alone at the 22 week time point (Figures 13D and 13E). Notably, total HBV DNA levels dropped to less than one copy per cell as a result of the combination treatment (Figure 13D). Mice treated with the combined siRNA-vaccine protocol also demonstrated a significant decrease level of HBV 3.5 kb RNA expression relative to GAPDH RNA expression as compared to all other treatment regimens (Figure 13F). A significant decrease in total HBV RNA relative to GAPDH RNA expression was observed as compared to treatment with vaccine or siRNA alone (Figure 13G). These data suggest that a short course of administration of siRNA alone or a therapeutic vaccine against HBV is insufficient to durably suppress HBV infection. Immune mediated control of HBV after siRNA knockdown of HBV expression is long lasting. At 22 weeks after the last siRNA dose, the effect of the siRNA was waning as seen in the groups which had only received the HBV siRNAs without vaccination. Mice treated with the siRNA-vaccine protocol maintained HBV DNA and RNA suppression long after the end of siRNA administration.

No immune responses were observed in these fully immune competent mice under siRNA treatment alone, but vaccine treatment resulted in anti-HBs seroconversion in all vaccinated animals (Figures 14A and 14B). siRNA-pretreated animals, however, developed 10-fold higher and more constant anti-HBs titers and were able to completely and persistently clear serum HBsAg and HBeAg. In contrast, anti-HBe seroconversion was only observed in antimals pretreated with HBV siRNAs. Interestingly, three of the 12 mice vaccinated after HBV siRNA treatment showed a transient relapse of HBeAg between week 15 and 22 co-inciding with decreased levels of anti-HBe (Figure 13C). Without being bound by mechanism, it is proposed that theHBeAg relapse was controlled by a memory immune response induced by the vaccine. Taken together, suppression of HBV antigen expression by an siRNA in combination with a heterologous prime-boost vaccine is sufficient to break immune tolerance to HBV antigens. The sequential therapy achieved long-term functional cure in a mouse model of persistent HBV infection without causing significant liver damage.

Figures 14A and 14B show that animals treated with HBV siRNA plus the vaccine regimen developed high titers of anti-HBs antibodies and anti-HBe antibodies. The level of anti-HBs antibodies continued to increase after the last vaccine dose. Although anti-HBs antibodies could also be measured in animals that received the control siRNA plus the vaccine regimen, the levels were significantly lower. Further, only animals that received HBV siRNA plus the vaccine regimen developed anti-HBe antibodies and achieved anti-HBe seroconversion. The combinatorial therapy using siRNA and vaccine appeared to be well tolerated. All mice equally gained weight and only a mild ALT elevation (<2-fold upper limit of normal) was observed (Figures 15A and 15B). The loss of antigenemia concided with slight increases of ALT activity seen in treatment groups which had received HBV siRNA in conjunction with the vaccination regimen (Figure 15A). These groups showed significant but mild increases (both $p>0.05$ or smaller by repeated measure two-way ANOVA; only comparing time points after start of vaccination) as compared to all other treatment groups that did not receive the combination HBVsiRNA-vaccine regimen. There was a steady increase in body weight in all animals throughout the experiment independent of siRNA treatment.

Animals that were vaccinated showed a slight and transient decrease (approximately 5%) of body weight after vaccination, but rebounded to normal levels within nine days and subsequently gained weight comparable to the control groups (Figure 15B).

Without being bound by mechanism, the data provided herein strongly suggest that the high level of HBV antigen expression routinely detected as circulating HBsAg and HBeAg prevents HBV-specific CD8+ T cell responses, which has far reaching consequences for future immune therapy of chronic hepatitis B. Using 2 different mouse models for chronic hepatitis B, it is proposed that HBV-specific immunomodulation can be reverted by suppressing HBV protein expression in hepatocytes using an RNAi-based therapy. Such reduction of HBV antigens by RNAi, in contrast to standard-of-care nucleo(t)side analogues, allows for induction of strong HBV-specific CD8+ T cell responses by therapeutic vaccination that are required for control of HBV infection.

APPENDIX A

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that, unless otherwise indicated, these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage

Abbreviation	Nucleotide(s)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(dT)	2`-deoxythymidine-3`-phosphate
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	2-hydroxymethyl-tetrahydrofurane-5-phosphate
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA)
P	Phosphate
VP	Vinyl-phosphate

Table 2. Exemplary Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-61522	A-123463	AGUUAUAUGGAUGAUGUGGUA	47	A-123464	UACCACAUCAUCCAUAUAACUGA	263	731_753
AD-61547	A-123487	GGAUGUGUCUGCGGCGUUUUA	48	A-123488	UAAAACGCCGCAGACACAUCCAG	264	373_395
AD-63938	A-127896	ACUCGUGGUGGACUUCUCUCA	49	A-127897	UGAGAGAAGUCCACCACGAGUCU	265	250_272
AD-63939	A-127909	ACUCGUGGUGGACUUCUCUCA	50	A-127906	UGAGAGAAGUCCACCACGAGUCU	266	250_272
AD-63940	A-127917	ACUCGUGGUGGACUUCTCUCA	51	A-127906	UGAGAGAAGUCCACCACGAGUCU	267	250_272
AD-63941	A-127905	ACUCGUGGUGGACUUCUCUCA	52	A-127925	UGAGAGAAGUCCACCACGAGUCU	268	250_272
AD-63942	A-127933	UCGUGGUGGACUUCUCUCA	53	A-127934	UGAGAGAAGUCCACCACGAGU	269	252_274
AD-63943	A-127944	ACUCGUGGUGGACUUCUCUCA	54	A-127942	UGAGAGAAGUCCACCACGAGUCU	270	250_272
AD-63945	A-127910	ACUCGUGGUGGACUUCUCUCA	55	A-127906	UGAGAGAAGUCCACCACGAGUCU	271	250_272
AD-63946	A-127918	ACUCGUGGUGGACUUCUCUCA	56	A-127906	UGAGAGAAGUCCACCACGAGUCU	272	250_272
AD-63947	A-127905	ACUCGUGGUGGACUUCUCUCA	57	A-127926	UGAGAGAAGUCCACCACGAGUCU	273	250_272
AD-63948	A-127935	GUGGUGGACUUCUCUCA	58	A-127936	UGAGAGAAGUCCACCACGA	274	254_276
AD-63949	A-127944	ACUCGUGGUGGACUUCUCUCA	59	A-127906	UGAGAGAAGUCCACCACGAGUCU	275	250_272
AD-63950	A-127900	UCGUGGUGGACUUCUCUCAUU	60	A-127901	UGAGAGAAGUCCACCACGAUU	276	252_274
AD-63951	A-127911	ACUCGUGGUGGACUUCUCUCA	61	A-127906	UGAGAGAAGUCCACCACGAGUCU	277	250_272
AD-63952	A-127905	ACUCGUGGUGGACUUCUCUCA	62	A-127919	UGAGAGAAGUCCACCACGAGUCU	278	250_272
AD-63953	A-127905	ACUCGUGGUGGACUUCUCUCA	63	A-127927	UGAGAGAAGUCCACCACGAGUCU	279	250_272
AD-63955	A-127945	ACUCGUGGUGGACUUCUCUCA	64	A-127940	UGAGAGAAGUCCACCACGAGUCU	280	250_272
AD-63956	A-127902	UCGUGGUGGACUUCUCUCA	65	A-127903	UGAGAGAAGUCCACCACGAUU	281	252_274
AD-63957	A-127912	ACUCGUGGUGGACUUCUCUCA	66	A-127906	UGAGAGAAGUCCACCACGAGUCU	282	250_272
AD-63958	A-127905	ACUCGUGGUGGACUUCUCUCA	67	A-127920	UGAGAGAAGUCCACCACGAGUCU	283	250_272
AD-63959	A-127905	ACUCGUGGUGGACUUCUCUCA	68	A-127928	UGAGAGAAGUCCACCACGAGUCU	284	250_272
AD-63960	A-126619	UAUUUCCUAGGGUACAA	69	A-127938	UGAGAGAAGUCCACCACGA	285	254_276
AD-63961	A-127945	ACUCGUGGUGGACUUCUCUCA	70	A-127942	UGAGAGAAGUCCACCACGAGUCU	286	250_272

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-63962	A-127902	UCGUGGUGGACUUCUCUCA	71	A-127904	UGAGAGAAGUCCACCACGAUU	287	252_274
AD-63963	A-127913	ACUCGUGGUGGACUUCUCUCA	72	A-127906	UGAGAGAAGUCCACCACGAGUCU	288	250_272
AD-63964	A-127905	ACUCGUGGUGGACUUCUCUCA	73	A-127921	UGAGAGAAGUCCACCACGAGUCU	289	250_272
AD-63965	A-127905	ACUCGUGGUGGACUUCUCUCA	74	A-127929	UGAGAGAAGUCCACCACGAGUCU	290	250_272
AD-63966	A-127939	ACUCGUGGUGGACUUCUCUCA	75	A-127940	UGAGAGAAGUCCACCACGAGUCU	291	250_272
AD-63967	A-127945	ACUCGUGGUGGACUUCUCUCA	76	A-127906	UGAGAGAAGUCCACCACGAGUCU	292	250_272
AD-63968	A-127905	ACUCGUGGUGGACUUCUCUCA	77	A-127906	UGAGAGAAGUCCACCACGAGUCU	293	250_272
AD-63968	A-127905	ACUCGUGGUGGACUUCUCUCA	78	A-127906	UGAGAGAAGUCCACCACGAGUCU	294	250_272
AD-63968	A-127905	ACUCGUGGUGGACUUCUCUCA	79	A-127906	UGAGAGAAGUCCACCACGAGUCU	295	250_272
AD-63969	A-127914	ACUCGUGGUGGACUUCUCUCA	80	A-127906	UGAGAGAAGUCCACCACGAGUCU	296	250_272
AD-63970	A-127905	ACUCGUGGUGGACUUCUCUCA	81	A-127922	UGAGAGAAGUCCACCACGAGUCU	297	250_272
AD-63971	A-127905	ACUCGUGGUGGACUUCUCUCA	82	A-127930	UGAGAGAAGUCCACCACGAGUCU	298	250_272
AD-63972	A-127941	ACUCGUGGUGGACUUCUCUCA	83	A-127942	UGAGAGAAGUCCACCACGAGUCU	299	250_272
AD-63973	A-127946	ACUCGUGGUGGACUUCUCUCA	84	A-127947	UGAGAGAAGTCCACCACGAGUCU	300	250_272
AD-63975	A-127915	ACUCGUGGUGGACUUCTCUCA	85	A-127906	UGAGAGAAGUCCACCACGAGUCU	301	250_272
AD-63976	A-127905	ACUCGUGGUGGACUUCUCUCA	86	A-127923	UGAGAGAAGUCCACCACGAGUCU	302	250_272
AD-63977	A-127917	ACUCGUGGUGGACUUCTCUCA	87	A-127931	UGAGAGAAGUCCACCACGAGUCU	303	250_272
AD-63978	A-127943	ACUCGUGGUGGACUUCUCUCA	88	A-127906	UGAGAGAAGUCCACCACGAGUCU	304	250_272
AD-63979	A-127908	ACUCGUGGUGGACUUCUCUCA	89	A-127906	UGAGAGAAGUCCACCACGAGUCU	305	250_272
AD-63980	A-127916	ACUCGUGGUGGACUUCTCUCA	90	A-127906	UGAGAGAAGUCCACCACGAGUCU	306	250_272
AD-63981	A-127905	ACUCGUGGUGGACUUCUCUCA	91	A-127924	UGAGAGAAGUCCACCACGAGUCU	307	250_272
AD-63982	A-127917	ACUCGUGGUGGACUUCTCUCA	92	A-127932	UGAGAGAAGUCCACCACGAGUCU	308	250_272
AD-63983	A-127944	ACUCGUGGUGGACUUCUCUCA	93	A-127940	UGAGAGAAGUCCACCACGAGUCU	309	250_272
AD-63985	A-127961	GUGGUGGACUUCUCUCAAUUU	94	A-127956	AAAUUGAGAGAAGUCCACCACGA	310	254_276
AD-63986	A-127969	GUGGUGGACUUCUCUCAAUUU	95	A-127956	AAAUUGAGAGAAGUCCACCACGA	311	254_276
AD-63987	A-127955	GUGGUGGACUUCUCUCAAUUU	96	A-127977	AAAUUGAGAGAAGUCCACCACGA	312	254_276
AD-63988	A-127986	UGGACUUCUCUCAAUUU	97	A-127987	AAAUUGAGAGAAGUCCACC	313	258_280

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-63989	A-127996	GUGGUGGACUUCUCUCAAUUU	98	A-127992	AAAUUGAGAGAAGUCCACCACGA	314	254_276
AD-63990	A-127950	GGUGGACUUCUCUCAAUUUUU	99	A-127951	AAAUUGAGAGAAGUCCACCUU	315	256_278
AD-63991	A-127962	GUGGUGGACUUCUCUCAAUUU	100	A-127956	AAAUUGAGAGAAGUCCACCACGA	316	254_276
AD-63992	A-127955	GUGGUGGACUUCUCUCAAUUU	101	A-127970	AAAUUGAGAGAAGUCCACCACGA	317	254_276
AD-63993	A-127955	GUGGUGGACUUCUCUCAAUUU	102	A-127978	AAAUUGAGAGAAGUCCACCACGA	318	254_276
AD-63994	A-127984	GGUGGACUUCUCUCAAUUU	103	A-127988	AAAUUGAGAGAAGUCCACCAC	319	256_278
AD-63995	A-127996	GUGGUGGACUUCUCUCAAUUU	104	A-127993	AAAUUGAGAGAAGUCCACCACGA	320	254_276
AD-63996	A-127952	GGUGGACUUCUCUCAAUUU	105	A-127953	AAAUUGAGAGAAGUCCACCUU	321	256_278
AD-63997	A-127963	GUGGUGGACUUCUCUCAAUUU	106	A-127956	AAAUUGAGAGAAGUCCACCACGA	322	254_276
AD-63999	A-127955	GUGGUGGACUUCUCUCAAUUU	107	A-127979	AAAUUGAGAGAAGUCCACCACGA	323	254_276
AD-64000	A-127986	UGGACUUCUCUCAAUUU	108	A-127989	AAAUUGAGAGAAGUCCACC	324	258_280
AD-64001	A-127996	GUGGUGGACUUCUCUCAAUUU	109	A-127994	AAAUUGAGAGAAGUCCACCACGA	325	254_276
AD-64002	A-127952	GGUGGACUUCUCUCAAUUU	110	A-127954	AAAUUGAGAGAAGUCCACCUU	326	256_278
AD-64003	A-127964	GUGGUGGACUUCUCUCAAUUU	111	A-127956	AAAUUGAGAGAAGUCCACCACGA	327	254_276
AD-64004	A-127955	GUGGUGGACUUCUCUCAAUUU	112	A-127972	AAAUUGAGAGAAGUCCACCACGA	328	254_276
AD-64005	A-127955	GUGGUGGACUUCUCUCAAUUU	113	A-127980	AAAUUGAGAGAAGUCCACCACGA	329	254_276
AD-64006	A-127990	GUGGUGGACUUCUCUCAAUUU	114	A-127991	AAAUUGAGAGAAGUCCACCACGA	330	254_276
AD-64007	A-127996	GUGGUGGACUUCUCUCAAUUU	115	A-127995	AAAUUGAGAGAAGUCCACCACGA	331	254_276
AD-64008	A-127955	GUGGUGGACUUCUCUCAAUUU	116	A-127956	AAAUUGAGAGAAGUCCACCACGA	332	254_276
AD-64008	A-127955	GUGGUGGACUUCUCUCAAUUU	117	A-127956	AAAUUGAGAGAAGUCCACCACGA	333	254_276
AD-64009	A-127965	GUGGUGGACUUCUCUCAAUUU	118	A-127956	AAAUUGAGAGAAGUCCACCACGA	334	254_276
AD-64010	A-127955	GUGGUGGACUUCUCUCAAUUU	119	A-127973	AAAUUGAGAGAAGUCCACCACGA	335	254_276
AD-64011	A-127955	GUGGUGGACUUCUCUCAAUUU	120	A-127981	AAAUUGAGAGAAGUCCACCACGA	336	254_276
AD-64012	A-127990	GUGGUGGACUUCUCUCAAUUU	121	A-127992	AAAUUGAGAGAAGUCCACCACGA	337	254_276
AD-64013	A-127997	GUGGUGGACTTCUCUCAAUUU	122	A-127998	AAAUUGAGAGAAGTCCACCACGA	338	254_276
AD-64014	A-127957	GUGGUGGACUUCUCUCAAUUU	123	A-127958	AAAUUGAGAGAAGUCCACCACGA	339	254_276
AD-64015	A-127966	GUGGUGGACUUCUCUCAAUUU	124	A-127956	AAAUUGAGAGAAGUCCACCACGA	340	254_276

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-64016	A-127955	GUGGUGGACUUCUCUCAAUUU	125	A-127974	AAAUUGAGAGAAGUCCACCACGA	341	254_276
AD-64017	A-127968	GUGGUGGACUTCUCUCAAUUU	126	A-127982	AAAUUGAGAGAAGTCCACCACGA	342	254_276
AD-64018	A-127990	GUGGUGGACUUCUCUCAAUUU	127	A-127993	AAAUUGAGAGAAGUCCACCACGA	343	254_276
AD-64019	A-127959	GUGGUGGACUUCUCUCAAUUU	128	A-127956	AAAUUGAGAGAAGUCCACCACGA	344	254_276
AD-64020	A-127967	GUGGUGGACUUCUCUCAAUUU	129	A-127956	AAAUUGAGAGAAGUCCACCACGA	345	254_276
AD-64021	A-127955	GUGGUGGACUUCUCUCAAUUU	130	A-127975	AAAUUGAGAGAAGUCCACCACGA	346	254_276
AD-64022	A-127968	GUGGUGGACUTCUCUCAAUUU	131	A-127983	AAAUUGAGAGAAGTCCACCACGA	347	254_276
AD-64023	A-127990	GUGGUGGACUUCUCUCAAUUU	132	A-127994	AAAUUGAGAGAAGUCCACCACGA	348	254_276
AD-64024	A-127960	GUGGUGGACUUCUCUCAAUUU	133	A-127956	AAAUUGAGAGAAGUCCACCACGA	349	254_276
AD-64025	A-127968	GUGGUGGACUTCUCUCAAUUU	134	A-127956	AAAUUGAGAGAAGUCCACCACGA	350	254_276
AD-64026	A-127955	GUGGUGGACUUCUCUCAAUUU	135	A-127976	AAAUUGAGAGAAGUCCACCACGA	351	254_276
AD-64027	A-127984	GGUGGACUUCUCUCAAUUU	136	A-127985	AAAUUGAGAGAAGUCCACCAC	352	256_278
AD-64028	A-127990	GUGGUGGACUUCUCUCAAUUU	137	A-127995	AAAUUGAGAGAAGUCCACCACGA	353	254_276
AD-64272	A-128001	GUGCACUUCGCUUCACCUCUG	138	A-128002	CAGAGGUGAAGCGAAGUGCACAC	354	1577_1599
AD-64274	A-128363	GUUGACAAAAAUCCUCACAAU	139	A-128364	AUUGUGAGGAUUUUUGUCAACAA	355	215_237
AD-64275	A-128377	UGUUGACAAAAAUCCUCACAA	140	A-128378	UUGUGAGGAUUUUUGUCAACAAG	356	214_236
AD-64276	A-128393	GGUGGACUUCUCUCAAUUUUA	141	A-128394	UAAAAUUGAGAGAAGUCCACCAC	357	256_278
AD-64277	A-128407	UCUUUUGGAGUGUGGAUUCGA	142	A-128408	UCGAAUCCACACUCCAAAAGACA	358	2259_2281
AD-64277	A-128407	UCUUUUGGAGUGUGGAUUCGA	143	A-128408	UCGAAUCCACACUCCAAAAGACA	359	2259_2281
AD-64278	A-128423	ACUGUUCAAGCCUCCAAGCUA	144	A-128424	UAGCUUGGAGGCUUGAACAAGAC	360	1857_1879
AD-64279	A-128435	UCUGCCGAUCCAUACUGCGGA	145	A-128436	UCCGCAGUAUGGAUCGGCAGAGG	361	1255_1277
AD-64280	A-128379	AUGUGUCUGCGGCGUUUUAUA	146	A-128380	UAUAAAACGCCGCAGACACAUCC	362	375_397
AD-64281	A-128395	CCCCGUCUGUGCCUUCUCAUA	147	A-128396	UAUGAGAAGGCACAGACGGGGAG	363	1545_1567
AD-64282	A-128409	GCCUAAUCAUCUCUUGUUCAU	148	A-128410	AUGAACAAGAGAUGAUUAGCGAG	364	1831_1853
AD-64283	A-128425	UCUAGACUCGUGGUGGACUUC	149	A-128426	GAAGUCCACCACGAGUCUAGACU	365	245_267
AD-64284	A-128437	CUGCCGAUCCAUACUGCGGAA	150	A-128438	UUCCGCAGUAUGGAUCGGCAGAG	366	1256_1278
AD-64285	A-128365	UUUUUCUUGUUGACAAAAAUA	151	A-128366	UAUUUUUGUCAACAAGAAAAACC	367	207_229

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-64286	A-128381	AUCUUCUUGUUGGUUCUUCUA	152	A-128382	UAGAAGAACCAACAAGAAGAUGA	368	426_448
AD-64289	A-128367	GUUUUUCUUGUUGACAAAAAU	153	A-128368	AUUUUUGUCAACAAGAAAAACCC	369	206_228
AD-64290	A-128383	CUGCCUAAUCAUCUCUUGUUA	154	A-128384	UAACAAGAGAUGAUUAGGCAGAG	370	1829_1851
AD-64291	A-128399	UCCUCACAAUACCACAGAGUA	155	A-128400	UACUCUGUGGUAUUGUGAGGAUU	371	226_248
AD-64292	A-128413	CUUGUUGACAAAAAUCCUCAA	156	A-128414	UUGAGGAUUUUUGUCAACAAGAA	372	212_234
AD-64293	A-128439	GCAACUUUUUCACCUCUGCCU	157	A-128440	AGGCAGAGGUGAAAAAGUUGCAU	373	1814_1836
AD-64294	A-128369	GGGAACAAGAGCUACAGCAUA	158	A-128370	UAUGCUGUAGCUCUUGUUCCCAA	374	2828_2850
AD-64295	A-128385	CGUGGUGGACUUCUCUCAAUU	159	A-128386	AAUUGAGAGAAGUCCACCAGCAG	375	253_275
AD-64297	A-128415	CUGCUGCUAUGCCUCAUCUUA	160	A-128416	UAAGAUGAGGCAUAGCAGCAGGA	376	411_433
AD-64298	A-128427	GUUGGAUGUGUCUGCGGCGUU	161	A-128428	AACGCCGCAGACACAUCCAACGA	377	370_392
AD-64299	A-128441	UUCAUCCUGCUGCUAUGCCUA	162	A-128442	UAGGCAUAGCAGCAGGAUGAAGA	378	405_427
AD-64300	A-128371	UUCUUGUUGACAAAAAUCCUA	163	A-128372	UAGGAUUUUUGUCAACAAGAAAA	379	210_232
AD-64302	A-128417	UAUAUGGAUGAUGUGGUAUUA	164	A-128418	UAAUACCACAUCAUCCAUAUAAC	380	734_756
AD-64303	A-128429	UUCAUCCUGCUGCUAUGCCUC	165	A-128430	GAGGCAUAGCAGCAGGAUGAAGA	381	405_427
AD-64304	A-128443	GUGCACUUCGCUUCACCUCUA	166	A-128444	UAGAGGUGAAGCGAAGUGCACAC	382	1577_1599
AD-64305	A-128373	UUGACAAAAAUCCUCACAAUA	167	A-128374	UAUUGUGAGGAUUUUUGUCAACA	383	216_238
AD-64307	A-128403	AAGCCUCCAAGCUGUGCCUUA	168	A-128404	UAAGGCACAGCUUGGAGGCUUGA	384	1864_1886
AD-64308	A-128419	CCUCUUCAUCCUGCUGCUAUA	169	A-128420	UAUAGCAGCAGGAUGAAGAGGAA	385	401_423
AD-64309	A-128431	CCUGCUGCUAUGCCUCAUCUU	170	A-128432	AAGAUGAGGCAUAGCAGCAGGAU	386	410_432
AD-64310	A-128375	CAUCUUCUUGUUGGUUCUUCU	171	A-128376	AGAAGAACCAACAAGAAGAUGAG	387	425_447
AD-64311	A-128391	CCGUCUGUGCCUUCUCAUCUA	172	A-128392	UAGAUGAGAAGGCACAGACGGGG	388	1547_1569
AD-64312	A-128405	CCUCAUCUUCUUGUUGGUUCU	173	A-128406	AGAACCAACAAGAAGAUGAGGCA	389	422_444
AD-64313	A-128421	CCACCAAAUGCCCCUAUCUUA	174	A-128422	UAAGAUAGGGGCAUUUGGUGGUC	390	2298_2320
AD-64314	A-128433	GCUCCUCUGCCGAUCCAUACU	175	A-128434	AGUAUGGAUCGGCAGAGGAGCCA	391	1250_1272
AD-64315	A-128363	GUUGACAAAAAUCCUCACAAU	176	A-128445	AUUGUGAGGAUUUUUGUCAACAA	392	215_237
AD-64316	A-128377	UGUUGACAAAAAUCCUCACAA	177	A-128453	UUGUGAGGAUUUUUGUCAACAAG	393	214_236
AD-64317	A-128393	GGUGGACUUCUCUCAAUUUUA	178	A-128461	UAAAAUUGAGAGAAGUCCACCAC	394	256_278

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-64318	A-128407	UCUUUUGGAGUGUGGAUUCGA	179	A-128469	UCGAAUCCACACUCCAAAAGACA	395	2259_2281
AD-64318	A-128407	UCUUUUGGAGUGUGGAUUCGA	180	A-128469	UCGAAUCCACACUCCAAAAGACA	396	2259_2281
AD-64319	A-128423	ACUGUUCAAGCCUCCAAGCUA	181	A-128477	UAGCUUGGAGGCUUGAACAAGAC	397	1857_1879
AD-64320	A-128435	UCUGCCGAUCCAUACUGCGGA	182	A-128483	UCCGCAGUAUGGAUCGGCAGAGG	398	1255_1277
AD-64321	A-123463	AGUUAUAUGGAUGAUGUGGUA	183	A-128446	UACCACAUCAUCCAUAUAACUGA	399	731_753
AD-64322	A-128379	AUGUGUCUGCGGCGUUUUAUA	184	A-128454	UAUAAAACGCCGCAGACACAUCC	400	375_397
AD-64323	A-128395	CCCCGUCUGUGCCUUCUCAUA	185	A-128462	UAUGAGAAGGCACAGACGGGGAG	401	1545_1567
AD-64324	A-128409	GCCUAAUCAUCUCUUGUUCAU	186	A-128470	AUGAACAAGAGAUGAUUAGCGAG	402	1831_1853
AD-64325	A-128425	UCUAGACUCGUGGUGGACUUC	187	A-128478	GAAGUCCACCACGAGUCUAGACU	403	245_267
AD-64326	A-128437	CUGCCGAUCCAUACUGCGGAA	188	A-128484	UUCCGCAGUAUGGAUCGGCAGAG	404	1256_1278
AD-64328	A-128381	AUCUUCUUGUUGGUUCUUCUA	189	A-128455	UAGAAGAACCAACAAGAAGAUGA	405	426_448
AD-64330	A-128411	UUCUCUCAAUUUUCUAGGGGA	190	A-128471	UCCCCUAGAAAAUUGAGAGAAGU	406	263_285
AD-64331	A-127905	ACUCGUGGUGGACUUCUCUCA	191	A-127907	UGAGAGAAGUCCACCACGAGUCU	407	250_272
AD-64332	A-128001	GUGCACUUCGCUUCACCUCUG	192	A-128485	CAGAGGUGAAGCGAAGUGCACAC	408	1577_1599
AD-64333	A-128367	GUUUUUCUUGUUGACAAAAAU	193	A-128448	AUUUUUGUCAACAAGAAAAACCC	409	206_228
AD-64334	A-128383	CUGCCUAAUCAUCUCUUGUUA	194	A-128456	UAACAAGAGAUGAUUAGGCAGAG	410	1829_1851
AD-64335	A-128399	UCCUCACAAUACCACAGAGUA	195	A-128464	UACUCUGUGGUAUUGUGAGGAUU	411	226_248
AD-64336	A-128413	CUUGUUGACAAAAAUCCUCAA	196	A-128472	UUGAGGAUUUUUGUCAACAAGAA	412	212_234
AD-64337	A-127955	GUGGUGGACUUCUCUCAAUUU	197	A-127958	AAAUUGAGAGAAGUCCACCACGA	413	254_276
AD-64338	A-128439	GCAACUUUUUCACCUCUGCCU	198	A-128486	AGGCAGAGGUGAAAAAGUUGCAU	414	1814_1836
AD-64339	A-128369	GGGAACAAGAGCUACAGCAUA	199	A-128449	UAUGCUGUAGCUCUUGUUCCCAA	415	2828_2850
AD-64341	A-128401	UCAUCUUCUUGUUGGUUCUUA	200	A-128465	UAAGAACCAACAAGAAGAUGAGG	416	424_446
AD-64342	A-128415	CUGCUGCUAUGCCUCAUCUUA	201	A-128473	UAAGAUGAGGCAUAGCAGCAGGA	417	411_433
AD-64343	A-128427	GUUGGAUGUGUCUGCGGCGUU	202	A-128479	AACGCCGCAGACACAUCCAACGA	418	370_392
AD-64344	A-128441	UUCAUCCUGCUGCUAUGCCUA	203	A-128487	UAGGCAUAGCAGCAGGAUGAAGA	419	405_427
AD-64345	A-128371	UUCUUGUUGACAAAAAUCCUA	204	A-128450	UAGGAUUUUUGUCAACAAGAAAA	420	210_232
AD-64347	A-123487	GGAUGUGUCUGCGGCGUUUUA	205	A-128466	UAAAACGCCGCAGACACAUCCAG	421	373_395

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-64348	A-128417	UAUAUGGAUGAUGUGGUAUUA	206	A-128474	UAAUACCACAUCAUCCAUAUAAC	422	734_756
AD-64349	A-128429	UUCAUCCUGCUGCUAUGCCUC	207	A-128480	GAGGCAUAGCAGCAGGAUGAAGA	423	405_427
AD-64350	A-128443	GUGCACUUCGCUUCACCUCUA	208	A-128488	UAGAGGUGAAGCGAAGUGCACAC	424	1577_1599
AD-64351	A-128373	UUGACAAAAAUCCUCACAAUA	209	A-128451	UAUUGUGAGGAUUUUUGUCAACA	425	216_238
AD-64352	A-128389	CCAAGUGUUUGCUGACGCAAA	210	A-128459	UUUGCGUCAGCAAACACUUGGCA	426	1174_1196
AD-64352	A-128389	CCAAGUGUUUGCUGACGCAAA	211	A-128459	UUUGCGUCAGCAAACACUUGGCA	427	1174_1196
AD-64353	A-128403	AAGCCUCCAAGCUGUGCCUUA	212	A-128467	UAAGGCACAGCUUGGAGGCUUGA	428	1864_1886
AD-64354	A-128419	CCUCUUCAUCCUGCUGCUAUA	213	A-128475	UAUAGCAGCAGGAUGAAGAGGAA	429	401_423
AD-64355	A-128431	CCUGCUGCUAUGCCUCAUCUU	214	A-128481	AAGAUGAGGCAUAGCAGCAGGAU	430	410_432
AD-64356	A-128375	CAUCUUCUUGUUGGUUCUUCU	215	A-128452	AGAAGAACCAACAAGAAGAUGAG	431	425_447
AD-64357	A-128391	CCGUCUGUGCCUUCUCAUCUA	216	A-128460	UAGAUGAGAAGGCACAGACGGGG	432	1547_1569
AD-64358	A-128405	CCUCAUCUUCUUGUUGGUUCU	217	A-128468	AGAACCAACAAGAAGAUGAGGCA	433	422_444
AD-64359	A-128421	CCACCAAAUGCCCCUAUCUUA	218	A-128476	UAAGAUAGGGGCAUUUGGUGGUC	434	2298_2320
AD-64360	A-128433	GCUCCUCUGCCGAUCCAUACU	219	A-128482	AGUAUGGAUCGGCAGAGGAGCCA	435	1250_1272
AD-64700	A-129379	ACUCGUGGUGTACUUCUCUCA	220	A-127906	UGAGAGAAGUCCACCACGAGUCU	436	250_272
AD-64701	A-127905	ACUCGUGGUGGACUUCUCUCA	221	A-129387	UGAGAGAAGTCCACCACGAGUCU	437	250_272
AD-64702	A-127905	ACUCGUGGUGGACUUCUCUCA	222	A-129395	UGAGAGAAGUCCACCACGAGUCU	438	250_272
AD-64703	A-129376	ACUCGUGGUGGACUUCACUCA	223	A-129385	UGAGAGAAGTCCACCACGAGUCU	439	250_272
AD-64704	A-129381	ACUCGUGGTGTACUUCACUCA	224	A-129389	UGAGAGAAGUCCACCACGAGUCU	440	250_272
AD-64705	A-129380	ACUCGUGGUGTACUUCACUCA	225	A-127906	UGAGAGAAGUCCACCACGAGUCU	441	250_272
AD-64706	A-127905	ACUCGUGGUGGACUUCUCUCA	226	A-129388	UGAGAGAAGUCCACCACGAGUCU	442	250_272
AD-64707	A-127905	ACUCGUGGUGGACUUCUCUCA	227	A-129396	UGAGAGAAGTCCACCACGAGUCU	443	250_272
AD-64708	A-129382	ACUCGUGGTGGACUUCTCUCA	228	A-129385	UGAGAGAAGTCCACCACGAGUCU	444	250_272
AD-64709	A-129373	ACUCGUGGUGGACUUCUCUCA	229	A-129391	UGAGAGAAGTCCACCACGAGUCU	445	250_272
AD-64710	A-129373	ACUCGUGGUGGACUUCUCUCA	230	A-127906	UGAGAGAAGUCCACCACGAGUCU	446	250_272
AD-64711	A-129381	ACUCGUGGTGTACUUCACUCA	231	A-127906	UGAGAGAAGUCCACCACGAGUCU	447	250_272
AD-64712	A-127905	ACUCGUGGUGGACUUCUCUCA	232	A-129389	UGAGAGAAGUCCACCACGAGUCU	448	250_272

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-64713	A-127905	ACUCGUGGUGGACUUCUCUCA	233	A-129397	UGAGAGAAGTCCACCACGAGUCU	449	250_272
AD-64714	A-129384	ACUCGUGGTGGACUUCACUCA	234	A-129385	UGAGAGAAGTCCACCACGAGUCU	450	250_272
AD-64715	A-129376	ACUCGUGGUGGACUUCACUCA	235	A-129391	UGAGAGAAGTCCACCACGAGUCU	451	250_272
AD-64716	A-129374	ACUCGUGGUGGACUUCUCUCA	236	A-127906	UGAGAGAAGUCCACCACGAGUCU	452	250_272
AD-64717	A-129382	ACUCGUGGTGGACUUCTCUCA	237	A-127906	UGAGAGAAGUCCACCACGAGUCU	453	250_272
AD-64718	A-127905	ACUCGUGGUGGACUUCUCUCA	238	A-129390	UGAGAGAAGUCCACCACGAGUCU	454	250_272
AD-64719	A-127917	ACUCGUGGUGGACUUCTCUCA	239	A-129385	UGAGAGAAGTCCACCACGAGUCU	455	250_272
AD-64720	A-129381	ACUCGUGGTGTACUUCACUCA	240	A-129385	UGAGAGAAGTCCACCACGAGUCU	456	250_272
AD-64721	A-129382	ACUCGUGGTGGACUUCTCUCA	241	A-129391	UGAGAGAAGTCCACCACGAGUCU	457	250_272
AD-64722	A-129375	ACUCGUGGUGGACUUCCUCA	242	A-127906	UGAGAGAAGUCCACCACGAGUCU	458	250_272
AD-64723	A-129383	ACUCGUGGUGGACUUCTCUCA	243	A-127906	UGAGAGAAGUCCACCACGAGUCU	459	250_272
AD-64725	A-127917	ACUCGUGGUGGACUUCTCUCA	244	A-129398	UGAGAGAAGTCCACCACGAGUCU	460	250_272
AD-64726	A-129373	ACUCGUGGUGGACUUCUCUCA	245	A-129389	UGAGAGAAGUCCACCACGAGUCU	461	250_272
AD-64727	A-129384	ACUCGUGGTGGACUUCACUCA	246	A-129391	UGAGAGAAGTCCACCACGAGUCU	462	250_272
AD-64728	A-129376	ACUCGUGGUGGACUUCACUCA	247	A-127906	UGAGAGAAGUCCACCACGAGUCU	463	250_272
AD-64729	A-129384	ACUCGUGGTGGACUUCACUCA	248	A-127906	UGAGAGAAGUCCACCACGAGUCU	464	250_272
AD-64730	A-127905	ACUCGUGGUGGACUUCUCUCA	249	A-129392	UGAGAGAAGTCCACCACGAGUCU	465	250_272
AD-64731	A-129399	ACUCGUGGUGGACUUCTCUCA	250	A-129385	UGAGAGAAGTCCACCACGAGUCU	466	250_272
AD-64732	A-129376	ACUCGUGGUGGACUUCACUCA	251	A-129389	UGAGAGAAGUCCACCACGAGUCU	467	250_272
AD-64733	A-129381	ACUCGUGGTGTACUUCACUCA	252	A-129391	UGAGAGAAGTCCACCACGAGUCU	468	250_272
AD-64734	A-129377	ACUCGUGGUGGACUUCCCUCA	253	A-127906	UGAGAGAAGUCCACCACGAGUCU	469	250_272
AD-64735	A-127905	ACUCGUGGUGGACUUCUCUCA	254	A-129385	UGAGAGAAGTCCACCACGAGUCU	470	250_272
AD-64736	A-127905	ACUCGUGGUGGACUUCUCUCA	255	A-129393	UGAGAGAAGTCCACCACGAGUCU	471	250_272
AD-64737	A-129399	ACUCGUGGUGGACUUCTCUCA	256	A-129398	UGAGAGAAGTCCACCACGAGUCU	472	250_272
AD-64738	A-129382	ACUCGUGGTGGACUUCTCUCA	257	A-129389	UGAGAGAAGUCCACCACGAGUCU	473	250_272
AD-64739	A-129378	ACUCGUGGUGGACUUCGCUCA	258	A-127906	UGAGAGAAGUCCACCACGAGUCU	474	250_272
AD-64740	A-127905	ACUCGUGGUGGACUUCUCUCA	259	A-129386	UGAGAGAAGTCCACCACGAGUCU	475	250_272

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-64741	A-127905	ACUCGUGGUGGACUUCUCUCA	260	A-129394	UGAGAGAAGTCCACCACGAGUCU	476	250_272
AD-64742	A-129373	ACUCGUGGUGGACUUCUCUCA	261	A-129385	UGAGAGAAGTCCACCACGAGUCU	477	250_272
AD-64743	A-129384	ACUCGUGGTGGACUUCACUCA	262	A-129389	UGAGAGAAGUCCACCACGAGUCU	478	250_272

Table 3. Exemplary Modified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-61522	A-123463	AfsgsUfuAfuAfuGfGfAfuGfaUfgUfgGfuAfL96	479	A-123464	usAfscCfaCfaUfcAfuccAfuAfuAfaCfusgsa	694
AD-61547	A-123487	GfsgsAfuGfuGfuCfUfGfcGfgCfgUfuUfuAfL96	480	A-123488	usAfsaAfaCfgCfcGfcagAfcAfcAfuCfcsasg	695
AD-63938	A-127896	Y44ACUCGUGGUGGACUUCUCUCA	481	A-127897	UGAGAGAAGUCCACCACGAGUCU	696
AD-63939	A-127909	ascsucGfuGfgUfGfGfaCfuucUfcucaL96	482	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	697
AD-63940	A-127917	ascsucguggugdGacuuc(Tgn)cucaL96	483	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	698
AD-63941	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	484	A-127925	usGfsaGfagaAfguccaCfcAfcgaGfuscsu	699
AD-63942	A-127933	uscsGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	485	A-127934	usGfsaGfaGfaAfgUfccaCfcAfcGfasgsu	700
AD-63943	A-127944	ascsucGfuGfguGfGfaCfuucucucaL96	486	A-127942	usGfsAfgaGfaAfgUfccaCfcAfcGfaguscsu	701
AD-63945	A-127910	ascsucguGfgUfGfGfaCfuucUfcucaL96	487	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	702
AD-63946	A-127918	ascsucguGfgUfGfGfacuuCfucucaL96	488	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	703
AD-63947	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	489	A-127926	usGfsaGfagaagUfccaCfcAfcgaGfuscsu	704
AD-63948	A-127935	gsusGfgUfGfGfaCfuUfcUfcUfcAfL96	490	A-127936	usGfsaGfaGfaAfgUfccaCfcAfcsgsa	705
AD-63949	A-127944	ascsucGfuGfguGfGfaCfuucucucaL96	491	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	706
AD-63950	A-127900	Y44UfcGfuGfgUfgGfaCfuUfcUfcUfcAfusuY44	492	A-127901	usGfsasGfaGfaAfgUfcCfaCfcAfcGfausu	707
AD-63951	A-127911	ascsucguGfgUfGfGfaCfuucucucaL96	493	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	708
AD-63952	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	494	A-127919	usGfsaGfaGfaagUfccaCfcAfcGfaGfuscsu	709
AD-63953	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	495	A-127927	usGfsagagaAfgUfccaCfcAfcgaguscsu	710

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-63955	A-127945	ascsucgugguGfGfacuucucucaL96	496	A-127940	usGfsAfgAfgAfaGfuccaCfCfaCfgAfguscsu	711
AD-63956	A-127902	Y44uscsGfuGfgUfgGfaCfuUfcUfcUfcAfY44	497	A-127903	usGfsaGfaGfaAfgUfcCfaCfcAfcGfasusu	712
AD-63957	A-127912	ascsucguGfgUfGfGfacuucucucaL96	498	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	713
AD-63958	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	499	A-127920	usGfsagaGfaAfgUfccaCfcAfcgaGfuscsu	714
AD-63959	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	500	A-127928	usGfsaGfagaAfguccaCfcAfcgaguscsu	715
AD-63960	A-126619	usasUfuUfCfCfuAfgGfgUfaCfaAfL96	501	A-127938	PusGfsaGfaGfaAfgUfccaCfcAfcsgsa	716
AD-63961	A-127945	ascsucgugguGfGfacuucucucaL96	502	A-127942	usGfsAfgaGfaAfgUfccaCfcAfcGfaguscsu	717
AD-63962	A-127902	Y44uscsGfuGfgUfgGfaCfuUfcUfcUfcAfY44	503	A-127904	PusGfsaGfaGfaAfgUfcCfaCfcAfcGfasusu	718
AD-63963	A-127913	ascsucguggUfgGfacuucucucaL96	504	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	719
AD-63964	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	505	A-127921	usGfsaGfaGfaAfgUfccaCfcAfcgaguscsu	720
AD-63965	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	506	A-127929	usGfsagaGfaaGfuccaCfcAfcgaguscsu	721
AD-63966	A-127939	ascsUfcGfugguGfGfaCfuuCfuCfucaL96	507	A-127940	usGfsAfgAfgAfaGfuccaCfCfaCfgAfguscsu	722
AD-63967	A-127945	ascsucgugguGfGfacuucucucaL96	508	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	723
AD-63968	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	509	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	724
AD-63968	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	510	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	725
AD-63968	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	511	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	726
AD-63969	A-127914	ascsucguggugGfacuucucucaL96	512	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	727
AD-63970	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	513	A-127922	usGfsagaGfaagUfccaCfcAfcgaGfuscsu	728
AD-63971	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	514	A-127930	usGfsagaGfaaguccaCfcAfcgaguscsu	729
AD-63972	A-127941	ascsUfcGfuGfguGfGfaCfuuCfuCfucaL96	515	A-127942	usGfsAfgaGfaAfgUfccaCfcAfcGfaguscsu	730
AD-63973	A-127946	ascsucguggudGdGacuucucucaL96	516	A-127947	usdGsaGfaGfaAfgdTccadCcAfcGfaguscsu	731
AD-63975	A-127915	ascsucguggUfgGfacuuc(Tgn)cucaL96	517	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	732
AD-63976	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	518	A-127923	usGfsagaGfaAfgUfccaCfcAfcgaguscsu	733
AD-63977	A-127917	ascsucguggugdGacuuc(Tgn)cucaL96	519	A-127931	usdGsagagaaguccadCcacgaguscsu	734
AD-63978	A-127943	ascsUfcGfuGfguGfGfaCfuUfcUfcUfcaL96	520	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	735
AD-63979	A-127908	ascsucGfuGfgUfGfGfaCfuucUfcucAfL96	521	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	736

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-63980	A-127916	ascsucguggugGfacuuc(Tgn)cucaL96	522	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	737
AD-63981	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	523	A-127924	usGfsaGfagaAfgUfccaCfcAfcgaGfuscsu	738
AD-63982	A-127917	ascsucguggugdGacuuc(Tgn)cucaL96	524	A-127932	PusdGsagagaaguccadCcacgaguscsu	739
AD-63983	A-127944	ascsucGfuGfguGfGfaCfuucucucaL96	525	A-127940	usGfsAfgAfgAfaGfuccaCfCfaCfgAfguscsu	740
AD-63985	A-127961	gsusggugGfaCfUfUfcUfcucAfauuuL96	526	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	741
AD-63986	A-127969	gsusggugGfaCfUfUfcucuCfaauuuL96	527	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	742
AD-63987	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	528	A-127977	asAfsaUfugagaGfaagUfcCfaccAfcsgsa	743
AD-63988	A-127986	usgsGfaCfUfUfcUfcUfcAfaUfuUfL96	529	A-127987	asAfsaUfuGfaGfaGfaagUfcCfascsc	744
AD-63989	A-127996	gsusgguggacUfUfcucucaauuuL96	530	A-127992	asAfsAfUfuGfaGfaGfaagUfcCfaCfcacsgsa	745
AD-63990	A-127950	Y44GfgUfgGfaCfuUfcUfcUfcAfaUfuUfusuY44	531	A-127951	asAfsasUfuGfaGfaGfaAfgUfcCfaCfcusu	746
AD-63991	A-127962	gsusggugGfaCfUfUfcUfcucaauuuL96	532	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	747
AD-63992	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	533	A-127970	asAfsaUfuGfagaGfaagUfcCfaCfcAfcsgsa	748
AD-63993	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	534	A-127978	asAfsauugaGfaGfaagUfcCfaccacsgsa	749
AD-63994	A-127984	gsgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	535	A-127988	PasAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	750
AD-63995	A-127996	gsusgguggacUfUfcucucaauuuL96	536	A-127993	asAfsAfuuGfaGfaGfaagUfCfcaCfcacsgsa	751
AD-63996	A-127952	Y44gsgsUfgGfaCfuUfcUfcUfcAfaUfuUfY44	537	A-127953	asAfsaUfuGfaGfaGfaAfgUfcCfaCfcsusu	752
AD-63997	A-127963	gsusggugGfaCfUfUfcucucaauuuL96	538	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	753
AD-63999	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	539	A-127979	asAfsaUfugaGfagaagUfcCfaccacsgsa	754
AD-64000	A-127986	usgsGfaCfUfUfcUfcUfcAfaUfuUfL96	540	A-127989	PasAfsaUfuGfaGfaGfaagUfcCfascsc	755
AD-64001	A-127996	gsusgguggacUfUfcucucaauuuL96	541	A-127994	asAfsAfUfuGfaGfaGfaagUfCfcaCfcacsgsa	756
AD-64002	A-127952	Y44gsgsUfgGfaCfuUfcUfcUfcAfaUfuUfY44	542	A-127954	PasAfsaUfuGfaGfaGfaAfgUfcCfaCfcsusu	757
AD-64003	A-127964	gsusgguggaCfuUfcucucaauuuL96	543	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	758
AD-64004	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	544	A-127972	asAfsaUfuGfaGfaGfaagUfcCfaccacsgsa	759
AD-64005	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	545	A-127980	asAfsauuGfagAfgaagUfcCfaccacsgsa	760
AD-64006	A-127990	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	546	A-127991	asAfsaUfuGfaGfaGfaagUfcCfaCfcacsgsa	761
AD-64007	A-127996	gsusgguggacUfUfcucucaauuuL96	547	A-127995	asAfsAfUfugaGfaGfaagUfCfcaCfcacsgsa	762

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64008	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	548	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	763
AD-64008	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	549	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	764
AD-64009	A-127965	gsusgguggacuUfcucucaauuuL96	550	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	765
AD-64010	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	551	A-127973	asAfsauuGfagaGfaagUfcCfaccAfcsgsa	766
AD-64011	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	552	A-127981	asAfsauuGfagagaagUfcCfaccacsgsa	767
AD-64012	A-127990	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	553	A-127992	asAfsAfUfuGfaGfaGfaagUfcCfaCfcacsgsa	768
AD-64013	A-127997	gsusgguggacdTdTcucucaauuuL96	554	A-127998	asdAsAfuugaGfaGfaagdTdCcaCfcacsgsa	769
AD-64014	A-127957	Y44GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	555	A-127958	PasAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	770
AD-64015	A-127966	gsusgguggaCfuUfcucuc(Agn)auuuL96	556	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	771
AD-64016	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	557	A-127974	asAfsauuGfaGfaGfaagUfcCfaccacsgsa	772
AD-64017	A-127968	gsusgguggacudTcucuc(Agn)auuuL96	558	A-127982	asdAsauugagagaagdTccaccacsgsa	773
AD-64018	A-127990	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	559	A-127993	asAfsAfuuGfaGfaGfaagUfCfcaCfcacsgsa	774
AD-64019	A-127959	gsusggUfgGfaCfUfUfcUfcucAfauuUfL96	560	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	775
AD-64020	A-127967	gsusgguggacuUfcucuc(Agn)auuuL96	561	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	776
AD-64021	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	562	A-127975	asAfsaUfugaGfaGfaagUfcCfaccAfcsgsa	777
AD-64022	A-127968	gsusgguggacudTcucuc(Agn)auuuL96	563	A-127983	PasdAsauugagagaagdTccaccacsgsa	778
AD-64023	A-127990	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	564	A-127994	asAfsAfUfuGfaGfaGfaagUfCfcaCfcacsgsa	779
AD-64024	A-127960	gsusggUfgGfaCfUfUfcUfcucAfauuuL96	565	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	780
AD-64025	A-127968	gsusgguggacudTcucuc(Agn)auuuL96	566	A-127956	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	781
AD-64026	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	567	A-127976	asAfsaUfugaGfagaagUfcCfaccAfcsgsa	782
AD-64027	A-127984	gsgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	568	A-127985	asAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	783
AD-64028	A-127990	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	569	A-127995	asAfsAfUfugaGfaGfaagUfCfcaCfcacsgsa	784
AD-64272	A-128001	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	570	A-128002	csAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	785
AD-64274	A-128363	GfsusUfgAfcAfaAfAfAfuCfcUfcAfcAfaUfL96	571	A-128364	asUfsuGfuGfaGfgAfuuuUfuGfuCfaAfcsasa	786
AD-64275	A-128377	UfsgsUfuGfaCfaAfAfAfaUfcCfuCfaCfaAfL96	572	A-128378	usUfsgUfgAfgGfaUfuuuUfgUfcAfaCfasasg	787
AD-64276	A-128393	GfsgsUfgGfaCfuUfCfUfcUfcAfaUfuUfuAfL96	573	A-128394	usAfsaAfaUfuGfaGfagaAfgUfcCfaCfcsasc	788

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64277	A-128407	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	574	A-128408	usCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	789
AD-64277	A-128407	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	575	A-128408	usCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	790
AD-64278	A-128423	AfscsUfgUfuCfaAfGfCfcUfcCfaAfgCfuAfL96	576	A-128424	usAfsgCfuUfgGfaGfgcuUfgAfaCfaAfgsasc	791
AD-64279	A-128435	UfscsUfgCfcGfaUfCfCfaUfaCfuGfcGfgAfL96	577	A-128436	usCfscGfcAfgUfaUfggaUfcGfgCfaGfasgsg	792
AD-64280	A-128379	AfsusGfuGfuCfuGfCfGfgCfgUfuUfuAfuAfL96	578	A-128380	usAfsuAfaAfaCfgCfcgcAfgAfcAfcAfuscsc	793
AD-64281	A-128395	CfscsCfcGfuCfuGfUfGfcCfuUfcUfcAfuAfL96	579	A-128396	usAfsuGfaGfaAfgGfcacAfgAfcGfgGfgsasg	794
AD-64282	A-128409	GfscsCfuAfaUfcAfUfCfuCfuUfgUfuCfaUfL96	580	A-128410	asUfsgAfaCfaAfgAfgauGfaUfuAfgCfgsasg	795
AD-64283	A-128425	UfscsUfaGfaCfuCfGfUfgGfuGfgAfcUfuCfL96	581	A-128426	gsAfsaGfuCfcAfcCfacgAfgUfcUfaGfascsu	796
AD-64284	A-128437	CfsusGfcCfgAfuCfCfAfuAfcUfgCfgGfaAfL96	582	A-128438	usUfscCfgCfaGfuAfuggAfuCfgGfcAfgsasg	797
AD-64285	A-128365	UfsusUfuUfcUfuGfUfUfgAfcAfaAfaAfuAfL96	583	A-128366	usAfsuUfuUfuGfuCfaacAfaGfaAfaAfascsc	798
AD-64286	A-128381	AfsusCfuUfcUfuGfUfUfgGfuUfcUfuCfuAfL96	584	A-128382	usAfsgAfaGfaAfcCfaacAfaGfaAfgAfusgsa	799
AD-64289	A-128367	GfsusUfuUfuCfuUfGfUfuGfaCfaAfaAfaUfL96	585	A-128368	asUfsuUfuUfgUfcAfacaAfgAfaAfaAfcscsc	800
AD-64290	A-128383	CfsusGfcCfuAfaUfCfAfuCfuCfuUfgUfuAfL96	586	A-128384	usAfsaCfaAfgAfgAfugaUfuAfgGfcAfgsasg	801
AD-64291	A-128399	UfscsCfuCfaCfaAfUfAfcCfaCfaGfaGfuAfL96	587	A-128400	usAfscUfcUfgUfgGfuauUfgUfgAfgGfasusu	802
AD-64292	A-128413	CfsusUfgUfuGfaCfAfAfaAfaUfcCfuCfaAfL96	588	A-128414	usUfsgAfgGfaUfuUfuugUfcAfaCfaAfgsasa	803
AD-64293	A-128439	GfscsAfaCfuUfuUfUfCfaCfcUfcUfgCfcUfL96	589	A-128440	asGfsgCfaGfaGfgUfgaaAfaAfgUfuGfcsasu	804
AD-64294	A-128369	GfsgsGfaAfcAfaGfAfGfcUfaCfaGfcAfuAfL96	590	A-128370	usAfsuGfcUfgUfaGfcucUfuGfuUfcCfcsasa	805
AD-64295	A-128385	CfsgsUfgGfuGfgAfCfUfuCfuCfuCfaAfuUfL96	591	A-128386	asAfsuUfgAfgAfgAfaguCfcAfcCfaGfcsasg	806
AD-64297	A-128415	CfsusGfcUfgCfuAfUfGfcCfuCfaUfcUfuAfL96	592	A-128416	usAfsaGfaUfgAfgGfcauAfgCfaGfcAfgsgsa	807
AD-64298	A-128427	GfsusUfgGfaUfgUfGfUfcUfgCfgGfcGfuUfL96	593	A-128428	asAfscGfcCfgCfaGfacaCfaUfcCfaAfcsgsa	808
AD-64299	A-128441	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuAfL96	594	A-128442	usAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	809
AD-64300	A-128371	UfsusCfuUfgUfuGfAfCfaAfaAfaUfcCfuAfL96	595	A-128372	usAfsgGfaUfuUfuUfgucAfaCfaAfgAfasasa	810
AD-64302	A-128417	UfsasUfaUfgGfaUfGfAfuGfuGfgUfaUfuAfL96	596	A-128418	usAfsaUfaCfcAfcAfucaUfcCfaUfaUfasasc	811
AD-64303	A-128429	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuCfL96	597	A-128430	gsAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	812
AD-64304	A-128443	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuAfL96	598	A-128444	usAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	813
AD-64305	A-128373	UfsusGfaCfaAfaAfAfUfcCfuCfaCfaAfuAfL96	599	A-128374	usAfsuUfgUfgAfgGfauuUfuUfgUfcAfascsa	814

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64307	A-128403	AfsasGfcCfuCfcAfAfGfcUfgUfgCfcUfuAfL96	600	A-128404	usAfsaGfgCfaCfaGfcuuGfgAfgGfcUfusgsa	815
AD-64308	A-128419	CfscsUfcUfuCfaUfCfCfuGfcUfgCfuAfuAfL96	601	A-128420	usAfsuAfgCfaGfcAfggaUfgAfaGfaGfgsasa	816
AD-64309	A-128431	CfscsUfgCfuGfcUfAfUfgCfcUfcAfuCfuUfL96	602	A-128432	asAfsgAfuGfaGfgCfauaGfcAfgCfaGfgsasu	817
AD-64310	A-128375	CfsasUfcUfuCfuUfGfUfuGfgUfuCfuUfcUfL96	603	A-128376	asGfsaAfgAfaCfcAfacaAfgAfaGfaUfgsasg	818
AD-64311	A-128391	CfscsGfuCfuGfuGfCfCfuUfcUfcAfuCfuAfL96	604	A-128392	usAfsgAfuGfaGfaAfggcAfcAfgAfcGfgsgsg	819
AD-64312	A-128405	CfscsUfcAfuCfuUfCfUfuGfuUfgGfuUfcUfL96	605	A-128406	asGfsaAfcCfaAfcAfagaAfgAfuGfaGfgscsa	820
AD-64313	A-128421	CfscsAfcCfaAfaUfGfCfcCfcUfaUfcUfuAfL96	606	A-128422	usAfsaGfaUfaGfgGfgcaUfuUfgGfuGfgsusc	821
AD-64314	A-128433	GfscsUfcCfuCfuGfCfCfgAfuCfcAfuAfcUfL96	607	A-128434	asGfsuAfuGfgAfuCfggcAfgAfgGfaGfcscsa	822
AD-64315	A-128363	GfsusUfgAfcAfaAfAfAfuCfcUfcAfcAfaUfL96	608	A-128445	PasUfsuGfuGfaGfgAfuuuUfuGfuCfaAfcsasa	823
AD-64316	A-128377	UfsgsUfuGfaCfaAfAfAfaUfcCfuCfaCfaAfL96	609	A-128453	PusUfsgUfgAfgGfaUfuuuUfgUfcAfaCfasasg	824
AD-64317	A-128393	GfsgsUfgGfaCfuUfCfUfcUfcAfaUfuUfuAfL96	610	A-128461	PusAfsaAfaUfuGfaGfagaAfgUfcCfaCfcsasc	825
AD-64318	A-128407	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	611	A-128469	PusCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	826
AD-64319	A-128423	AfscsUfgUfuCfaAfGfCfcUfcCfaAfgCfuAfL96	612	A-128477	PusAfsgCfuUfgGfaGfgcuUfgAfaCfaAfgsasc	827
AD-64320	A-128435	UfscsUfgCfcGfaUfCfCfaUfaCfuGfcGfgAfL96	613	A-128483	PusCfscGfcAfgUfaUfggaUfcGfgCfaGfasgsg	828
AD-64321	A-123463	AfsgsUfuAfuAfuGfGfAfuGfaUfgUfgGfuAfL96	614	A-128446	PusAfscCfaCfaUfcAfuccAfuAfuAfaCfusgsa	829
AD-64322	A-128379	AfsusGfuGfuCfuGfCfGfgCfgUfuUfuAfuAfL96	615	A-128454	PusAfsuAfaAfaCfgCfcgcAfgAfcAfcAfuscsc	830
AD-64323	A-128395	CfscsCfcGfuCfuGfUfGfcCfuUfcUfcAfuAfL96	616	A-128462	PusAfsuGfaGfaAfgGfcacAfgAfcGfgGfgsasg	831
AD-64324	A-128409	GfscsCfuAfaUfcAfUfCfuCfuUfgUfuCfaUfL96	617	A-128470	PasUfsgAfaCfaAfgAfgauGfaUfuAfgCfgsasg	832
AD-64325	A-128425	UfscsUfaGfaCfuCfGfUfgGfuGfgAfcUfuCfL96	618	A-128478	PgsAfsaGfuCfcAfcCfacgAfgUfcUfaGfascsu	833
AD-64326	A-128437	CfsusGfcCfgAfuCfCfAfuAfcUfgCfgGfaAfL96	619	A-128484	PusUfscCfgCfaGfuAfuggAfuCfgGfcAfgsasg	834
AD-64328	A-128381	AfsusCfuUfcUfuGfUfUfgGfuUfcUfuCfuAfL96	620	A-128455	PusAfsgAfaGfaAfcCfaacAfaGfaAfgAfusgsa	835
AD-64330	A-128411	UfsusCfuCfuCfaAfUfUfuUfcUfaGfgGfgAfL96	621	A-128471	PusCfscCfcUfaGfaAfaauUfgAfgAfgAfasgsu	836
AD-64331	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	622	A-127907	PusGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	837
AD-64332	A-128001	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	623	A-128485	PcsAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	838
AD-64333	A-128367	GfsusUfuUfuCfuUfGfUfuGfaCfaAfaAfaUfL96	624	A-128448	PasUfsuUfuUfgUfcAfacaAfgAfaAfaAfcscsc	839
AD-64334	A-128383	CfsusGfcCfuAfaUfCfAfuCfuCfuUfgUfuAfL96	625	A-128456	PusAfsaCfaAfgAfgAfugaUfuAfgGfcAfgsasg	840

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64335	A-128399	UfscsCfuCfaCfaAfUfAfcCfaCfaGfaGfuAfL96	626	A-128464	PusAfscUfcUfgUfgGfuauUfgUfgAfgGfasusu	841
AD-64336	A-128413	CfsusUfgUfuGfaCfAfAfaAfaUfcCfuCfaAfL96	627	A-128472	PusUfsgAfgGfaUfuUfuugUfcAfaCfaAfgsasa	842
AD-64337	A-127955	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	628	A-127958	PasAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	843
AD-64338	A-128439	GfscsAfaCfuUfuUfUfCfaCfcUfcUfgCfcUfL96	629	A-128486	PasGfsgCfaGfaGfgUfgaaAfaAfgUfuGfcsasu	844
AD-64339	A-128369	GfsgsGfaAfcAfaGfAfGfcUfaCfaGfcAfuAfL96	630	A-128449	PusAfsuGfcUfgUfaGfcucUfuGfuUfcCfcsasa	845
AD-64341	A-128401	UfscsAfuCfuUfcUfUfGfuUfgGfuUfcUfuAfL96	631	A-128465	PusAfsaGfaAfcCfaAfcaaGfaAfgAfuGfasgsg	846
AD-64342	A-128415	CfsusGfcUfgCfuAfUfGfcCfuCfaUfcUfuAfL96	632	A-128473	PusAfsaGfaUfgAfgGfcauAfgCfaGfcAfgsgsa	847
AD-64343	A-128427	GfsusUfgGfaUfgUfGfUfcUfgCfgGfcGfuUfL96	633	A-128479	PasAfscGfcCfgCfaGfacaCfaUfcCfaAfcsgsa	848
AD-64344	A-128441	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuAfL96	634	A-128487	PusAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	849
AD-64345	A-128371	UfsusCfuUfgUfuGfAfCfaAfaAfaUfcCfuAfL96	635	A-128450	PusAfsgGfaUfuUfuUfgucAfaCfaAfgAfasasa	850
AD-64347	A-123487	GfsgsAfuGfuGfuCfUfGfcGfgCfgUfuUfuAfL96	636	A-128466	PusAfsaAfaCfgCfcGfcagAfcAfcAfuCfcsasg	851
AD-64348	A-128417	UfsasUfaUfgGfaUfGfAfuGfuGfgUfaUfuAfL96	637	A-128474	PusAfsaUfaCfcAfcAfucaUfcCfaUfaUfasasc	852
AD-64349	A-128429	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuCfL96	638	A-128480	PgsAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	853
AD-64350	A-128443	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuAfL96	639	A-128488	PusAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	854
AD-64351	A-128373	UfsusGfaCfaAfaAfAfUfcCfuCfaCfaAfuAfL96	640	A-128451	PusAfsuUfgUfgAfgGfauuUfuUfgUfcAfascsa	855
AD-64352	A-128389	CfscsAfaGfuGfuUfUfGfcUfgAfcGfcAfaAfL96	641	A-128459	PusUfsuGfcGfuCfaGfcaaAfcAfcUfuGfgscsa	856
AD-64352	A-128389	CfscsAfaGfuGfuUfUfGfcUfgAfcGfcAfaAfL96	642	A-128459	PusUfsuGfcGfuCfaGfcaaAfcAfcUfuGfgscsa	857
AD-64353	A-128403	AfsasGfcCfuCfcAfAfGfcUfgUfgCfcUfuAfL96	643	A-128467	PusAfsaGfgCfaCfaGfcuuGfgAfgGfcUfusgsa	858
AD-64354	A-128419	CfscsUfcUfuCfaUfCfCfuGfcUfgCfuAfuAfL96	644	A-128475	PusAfsuAfgCfaGfcAfggaUfgAfaGfaGfgsasa	859
AD-64355	A-128431	CfscsUfgCfuGfcUfAfUfgCfcUfcAfuCfuUfL96	645	A-128481	PasAfsgAfuGfaGfgCfauaGfcAfgCfaGfgsasu	860
AD-64356	A-128375	CfsasUfcUfuCfuUfGfUfuGfgUfuCfuUfcUfL96	646	A-128452	PasGfsaAfgAfaCfcAfacaAfgAfaGfaUfgsasg	861
AD-64357	A-128391	CfscsGfuCfuGfuGfCfCfuUfcUfcAfuCfuAfL96	647	A-128460	PusAfsgAfuGfaGfaAfggcAfcAfgAfcGfgsgsg	862
AD-64358	A-128405	CfscsUfcAfuCfuUfCfUfuGfuUfgGfuUfcUfL96	648	A-128468	PasGfsaAfcCfaAfcAfagaAfgAfuGfaGfgscsa	863
AD-64359	A-128421	CfscsAfcCfaAfaUfGfCfcCfcUfaUfcUfuAfL96	649	A-128476	PusAfsaGfaUfaGfgGfgcaUfuUfgGfuGfgsusc	864
AD-64360	A-128433	GfscsUfcCfuCfuGfCfCfgAfuCfcAfuAfcUfL96	650	A-128482	PasGfsuAfuGfgAfuCfggcAfgAfgGfaGfcscsa	865
AD-64700	A-129379	ascsucguggugdTacuu(Cgn)ucucaL96	651	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	866

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64701	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	652	A-129387	PusgsagagaagdTccadCcacgaguscsu	867
AD-64702	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	653	A-129395	usGsagadGaaguccaCcacgaguscsu	868
AD-64703	A-129376	ascsucguggugdGacuucdAcucaL96	654	A-129385	usdGsagagaagdTccadCcacgaguscsu	869
AD-64704	A-129381	ascsucguggdTgdTacuucdAcucaL96	655	A-129389	usdGsagadGaaguccadCcacgaguscsu	870
AD-64705	A-129380	ascsucguggugdTacuucdAcucaL96	656	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	871
AD-64706	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	657	A-129388	usdGsadGagaaguccadCcacgaguscsu	872
AD-64707	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	658	A-129396	usgsagadGaagdTccadCcacgaguscsu	873
AD-64708	A-129382	ascsucguggdTgdGacuuc(Tgn)cucaL96	659	A-129385	usdGsagagaagdTccadCcacgaguscsu	874
AD-64709	A-129373	ascsucguggugdGacuu(Cgn)ucucaL96	660	A-129391	usdGsagadGaagdTccadCcacgaguscsu	875
AD-64710	A-129373	ascsucguggugdGacuu(Cgn)ucucaL96	661	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	876
AD-64711	A-129381	ascsucguggdTgdTacuucdAcucaL96	662	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	877
AD-64712	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	663	A-129389	usdGsagadGaaguccadCcacgaguscsu	878
AD-64713	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	664	A-129397	PusgsagadGaagdTccadCcacgaguscsu	879
AD-64714	A-129384	ascsucguggdTgdGacuucdAcucaL96	665	A-129385	usdGsagagaagdTccadCcacgaguscsu	880
AD-64715	A-129376	ascsucguggugdGacuucdAcucaL96	666	A-129391	usdGsagadGaagdTccadCcacgaguscsu	881
AD-64716	A-129374	ascsucguggugdGacuucu(Cgn)ucaL96	667	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	882
AD-64717	A-129382	ascsucguggdTgdGacuuc(Tgn)cucaL96	668	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	883
AD-64718	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	669	A-129390	usdGsagagadAguccadCcacgaguscsu	884
AD-64719	A-127917	ascsucguggugdGacuuc(Tgn)cucaL96	670	A-129385	usdGsagagaagdTccadCcacgaguscsu	885
AD-64720	A-129381	ascsucguggdTgdTacuucdAcucaL96	671	A-129385	usdGsagagaagdTccadCcacgaguscsu	886
AD-64721	A-129382	ascsucguggdTgdGacuuc(Tgn)cucaL96	672	A-129391	usdGsagadGaagdTccadCcacgaguscsu	887
AD-64722	A-129375	ascsucguggugdGacuucY34cucaL96	673	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	888
AD-64723	A-129383	ascsucguggugdGdAcuuc(Tgn)cucaL96	674	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	889
AD-64725	A-127917	ascsucguggugdGacuuc(Tgn)cucaL96	675	A-129398	PusdGsagagaagdTccadCcacgaguscsu	890
AD-64726	A-129373	ascsucguggugdGacuu(Cgn)ucucaL96	676	A-129389	usdGsagadGaaguccadCcacgaguscsu	891
AD-64727	A-129384	ascsucguggdTgdGacuucdAcucaL96	677	A-129391	usdGsagadGaagdTccadCcacgaguscsu	892

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64728	A-129376	ascsucguggugdGacuucdAcucaL96	678	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	893
AD-64729	A-129384	ascsucguggdTgdGacuucdAcucaL96	679	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	894
AD-64730	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	680	A-129392	usGsagagaagdTccadCcacgaguscsu	895
AD-64731	A-129399	Y34ascsucguggugdGacuuc(Tgn)cucaL96	681	A-129385	usdGsagagaagdTccadCcacgaguscsu	896
AD-64732	A-129376	ascsucguggugdGacuucdAcucaL96	682	A-129389	usdGsagadGaaguccadCcacgaguscsu	897
AD-64733	A-129381	ascsucguggdTgdTacuucdAcucaL96	683	A-129391	usdGsagadGaagdTccadCcacgaguscsu	898
AD-64734	A-129377	ascsucguggugdGacuucdCcucaL96	684	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	899
AD-64735	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	685	A-129385	usdGsagagaagdTccadCcacgaguscsu	900
AD-64736	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	686	A-129393	usdGsagagaagdTccaCcacgaguscsu	901
AD-64737	A-129399	Y34ascsucguggugdGacuuc(Tgn)cucaL96	687	A-129398	PusdGsagagaagdTccadCcacgaguscsu	902
AD-64738	A-129382	ascsucguggdTgdGacuuc(Tgn)cucaL96	688	A-129389	usdGsagadGaaguccadCcacgaguscsu	903
AD-64739	A-129378	ascsucguggugdGacuucdGcucaL96	689	A-127906	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	904
AD-64740	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	690	A-129386	usgsagagaagdTccadCcacgaguscsu	905
AD-64741	A-127905	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	691	A-129394	usGsagagaagdTccaCcacgaguscsu	906
AD-64742	A-129373	ascsucguggugdGacuu(Cgn)ucucaL96	692	A-129385	usdGsagagaagdTccadCcacgaguscsu	907
AD-64743	A-129384	ascsucguggdTgdGacuucdAcucaL96	693	A-129389	usdGsagadGaaguccadCcacgaguscsu	908

Table 4. Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

Duplex ID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65369	UCGUGGUGGACUUCUCUCA	909	UGAGAGAAGUCCACCACGAUU	938
AD-65381	UCGUGGUGGACUUCUCUCA	910	UGAGAGAAGUCCACCACGAUU	939
AD-63962	UCGUGGUGGACUUCUCUCA	911	UGAGAGAAGUCCACCACGAUU	940

Duplex ID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-63938	ACUCGUGGUGGACUUCUCUCA	912	UGAGAGAAGUCCACCACGAGUCU	941
AD-65561	UCGUGGUGGACUUCUCUCA	913	UGAGAGAAGUCCACCACGAUU	942
AD-65566	UCGUGGUGGACUUCUCUCA	914	UGAGAGAAGUCCACCACGAUU	943
AD-63944	UCGUGGUGGACUUCUCUCAUU	915	UGAGAGAAGUCCACCACGAUU	944
AD-63968	ACUCGUGGUGGACUUCUCUCA	916	UGAGAGAAGUCCACCACGAGUCU	945
AD-65406	UCGUGGUGGACUUCUCUCA	917	UGAGAGAAGUCCACCACGAUU	946
AD-65396	ACUCGUGGUGGACUUCUCUCA	918	UGAGAGAAGUCCACCACGAGUUU	947
AD-65427	GUGCACUUCGCUUCACCUCUA	919	UAGAGGUGAAGCGAAGUGCACUU	948
AD-65573	GUGCACUUCGCUUCACCUCUA	920	UAGAGGUGAAGCGAAGUGCACAC	949
AD-65432	GCACUUCGCUUCACCUCUA	921	UAGAGGUGAAGCGAAGUGCAC	950
AD-64332	GUGCACUUCGCUUCACCUCUG	922	CAGAGGUGAAGCGAAGUGCACAC	951
AD-64322	AUGUGUCUGCGGCGUUUUAUA	923	UAUAAAACGCCGCAGACACAUCC	952
AD-64272	GUGCACUUCGCUUCACCUCUG	924	CAGAGGUGAAGCGAAGUGCACAC	953
AD-65583	GCACUUCGCUUCACCUCUA	925	UAGAGGUGAAGCGAAGUGCUU	954
AD-63994	GGUGGACUUCUCUCAAUUU	926	AAAUUGAGAGAAGUCCACCAC	955
AD-65370	CGUGGUGGACUUCUCUCAAUU	927	AAUUGAGAGAAGUCCACCAGCAG	956
AD-65265	GUGGUGGACUUCUCUCAAUUU	928	AAAUUGAGAGAAGUCCACCACGA	957
AD-65407	CGUGGUGGACUUCUCUCAAUU	929	AAUUGAGAGAAGUCCACCAGCAG	958
AD-64027	GGUGGACUUCUCUCAAUUU	930	AAAUUGAGAGAAGUCCACCAC	959
AD-65266	GUGGUGGACUUCUCUCAAUUU	931	AAAUUGAGAGAAGUCCACCACGA	960
AD-65389	UGGUGGUCTUCUCUAAAUU	932	AAUUGAGAGAAGUCCACCAUU	961
AD-64008	GUGGUGGACUUCUCUCAAUUU	933	AAAUUGAGAGAAGUCCACCACGA	962
AD-65377	CGUGGUGGUCTUCUCUAAAUU	934	AAUUGAGAGAAGUCCACCAGCUU	963
AD-65409	GGUGGACUUCUCUCAAUUUUA	935	UAAAAUUGAGAGAAGUCCACCAC	964
AD-65403	GGUGGACUUCUCUCAAUUUUA	936	UAAAAUUGAGAGAAGUCCACCAC	965
AD-65385	UGGACUACTCUCAAAUUUA	937	UAAAAUUGAGAGAAGUCCAUU	966

Table 5. Exemplary Modified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

DuplexID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65369	uscsguGfgUfGfGfacuuCfUfcucaL96	967	PusGfsagaGfaAfGfuccaCfcAfcgasusu	996
AD-65381	uscsguGfgUfGfGfacuucucucaL96	968	PusGfsagaGfaAfGfuccaCfcAfcgasusu	997
AD-63962	Y44uscsGfuGfgUfgGfaCfuUfcUfcUfcAfY44	969	PusGfsaGfaGfaAfgUfcCfaCfcAfcGfasusu	998
AD-63938	Y44ACUCGUGGUGGACUUCUCUCA	970	UGAGAGAAGUCCACCACGAGUCU	999
AD-65561	uscsguGfgUfGfGfacuuCfUfcucaL96	971	UfsGfsagaGfaAfGfuccaCfcAfcgasusu	1000
AD-65566	uscsguGfgUfGfGfacuucucucaL96	972	UfsGfsagaGfaAfGfuccaCfcAfcgasusu	1001
AD-63944	Y44ucGuGGuGGAcuucucucAusuY44	973	UfGfagAfgAfAfGUfccaCfCAfcgAusu	1002
AD-63968	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	974	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1003
AD-65406	uscsguGfgUfGfGfacuuCfUfcucaL96	975	usGfsagaGfaAfGfuccaCfcAfcgasusu	1004
AD-65396	ascsucguGfgUfGfGfacuucucucaL96	976	usGfsagaGfaaguccaCfcAfcgagususu	1005
AD-65427	gsusgcacUfuCfGfCfuucaccucuaL96	977	PusAfsgagGfugaagcgAfaGfugcacsusu	1006
AD-65573	gsusgcacUfuCfGfCfuucaCfCfucuaL96	978	UfsAfsgagGfuGfAfagcgAfaGfugcacsasc	1007
AD-65432	gscsacUfucGfCfuucacCfucuaL96	979	PusAfsgagGfuGfAfagcgAfaGfugcsasc	1008
AD-64332	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	980	PcsAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1009
AD-64322	AfsusGfuGfuCfuGfCfGfgCfgUfuUfuAfuAfL96	981	PusAfsuAfaAfaCfgCfcgcAfgAfcAfcAfuscsc	1010
AD-64272	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	982	csAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1011
AD-65583	gscsacuucgdCuucac(Cgn)ucuaL96	983	usdAsgagdGugaagcgdAagugcsusu	1012
AD-63994	gsgsUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	984	PasAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	1013
AD-65370	csgsugguGfgAfCfUfucucUfCfaauuL96	985	asAfsuugAfgAfGfaaguCfcAfccagcsasg	1014
AD-65265	gsusggugGfaCfUfUfcUfcucaauuuL96	986	asAfsaUfugagaGfaagUfcCfaccAfcsgsa	1015
AD-65407	csgsugguGfgAfCfUfucucUfCfaauuL96	987	asAfsuugAfgAfgAfaguCfcAfccagcsasg	1016
AD-64027	gsgsUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	988	asAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	1017
AD-65266	gsusggugGfaCfUfUfcucuCfaauuuL96	989	asAfsaUfugagaGfaagUfcCfaccAfcsgsa	1018

DuplexID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65389	usgsgudGgucdTucucuaaauuL96	990	asdAsuugagagdAagudCcaccasusu	1019
AD-64008	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	991	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1020
AD-65377	csgsuggudGgucdTucucuaaauuL96	992	asdAsuugagagdAagudCcaccagcsusu	1021
AD-65409	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	993	PusAfsaaaUfuGfAfgagaAfgUfccaccsasc	1022
AD-65403	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	994	usAfsaaaUfuGfAfgagaAfgUfccaccsasc	1023
AD-65385	usgsgacuacdTcucaaauuuaL96	995	usdAsaaauugadGagadAguccasusu	1024

Table 6. Exemplary Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

Duplex ID	Sense ID	Sense Sequence Unmodified (5' to 3')	SEQ ID NO:	Antisense ID	Antisense Sequence Unmodified (5' to 3')	SEQ ID NO:
AD-65381	A-130366	UCGUGGUGGACUUCUCUCA	1025	A-131904	UGAGAGAAGUCCACCACGAUU	1036
AD-66019	A-130366	UCGUGGUGGACUUCUCUCA	1026	A-131904	UGAGAGAAGUCCACCACGAUU	1037
AD-65375	A-130366	UCGUGGUGGACUUCUCUCA	1027	A-130364	UGAGAGAAGUCCACCACGAUU	1038
AD-65427	A-130441	GUGCACUUCGCUUCACCUCUA	1028	A-131905	UAGAGGUGAAGCGAAGUGCACUU	1039
AD-66110	A-130441	GUGCACUUCGCUUCACCUCUA	1029	A-131905	UAGAGGUGAAGCGAAGUGCACUU	1040
AD-65421	A-130441	GUGCACUUCGCUUCACCUCUA	1030	A-130442	UAGAGGUGAAGCGAAGUGCACUU	1041
AD-65407	A-130371	CGUGGUGGACUUCUCUCAAUU	1031	A-130372	AAUUGAGAGAAGUCCACCAGCAG	1042
AD-65377	A-130384	CGUGGUGGUCTUCUCUAAAUU	1032	A-130748	AAUUGAGAGAAGUCCACCAGCUU	1043

Duplex ID	Sense ID	Sense Sequence Unmodified (5' to 3')	SEQ ID NO:	Antisense ID	Antisense Sequence Unmodified (5' to 3')	SEQ ID NO:
AD-65409	A-130388	GGUGGACUUCUCUCAAUUUUA	1033	A-131906	UAAAAUUGAGAGAAGUCCACCAC	1044
AD-66111	A-130388	GGUGGACUUCUCUCAAUUUUA	1034	A-131906	UAAAAUUGAGAGAAGUCCACCAC	1045
AD-65403	A-130388	GGUGGACUUCUCUCAAUUUUA	1035	A-130389	UAAAAUUGAGAGAAGUCCACCAC	1046

Table 7. Exemplary Modified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

Duplex ID	Sense ID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense ID	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65381	A-130366	uscsguGfgUfGfGfacuucucucaL96	1047	A-131904	PusGfsagaGfaAfGfuccaCfcAfcgasusu	1058
AD-66019	A-130366	uscsguGfgUfGfGfacuucucucaL96	1048	A-131904	VPusGfsagaGfaAfGfuccaCfcAfcgasusu	1059
AD-65375	A-130366	uscsguGfgUfGfGfacuucucucaL96	1049	A-130364	usGfsagaGfaAfGfuccaCfcAfcgasusu	1060
AD-65427	A-130441	gsusgcacUfuCfGfCfuucaccucuaL96	1050	A-131905	PusAfsgagGfugaagcgAfaGfugcacsusu	1061
AD-66110	A-130441	gsusgcacUfuCfGfCfuucaccucuaL96	1051	A-131905	VPusAfsgagGfugaagcgAfaGfugcacsusu	1062
AD-65421	A-130441	gsusgcacUfuCfGfCfuucaccucuaL96	1052	A-130442	usAfsgagGfugaagcgAfaGfugcacsusu	1063
AD-65407	A-130371	csgsugguGfgAfCfUfucucUfCfaauuL96	1053	A-130372	asAfsuugAfgAfgAfaguCfcAfccagcsasg	1064
AD-65377	A-130384	csgsuggudGgucdTucucuaaauuL96	1054	A-130748	asdAsuugagagdAagudCcaccagcsusu	1065

Duplex ID	Sense ID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense ID	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65409	A-130388	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1055	A-131906	PusAfsaaaUfuGfAfgagaAfgUfccaccsasc	1066
AD-66111	A-130388	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1056	A-131906	VPusAfsaaaUfuGfAfgagaAfgUfccaccsasc	1067
AD-65403	A-130388	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1057	A-130389	usAfsaaaUfuGfAfgagaAfgUfccaccsasc	1068

Table 8. Exemplary Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

DuplexID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65776	A-131859	UGUGCACUUCGCUUCACCUCU	1069	A-131860	AGAGGUGAAGCGAAGUGCACACG	1115
AD-65782	A-131877	UGCACUUCGCUUCACCUCUGA	1070	A-131878	UCAGAGGUGAAGCGAAGUGCACA	1116
AD-65792	A-131865	GUGUGCACUUCGCUUCACCUA	1071	A-131866	UAGGUGAAGCGAAGUGCACACGG	1117
AD-65781	A-131861	CGUGUGCACUUCGCUUCACCU	1072	A-131862	AGGUGAAGCGAAGUGCACACGGU	1118
AD-64304	A-128443	GUGCACUUCGCUUCACCUCUA	1073	A-128444	UAGAGGUGAAGCGAAGUGCACAC	1119
AD-65771	A-131857	CCGUGUGCACUUCGCUUCACA	1074	A-131858	UGUGAAGCGAAGUGCACACGGUC	1120
AD-65758	A-131867	CACUUCGCUUCACCUCUGCAA	1075	A-131868	UUGCAGAGGUGAAGCGAAGUGCA	1121
AD-65777	A-131875	ACUUCGCUUCACCUCUGCACA	1076	A-131876	UGUGCAGAGGUGAAGCGAAGUGC	1122
AD-61567	A-123525	GGCUGUAGGCAUAAAUUGGUA	1077	A-123526	UACCAAUUUAUGCCUACAGCCUC	1123
AD-65772	A-131873	UUCGCUUCACCUCUGCACGUA	1078	A-131874	UACGUGCAGAGGUGAAGCGAAGU	1124
AD-65767	A-131871	UCGCUUCACCUCUGCACGUCA	1079	A-131872	UGACGUGCAGAGGUGAAGCGAAG	1125
AD-65763	A-131869	CUUCGCUUCACCUCUGCACGU	1080	A-131870	ACGUGCAGAGGUGAAGCGAAGUG	1126
AD-64281	A-128395	CCCCGUCUGUGCCUUCUCAUA	1081	A-128396	UAUGAGAAGGCACAGACGGGGAG	1127
AD-64311	A-128391	CCGUCUGUGCCUUCUCAUCUA	1082	A-128392	UAGAUGAGAAGGCACAGACGGGG	1128

DuplexID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65790	A-131837	CCAGCACCAUGCAACUUUUUA	1083	A-131838	UAAAAAGUUGCAUGGUGCUGGUG	1129
AD-65761	A-131841	CACCAGCACCAUGCAACUUUU	1084	A-131842	AAAAGUUGCAUGGUGCUGGUGCG	1130
AD-65786	A-131849	CACCAUGCAACUUUUUCACCU	1085	A-131850	AGGUGAAAAAGUUGCAUGGUGCU	1131
AD-65785	A-131835	CAAUGUCAACGACCGACCUUA	1086	A-131836	UAAGGUCGGUCGUUGACAUUGCA	1132
AD-65787	A-131863	CGCUUCACCUCUGCACGUCGA	1087	A-131864	UCGACGUGCAGAGGUGAAGCGAA	1133
AD-65770	A-131845	ACCUUGAGGCAUACUUCAAAG	1088	A-131846	CUUUGAAGUAUGCCUCAAGGUCG	1134
AD-65766	A-131843	CCGACCUUGAGGCAUACUUCA	1089	A-131844	UGAAGUAUGCCUCAAGGUCGGUC	1135
AD-61555	A-123521	GACCUUGAGGCAUACUUCAAA	1090	A-123522	UUUGAAGUAUGCCUCAAGGUCGG	1136
AD-65762	A-131855	ACCGACCUUGAGGCAUACUUA	1091	A-131856	UAAGUAUGCCUCAAGGUCGGUCG	1137
AD-65755	A-131827	UCGCAUGGAGACCACCGUGAA	1092	A-131828	UUCACGGUGGUCUCCAUGCGACG	1138
AD-65788	A-131811	UUACAUAAGAGGACUCUUGGA	1093	A-131812	UCCAAGAGUCCUCUUAUGUAAGA	1139
AD-65768	A-131803	UCUUACAUAAGAGGACUCUUA	1094	A-131804	UAAGAGUCCUCUUAUGUAAGACC	1140
AD-61561	A-123523	ACUUCAAAGACUGUUUGUUUA	1095	A-123524	UAAACAAACAGUCUUUGAAGUAU	1141
AD-65764	A-131801	UACUUCAAAGACUGUUUGUUU	1096	A-131802	AAACAAACAGUCUUUGAAGUAUG	1142
AD-65753	A-131799	AUACUUCAAAGACUGUUUGUU	1097	A-131800	AACAAACAGUCUUUGAAGUAUGC	1143
AD-65765	A-131817	UUGUUUAAAGACUGGGAGGAA	1098	A-131818	UUCCUCCCAGUCUUUAAACAAAC	1144
AD-65769	A-131819	GCAUACUUCAAAGACUGUUUA	1099	A-131820	UAAACAGUCUUUGAAGUAUGCCU	1145
AD-65759	A-131815	CAAAGACUGUUUGUUUAAAGA	1100	A-131816	UCUUUAAACAAACAGUCUUUGAA	1146
AD-65774	A-131831	AGACUGUUUGUUUAAAGACUA	1101	A-131832	UAGUCUUUAAACAAACAGUCUUU	1147
AD-65778	A-131807	GUUUGUUUAAAGACUGGGAGA	1102	A-131808	UCUCCCAGUCUUUAAACAAACAG	1148
AD-65773	A-131805	GGGGGAGGAGAUUAGAUUAAA	1103	A-131806	UUUAAUCUAAUCUCCUCCCCCAA	1149
AD-65789	A-131825	GGGGAGGAGAUUAGAUUAAAG	1104	A-131826	CUUUAAUCUAAUCUCCUCCCCCA	1150
AD-65783	A-131809	GUUGGGGGAGGAGAUUAGAUU	1105	A-131810	AAUCUAAUCUCCUCCCCCAACUC	1151
AD-65754	A-131813	UUGGGGGAGGAGAUUAGAUUA	1106	A-131814	UAAUCUAAUCUCCUCCCCCAACU	1152
AD-65779	A-131821	GGGAGGAGAUUAGAUUAAAGA	1107	A-131822	UCUUUAAUCUAAUCUCCUCCCCC	1153
AD-65791	A-131851	UUAGAUUAAAGGUCUUUGUAA	1108	A-131852	UUACAAAGACCUUUAAUCUAAUC	1154

DuplexID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65760	A-131829	UAGAUUAAAGGUCUUUGUACU	1109	A-131830	AGUACAAAGACCUUUAAUCUAAU	1155
AD-65784	A-131823	AUUAGAUUAAAGGUCUUUGUA	1110	A-131824	UACAAAGACCUUUAAUCUAAUCU	1156
AD-65757	A-131853	GAGGAGAUUAGAUUAAAGGUA	1111	A-131854	UACCUUUAAUCUAAUCUCCUCCC	1157
AD-65775	A-131847	GGACUCUUGGACUCUCUGCAA	1112	A-131848	UUGCAGAGAGUCCAAGAGUCCUC	1158
AD-65780	A-131833	ACUCUUGGACUCUCUGCAAUA	1113	A-131834	UAUUGCAGAGAGUCCAAGAGUCC	1159
AD-65756	A-131839	AGAUUAAAGGUCUUUGUACUA	1114	A-131840	UAGUACAAAGACCUUUAAUCUAA	1160

Table 9. Exemplary Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs (Activity data available in WO2016/077321, incorporated herein by reference)

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65776	A-131859	UfsgsUfgCfaCfuUfCfGfcUfuCfaCfcUfcUfL96	1161	A-131860	asGfsaGfgUfgAfaGfcgaAfgUfgCfaCfascsg	1207
AD-65782	A-131877	UfsgsCfaCfuUfcGfCfUfuCfaCfcUfcUfgAfL96	1162	A-131878	usCfsaGfaGfgUfgAfagcGfaAfgUfgCfascsa	1208
AD-65792	A-131865	GfsusGfuGfcAfcUfUfCfgCfuUfcAfcCfuAfL96	1163	A-131866	usAfsgGfuGfaAfgCfgaaGfuGfcAfcAfcsgsg	1209
AD-65781	A-131861	CfsgsUfgUfgCfaCfUfUfcGfcUfuCfaCfcUfL96	1164	A-131862	asGfsgUfgAfaGfcGfaagUfgCfaCfaCfgsgsu	1210
AD-64304	A-128443	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuAfL96	1165	A-128444	usAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1211
AD-65771	A-131857	CfscsGfuGfuGfcAfCfUfuCfgCfuUfcAfcAfL96	1166	A-131858	usGfsuGfaAfgCfgAfaguGfcAfcAfcGfgsusc	1212
AD-65758	A-131867	CfsasCfuUfcGfcUfUfUfCfaCfcUfcUfgCfaAfL96	1167	A-131868	usUfsgCfaGfaGfgUfgaaGfcGfaAfgUfgscsa	1213
AD-65777	A-131875	AfscsUfuCfgCfuUfCfAfcCfuCfuGfcAfcAfL96	1168	A-131876	usGfsuGfcAfgAfgGfugaAfgCfgAfaGfusgsc	1214
AD-61567	A-123525	GfsgsCfuGfuAfgGfCfAfuAfaAfuUfgGfuAfL96	1169	A-123526	usAfscCfaAfuUfuAfugcCfuAfcAfgCfcsusc	1215
AD-65772	A-131873	UfsusCfgCfuUfcAfCfCfuCfuGfcAfcGfuAfL96	1170	A-131874	usAfscGfuGfcAfgAfgguGfaAfgCfgAfasgsu	1216
AD-65767	A-131871	UfscsGfcUfuCfaCfCfUfcUfgCfaCfgUfcAfL96	1171	A-131872	usGfsaCfgUfgCfaGfaggUfgAfaGfcGfasasg	1217
AD-65763	A-131869	CfsusUfcGfcUfuCfAfCfcUfcUfgCfaCfgUfL96	1172	A-131870	asCfsgUfgCfaGfaGfgugAfaGfcGfaAfgsusg	1218
AD-64281	A-128395	CfscsCfcGfuCfuGfUfGfcCfuUfcUfcAfuAfL96	1173	A-128396	usAfsuGfaGfaAfgGfcacAfgAfcGfgGfgsasg	1219

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64311	A-128391	CfscsGfuCfuGfuGfCfCfuUfcUfcAfuCfuAfL96	1174	A-128392	usAfsgAfuGfaGfaAfggcAfcAfgAfcGfgsgsg	1220
AD-65790	A-131837	CfscsAfgCfaCfcAfUfGfcAfaCfuUfuUfuAfL96	1175	A-131838	usAfsaAfaAfgUfuGfcauGfgUfgCfuGfgsusg	1221
AD-65761	A-131841	CfsasCfcAfgCfaCfCfAfuGfcAfaCfuUfuUfL96	1176	A-131842	asAfsaAfgUfuGfcAfuggUfgCfuGfgUfgscsg	1222
AD-65786	A-131849	CfsasCfcAfuGfcAfAfCfuUfuUfuCfaCfcUfL96	1177	A-131850	asGfsgUfgAfaAfaAfguuGfcAfuGfgUfgscsu	1223
AD-65785	A-131835	CfsasAfuGfuCfaAfCfGfaCfcGfaCfcUfuAfL96	1178	A-131836	usAfsaGfgUfcGfgUfcguUfgAfcAfuUfgscsa	1224
AD-65787	A-131863	CfsgsCfuUfcAfcCfUfCfuGfcAfcGfuCfgAfL96	1179	A-131864	usCfsgAfcGfuGfcAfgagGfuGfaAfgCfgsasa	1225
AD-65770	A-131845	AfscsCfuUfgAfgGfCfAfuAfcUfuCfaAfaGfL96	1180	A-131846	csUfsuUfgAfaGfuAfugcCfuCfaAfgGfuscsg	1226
AD-65766	A-131843	CfscsGfaCfcUfuGfAfGfgCfaUfaCfuUfcAfL96	1181	A-131844	usGfsaAfgUfaUfgCfcucAfaGfgUfcGfgsusc	1227
AD-61555	A-123521	GfsasCfcUfuGfaGfGfCfaUfaCfuUfcAfaAfL96	1182	A-123522	usUfsuGfaAfgUfaUfgccUfcAfaGfgUfcsgsg	1228
AD-65762	A-131855	AfscsCfgAfcCfuUfGfAfgGfcAfuAfcUfuAfL96	1183	A-131856	usAfsaGfuAfuGfcCfucaAfgGfuCfgGfuscsg	1229
AD-65755	A-131827	UfscsGfcAfuGfgAfGfAfcCfaCfcGfuGfaAfL96	1184	A-131828	usUfscAfcGfgUfgGfucuCfcAfuGfcGfascsg	1230
AD-65788	A-131811	UfsusAfcAfuAfaGfAfGfgAfcUfcUfuGfgAfL96	1185	A-131812	usCfscAfaGfaGfuCfcucUfuAfuGfuAfasgsa	1231
AD-65768	A-131803	UfscsUfuAfcAfuAfAfGfaGfgAfcUfcUfuAfL96	1186	A-131804	usAfsaGfaGfuCfcUfcuuAfuGfuAfaGfascsc	1232
AD-61561	A-123523	AfscsUfuCfaAfaGfAfCfuGfuUfuGfuUfuAfL96	1187	A-123524	usAfsaAfcAfaAfcAfgucUfuUfgAfaGfusasu	1233
AD-65764	A-131801	UfsasCfuUfcAfaAfGfAfcUfgUfuUfgUfuUfL96	1188	A-131802	asAfsaCfaAfaCfaGfucuUfuGfaAfgUfasusg	1234
AD-65753	A-131799	AfsusAfcUfuCfaAfAfGfaCfuGfuUfuGfuUfL96	1189	A-131800	asAfscAfaAfcAfgUfcuuUfgAfaGfuAfusgsc	1235
AD-65765	A-131817	UfsusGfuUfuAfaAfGfAfcUfgGfgAfgGfaAfL96	1190	A-131818	usUfscCfuCfcCfaGfucuUfuAfaAfcAfasasc	1236
AD-65769	A-131819	GfscsAfuAfcUfuCfAfAfaGfaCfuGfuUfuAfL96	1191	A-131820	usAfsaAfcAfgUfcUfuugAfaGfuAfuGfcscsu	1237
AD-65759	A-131815	CfsasAfaGfaCfuGfUfUfuGfuUfuAfaAfgAfL96	1192	A-131816	usCfsuUfuAfaAfcAfaacAfgUfcUfuUfgsasa	1238
AD-65774	A-131831	AfsgsAfcUfgUfuUfGfUfuUfaAfaGfaCfuAfL96	1193	A-131832	usAfsgUfcUfuUfaAfacaAfaCfaGfuCfususu	1239
AD-65778	A-131807	GfsusUfuGfuUfuAfAfAfgAfcUfgGfgAfgAfL96	1194	A-131808	usCfsuCfcCfaGfuCfuuuAfaAfcAfaAfcsasg	1240
AD-65773	A-131805	GfsgsGfgGfaGfgAfGfAfuUfaGfaUfuAfaAfL96	1195	A-131806	usUfsuAfaUfcUfaAfucuCfcUfcCfcCfcsasa	1241
AD-65789	A-131825	GfsgsGfgAfgGfaGfAfUfuAfgAfuUfaAfaGfL96	1196	A-131826	csUfsuUfaAfuCfuAfaucUfcCfuCfcCfcscsa	1242
AD-65783	A-131809	GfsusUfgGfgGfgAfGfGfaGfaUfuAfgAfuUfL96	1197	A-131810	asAfsuCfuAfaUfcUfccuCfcCfcCfaAfcsusc	1243
AD-65754	A-131813	UfsusGfgGfgGfaGfGfAfgAfuUfaGfaUfuAfL96	1198	A-131814	usAfsaUfcUfaAfuCfuccUfcCfcCfcAfascsu	1244
AD-65779	A-131821	GfsgsGfaGfgAfgAfUfUfaGfaUfuAfaAfgAfL96	1199	A-131822	usCfsuUfuAfaUfcUfaauCfuCfcUfcCfcscsc	1245

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65791	A-131851	UfsusAfgAfuUfaAfAfGfgUfcUfuUfgUfaAfL96	1200	A-131852	usUfsaCfaAfaGfaCfcuuUfaAfuCfuAfasusc	1246
AD-65760	A-131829	UfsasGfaUfuAfaAfGfGfuCfuUfuGfuAfcUfL96	1201	A-131830	asGfsuAfcAfaAfgAfccuUfuAfaUfcUfasasu	1247
AD-65784	A-131823	AfsusUfaGfaUfuAfAfAfgGfuCfuUfuGfuAfL96	1202	A-131824	usAfscAfaAfgAfcCfuuuAfaUfcUfaAfuscsu	1248
AD-65757	A-131853	GfsasGfgAfgAfuUfAfGfaUfuAfaAfgGfuAfL96	1203	A-131854	usAfscCfuUfuAfaUfcuaAfuCfuCfcUfcscsc	1249
AD-65775	A-131847	GfsgsAfcUfcUfuGfGfAfcUfcUfcUfgCfaAfL96	1204	A-131848	usUfsgCfaGfaGfaGfuccAfaGfaGfuCfcsusc	1250
AD-65780	A-131833	AfscsUfcUfuGfgAfCfUfcUfcUfgCfaAfuAfL96	1205	A-131834	usAfsuUfgCfaGfaGfaguCfcAfaGfaGfuscsc	1251
AD-65756	A-131839	AfsgsAfuUfaAfaGfGfUfcUfuUfgUfaCfuAfL96	1206	A-131840	usAfsgUfaCfaAfaGfaccUfuUfaAfuCfusasa	1252

Table 10. Exemplary Unmodified HBV X ORF Sense and Antisense Sequences. (Activity data available in WO2016/077321, incorporated herein by reference)

DuplexID	Sense Sequence Unmodified (5' to 3')	SEQ ID NO:	Antisense Sequence Umodified (5' to 3')	SEQ ID NO:
AD-66808	GUCUGUGCCUUCUCAUCUA	1253	UAGAUGAGAAGGCACAGACUU	1263
AD-66809	GUCUGUGCCUUCUCAUCUA	1254	UAGAUGAGAAGGCACAGACUU	1264
AD-66810	GUGUGCACUUCGCUUCACA	1255	UGUGAAGCGAAGUGCACACUU	1265
AD-66811	GUGUGCACUUCGCUUCACA	1256	UGUGAAGCGAAGUGCACACUU	1266
AD-66812	UGUGCACUUCGCUUCACCUCU	1257	AGAGGUGAAGCGAAGUGCACAUU	1267
AD-66813	UGUGCACUUCGCUUCACCUCU	1258	AGAGGUGAAGCGAAGUGCACAUU	1268
AD-66814	CACCAGCACCAUGCAACUUUU	1259	AAAAGUUGCAUGGUGCUGGUGUU	1269
AD-66815	CACCAGCACCAUGCAACUUUU	1260	AAAAGUUGCAUGGUGCUGGUGUU	1270
AD-66816	CACCAUGCAACUUUUUCACCU	1261	AGGUGAAAAAGUUGCAUGGUGUU	1271
AD-66817	CACCAUGCAACUUUUUCACCU	1262	AGGUGAAAAAGUUGCAUGGUGUU	1272

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Table 11. Exemplary Modified HBV X ORF Sense and Antisense Sequences. (Activity data available in WO2016/077321, incorporated herein by reference)

DuplexID	Sense Sequence Modified (5' to 3')	SEQ ID NO:	Antisense Sequence Modified (5' to 3')	SEQ ID NO:
AD-66808	gsuscuGfuGfCfCfuucucaucuaL96	1273	usAfsgauGfaGfAfaggcAfcAfgacsusu	1283
AD-66809	gsuscuGfuGfCfCfuucucaucuaL96	1274	UfsAfsgauGfaGfAfaggcAfcAfgacsusu	1284
AD-66810	gsusguGfcAfCfUfucgcuucacaL96	1275	usGfsugaAfgCfGfaaguGfcAfcacsusu	1285
AD-66811	gsusguGfcAfCfUfucgcuucacaL96	1276	UfsGfsugaAfgCfGfaaguGfcAfcacsusu	1286
AD-66812	usgsugcaCfuUfCfGfcuucaccucuL96	1277	asGfsaggUfgAfAfgcgaAfgUfgcacasusu	1287
AD-66813	usgsugcaCfuUfCfGfcuucaccucuL96	1278	AfsGfsaggUfgAfAfgcgaAfgUfgcacasusu	1288
AD-66814	csasccagCfaCfCfAfugcaacuuuuL96	1279	asAfsaagUfuGfCfauggUfgCfuggugsusu	1289
AD-66815	csasccagCfaCfCfAfugcaacuuuuL96	1280	AfsAfsaagUfuGfCfauggUfgCfuggugsusu	1290
AD-66816	csasccauGfcAfAfCfuuuuucaccuL96	1281	asGfsgugAfaAfAfaguuGfcAfuggugsusu	1291
AD-66817	csasccauGfcAfAfCfuuuuucaccuL96	1282	AfsGfsgugAfaAfAfaguuGfcAfuggugsusu	1292

Table 12: HBV Target Sequences, noting target sites on Accession No. X02763.1. (Activity data and exemplary chemical modifications available at WO2012/024170, incorporated herein by reference)

Target Sequence	Target Site	SEQ ID NO:
UCGUGGUGGACUUCUCUCA	1663	1293
GUGGUGGACUUCUCUCAAU	1665	1294
GCCGAUCCAUACUGCGGAA	2669	1295
CCGAUCCAUACUGCGGAAC	2670	1296
CAUCCUGCUGCUAUGCCUC	1818	1297
UGCUGCUAUGCCUCAUCUU	1823	1298
GGUGGACUUCUCUCAAUUU	1667	1299
UGGUGGACUUCUCUCAAUU	1666	1300
UAGACUCGUGGUGGACUUC	1658	1301
UCCUCUGCCGAUCCAUACU	2663	1302
UGCCGAUCCAUACUGCGGA	2668	1303
UGGAUGUGUCUGCGGCGUU	1783	1304
CGAUCCAUACUGCGGAACU	2671	1305
CGCACCUCUCUUUACGCGG	2934	1306
CUGCCGAUCCAUACUGCGG	2667	1307
CGUGGUGGACUUCUCUCAA	1664	1308
CUGCUGCUAUGCCUCAUCU	1822	1309
CCUGCUGCUAUGCCUCAUC	1821	1310
CUAGACUCGUGGUGGACUU	1657	1311
UCCUGCUGCUAUGCCUCAU	1820	1312
GACUCGUGGUGGACUUCUC	1660	1313
AUCCAUACUGCGGAACUCC	2673	1314
CUCUGCCGAUCCAUACUGC	2665	1315
GAUCCAUACUGCGGAACUC	2672	1316

Target Sequence	Target Site	SEQ ID NO:
GAAGAACUCCCUCGCCUCG	567	1317
AAGCCUCCAAGCUGUGCCU	54	1318
AGAAGAACUCCCUCGCCUC	566	1319
GGAGUGUGGAUUCGCACUC	455	1320
CCUCUGCCGAUCCAUACUG	2664	1321
CAAGCCUCCAAGCUGUGCC	53	1322
UCCAUACUGCGGAACUCCU	2674	1323
CAGAGUCUAGACUCGUGGU	1651	1324
AAGAAGAACUCCCUCGCCU	565	1325
GAGUGUGGAUUCGCACUCC	456	1326
UCUAGACUCGUGGUGGACU	1656	1327
GCUGCUAUGCCUCAUCUUC	1824	1328
AGUCUAGACUCGUGGUGGA	1654	1329
CUCCUCUGCCGAUCCAUAC	2662	1330
UGGCUCAGUUUACUAGUGC	2077	1331
GUCUAGACUCGUGGUGGAC	1655	1332
UUCAAGCCUCCAAGCUGUG	51	1333
CUAUGGGAGUGGGCCUCAG	2047	1334
CUCGUGGUGGACUUCUCUC	1662	1335
CCUAUGGGAGUGGGCCUCA	2046	1336
AAGAACUCCCUCGCCUCGC	568	1337
UCUGCCGAUCCAUACUGCG	2666	1338
AGAGUCUAGACUCGUGGUG	1652	1339
GAAGAAGAACUCCCUCGCC	564	1340
UCAAGCCUCCAAGCUGUGC	52	1341
AGCCUCCAAGCUGUGCCUU	55	1342

Target Sequence	Target Site	SEQ ID NO:
AGACUCGUGGUGGACUUCU	1659	1343

Table 13. Various HBV siNA sense and antisense sequences corresponding to the identified target sequences in **Table 1a**. (Activity data and exemplary chemical modifications available at WO2012/024170, incorporated herein by reference)

Target Site	SEQ ID NO:	Sense Sequence	Antisense Sequence	SEQ ID NO:
1663	1344	UCGUGGUGGACUUCUCUCA	UGAGAGAAGUCCACCACGA	1395
1665	1345	GUGGUGGACUUCUCUCAAU	AUUGAGAGAAGUCCACCAC	1396
2669	1346	GCCGAUCCAUACUGCGGAA	UUCCGCAGUAUGGAUCGGC	1397
2670	1347	CCGAUCCAUACUGCGGAAC	GUUCCGCAGUAUGGAUCGG	1398
1818	1348	CAUCCUGCUGCUAUGCCUC	GAGGCAUAGCAGCAGGAUG	1399
1823	1349	UGCUGCUAUGCCUCAUCUU	AAGAUGAGGCAUAGCAGCA	1400
1667	1350	GGUGGACUUCUCUCAAUUU	AAAUUGAGAGAAGUCCACC	1401
1666	1351	UGGUGGACUUCUCUCAAUU	AAUUGAGAGAAGUCCACCA	1402
1658	1352	UAGACUCGUGGUGGACUUC	GAAGUCCACCACGAGUCUA	1403
2663	1353	UCCUCUGCCGAUCCAUACU	AGUAUGGAUCGGCAGAGGA	1404
2668	1354	UGCCGAUCCAUACUGCGGA	UCCGCAGUAUGGAUCGGCA	1405
1783	1355	UGGAUGUGUCUGCGGCGUU	AACGCCGCAGACACAUCCA	1406
2671	1356	CGAUCCAUACUGCGGAACU	AGUUCCGCAGUAUGGAUCG	1407
2934	1357	CGCACCUCUCUUUACGCGG	CCGCGUAAAGAGAGGUGCG	1408
2667	1358	CUGCCGAUCCAUACUGCGG	CCGCAGUAUGGAUCGGCAG	1409
1664	1359	CGUGGUGGACUUCUCUCAA	UUGAGAGAAGUCCACCACG	1410
1822	1360	CUGCUGCUAUGCCUCAUCU	AGAUGAGGCAUAGCAGCAG	1411
1821	1361	CCUGCUGCUAUGCCUCAUC	GAUGAGGCAUAGCAGCAGG	1412
1657	1362	CUAGACUCGUGGUGGACUU	AAGUCCACCACGAGUCUAG	1413
1820	1363	UCCUGCUGCUAUGCCUCAU	AUGAGGCAUAGCAGCAGGA	1414
1660	1364	GACUCGUGGUGGACUUCUC	GAGAAGUCCACCACGAGUC	1415

Target Site	SEQ ID NO:	Sense Sequence	Antisense Sequence	SEQ ID NO:
2673	1365	AUCCAUACUGCGGAACUCC	GGAGUUCCGCAGUAUGGAU	1416
2665	1366	CUCUGCCGAUCCAUACUGC	GCAGUAUGGAUCGGCAGAG	1417
2672	1367	GAUCCAUACUGCGGAACUC	GAGUUCCGCAGUAUGGAUC	1418
567	1368	GAAGAACUCCCUCGCCUCG	CGAGGCGAGGGAGUUCUUC	1419
54	1369	AAGCCUCCAAGCUGUGCCU	AGGCACAGCUUGGAGGCUU	1420
566	1370	AGAAGAACUCCCUCGCCUC	GAGGCGAGGGAGUUCUUCU	1421
455	1371	GGAGUGUGGAUUCGCACUC	GAGUGCGAAUCCACACUCC	1422
2664	1372	CCUCUGCCGAUCCAUACUG	CAGUAUGGAUCGGCAGAGG	1423
53	1373	CAAGCCUCCAAGCUGUGCC	GGCACAGCUUGGAGGCUUG	1424
2674	1374	UCCAUACUGCGGAACUCCU	AGGAGUUCCGCAGUAUGGA	1425
1651	1375	CAGAGUCUAGACUCGUGGU	ACCACGAGUCUAGACUCUG	1426
565	1376	AAGAAGAACUCCCUCGCCU	AGGCGAGGGAGUUCUUCUU	1427
456	1377	GAGUGUGGAUUCGCACUCC	GGAGUGCGAAUCCACACUC	1428
1656	1378	UCUAGACUCGUGGUGGACU	AGUCCACCACGAGUCUAGA	1429
1824	1379	GCUGCUAUGCCUCAUCUUC	GAAGAUGAGGCAUAGCAGC	1430
1654	1380	AGUCUAGACUCGUGGUGGA	UCCACCACGAGUCUAGACU	1431
2662	1381	CUCCUCUGCCGAUCCAUAC	GUAUGGAUCGGCAGAGGAG	1432
2077	1382	UGGCUCAGUUUACUAGUGC	GCACUAGUAAACUGAGCCA	1433
1655	1383	GUCUAGACUCGUGGUGGAC	GUCCACCACGAGUCUAGAC	1434
51	1384	UUCAAGCCUCCAAGCUGUG	CACAGCUUGGAGGCUUGAA	1435
2047	1385	CUAUGGGAGUGGGCCUCAG	CUGAGGCCCACUCCCAUAG	1436
1662	1386	CUCGUGGUGGACUUCUCUC	GAGAGAAGUCCACCACGAG	1437
2046	1387	CCUAUGGGAGUGGGCCUCA	UGAGGCCCACUCCCAUAGG	1438
568	1388	AAGAACUCCCUCGCCUCGC	GCGAGGCGAGGGAGUUCUU	1439
2666	1389	UCUGCCGAUCCAUACUGCG	CGCAGUAUGGAUCGGCAGA	1440
1652	1390	AGAGUCUAGACUCGUGGUG	CACCACGAGUCUAGACUCU	1441
564	1391	GAAGAAGAACUCCCUCGCC	GGCGAGGGAGUUCUUCUUC	1442

Target Site	SEQ ID NO:	Sense Sequence	Antisense Sequence	SEQ ID NO:
52	1392	UCAAGCCUCCAAGCUGUGC	GCACAGCUUGGAGGCUUGA	1443
55	1393	AGCCUCCAAGCUGUGCCUU	AAGGCACAGCUUGGAGGCU	1444
1659	1394	AGACUCGUGGUGGACUUCU	AGAAGUCCACCACGAGUCU	1445

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. Use of an RNAi agent in the manufacture of a first medicament, use of a protein-based HBV vaccine in the manufacture of a second medicament, and use of a nucleic acid-based HBV vaccine in the manufacture of a third medicament, wherein the first, second and third medicaments are to be used for a combination therapy 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, and (i) the sense strand comprises the nucleotide sequence of 5'-GUGUGCACUUCGCUUCACA-3' (SEQ ID NO: 27) and the antisense strand comprises the nucleotide sequence of 5'-UGUGAAGCGAAGUGCACACUU-3' (SEQ ID NO: 25), or (ii) the sense strand comprises the nucleotide sequence of 5'-CACCAUGCAACUUUUUCACCU-3' (SEQ ID NO: 28) and the antisense strand comprises the nucleotide sequence of 5'-AGGUGAAAAAGUUGCAUGGUGUU-3' (SEQ ID NO: 26);

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 is to be administered to a subject having an HBV infection prior to the protein-based HBV vaccine that is to be administered to the subject, and the protein-based HBV vaccine is to be administered to the subject prior to the nucleic acid-based HBV vaccine that is to be administered to the subject.

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 at least one HBsAg determinant of the first HBsAg polypeptide and/or the at least one HBsAg determinant of the second HBsAg polypeptide 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 at least one HBcAg determinant of the first HBcAg polypeptide and/or the at least one HBcAg determinant of the second HBcAg polypeptide 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 0.1 μg to 1.0 mg of the first HBcAg polypeptide, or immunogenic fragment thereof, and a dose of 0.1 μg to 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, a STING agonist, c-di-AMP, c-di-GMP, c-di-CMP; short, blunt-ended 5′-triphosphate dsRNA (3pRNA) Rig-I ligand, poly[di(sodiumcarboxylatoethylphenoxy)phosphazene] (PCEP)), alum, virosomes, cytokines, IL-12, AS02, AS03, AS04, MF59, 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

(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

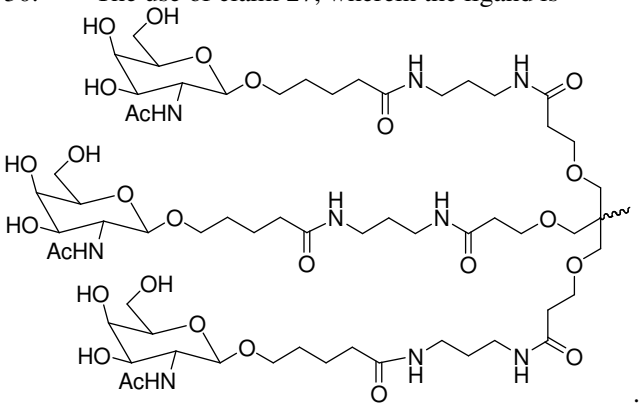
(e) each strand of the RNAi agent has 15-30 or 19-30 nucleotides.

28. The use of claim 27, wherein the ligand is one or more GalNAc derivatives attached through a monovalent linker, bivalent branched linker, or trivalent branched linker.

29. The use of claim 28, 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

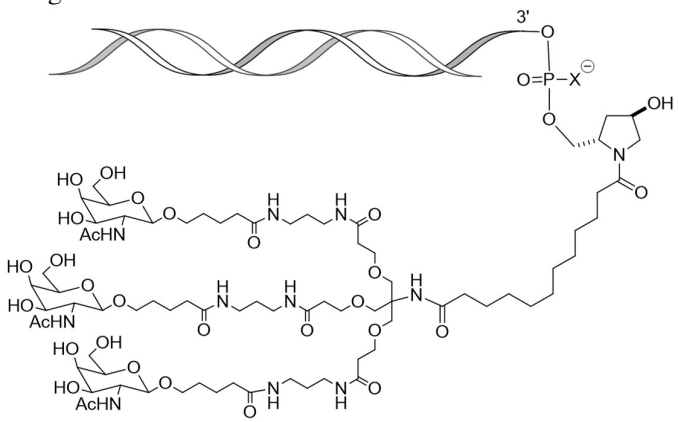
nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified 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.

30. The use of claim 27, wherein the ligand is



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31. The use of claim 30, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

32. The use of any of claims 1-26, wherein (i) the sense strand comprises the nucleotide sequence of 5'-gsusguGfcAfCfUfucgcuucacaL96-3' (SEQ ID NO: 37) and the antisense strand comprises the nucleotide sequence of 5'-usGfsugaAfgCfGfaaguGfcAfcacsusu-3' (SEQ ID NO: 30), or (ii) the sense strand comprises the nucleotide sequence of 5'-csasccauGfcAfAfCfuuuuucaccuL96-3' (SEQ ID NO: 38) and the antisense strand comprises the nucleotide sequence of 5'-asGfsgugAfaAfAfaguuGfcAfuggugsusu-3' (SEQ ID NO: 32);

wherein a, c, g, and u are 2'-O-methyl (2'-OMe) A, 2'-OMe C, 2'-OMe G, and 2'-OMe U, respectively; Af, Cf, Gf, and Uf are 2'-fluoro A, 2'-fluoro C, 2'-fluoro G, and 2'-fluoro U, respectively; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

33. The use of any of claims 1-32, 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.