Therefore, in the absence of any current treatment regimen which can restore immunological control of HBV in a large proportion of patients, there is a need for an effective treatment against HBV infection which can inhibit viral replication as well as restore immunological control in the majority of patients. Accordingly, there is a need in the art for alternative therapies and combination therapies for subjects infected with HBV and/or having an HBV-associated disease.

Summary of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a Hepatitis B virus (HBV) gene. The HBV gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

The present disclosure also provides methods and therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an HBV gene, *e.g.,* an HBV infection and/or an HBV-associated disease, such as chronic Hepatitis B infection (CHB), cirrhosis, liver failure, and hepatocellular carcinoma (HCC), using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an HBV gene for inhibiting the expression of an HBV gene.

The RNAi agents of the disclosure have been designed to target regions in the HBV genome that are conserved across all 8 serotypes of HBV. In addition, the RNAi agents of the disclosure have been designed to inhibit all steps of the HBV life cycle, *e.g.,* replication, assembly, secretion of virus, and secretion of sub-viral antigens, by inhibiting expression of more than one HBV gene. In particular, since transcription of the HBV genome results in polycistronic, overlapping RNAs, an RNAi agent of the disclosure targeting a single HBV gene results in significant inhibition of expression of most or all HBV transcripts. For example, because the HBV genome is transcribed into a single mRNA, an RNAi agent of the disclosure targeting the S gene will result in inhibition of not only S gene expression but also the expression of the "downstream" reverse transcriptase gene. Furthermore, the RNAi agents of the disclosure have been designed to inhibit HBV viral replication by targeting HBV structural genes, and the HBV X gene thereby permitting a subject's immune system to detect and respond to the presence of HBsAg to produce anti-HBV antibodies to clear an HBV infection. 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 disclosure improved efficacy, stability, safety, potency, and durability.

Accordingly, also disclosed are double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region,

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one aspect, the present invention provides, a double stranded RNAi agent for inhibiting expression of hepatitis B virus (HBV) in a cell, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region of complementarity which comprises at least 15 contiguous nucleotides, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:39 (GUGUGCACUUCGCUUCACA), and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:40 (UGUGAAGCGAAGUGCACACUU), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the antisense strand.

In another example, the one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the sense strand.

In one example, all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

In one example, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the sequences listed in any one of Tables 3, 4, 6, 7, 12, 13, 22, 23, 25, and 26.

In one example, 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.

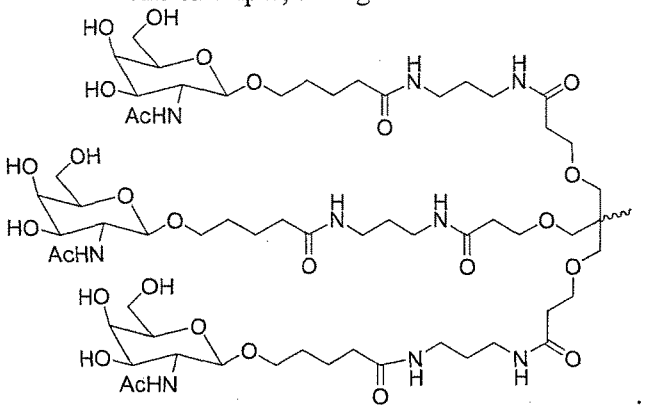
In one example, the at least one strand comprises a 3' overhang of at least 1 nucleotide. In another example, the at least one strand comprises a 3' overhang of at least 2 nucleotides.

In one example, the double-stranded region is 15-30 nucleotide pairs in length. In another example, the double-stranded region is 17-23 nucleotide pairs in length. In yet another example, the double-stranded region is 17-25 nucleotide pairs in length. In one example, the e double-stranded region is 23-27 nucleotide pairs in length. In another

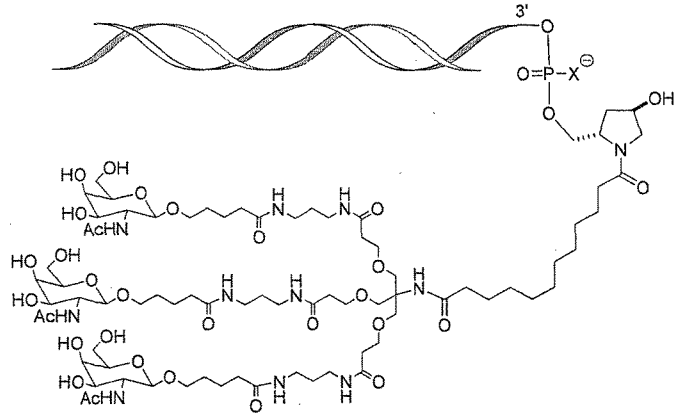
example, the double-stranded region is 19-21 nucleotide pairs in length. In yet another example, the double-stranded region is 21-23 nucleotide pairs in length.

In one example, each strand has 15-30 nucleotides. In another example, each strand has 19-30 nucleotides.

In one example, the ligand is



In one example, the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the RNAi agent is selected from the group of RNAi agents listed in any one of Tables 3, 4, 6, 7, 12, 13, 22, 23, 25, and 26.

Also disclosed are RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'-UCGUGGUGGACUUCUCUCA -3' (SEQ ID NO:5), and said antisense strand comprises 5'-UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

The present disclosure also provides RNAi agents comprising sense and antisense nucleotide sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over their entire length to the foregoing sense and antisense nucleotide sequences.

Also disclosed are double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'-GUGCACUUCGCUUCACCUCUA -3' (SEQ ID NO:7), and said antisense strand comprises 5'-UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. The present disclosure also provides RNAi agents comprising sense and antisense nucleotide sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over their entire length to the foregoing sense and antisense nucleotide sequences.

Also disclosed are double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'-CGUGGUGGACUUCUCUCAAUU -3' (SEQ ID NO:9), and said antisense strand comprises 5'-AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. The present disclosure also provides RNAi agents comprising sense and antisense nucleotide sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over their entire length to the foregoing sense and antisense nucleotide sequences.

Also disclosed are double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region, wherein the sense strand comprises 5'-CGUGGUGGUCUUCUCUAAAUU -3' (SEQ ID NO:37), and the antisense strand comprises 5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein said sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. The present

disclosure also provides RNAi agents comprising sense and antisense nucleotide sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over their entire length to the foregoing sense and antisense nucleotide sequences.

Also disclosed are double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'-GGUGGACUUCUCUCAAUUUUA -3' (SEQ ID NO:11), and said antisense strand comprises 5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. The present disclosure also provides RNAi agents comprising sense and antisense nucleotide sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over their entire length to the foregoing sense and antisense nucleotide sequences.

Also disclosed are double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell. The double stranded RNAi agents include a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'-GUGUGCACUUCGCUUCACA -3' (SEQ ID NO:39), and said antisense strand comprises 5'-UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. The present disclosure also provides RNAi agents comprising sense and antisense nucleotide sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over their entire length to the foregoing sense and antisense nucleotide sequences.

In another aspect, the present invention provides A double stranded RNAi agent for inhibiting expression of hepatitis B virus (HBV) in a cell, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39), and said antisense strand comprises 5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

In one example, at least one of said modified nucleotides is selected from the group consisting of 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 one example, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

In one example, the sense strand comprises 5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:13) and the antisense strand comprises 5'-usGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:14), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In another example, the sense strand comprises 5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15) and the antisense strand comprises 5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In one example, the sense strand comprises 5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:17) and the antisense strand comprises 5'-usAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:18), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In another example, the sense strand comprises 5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:19) and the antisense strand comprises 5'-PusAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:20) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In one example, the sense strand comprises 5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21) and the antisense strand comprises 5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In another example, the sense strand comprises 5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:23) and the antisense strand comprises 5'-PasAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:24) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

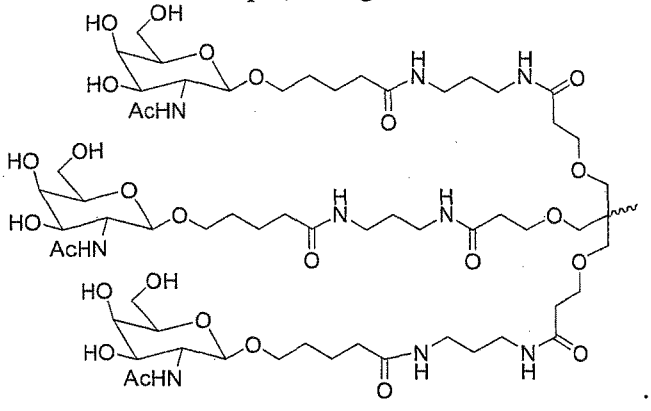
In another example, the sense strand comprises 5'-csgsuggudGgucdTucucuaaauu – 3' (SEQ ID NO:35) and the antisense strand comprises 5'- asdAsuugagagdAagudCcaccagcsusu – 3' (SEQ ID NO:36), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; dA, dC, dG, and dT are deoxyribose A, C, G, and T; and s is a phosphorothioate linkage.

In one example, the sense strand comprises 5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25) and the antisense strand comprises 5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

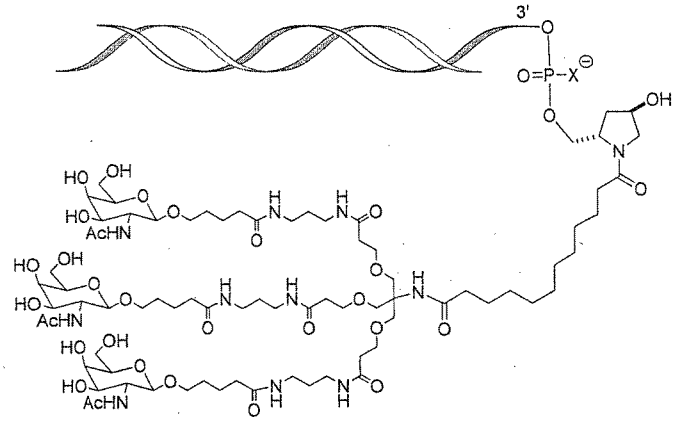
In another example, the sense strand comprises 5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:27) and the antisense strand comprises 5'- PusAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:28) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In another example, the sense strand comprises 5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41) and the antisense strand comprises 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In one example, the ligand is



In one example, the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the P is a 5'-phosphate mimic. In one example, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

Also disclosed are compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis B virus (HBV) in a cell, wherein each double stranded RNAi agent independently comprises a sense strand and an antisense strand forming a double-stranded region, wherein each of said sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and each of said antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of each of said sense strands and substantially all of the nucleotides of each of said antisense strands are independently modified nucleotides, wherein each of said sense strands are independently conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the antisense strand. In another example, the one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the sense strand.

In one example, all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

In one example, the sense strand and said antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the sequences listed in any one of Tables 3, 4, 6, 7, 12, 13, 22, 23, 25, and 26.

In one example, 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.

Also disclosed are compositions for inhibiting expression of hepatitis B virus (HBV) in a cell, the composition comprising (a) a first double-stranded RNAi agent comprising a first sense strand and a first antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and (b) a second double-stranded RNAi agent comprising a second sense strand and a second antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of said second sense strand and substantially all of the nucleotides of said second antisense strand are modified nucleotides, wherein said second sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; wherein the first and second sense strands each independently comprise a sequence selected from the group consisting of
5'- UCGUGGUGGACUUCUCUCA -3' (SEQ IDNO:5),
5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ IDNO:7),
5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ IDNO:9),
5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ IDNO:37),
5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ IDNO:11), and
5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequences), and wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of
5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6),
5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8),

5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10),
5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38),
5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12), and
5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequences).

In one example, the first and second sense strand and/or all of the nucleotides of the first and second antisense strand comprise a modification.

In one example, 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 one example, the first and second RNAi agents are selected from the group consisting of:
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:13)
5'-usGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:14);
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15)
5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16);
5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:17)
5'-usAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:18);
5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:19)
5'-PusAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:20);
5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21)
5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22);
5'- csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:23)
5'-PasAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:24);
5'-csgsuggudGgucdTucucuaaaauu – 3' (SEQ ID NO:35)
5'- asdAsuugagagdAagudCcaccagcsusu – 3' (SEQ ID NO:36);
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25)
5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26);

5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:27)
5'- PusAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:28); and
5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41)
5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; dA, dC, dG, and dT are deoxyribose A, C, G, and T; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In one example, the first and second RNAi agents are
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15)
5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16);
5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21)
5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In another example, the first and second RNAi agents are
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25)
5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26); and
5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41)
5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

Also disclosed is a double stranded RNAi agent comprising the RNAi agents listed in any one of Tables 3, 4, 6, 7, 12, 13, 22, 23, 25, and 26.

The present disclosure also provides vectors and cells comprising the double stranded RNAi agent of the disclosure.

Also disclosed are pharmaceutical compositions comprising the double stranded RNAi agents of the disclosure, or the compositions of the disclosure, or the vectors of the disclosure.

In yet another aspect, the present invention provides a pharmaceutical composition comprising the double stranded RNAi agent disclosed herein.

In one example, the double stranded RNAi agent is administered in an unbuffered solution. In one example, the unbuffered solution is saline or water.

In another example, the double stranded RNAi agent is administered with a buffer solution. In one example, the buffer solution comprises acetate, citrate,

prolamine, carbonate, or phosphate or any combination thereof. In another example, the buffer solution is phosphate buffered saline (PBS).

Also disclosed are methods of inhibiting Hepatitis B virus (HBV) gene expression in a cell. The methods include contacting the cell with the double

stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure; and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of an HBV gene, thereby inhibiting expression of the HBV gene in the cell.

In one example, the HBV gene is selected from the group consisting of C, X, P, S, and a combination thereof.

In yet another aspect, the present invention provides the use of a double stranded RNAi agent disclosed herein, or a pharmaceutical composition disclosed herein in the manufacture of a medicament for inhibiting Hepatitis B virus (HBV) gene expression or replication of HBV in a cell, wherein: (a) the agent or the composition is to be contacted with the the cell; and (b) the cell produced in step (a) is to be maintained for a time sufficient to obtain degradation of the mRNA transcript of an HBV gene, thereby expression of the HBV gene in the cell is inhibited, wherein the HBV gene is optionally selected from the group consisting of C, X, P, S, and a combination thereof.

Also disclosed are methods of inhibiting replication of a Hepatitis B virus (HBV) in a cell. The methods include contacting the cell with the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure; and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of an HBV gene, thereby inhibiting replication of the HBV in the cell.

In one example, the cell is within a subject. In one example, the subject is a human.

In one example, the subject suffers from an HBV-associated disease.

In one example, HBV gene expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In one example, replication of HBV in the cell is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

Also disclosed are methods of reducing the level of Hepatitis B virus (HBV) DNA in a subject infected with HBV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby reducing the level of HBV ccc DNA in the subject.

Also disclosed are methods of reducing the level of a Hepatitis B virus (HBV) antigen in a subject infected with HBV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby reducing the level of the HBV antigen in the subject.

In one example, the HBV antigen is HBsAg. In abother example, the HBV antigen is HBeAg.

Also disclosed are methods of reducing the viral load of Hepatitis B virus (HBV) in a subject infected with HBV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the

disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby reducing the viral load of HBV in the subject.

In yet another aspect, the present invention provides the use of a therapeutically effective amount of the double stranded RNAi agent disclosed herein, or the composition disclosed herein in the manufacture of a medicament for reducing the viral load of Hepatitis B virus (HBV) in a subject infected with HBV, wherein the agent or the composition is to be administered to the subject, thereby the viral load of HBV in the subject is reduced.

Also disclosed are methods of reducing the level of alanine aminotransferase (ALT) in a subject infected with HBV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby reducing the level of ALT in the subject.

Also disclosed are methods of reducing the level of aspartate aminotransferase (AST) in a subject infected with HBV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby reducing the level of AST in the subject.

Also disclosed are methods of increasing the level of anti-Hepatitis B virus (HBV) antibodies in a subject infected with HBV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby increasing the level of anti- HBV antibodies in the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby treating said subject.

In yet another aspect, the present invention provides the use of a therapeutically effective amount of the double stranded RNAi agent disclosed herein, or the pharmaceutical composition disclosed herein for treating a subject having a Hepatitis B virus (HBV) infection, wherein the agent or the composition is to be administered to the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the disclosure, or the composition of the disclosure, or the vector of the disclosure, or the pharmaceutical composition of the disclosure, thereby treating said subject.

In one example, the HBV-associated disorder is selected from the group consisting of hepatitis D virus infection, delta hepatitis, acute hepatitis B; acute fulminant hepatitis B; chronic hepatitis B; liver fibrosis; end-stage liver disease; hepatocellular carcinoma.

In one example, the HBV-associated disorder is chronic hepatitis and the subject is HBeAg positive. In another example, the HBV-associated disorder is chronic hepatitis and the subject is HBeAg negative.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- UCGUGGUGGACUUCUCUCA -3' (SEQ ID NO:5) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- UCGUGGUGGACUUCUCUCA -3' (SEQ ID NO:5) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ ID NO:7) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and

said antisense strand comprises 5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are provides methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region,

wherein said sense strand comprises 5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ ID NO:7) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region,

wherein said sense strand comprises 5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ ID NO:9) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more

GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ ID NO:9) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein the sense strand comprises 5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ ID NO:37), (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and the antisense strand comprises 5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a

double-stranded region, wherein the sense strand comprises 5'-CGUGGUGGUCUUCUCUAAAUU -3' (SEQ ID NO:37) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and the antisense strand comprises 5'-AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein said sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ ID NO:11) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ ID NO:11) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence),

wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- GUGUGCACUUCGCUUCACA -3' (SEQ ID NO:39) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region, wherein said sense strand comprises 5'- GUGUGCACUUCGCUUCACA -3' (SEQ ID NO:39) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), and said antisense strand comprises 5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequence), wherein 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, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

In one example, all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

In one example, 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 one example, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

In one example, the sense strand comprises 5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:13) and the antisense strand comprises 5'-usGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:14) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In another example, the sense strand comprises 5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15) and the antisense strand comprises 5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In one example, the sense strand comprises 5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:17) and the antisense strand comprises 5'-usAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:18) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In another example, the sense strand comprises 5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:19) and the antisense strand comprises 5'-PusAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:20) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In one example, the sense strand comprises 5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21) and the antisense strand comprises 5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In another example, the sense strand comprises 5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:23) and the antisense strand comprises 5'-PasAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:24) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

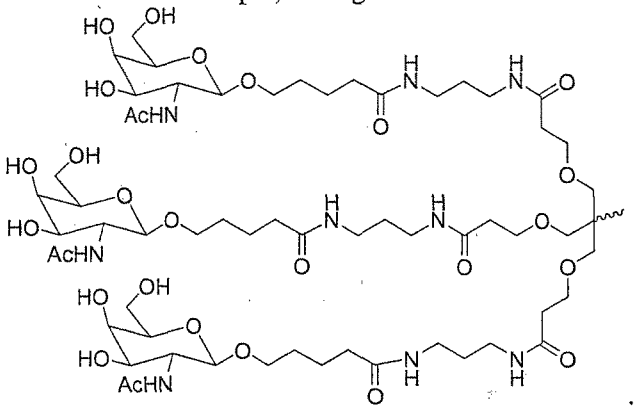
In another example, the sense strand comprises 5'-csgsuggudGgucdTucucuaaauu – 3' (SEQ ID NO:35) and the antisense strand comprises 5'- asdAsuugagagdAagudCcaccagcsusu – 3' (SEQ ID NO:36), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; dA, dC, dG, and dT are deoxyribose A, C, G, and T; and s is a phosphorothioate linkage.

In one example, the sense strand comprises 5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25) and the antisense strand comprises 5'-usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

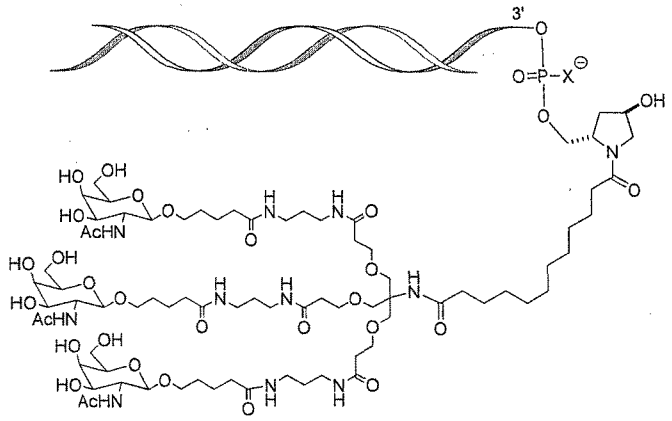
In another example, the sense strand comprises 5'-gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:27) and the antisense strand comprises 5'- PusAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:28) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In another example, the sense strand comprises
5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41) and the antisense strand comprises 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In one example, the ligand is



In one example, the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the HBV-associated disorder is selected from the group consisting of hepatitis D virus infection, delta hepatitis, acute hepatitis B; acute fulminant hepatitis B; chronic hepatitis B; liver fibrosis; end-stage liver disease; hepatocellular carcinoma.

In one example, the HBV-associated disorder is chronic hepatitis and the subject is HBeAg positive. In another example, the HBV-associated disorder is chronic hepatitis and the subject is HBeAg negative.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV) infection. The methods include administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis B virus (HBV) in a cell. The composition includes: (a) a first double-stranded RNAi agent comprising a first sense strand and a first antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of said first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and

(b) a second double-stranded RNAi agent comprising a second sense strand and a second antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of said second sense strand and substantially all of the nucleotides of said second antisense strand are modified nucleotides, wherein said second sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; wherein the first and second sense strands each independently comprise a sequence selected from the group consisting of
5'- UCGUGGUGGACUUCUCUCA -3' (SEQ IDNO:5),
5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ IDNO:7),
5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ IDNO:9),
5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ IDNO:37),
5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ IDNO:11), and
5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to any of the foregoing nucleotide sequences), and wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of
5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6);
5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8);
5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10);
5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38),
5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12), and
5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to any of the foregoing nucleotide sequences), thereby treating the subject.

Also disclosed are methods of treating a subject having a Hepatitis B virus (HBV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis B virus (HBV) in a cell. The composition includes: (a) a first double-stranded RNAi agent comprising a first sense strand and a first antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of said first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and (b) a second double-stranded RNAi agent comprising a second sense strand and a second antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of said second sense strand and substantially all of the nucleotides of said second antisense strand are modified nucleotides, wherein said second sense strand is conjugated to a

ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; wherein the first and second sense strands each independently comprise a sequence selected from the group consisting of
5'- UCGUGGUGGACUUCUCUCA -3' (SEQ IDNO:5),
5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ IDNO:7),
5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ IDNO:9),
5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ IDNO:37),
5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ IDNO:11), and
5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to any of the foregoing nucleotide sequences), and wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of
5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6);
5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8);
5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10);
5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38),
5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12), and
5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to any of the foregoing nucleotide sequences), thereby treating the subject.

In one example, all of the nucleotides of the first and second sense strand and all of the nucleotides of the first and second antisense strand comprise a modification.

In one example, 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.

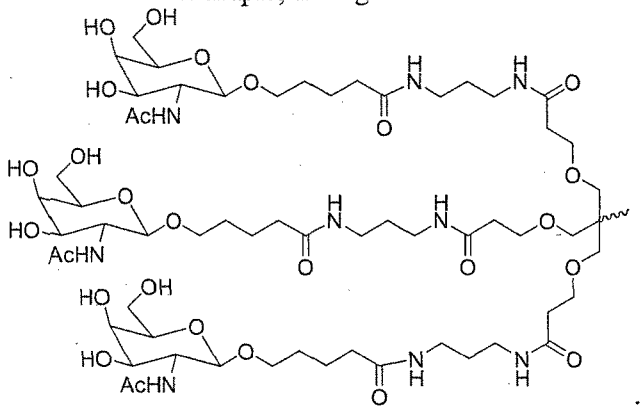
In one example, the first and second RNAi agent are selected from the group consisting of:
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:13)

5'-usGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:14);
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15)
5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16);
5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:17)
5'-usAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:18);
5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:19)
5'-PusAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:20);
5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21)
5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22);
5'- csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:23)
5'-PasAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:24);
5'-csgsuggudGgucdTucucuaaauu – 3' (SEQ ID NO:35)
5'- asdAsuugagagdAagudCcaccagcsusu – 3' (SEQ ID NO:36);
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25)
5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26);
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:27)
5'- PusAfsaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:28); and
5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41)
5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; dA, dC, dG, and dT are deoxyribose A, C, G, and T; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

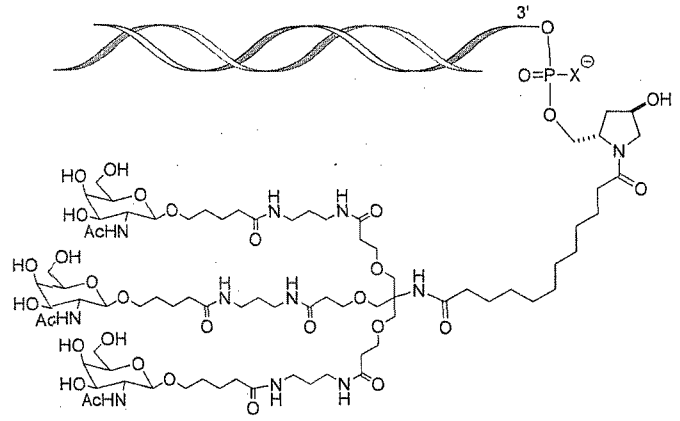
In one example, the first and second RNAi agents are
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15)
5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16); and
5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21)
5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In another example, the first and second RNAi agents are
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25)
5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26); and
5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41)
5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In one example, the ligand is



In one example, the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the subject is a human.

In one example, the HBV-associated disorder is selected from the group consisting of hepatitis D virus infection, delta hepatitis, acute hepatitis B; acute fulminant hepatitis B; chronic hepatitis B; liver fibrosis; end-stage liver disease; hepatocellular carcinoma.

In one example, the HBV-associated disorder is chronic hepatitis and the subject is HBeAg positive. In another example, the HBV-associated disorder is chronic hepatitis and the subject is HBeAg negative.

In one example, the double stranded RNAi agent is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

In one example, the double stranded RNAi agent is administered at a dose of about 10 mg/kg to about 30 mg/kg. In another example, the double stranded RNAi agent is administered at a dose of about 3 mg/kg. In one example, the double stranded RNAi agent is administered at a dose of about 10 mg/kg.

In one example, the double stranded RNAi agent is administered at a dose of about 0.5 mg/kg twice per week.

In one example, the double stranded RNAi agent is administered at a fixed dose of about 50 mg to 200 mg.

In one example, the double stranded RNAi agent is administered subcutaneously. In another example, the double stranded RNAi agent is administered intravenously.

In one example, the RNAi agent is administered in two or more doses.

In one example, the RNAi agent is administered at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours.

In one example, the RNAi agent is administered twice per week. In another example, the RNAi agent is administered every other week.

In one example, the methods of the disclosure further include administering to the subject an additional therapeutic agent.

In one example, the additional therapeutic agent is selected from the group consisting of an antiviral agent, a reverse transcriptase inhibitor, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, an oligonucleotide that inhibits the secretion or release of HbsAg, a capsid inhibitor, a cccDNA inhibitor, and a combination of any of the foregoing.

In another example, the methods of the disclosure further include administering administering to the subject a reverse transcriptase inhibitor. In yet another example, the methods of the disclosure further include administering to the subject a reverse transcriptase inhibitor and an immune stimulator.

In one example, the reverse transcriptase inhibitor is slected from the group consisting of Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide, Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, and AGX-1009.

In some examples, the methods of the disclosure further comprise treatment of hepatitis D virus (HDV) in the subject. Methods of treatment can include any methods of treatment known in the art. In certain examples, HDV is treated in the subject using one of more of the iRNA agents targeting HBV as described herein.

In some examples, the methods of the disclosure further include methods to modulate, *e.g.*, decrease, the expression of PD-L1. Compositions and methods to reduce the expression of PD-L1 are provided, for example, in PCT publication no. WO2011/127180, the entire contents of which are hereby incorporated herein by reference.

In one example, the immune stimulator is selected from the group consisting of pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and aToll-like receptor 7 (TLR7) agonist.

Also disclosed is a method of treating a subject having a Hepatitis B virus (HBV)-associated disorder, comprising administering to the subject a therapeutically effective amount of a double stranded RNAi agent,

wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:29, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:30,

wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides,

wherein the sense strand is conjugated to a ligand attached at the 3'-terminus, and

wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

Also disclosed is a method of treating a subject having a Hepatitis B virus (HBV) infection, comprising administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis B virus (HBV) in a cell, said composition comprising

(a) a first double-stranded RNAi agent comprising a first strand and a first antisense strand forming a double-stranded region,

wherein substantially all of the nucleotides of the first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides,

wherein said first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and

(b) a second double-stranded RNAi agent comprising a second sense strand and a second antisense strand forming a double-stranded region,

wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides,

wherein the second sense strand is conjugated to a ligand attached at the 3'-terminus, and

wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker;

wherein the first sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and said first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2,

wherein the sense second strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:29, and the second antisense strand comprises at least 15 contiguous nucleotides differing by no more

than 3 nucleotides from the nucleotide sequence of SEQ ID NO:30, thereby treating the subject.

In some examples, the first sense strand comprises a sequence selected from the group consisting of

5'- UCGUGGUGGACUUCUCUCA -3' (SEQ IDNO:5),

5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ IDNO:7),

5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ IDNO:9),

5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ IDNO:37)

5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ IDNO:11), and

5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39), (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequences), and the second antisense strand comprises a sequence selected from the group consisting of

5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6);

5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8);

5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10);

5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38);

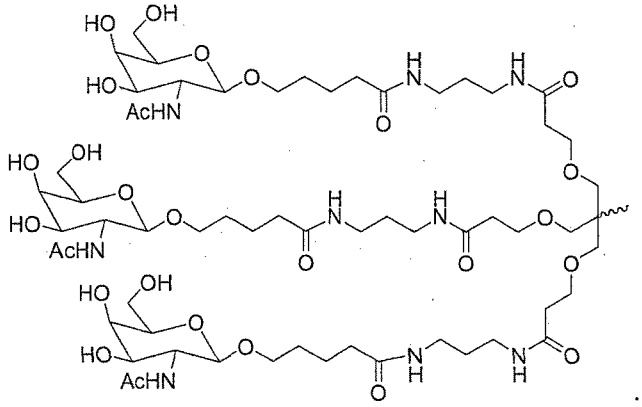
5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12); and

5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40) (or a nucleotide sequence which is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to the foregoing nucleotide sequences).

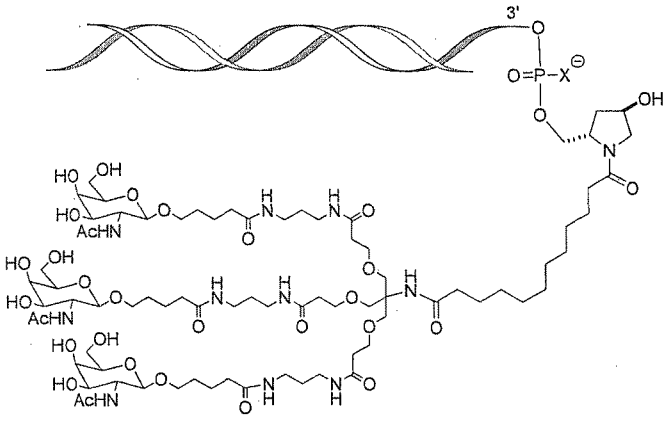
Also disclosed, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In certain examples, at least one of the 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 some examples, the ligand is



In a specific example, the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In certain examples, the double stranded RNAi agents and compositions provided herein are used for treatment of an HDV infection and/or an HDV-associated disorder.

Accordingly, the present disclosure provides methods of inhibiting replication of a Hepatitis D virus (HDV) in a cell. The methods include (a) contacting the cell with a double stranded RNAi agent, composition, vector, or the pharmaceutical composition provided herein; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an HBV gene, thereby inhibiting replication of the HDV in the cell. In certain examples, the cell is within a subject. In certain examples, the subject is a human.

The disclosure further provides methods of reducing the level of a Hepatitis D virus (HDV) antigen in a subject infected with HDV. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, composition, vector, or the pharmaceutical composition provided herein, thereby reducing the level of the HDV antigen, *e.g.*, S-HDAg or L-HDAg, in the subject.

In yet another aspect, the present invention provides the use of a therapeutically effective amount of the double stranded RNAi agent disclosed herein, or the pharmaceutical composition disclosed herein in the manufacture of a medicament for reducing the level of a Hepatitis D virus (HDV) antigen or reducing the viral load of HDV in a subject infected with HDV, wherein the agent or the composition is to be administered to the subject, thereby the level of the HDV antigen in the subject is reduced, wherein the HDV antigen is optionally S-HDAg or L-HDAg.

The disclosure also provides methods of reducing the viral load of Hepatitis D virus (HDV) in a subject infected with HDV. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, composition, vector, or pharmaceutical composition provided herein, thereby reducing the viral load of HDV in the subject.

The disclosure also provides methods of treating a subject having a Hepatitis D virus (HDV) infection, comprising administering to the subject a therapeutically effective amount of a double stranded RNAi agent, composition, vector, or pharmaceutical composition provided herein, thereby treating the subject.

In certain examples, the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region. Sense strand and antisense strands can be selected from the following RNAi agents wherein, the sense strand comprises 5'-UCGUGGUGGACUUCUCUCA -3' (SEQ ID NO:5), and the antisense strand comprises 5'-UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6); the sense strand comprises 5'-GUGCACUUCGCUUCACCUCUA -3' (SEQ ID NO:7), and the antisense strand comprises 5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8); the sense strand comprises 5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ IDNO:9), and the antisense strand comprises 5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10); the sense strand comprises 5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ ID NO:37), and the antisense strand comprises 5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38); the sense strand comprises 5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ ID NO:11), and the antisense strand comprises 5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12); or the sense strand comprises 5'- GUGUGCACUUCGCUUCACA -3' (SEQ ID NO:39), and the antisense strand comprises 5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40), wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, thereby treating the subject.

In certain examples, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification. In certain

examples, at least one of the 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,

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, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

In certain examples, the sense strand comprises 5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:13) and the antisense strand comprises 5'-usGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:14) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In certain examples, the sense strand comprises 5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15) and the antisense strand comprises 5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In certain examples, the sense strand comprises 5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:17) and the antisense strand comprises 5'-usAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:18) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In certain examples, the sense strand comprises 5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:19) and the antisense strand comprises 5'-PusAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:20) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In certain examples, sense strand comprises 5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21) and the antisense strand comprises 5'-AfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are
2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In certain examples, the sense strand comprises 5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:23) and the antisense strand comprises 5'-PasAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:24) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are

2′-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

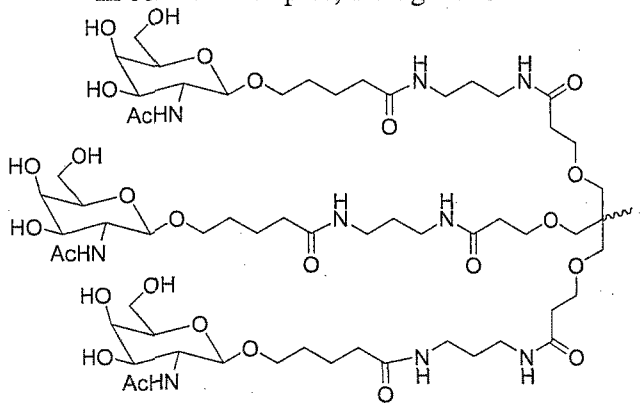
In certain examples, the sense strand comprises 5'-csgsuggudGgucdTucucuaaauu – 3' (SEQ ID NO:35) and the antisense strand comprises 5'- asdAsuugagagdAagudCcaccagcsusu – 3' (SEQ ID NO:36), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; dA, dC, dG, and dT are deoxyribose A, C, G, and T; and s is a phosphorothioate linkage.

In certain examples, the sense strand comprises 5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25) and the antisense strand comprises 5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; and s is a phosphorothioate linkage.

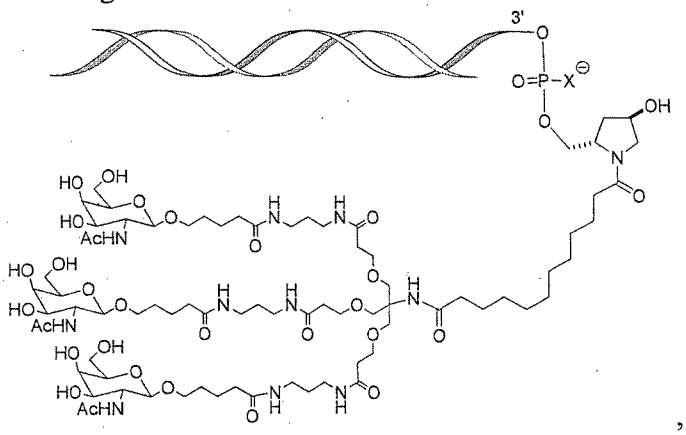
In certain examples, the sense strand comprises 5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:27) and the antisense strand comprises 5'- PusAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:28) , wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; and s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In certain examples, sense strand comprises 5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41) and the antisense strand comprises 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; and s is a phosphorothioate linkage.

In certain examples, the ligand is



In certain examples, the RNAi agent is conjugated to the ligand as shown in the following schematic



, wherein X is O or S.

The disclosure provides methods of treating a subject having a Hepatitis D virus (HDV) infection. The methods include administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis B virus (HBV) in a cell, the composition comprising (a) a first double-stranded RNAi agent comprising a first sense strand and a first antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of the first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein the first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and (b) a second double-stranded RNAi agent comprising a second sense strand and a second antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides, wherein the second sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; wherein the first and second sense strands each independently comprise a sequence selected from the group consisting of

5'- UCGUGGUGGACUUCUCUCA -3' (SEQ ID NO:5),

5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ ID NO:7),

5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ ID NO:9),

5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ ID NO:37),

5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ ID NO:11), and

5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39), and wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of

5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6);

5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8);

5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10);

5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38),
5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12), and
5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40), thereby treating the subject.

In certain examples, all of the nucleotides of the first and second sense strand and all of the nucleotides of the first and second antisense strand comprise a modification. In certain examples, at least one of the 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, the first and second RNAi agent are selected from the group:
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:13)
5'-usGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:14);
5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15)
5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16);
5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:17)
5'-usAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:18);
5'-gsusgcacUfuCfGfCfuucaccucua – 3' (SEQ ID NO:19)
5'-PusAfsgagGfugaagcgAfaGfugcacsusu – 3' (SEQ ID NO:20);
5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21)
5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22);
5'- csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:23)
5'-PasAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:24);
5'-csgsuggudGgucdTucucuaaaauu – 3' (SEQ ID NO:35)
5'- asdAsuugagagdAagudCcaccagcsusu – 3' (SEQ ID NO:36);
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25)
5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26);
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:27)
5'- PusAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:28); and
5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41)

5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; dA, dC, dG, and dT are deoxyribose A, C, G, and T; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In certain examples, the first and second RNAi agents are

5'-uscsguGfgUfGfGfacuucucuca – 3' (SEQ ID NO:15)

5'-PusGfsagaGfaAfGfuccaCfcAfcgasusu – 3' (SEQ ID NO:16); and

5'-csgsugguGfgAfCfUfucucUfCfaauu – 3' (SEQ ID NO:21)

5'-asAfsuugAfgAfgAfaguCfcAfccagcsasg – 3' (SEQ ID NO:22), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In certain examples, the first and second RNAi agents are

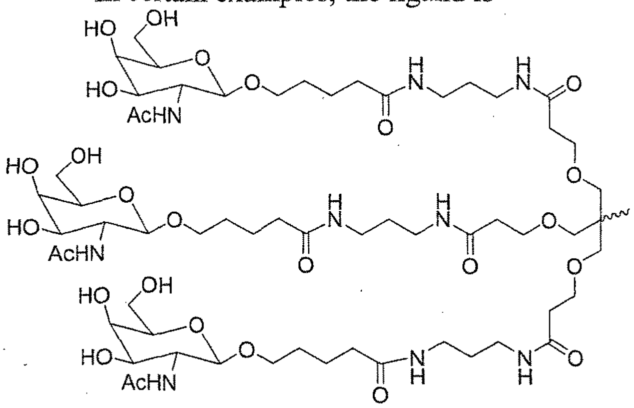
5'- gsgsuggaCfuUfCfUfcucaAfUfuuua – 3' (SEQ ID NO:25)

5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc – 3' (SEQ ID NO:26); and

5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41)

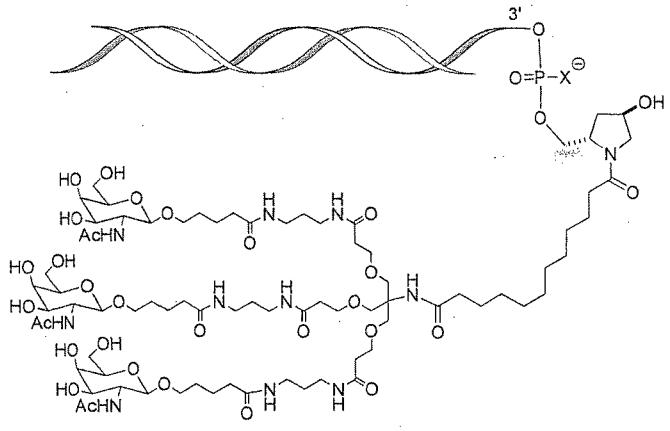
5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42), wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2′-O-methyl (2′-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2′-fluoro A, G, C or U; s is a phosphorothioate linkage; and P is a 5'-phosphate or 5'phosphate mimic.

In certain examples, the ligand is



.

In certain examples, the RNAi agent is conjugated to the ligand as shown in the following schematic



, wherein X is O or S.

In certain examples, the subject is a human.

In certain examples, the double stranded RNAi agent is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg. In certain examples, the double stranded RNAi agent is administered at a dose of about 10 mg/kg to about 30 mg/kg. In certain examples, the double stranded RNAi agent is administered at a dose of about 3 mg/kg. In certain examples, the double stranded RNAi agent is administered at a dose of about 10 mg/kg. In certain examples, the double stranded RNAi agent is administered at a dose of about 0.5 mg/kg twice per week. In certain examples, the double stranded RNAi agent is administered at a fixed dose of about 50 mg to 200 mg.

In certain examples, the double stranded RNAi agent is administered subcutaneously. In certain examples, the double stranded RNAi agent is administered intravenously.

In certain examples, the RNAi agent is administered in two or more doses. In certain examples, the RNAi agent is administered at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours. In certain examples, the RNAi agent is administered twice per week. In certain examples, the RNAi agent is administered every other week. In certain examples, the RNAi agent is administered once per month. In certain examples, the RNAi agent is administered once every other month. In certain examples, the RNAi agent is administered once every three months.

In certain examples, the RNAi agent is administered to the subject with an additional therapeutic agent. Additional therapeutic agents include, for example, an antiviral agent, a reverse transcriptase inhibitor, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, an oligonucleotide that inhibits the secretion or release of HbsAg, a capsid inhibitor, a covalently closed circular (ccc) HBV DNA inhibitor, and a combination of any of the foregoing.

In certain examples, the additional agent is a reverse transcriptase inhibitor. In certain examples, the additional agent is a reverse transcriptase inhibitor and an immune stimulator. Exemplary reverse transcriptase inhibitors include Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide, Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, and AGX-1009. Exemplary immune stimulators include pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and a Toll-like receptor 7 (TLR7) agonist.

The disclosure further provides methods of treating a subject having a Hepatitis D virus (HDV) infection, comprising administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis B virus (HBV) in a cell, the composition comprising (a) a first double-stranded RNAi agent comprising a first strand and a first antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of the first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein the first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and (b) a second double-stranded RNAi agent comprising a second sense strand and a second antisense strand forming a double-stranded region, wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides, wherein the second sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; wherein the first sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2, wherein the sense second strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:29, and the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:30, thereby treating the subject.

In certain examples, the first sense strand comprises a sequence selected from the group consisting of

5'- UCGUGGUGGACUUCUCUCA -3' (SEQ IDNO:5),

5'- GUGCACUUCGCUUCACCUCUA -3' (SEQ ID NO:7),

5'- CGUGGUGGACUUCUCUCAAUU -3' (SEQ ID NO:9),

5'- CGUGGUGGUCUUCUCUAAAUU -3' (SEQ ID NO:37),

5'- GGUGGACUUCUCUCAAUUUUA -3' (SEQ ID NO:11), and

5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39), and

the second antisense strand comprises a sequence selected from the group consisting of

5'- UGAGAGAAGUCCACCACGAUU -3' (SEQ ID NO:6);

5'- UAGAGGUGAAGCGAAGUGCACUU -3' (SEQ ID NO:8);

5'- AAUUGAGAGAAGUCCACCAGCAG -3' (SEQ ID NO:10);

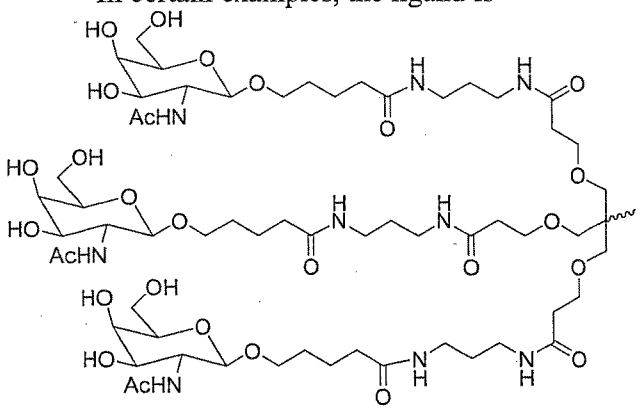
5'- AAUUGAGAGAAGUCCACCAGCUU -3' (SEQ ID NO:38),

5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:12), and

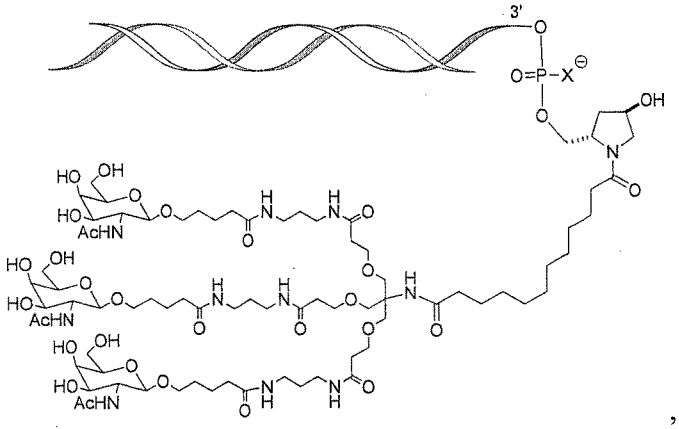
5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40).

In certain examples, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification. In certain examples, the additional agent is at least one of the modified nucleotides is selected from the group consisting of a dexoy-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, the ligand is



In certain examples, the RNAi agent is conjugated to the ligand as shown in the following schematic



, wherein X is O or S.

The present invention 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 (an about 3.5 kb transcript, an about 2.4 kb transcript, an about 2.1 kb transcript, and an about 0.7 kb transcript) encoding seven viral proteins (pre-S1, pre-S2, S, P, X, pre-C and C) translated across three reading frames.

Figure 2 is a graph depicting the log decrease of HBsAg serum levels normalized to pre-dose HBsAg serum levels following administration of a single 3 mg/kg dose of the indicated iRNA agents.

Figure 3 is a graph depicting the log decrease of HBsAg serum levels normalized to pre-dose HBsAg serum levels following administration of a single 3 mg/kg dose of the indicated iRNA agents.

Figure 4 is a graph depicting the percent of pre-dose HBsAg remaining at days 5 and 10 following administration of a single 3 mg/kg dose of the indicated iRNA agents. Figure 4 also depicts the percent of HBsAG remaining at day 10 post-dose relative to the percent of HBsAG remaining at day 10 post-dose in an animal administered 3 mg/kg of a control dsRNA targeting mouse/rat transtherytin (mrTTR).

Figure 5 is a graph depicting the log decrease of HBsAg serum levels normalized to pre-dose HBsAg serum levels following administration of a single 3 mg/kg dose of AD-65403.

Figure 6A is a graph depicting the decrease of HBsAg serum levels normalized to pre-dose HBsAg serum levels on a standard linear scale following administration of a single subcutaneous 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 9 mg/kg dose of AD-66810.

We claim:

1. A double stranded RNAi agent for inhibiting expression of hepatitis B virus (HBV) in a cell, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region of complementarity which comprises at least 15 contiguous nucleotides, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:39 (GUGUGCACUUCGCUUCACA), and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:40 (UGUGAAGCGAAGUGCACACUU),

wherein 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, and

wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

2. The double stranded RNAi agent of claim 1, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

3. The double stranded RNAi agent of claim 1 or 2, wherein 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.

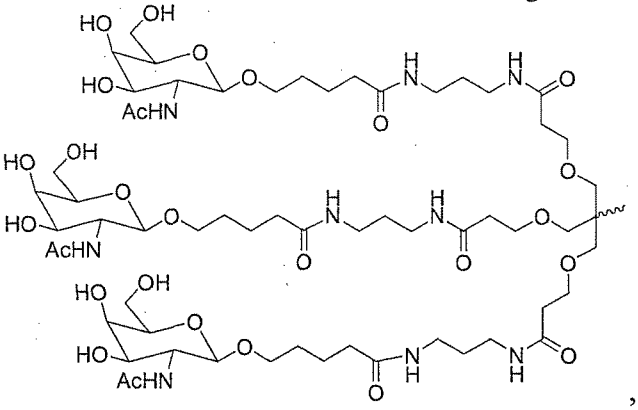
4. The double stranded RNAi agent of claim 1, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide or at least 2 nucleotides.

5. The double stranded RNAi agent of claim 1, wherein the double-stranded region is 15-30 nucleotide pairs in length, 17-23 nucleotide pairs in length, 17-25 nucleotide

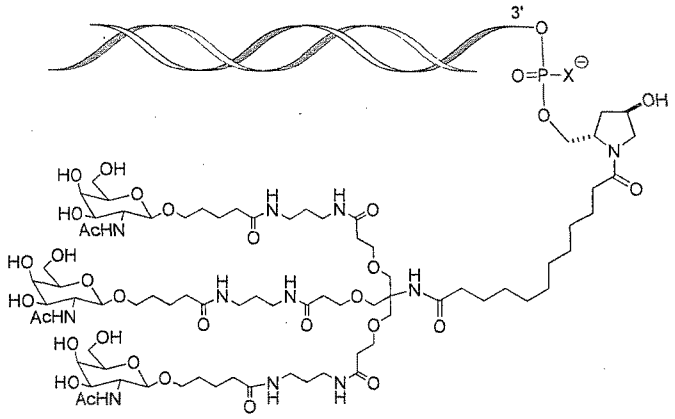
pairs in length, 23-27 nucleotide pairs in length, 19-21 nucleotide pairs in length, or 21-23 nucleotide pairs in length.

6. The double stranded RNAi agent of claim 1, wherein each strand has 15-30 nucleotides or 19-30 nucleotides.

7. The double stranded RNAi agent of claim 1, wherein the ligand is



, and wherein the RNAi agent is preferably conjugated to the ligand as shown in the following schematic



wherein X is O or S.

8. The double stranded RNAi agent of claim 1, wherein said RNAi agent has a sense sequence of GUGUGCACUUCGCUUCACA (SEQ ID NO: 39) and an antisense sequence of UGUGAAGCGAAGUGCACACUU (SEQ ID NO: 40).

9. A double stranded RNAi agent for inhibiting expression of hepatitis B virus (HBV) in a cell, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region,

wherein said sense strand comprises 5'- GUGUGCACUUCGCUUCACA -3' (SEQ IDNO:39), and said antisense strand comprises 5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:40),

wherein 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, and

wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

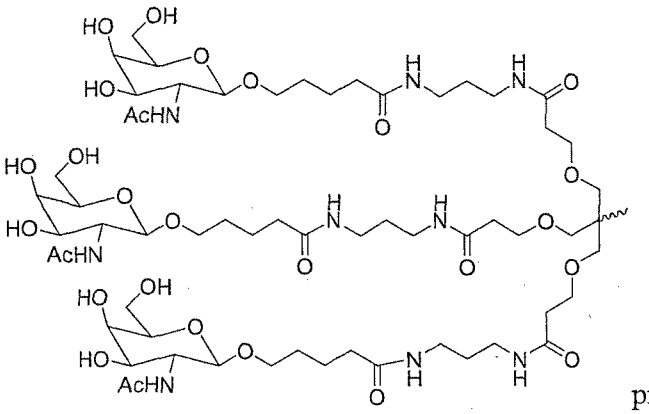
10. The double stranded RNAi agent of claim 9, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

11. The double stranded RNAi agent of claim 9, wherein 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.

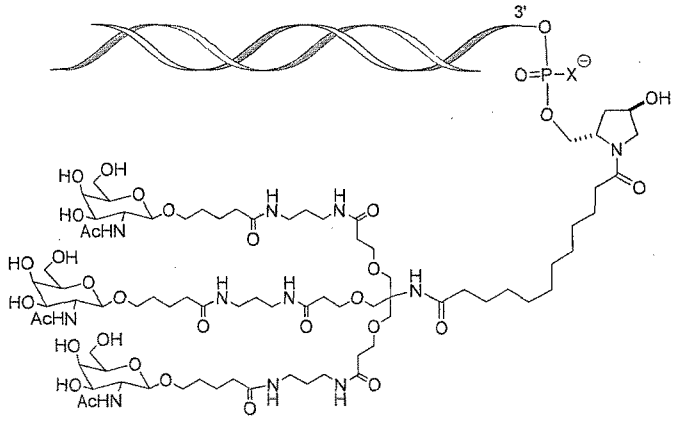
12. The double stranded RNAi agent of claim 9, wherein the sense strand comprises 5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41) and the antisense strand comprises 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42),

wherein A, C, G, and U are ribose A, C, G or U; a, g, c and u are 2'-O-methyl (2'-OMe) A, U, C, or G; Af, Cf, Gf or Uf are 2'-fluoro A, G, C or U; and s is a phosphorothioate linkage.

13. The double stranded RNAi agent of any one of claims 9-12, wherein the ligand is



preferably, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

14. A pharmaceutical composition comprising the double stranded RNAi agent of claim 1 or 9.

15. The use of a double stranded RNAi agent of claim 1 or 9, or a pharmaceutical composition of claim 14 in the manufacture of a medicament for inhibiting Hepatitis B virus (HBV) gene expression or replication of HBV in a cell, wherein:

(a) the agent or the composition is to be contacted with the the cell; and

(b) the cell produced in step (a) is to be maintained for a time sufficient to obtain degradation of the mRNA transcript of an HBV gene, thereby expression of the HBV gene in the cell is inhibited, wherein the HBV gene is optionally selected from the group consisting of C, X, P, S, and a combination thereof.

16. The use of claim 15, wherein the HBV gene expression or replication is to be inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100%.

17. The use of a therapeutically effective amount of the double stranded RNAi agent of claim 1 or 9, or the composition of claim 14 in the manufacture of a medicament for

reducing the viral load of Hepatitis B virus (HBV) in a subject infected with HBV, wherein the agent or the composition is to be administered to the subject, thereby the viral load of HBV in the subject is reduced.

18. The use of a therapeutically effective amount of the double stranded RNAi agent of claim 1 or 9, or the pharmaceutical composition of claim 14 in the manufacture of a medicament for treating a subject having a Hepatitis B virus (HBV) infection, wherein the agent or the composition is to be administered to the subject.

19. The use of claim 17 or 18, wherein the double stranded RNAi agent is to be administered 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, 3 mg/kg, 10 mg/kg, or at a fixed dose of 50 mg to 200 mg.

20. The use of any one of claims 17-19, wherein the double stranded RNAi agent is to be administered subcutaneously, or intravenously.

21. The use of any one of claims 17-20, wherein an additional therapeutic agent is to be further administered to the subject, optionally the additional therapeutic agent is selected from the group consisting of an antiviral agent, a reverse transcriptase inhibitor (e.g., Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide, Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, or AGX-1009), an immune stimulator (e.g., pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and a Toll-like receptor 7 (TLR7) agonist), a therapeutic vaccine, a viral entry inhibitor, an oligonucleotide that inhibits the secretion or release of HbsAg, a capsid inhibitor, a covalently closed circular (ccc) HBV DNA inhibitor, and a combination of any of the foregoing.

22. The use of a therapeutically effective amount of the double stranded RNAi agent of claim 1 or 9, or the pharmaceutical composition of claim 14 in the manufacture of a medicament for reducing the level of a Hepatitis D virus (HDV) antigen or reducing the viral load of HDV in a subject infected with HDV, wherein the agent or the composition is to be administered to the subject, thereby the level of the HDV antigen in the subject is reduced, wherein the HDV antigen is optionally S-HDAg or L-HDAg.