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

The present invention refers to subject matter as defined in the attached set of claims. Disclosed herein are 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,

Table 25. Unmodified HBV X ORF Sense and Antisense Sequences.

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

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,

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.

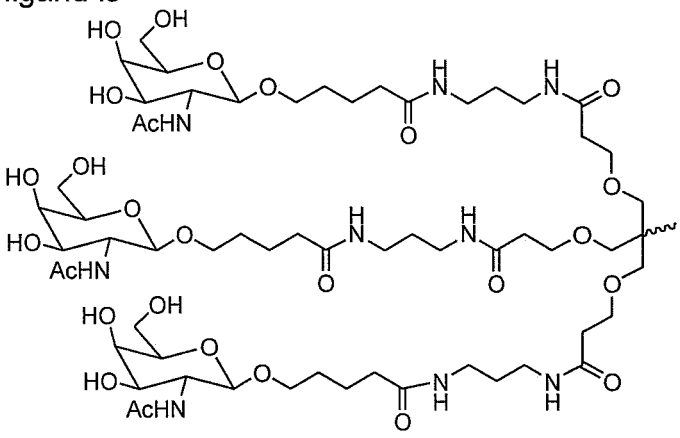
2. The double stranded RNAi agent of claim 1, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

3. The double stranded RNAi agent of claim 1, 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.

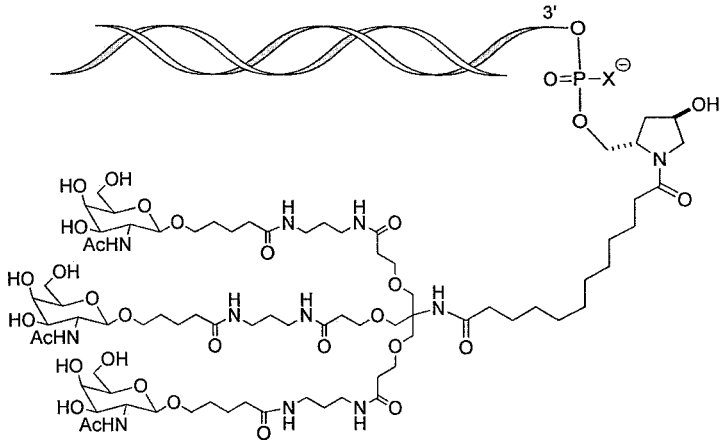
4. The double stranded RNAi agent of claim 1, wherein the sense strand comprises 5'- gsusguGfcAfCfUfucgcuucaca -3' (SEQ ID NO:41) and the antisense strand comprises 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:42),

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

5. The double stranded RNAi agent of any one of claims 1-4, 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.

6. The double stranded RNAi agent of claim 1, wherein the sense strand comprises 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:1275) and the antisense strand comprises 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:1285), wherein a, g, c, and u are 2'-O-methyl (2'-OMe) A, 2'-OMe G, 2'-OMe C, and 2'-OMe U, respectively; Af, Cf, Gf, and Uf are 2'-fluoro A, 2'-fluoro C, 2'-fluoro G, and 2'-fluoro U, respectively; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3.

7. A pharmaceutical composition comprising the double stranded RNAi agent of claim 1 or 6.

8. The use of a double stranded RNAi agent of claim 1 or 6, or a pharmaceutical composition of claim 7, 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.

9. The use of claim 8, 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%.

10. The use of a therapeutically effective amount of the double stranded RNAi agent of claim 1 or 6, or the composition of claim 7, 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.

11. The use of a therapeutically effective amount of the double stranded RNAi agent of claim 1 or 6, or the pharmaceutical composition of claim 7, 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.

12. The use of claim 10 or 11, 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.

13. The use of any one of claims 10-12, wherein the double stranded RNAi agent is to be administered subcutaneously, or intravenously.

14. The use of any one of claims 10-12, 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.

15. The use of a therapeutically effective amount of the double stranded RNAi agent of claim 1 or 6, or the pharmaceutical composition of claim 7, 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.