SUMMARY

In one aspect, the present disclosure refers to the use of an inhibitor of HBV gene expression or an agent that reduces HBV antigenic load and an anti-HBV antibody in the manufacture of a medicament for the treatment of a chronic HBV infection. In some examples, the present disclosure provides an inhibitor of HBV gene expression for use in the treatment of a chronic HBV infection in a subject, wherein the subject is subsequently administered an anti-HBV antibody.

The present disclosure also provides an agent that reduces HBV antigenic load for use in the treatment of a chronic HBV infection in a subject, wherein the subject is subsequently administered an anti-HBV antibody.

The present disclosure also provides a composition for use in the treatment of a chronic HBV infection in a subject, wherein (a) the composition comprises an anti-HBV antibody and the subject has been previously administered an inhibitor of gene expression; or (b) composition comprises an anti-HBV antibody and the subject has been previously administered an agent that reduces HBV antigenic load.

In another aspect, the present disclosure refers to the use of (a) an inhibitor of HBV gene expression or an agent that reduces HBV antigenic load and (b) an anti-HBV antibody, in the manufacture of a medicament for the treatment of a chronic HBV infection in a subject, wherein: (1) the anti-HBV antibody comprises a light chain amino acid sequence according to SEQ ID NO:73, and a heavy chain amino acid sequence according to SEQ ID NO:71; and (2) the inhibitor of HBV gene expression or agent that reduces HBV antigenic load is an siRNA having a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:7) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:8), wherein: (a) a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively; (b) Af, Cf, Gf, and Uf are 2'-fluoroadenosine-3'-phosphate, 2'-fluorocytidine-3'-phosphate, 2'-fluoroguanosine-3'-phosphate, and 2'-fluorouridine-3'-phosphate, respectively; (c) (Agn) is adenosine-glycol nucleic acid (GNA); (d) s is a phosphorothioate linkage; and (e) L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

In some examples, the present disclosure provides for the use of an inhibitor of HBV gene expression and an anti-HBV antibody in the manufacture of a medicament for the treatment of a chronic HBV infection. The present disclosure also provides for the use of agent that reduces HBV antigenic load and an anti-HBV antibody in the manufacture of a medicament for the treatment of a chronic HBV infection.

In some examples, the present disclosure provides a method of treating chronic HBV infection in a subject in need thereof, comprising: administering to the subject an agent that reduces HBV antigenic load; and administering to the subject an anti-HBV antibody. The present disclosure also provides a method of treating chronic HBV infection in a subject in need thereof, comprising: administering to the subject an inhibitor of HBV gene expression; and administering to the subject an anti-HBV antibody.

In some methods, compositions for use, or uses described herein, the anti-HBV antibody is HBC34 or a non-natural variant of HBC34.

In some methods, compositions for use, or uses described herein, the agent that reduces HBV antigenic load or the inhibitor of HBV gene expression is an RNAi agent (*e.g.*, an siRNA, such as HBV02).The present disclosure also provides, in some examples, kits comprising an RNAi agent and an anti-HBV antibody as disclosed herein, optionally with instructions for practicing a method described herein. In yet another aspect, the present disclosure refers to a kit comprising: a pharmaceutical composition comprising an RNAi agent that targets an mRNA encoded by an HBV gene, and a pharmaceutically acceptable excipient; and a pharmaceutical composition comprising an anti-HBV antibody, and a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the dosing schedule of a combination therapy study in a murine HBV model (an AAV-HBV model), as described in Exampe 1. Entecavir was administered orally once per day. An HBV-specific siRNA was administered subcutaneously once at the start of the study, and a mouse-chimeric anti-HBV antibody was administered intraperitoneally twice per week, during weeks three and four of the study. A subset of the mice were sacrificed at week four, and the other subset was sacrificed at week six of the study.

Figure 2A depicts results of assaying for HBV viral load in mouse serum samples, measured as HBV DNA copy number, of mice treated with HBV02 siRNA (squares, solid line); a mouse-chimeric HBC34 antibody (HBC34v7; at 15 mg/kg) (circles, solid line); or saline (triangles, solid line).

Figure 2B depicts results of assaying for HBV viral load in mouse serum samples, measured as HBV DNA copy number, of mice treated with a control siRNA and control antibody (squares, dashed line); entecavir alone ("ETV," diamonds, dashed line); HBV02 siRNA and the mouse-chimeric HBC34v7 antibody (at 15 mg/kg) (circles, dashed line); or HBV02 siRNA, HBC34v7 antibody (at 15 mg/kg), and entecavir (triangles, solid line).

Figure 3A depicts HBsAg levels measured from mouse serum following treatment with HBV02 siRNA (squares, solid line); mouse-chimeric HBC34v7 antibody at 15 mg/kg (circles, solid line); or saline (triangles, solid line).

Figure 3B depicts HBsAg levels measured from mouse serum following treatment with a control siRNA and control antibody (squares, dashed line); entecavir alone ("ETV," diamonds, dashed line); HBV02 siRNA and mouse-chimeric HBC34v7 antibody (at 15 mg/kg) (circles, dashed line); or HBV02 siRNA, mouse-chimeric HBC34v7 antibody (at 15 mg/kg), and entecavir (triangles, dashed line).

Figure 4 depicts levels of free antibody measured from mouse serum between Day 14 and 42 post siRNA administration. The following treatment groups are depicted: mouse-chimeric HBC34v7 antibody alone (circles); HBV02 siRNA, mouse-chimeric HBC34v7 antibody, and entecavir (squares); and HBV02 siRNA and mouse-chimeric HBC34v7 antibody (triangles).

Figure 5A depicts results of assaying for HBV viral load in mouse serum samples, measured as HBV DNA copy number, following siRNA, antibody, and/or control treatments. Mice were injected with AAV/HBV virus on day -28. AAV/HBV-infected C57Bl/6 mice were administered one of eleven different treatments on day 0: (1) an HBV-specific siRNA (HBV02, having an antisense strand of SEQ ID NO:8; *see* description in Example 1); (2)-(3) an anti-HBV antibody (a fully murinized HBC24), at one of two doses; (4)-(5) the HBV02 siRNA at one dose, and the fully murinized HBC24 at one of two doses; (6-9) the HBV02 siRNA at one of two doses, and a fully murinized anti-HBV antibody HBC34 (HBC34v35), at one of three antibody doses; (10) a control siRNA and a control antibody; or (11) PBS only, administered intraperitoneally. Results are shown for treatments 1-5, 10, and 11.

Figure 5B depicts results of assaying for HBV viral load in mouse serum samples, measured as HBV DNA copy number, following siRNA, antibody, and/or control treatments. Mice were injected with AAV/HBV virus on day -28. AAV/HBV-infected C57Bl/6 mice were administered one of eleven different treatments on day 0: (1) an HBV-specific siRNA (HBV02, having an antisense strand of SEQ ID NO:8; *see* description in Example 1); (2)-(3) an anti-HBV antibody (a fully murinized HBC24), at one of two doses; (4)-(5) the HBV02 siRNA at one dose, and the fully murinized HBC24 at one of two doses; (6-9) the HBV02 siRNA at one of two doses, and a fully murinized anti-HBV antibody HBC34 (HBC34v35), at one of three antibody doses; (10) a control siRNA and a control antibody; or (11) PBS only, administered intraperitoneally. Results are shown for treatments 1 and 6-11.

Figure 6A depicts HBsAg levels measured from mouse serum following siRNA, antibody, and/or control treatments, as described above for Figure 5A.

CLAIMS

That which is claimed is:

1. Use of an inhibitor of HBV gene expression or an agent that reduces HBV antigenic load and an anti-HBV antibody in the manufacture of a medicament for the treatment of a chronic HBV infection.

2. The use of claim 1, wherein (a) a therapeutically effective amount of the anti-HBV antibody is less than a therapeutically effective amount of the anti-HBV antibody delivered when the inhibitor of HBV gene expression or the agent that reduces HBV antigenic load has not been administered to the subject; or (b) administering the anti-HBV antibody begins at least 1 week after administering the inhibitor of HBV gene expression or the agent that reduces HBV antigenic load.

3. The use according to any one of the preceding claims, wherein administering the anti-HBV antibody comprises administering at least two doses of a therapeutically effective amount of the anti-HBV antibody.

4. The use according to claim 3, wherein the at least two doses are administered twice per week, once per week, every other week, every two weeks, or once a month.

5. The use according to any one of the preceding claims, wherein the anti-HBV antibody is administered subcutaneously.

6. The use according to any one of the preceding claims, wherein the anti-HBV antibody recognizes HBV genotypes A, B, C, D, E, F, G, H, I, and J.

7. The use according to any one of the preceding claims, wherein the anti-HBV antibody is a human antibody.

8. The use according to any one of the preceding claims, wherein the antibody is HBC34 or a non-natural variant of HBC34.

9. The use according to any one of the preceding claims, wherein the anti-HBV antibody comprises:

(i) CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NOs:44, 45 or 46, and 47, respectively; and

(ii) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NOs:48, 49 or 50, and 52 or 51, respectively.

10. The use according to any one of the preceding claims, wherein the anti-HBV antibody comprises:

(a) a light chain variable domain (V_L) that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs:55-69; and (b) a heavy chain variable domain (V_H) that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:53: or

(a) a light chain variable domain (V_L) that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs:55-69; and (b) a heavy chain variable domain (V_H) that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:54.

11. The use according to any one of the preceding claims, wherein the anti-HBV antibody comprises:

(a) a light chain that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:73, and (b) a heavy chain that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs:70-72 and 97; or

(a) a light chain that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:74, and (b) a heavy chain that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs:70-72 and 97; or

(a) a light chain that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NOs:83-95, and (b) a heavy chain that is at least 90%, at least 95%, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs:70-72, 97, and 98.

12. The use according to any one of claims 1-7, wherein the anti-HBV antibody comprises:

(a) CDRs having the amino acid sequences according to SEQ ID NOs:77-82; or

(b) (i) a light chain variable domain (VL) amino acid sequence according to SEQ ID NO:76; and (ii) a heavy chain variable domain (VH) amino acid sequence according to SEQ ID NO:75.

13. The use according to any one of the preceding claims, wherein (a) the anti-HBV antibody is a monoclonal antibody; (b) the anti-HBV antibody is a bispecific antibody, with a first specificity for HBsAg, and a second specificity that stimulates an immune effector; (c) the anti-HBV antibody is a bispecific antibody, with a first specificity for HBsAg, and a second specificity stimulates cytotoxicity or a vaccinal effect.

14. The use according to any one of the preceding claims, wherein the subject is a human and a therapeutically effective amount of the anti-HBV antibody is administered; wherein the therapeutically effective amount is from about 3 mg/kg to about 30 mg/kg.

15. The use according to any one of the preceding claims, wherein the inhibitor of HBV gene expression or the agent that reduces HBV antigenic load is an RNAi agent that inhibits expression of an HBV transcript.

16. The use according to claim 15, wherein the 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 nucleotides 1579-1597 of SEQ ID NO:1.

17. The use according to claim 15 or claim 16, wherein at least one strand of the RNAi agent comprises a 3' overhang of at least 1 nucleotide.

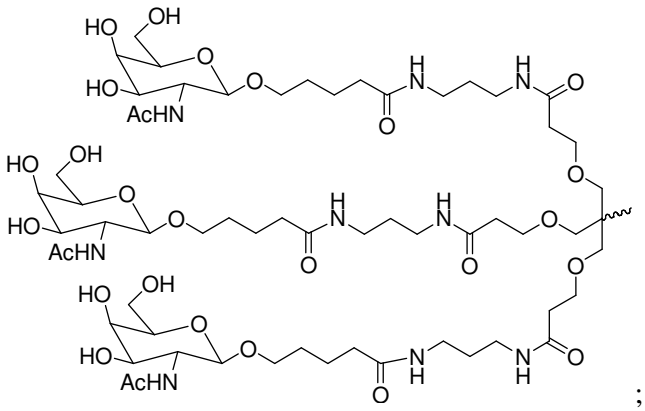
18. The use according to any one of claims 15-17, wherein the double-stranded region of the RNAi agent is 15-30 nucleotide pairs in length or wherein each strand of the RNAi agent has 15-30 nucleotides.

19. The use of any one of claims 15-18, wherein (a) the RNAi agent is an siRNA that inhibits expression of an HBV transcript that encodes an HBsAg protein, an HBcAg protein, an HBx protein, or an HBV DNA polymerase protein; (b) the RNAi agent is an siRNA that binds to at least 15 contiguous nucleotides of a target encoded by: P gene, nucleotides 2309-3182 and 1-1625 of NC_003977.2; S gene (encoding L, M, and S proteins), nucleotides 2850-3182 and 1-837 of NC_003977.2; HBx, nucleotides 1376-1840 of NC_003977.2; or C gene, nucleotides 1816-2454 of NC_003977.2; (c) the RNAi agent is an siRNA that comprises an antisense strand, wherein the antisense strand comprises at least 15 contiguous nucleotides of the nucleotide sequence of 5'- UGUGAAGCGAAGUGCACACUU -3' (SEQ ID NO:4); or (d) the RNAi agent is an siRNA that comprises an antisense strand, wherein the antisense strand comprises an at least 15 contiguous nucleotides of the nucleotide sequence of 5'- UAAAAUUGAGAGAAGUCCACCAC -3' (SEQ ID NO:107).

20. The use according to claim 19, wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and

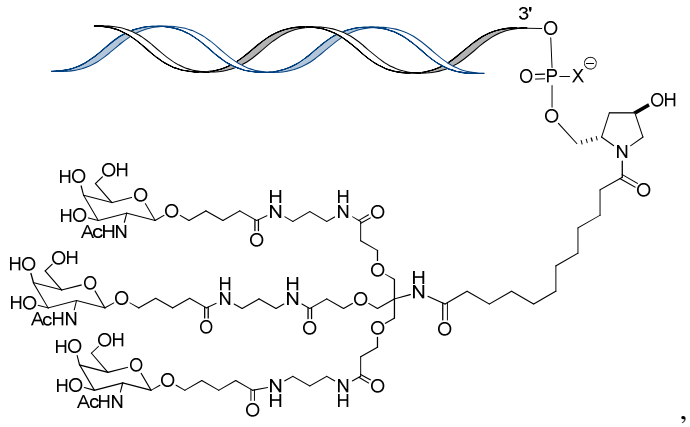
wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

21. The use according to claim 20, wherein (a) the ligand is one or more GalNAc derivatives attached through a monovalent linker, bivalent branched linker, or trivalent branched linker; (b) the ligand is



; or

(c) the siRNA is conjugated to the ligand as shown in the following structure:



,

wherein X is O or S.

22. The use according to any one of claims 19-21, wherein at least one nucleotide of the siRNA is a modified nucleotide comprising a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, an adenosine-glycol nucleic acid, or a nucleotide comprising a 5'-phosphate mimic.

23. The use according to any one of claims 19-22, wherein the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsugaAfgCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

Af, Cf, Gf, and Uf are 2'-fluoroadenosine-3'-phosphate, 2'-fluorocytidine-3'-phosphate, 2'-fluoroguanosine-3'-phosphate, and 2'-fluorouridine-3'-phosphate, respectively;

s is a phosphorothioate linkage; and

L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

24. The use according to any one of claims 19-22, wherein the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:7) and

an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:8)

wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

Af, Cf, Gf, and Uf are 2'-fluoroadenosine-3'-phosphate, 2'-fluorocytidine-3'-phosphate, 2'-fluoroguanosine-3'-phosphate, and 2'-fluorouridine-3'-phosphate, respectively;

(Agn) is adenosine-glycol nucleic acid (GNA);

s is a phosphorothioate linkage; and

L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

25. The use according to any one of claims 19-22, wherein the siRNA has a sense strand comprising 5'- gsgsuggaCfuUfCfUfcucaAfUfuuuaL96 -3' (SEQ ID NO:108) and an antisense strand comprising 5'- usAfsaaaUfuGfAfgagaAfgUfccaccsasc -3' (SEQ ID NO:109),

wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

Af, Cf, Gf, and Uf are 2'-fluoroadenosine-3'-phosphate, 2'-fluorocytidine-3'-phosphate, 2'-fluoroguanosine-3'-phosphate, and 2'-fluorouridine-3'-phosphate, respectively;

s is a phosphorothioate linkage; and

L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

26. The use according to any one of claims 15-25, wherein (a) the subject is a human and a therapeutically effective amount of RNAi agent or siRNA is administered to the subject; and wherein the effective amount of the RNAi agent or siRNA is from about 1 mg/kg to about 8 mg/kg; or (b) the RNAi agent or siRNA is

administered to the subject twice daily, once daily, every two days, every three days, twice per week, once per week, every other week, every four weeks, or once per month.

27. The use according to any one of claims 19-26, wherein two siRNAs each directed to an HBV gene are administered, and (a) the first siRNA has an antisense strand comprising SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8; and the second siRNA comprises an siRNA having a sense strand that comprises at least 15 contiguous nucleotides of nucleotides 2850-3182 of SEQ ID NO:1; (b) the two siRNAs comprise: an siRNA directed to an HBV X gene and an siRNA directed to an HBV S gene; (c) the first siRNA has an antisense strand comprising SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, and the second siRNA has an antisense strand that comprises SEQ ID NO:107 or SEQ ID NO:109; or (d) the two siRNAs are administered simultaneously.

28. The use according to any one of the preceding claims, further comprising administering a nucleot(s)ide analog to the subject, or wherein the subject is also administered a nucleot(s)ide analog.

29. Use of (a) an inhibitor of HBV gene expression or an agent that reduces HBV antigenic load and (b) an anti-HBV antibody, in the manufacture of a medicament for the treatment of a chronic HBV infection in a subject, wherein:

(1) the anti-HBV antibody comprises a light chain amino acid sequence according to SEQ ID NO:73, and a heavy chain amino acid sequence according to SEQ ID NO:71; and

(2) the inhibitor of HBV gene expression or agent that reduces HBV antigenic load is an siRNA having a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:7) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:8), wherein:

(a) a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

(b) Af, Cf, Gf, and Uf are 2'-fluoroadenosine-3'-phosphate, 2'-fluorocytidine-3'-phosphate, 2'-fluoroguanosine-3'-phosphate, and 2'-fluorouridine-3'-phosphate, respectively;

(c) (Agn) is adenosine-glycol nucleic acid (GNA);

(d) s is a phosphorothioate linkage; and

(e) L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

30. The use of claim 29, wherein the anti-HBV antibody is to be administered to the subject at least 1 week after administering the siRNA to the subject.

31. The use of claim 29 or 30, wherein the subject is also administered a nucleot(s)ide analog.

32. A kit comprising:

a pharmaceutical composition comprising an RNAi agent that targets an mRNA encoded by an HBV gene, and a pharmaceutically acceptable excipient; and

a pharmaceutical composition comprising an anti-HBV antibody, and a pharmaceutically acceptable excipient.