events, and treatment discontinuations than those observed in trials evaluating 48-week regimens (Lawitz E, et al., Sofosbuvir for previously untreated chronic hepatitis C infection, N Engl J Med. 2013, 368(20): 1878-1887); Hadziyannis SJ, et al., Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose, Ann Intern Med. 2004, 140(5): 346-355; Fried MW, et al., Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection, N Engl J Med. 2002, 347(13): 975-982). The high variability of response, in combination with an unfavorable safety and side effect profile, make a significant number of patients ineligible or unwilling to undergo PEG-IFNα treatment.

The failure of NRTI therapy to eradicate the virus, and the limitations of PEG-IFNα therapy, highlight the clinical need for new HBV therapies that are effective, well tolerated, and do not require lifelong administration.

**SUMMARY**

In another aspect, the present disclosure relates to a use of an siRNA in the manufacture of a first medicament and use of a PEG-INFα in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a chronic hepatitis B virus (HBV) infection or an HBV-associated disease in a subject, wherein:

(a) the siRNA has a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

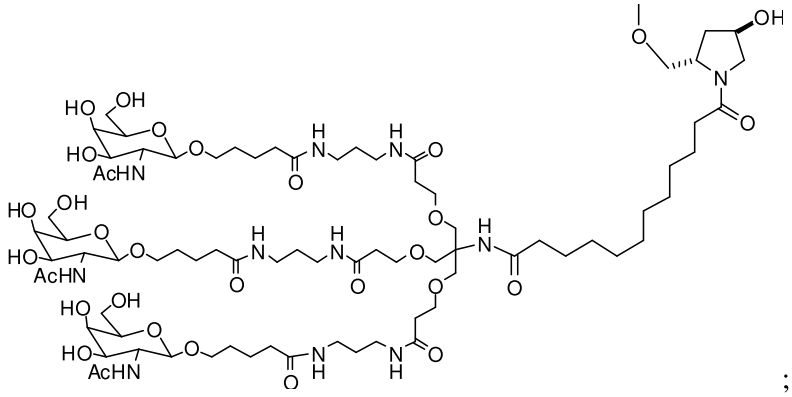
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

In another aspect, the present disclosure relates to a use of an siRNA in the manufacture of a medicament for use in combinatorial therapy for treating a chronic hepatitis B virus (HBV) infection or an HBV-associated disease in a subject, wherein said medicament is to be administered in combination with a PEG-INFα, wherein:

(a) the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

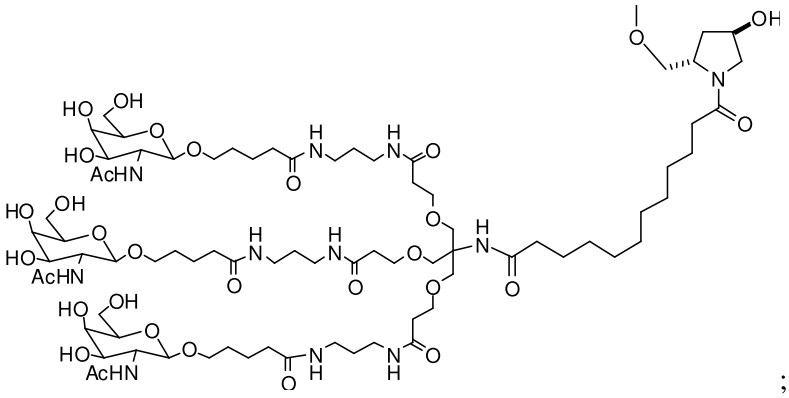
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

In another aspect, the present disclosure relates to a use of an siRNA in the manufacture of a first medicament, and use of a PEG-IFNα in the manufacture of a second medicament, and use of an NRTI in the manufacture of a third medicament, wherein the first, second and third medicament are to be used in combination therapy for treating a chronic HBV infection or an HBV-associated disease, wherein:

(a) the siRNA has a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

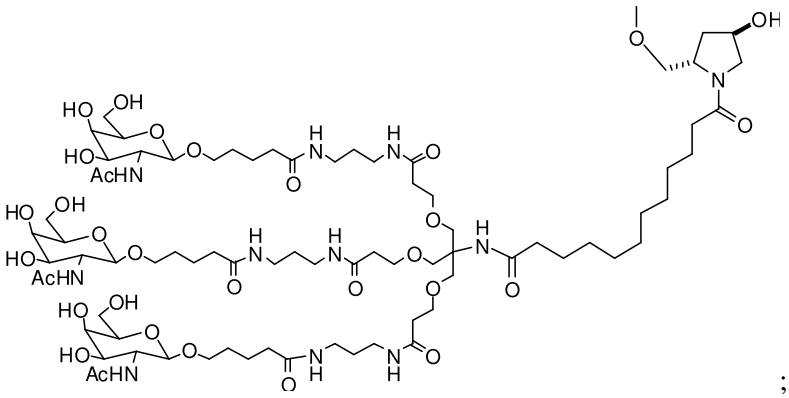
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

In another aspect, the present disclosure relates to a use of an siRNA in the manufacture of a medicament for use in combinatorial therapy for treating a chronic HBV infection or an HBV-associated disease, wherein the medicament is to be administered in combination with a PEG-IFNα and an NRTI, wherein:

(a) the siRNA has a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

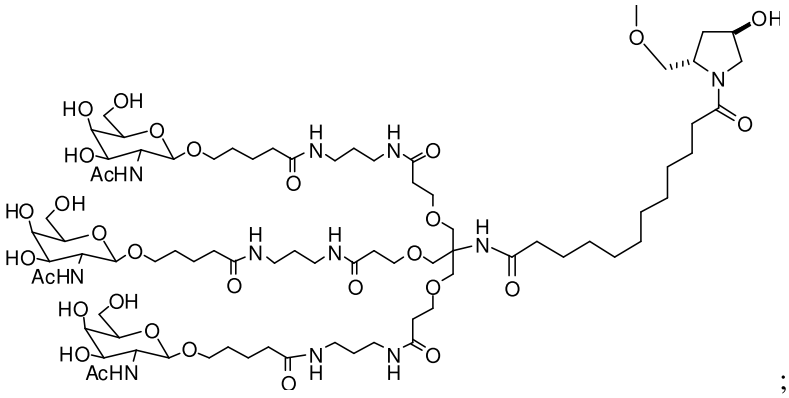
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

In yet another aspect, the present disclosure relates to a kit for treating chronic HBV infection or an HBV-associated disease comprising:

(a) a pharmaceutical composition comprising two or more doses of 200 mg of an siRNA, and a pharmaceutically acceptable excipient, wherein the siRNA has a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

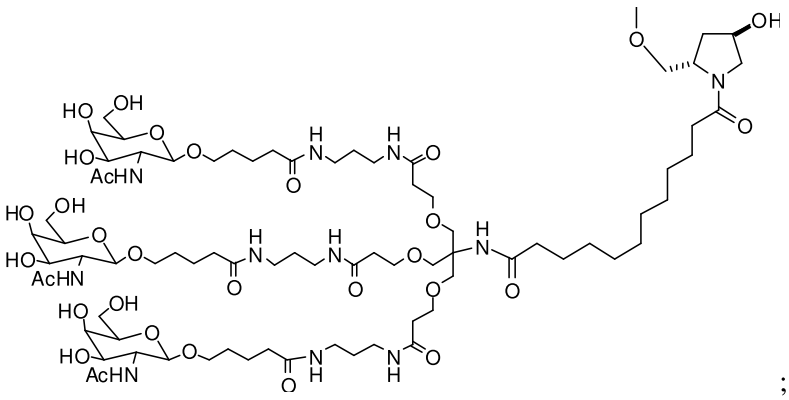
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is


; and

(b) a pharmaceutical composition comprising a dose of 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg of a PEG-IFNα, and a pharmaceutically acceptable excipient.

In some examples, the present disclosure relates to compositions and methods of treating HBV with siRNA, in particular HBV02. For example, in accordance with some examples, a method of treating an HBV infection in a subject by administering an siRNA is provided, wherein the siRNA has a sense strand that comprises SEQ ID NO: 5 and an antisense strand that comprises SEQ ID NO: 6. In some examples, the method of treating further comprises administering to the subject a pegylated interferon-alpha (PEG-INFα). In some examples the PEG-INFα is administered before, concurrently, or after the siRNA HBV02 is administered. In some examples, the HBV infection is chronic. In some further examples, the subject is administered a nucleoside/nucleotide reverse transcriptase inhibitor (NRTI). In some examples the NRTI is administered before, concurrently, or after the HBV02 is administered. In some examples the NRTI is administered for 2 to 6 months prior to the HBV02.

In some examples, the present disclosure also provides a siRNA for use in the treatment of an HBV infection in a subject, wherein the siRNA is HBV02 and has a sense strand that comprises SEQ ID NO: 5 and an antisense strand that comprises SEQ ID NO: 6. In some additional examples, the siRNA HBV02 is administered to a

subject that is also administered a PEG-INFα. In some examples, the PEG-INFα is administered before, concurrently, or after the siRNA HBV02 is administered. In some examples, the HBV infection is chronic. In some further examples, the subject is administered a NRTI. In some examples the NRTI is administered before, concurrently, or after the HBV02 is administered. In some examples the NRTI is administered for 2 to 6 months prior to the HBV02.

In some further examples, the present disclosure provides for the use of an siRNA in the manufacture of a medicament for the treatment of an HBV infection, wherein the siRNA is HBV02 and has a sense strand that comprises SEQ ID NO: 5 and an antisense strand that comprises SEQ ID NO: 6. In some examples, the use of the siRNA HBV02 is for use with PEG-IFNα. In some examples, the siRNA HBV02 is for use with PEG-IFNα and an NRTI.

In some of the aforementioned examples, the dose of the siRNA HBV02 is 0.8 mg/kg, 1.7 mg/kg, 3.3 mg/kg, 6.7 mg/kg, 10 mg/kg, or 15 mg/kg. In some of the aforementioned examples, the dose of the siRNA HBV02 is from 20 mg to 900 mg. In some of the aforementioned examples, the dose of the siRNA HBV02 is 20 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 400 mg, or 450 mg. In some of the aforementioned examples, the HBV02 is administered weekly. In some of the aforementioned examples, more than one dose of the siRNA is administered. In some of the aforementioned examples, two, three, four, five, six, or more doses of the siRNA are administered with each dose separated by 1, 2, 3, or 4 weeks. In some of the aforementioned examples, six 200-mg doses of the siRNA are administered. In some of the aforementioned examples, two 400-mg doses of the siRNA are administered. In some of the aforementioned examples, the siRNA is administered via subcutaneous injection; for example, in some examples, administering the siRNA HBV02 includes administering 1, 2, or 3 subcutaneous injections per dose.

In some of the aforementioned examples, the dose of PEG-IFNα is 50 μg, 100 μg, 150 μg, or 200 μg. In some of the aforementioned examples, the PEG-IFNα is administered weekly. In some of the aforementioned examples, the PEG-IFNα is administered via subcutaneous injection.

In some of the aforementioned examples, the NRTI is tenofovir, tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), lamivudine, adefovir, adefovir dipivoxil, entecavir (ETV), telbivudine, AGX-1009, emtricitabine (FTC), clevudine, ritonavir, dipivoxil, lobucavir, famvir, N-Acetyl-Cysteine (NAC), PC1323, theradigm-HBV, thymosin-alpha, ganciclovir, besifovir (ANA-380/LB-80380), or tenofvir-exaliades (TLX/CMX157).

In some of the aforementioned examples, the subject is HBeAg negative. In some examples, the subject is HBeAg positive.

In another aspect of the disclosure, a kit is provided comprising: a pharmaceutical composition comprising an siRNA as defined in any of the preceding examples, and a pharmaceutically acceptable excipient; and a pharmaceutical composition comprising PEG-IFNα, and a pharmaceutically acceptable excipient. The kit may also contain a NRTI, and a pharmaceutically acceptable excipient.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts characteristics of acute and chronic Hepatitis B infections.

Figure 2 depicts characteristics of chronic Hepatitis B infection. The disease is divided into 4 phases based on HBeAg status and laboratory or radiographic evidence of liver disease. Heterogeneity of disease could be due to differences in virus (*e.g.*, HBV genotypes, mutations), host (*e.g.*, immune responses, age at inflection, number of infected hepatocytes), and other factors (*e.g.*¸ co-infections (HDV, HCV, HIV), intercurrent infections, co-morbidities).

Figure 3 depicts the single ascending dose design for Part A of Example 2. [a]Subject discharge occurs after all assessments are completed on day 2.

Figure 4 depicts the multiple ascending dose design for Parts B and C of Example 2. [a]Additional HBsAg monitoring is required for subjects with HBsAg levels with a >10% decrease from the Day 1 predose level at the Week 16 visit. Visits occur every 4 weeks starting at Week 20 up to Week 48 or until the HBsAg level returns to ≥90% of the Day 1 perdose level.

## Claims

1. Use of an siRNA in the manufacture of a first medicament and use of a PEG-INFα in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a chronic hepatitis B virus (HBV) infection or an HBV-associated disease in a subject, wherein:

(a) the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

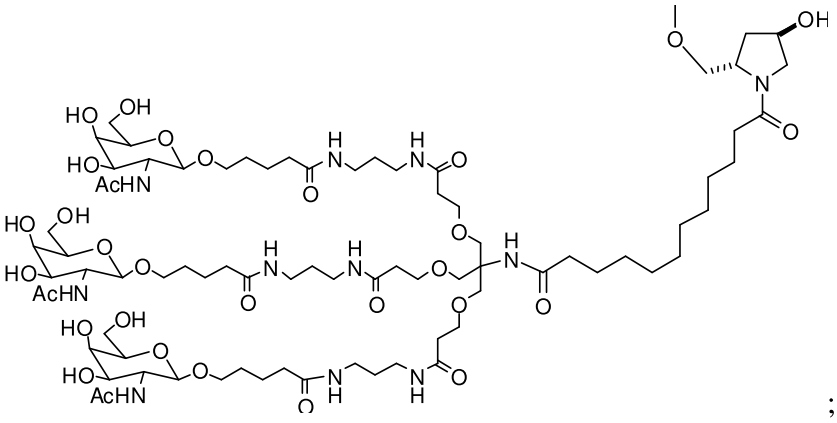
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is




;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

2. Use of an siRNA in the manufacture of a medicament for use in combinatorial therapy for treating a chronic hepatitis B virus (HBV) infection or an HBV-associated disease in a subject, wherein said medicament is to be administered in combination with a PEG-INFα, wherein:

(a) the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

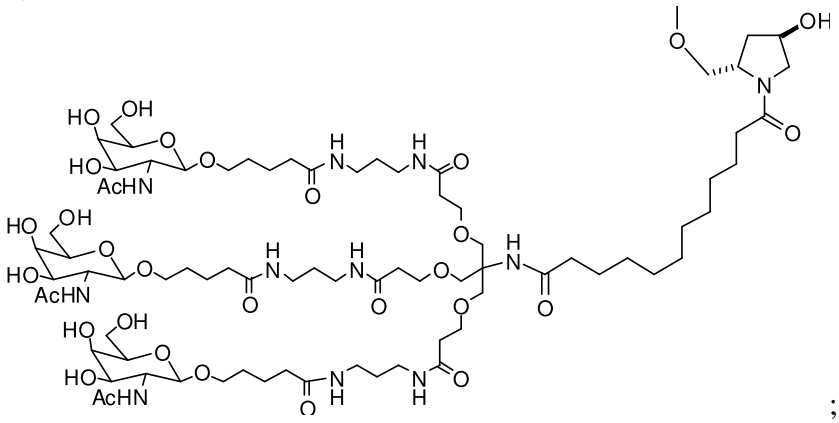
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

3. The use of claim 1 or 2, wherein the siRNA and the PEG-IFNα are to be administered to the subject on the same day.

4. The use of claim 1 or 2, wherein three doses of the siRNA are to be administered to the subject, with one dose administered every four weeks for 12 weeks, before the PEG-IFNα

is to be administered to the subject.

5. The use of claim 1 or 2, wherein the subject has been administered PEG-IFNα prior to the administration of the siRNA.

6. The use of any one of claims 1-5, wherein the subject is also to be administered a NRTI.

7. The use of any one of claims 1-6, wherein the subject has been administered a NRTI prior to the administration of the siRNA.

8. The use of any one of claims 1-7, wherein the subject has been administered a NRTI for at least 2 months or at least 6 months prior to the administration of the siRNA.

9. Use of an siRNA in the manufacture of a first medicament, and use of a PEG-IFNα in the manufacture of a second medicament, and use of an NRTI in the manufacture of a third medicament, wherein the first, second and third medicament are to be used in combination therapy for treating a chronic HBV infection or an HBV-associated disease, wherein:

(a) the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

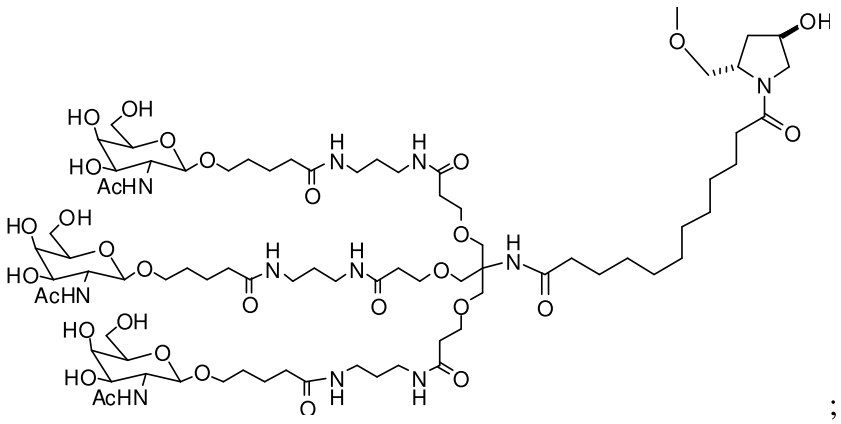
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

10. Use of an siRNA in the manufacture of a medicament for use in combinatorial therapy for treating a chronic HBV infection or an HBV-associated disease, wherein the medicament is to be administered in combination with a PEG-IFNα and an NRTI, wherein:

(a) the siRNA has a sense strand comprising 5'- gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'- usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

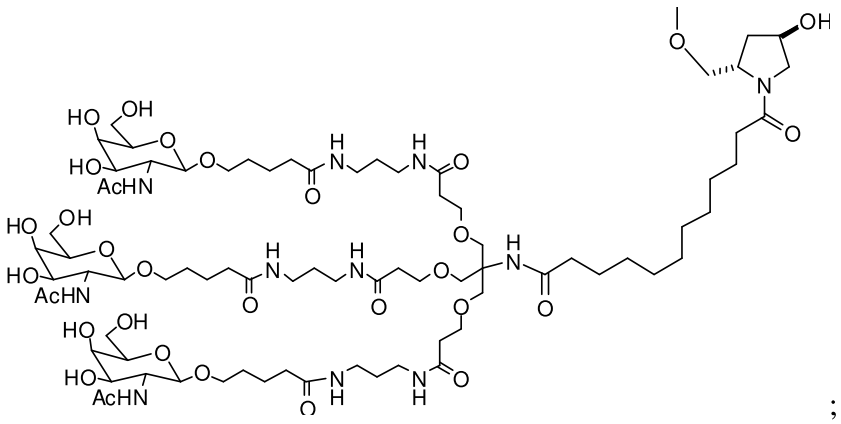
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is


;

(b) two or more doses of 200 mg of the siRNA are to be administered; and

(c) the dose of the PEG-IFNα is 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg.

11. The use according to any one of claims 1-3 and 5-10, wherein two, three, four, five, six, or more doses of the siRNA are to be administered with each dose separated by 1, 2, 3, or 4 weeks.

12. The use according to any one of claims 1-11, wherein six 200-mg doses of the siRNA are to be administered.

13. The use according to any one of claims 1-12, wherein the siRNA is to be administered via subcutaneous injection.

14. The use according to claim 13, wherein the siRNA to be administered comprises that 1, 2, or 3 subcutaneous injections are to be administered per dose.

15. The use according to any one of claims 1-14, wherein the PEG-IFNα is to be administered weekly.

16. The use according to any one of claims 1-14, wherein the PEG-IFNα is to be administered via subcutaneous injection.

17. The use according to any one of claims 6-16, wherein the NRTI is tenofovir, tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), lamivudine, adefovir, adefovir dipivoxil, entecavir (ETV), telbivudine, AGX-1009, emtricitabine (FTC), clevudine, ritonavir, dipivoxil, lobucavir, famvir, N-Acetyl-Cysteine (NAC), PC1323, theradigm-HBV, thymosin-alpha, ganciclovir, besifovir (ANA-380/LB-80380), or tenofvir-exaliades (TLX/CMX157).

18. The use according to claim 17, wherein the NRTI is entecavir (ETV).

19. The use according to claim 17, wherein the NRTI is tenofovir.

20. The use according to claim 17, wherein the NRTI is lamivudine.

21. The use according to claim 17, wherein the NRTI is adefovir or adefovir dipivoxil.

22. The use according to any one of claims 1-21, wherein the subject is HBeAg negative.

23. The use according to any one of claims 1-21, wherein the subject is HBeAg positive.

24. The use according to any one of claims 1-23, wherein the subject has a chronic HBV infection.

25. The use according to any one of claims 1-24, wherein the subject has an HBV-associated disease, and the HBV-associated disease is hepatitis D virus (HDV) infection.

26. A kit comprising:

a pharmaceutical composition comprising an siRNA as defined in any of the preceding claims, and a pharmaceutically acceptable excipient; and

a pharmaceutical composition comprising PEG-IFNα, and a pharmaceutically acceptable excipient.

27. The kit according to claim 26, further comprising a NRTI, and a pharmaceutically acceptable excipient.

28. A kit for treating chronic HBV infection or an HBV-associated disease comprising:

(a) a pharmaceutical composition comprising two or more doses of 200 mg of an siRNA, and a pharmaceutically acceptable excipient, wherein the siRNA has a sense strand comprising 5'-gsusguGfcAfCfUfucgcuucacaL96 -3' (SEQ ID NO:5) and an antisense strand comprising 5'-usGfsuga(Agn)gCfGfaaguGfcAfcacsusu -3' (SEQ ID NO:6),

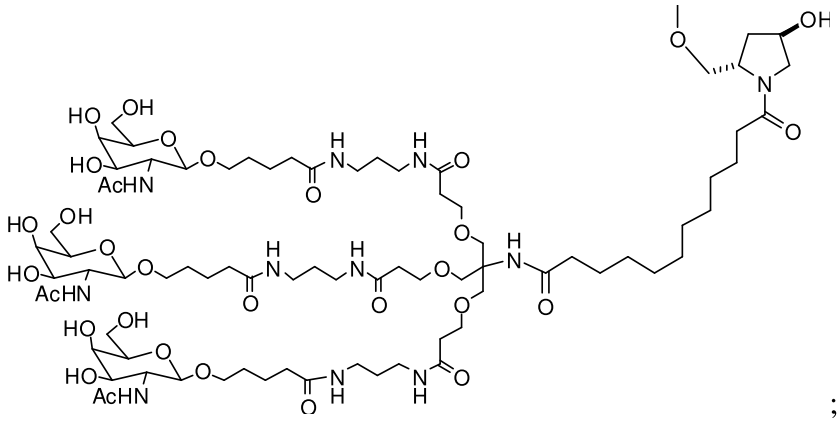
wherein a, c, g, and u are 2'-O-methyladenosine-3'-phosphate, 2'-O-methylcytidine-3'-phosphate, 2'-O-methylguanosine-3'-phosphate, and 2'-O-methyluridine-3'-phosphate, respectively;

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

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

s is a phosphorothioate linkage; and

L96 is



; and

(b) a pharmaceutical composition comprising a dose of 50 μg, 100 μg, 150 μg, 180 μg, or 200 μg of a PEG-IFNα, and a pharmaceutically acceptable excipient.

29. The kit according to claim 28, further comprising (c) a NRTI, and a pharmaceutically acceptable excipient.

30. The kit according to claim 28 or claim 29, wherein the HBV-associated disease is a hepatitis D (HDV) infection.