SUMMARY

[0009] In one aspect, the present invention refers to an isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27 and SEQ ID NO:29. In another aspect, the present invention refers to An isolated nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:30. In yet another aspect, the present invention refers to an isolated, double stranded, siRNA molecule selected from the group consisting of 1m (SEQ ID NO:1 and 2), 2m (SEQ ID NO:3 and 4), 3m (SEQ ID NO:5 and 6), 4m (SEQ ID NO:7 and 8), 5m (SEQ ID NO:9 and 10), 6m (SEQ ID NO:11 and 12), 7m (SEQ ID NO:13 and 14), 8m (SEQ ID NO:15 and 16), 9m (SEQ ID NO:17 and 18), 10m (SEQ ID NO:19 and 20), 11m (SEQ ID NO:21 and 22), 12m (SEQ ID NO:23 and 24), 13m (SEQ ID NO:25 and 26), 14m (SEQ ID NO:27 and 28) and 15m (SEQ ID NO:29 and 30). In a further aspect, the present invention refers to a composition comprising an isolated, double stranded siRNA molecule as described herein. In one aspect, the present invention refers to a nucleic acid-lipid particle comprising (a) one or more isolated, double stranded siRNA molecules selected from the isolated, double stranded siRNA molecules as described herein; (b) a cationic lipid; and (c) a non-cationic lipid. In another aspect, the present invention refers to a pharmaceutical composition comprising a nucleic acid-lipid particle as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present invention refers to use of a nucleic acid-lipid particle as described herein or a pharmaceutical composition as described herein in the manufacture of a medicament for silencing expression of a Hepatitis B virus gene in a cell. In one example, the present invention refers to a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein for use in silencing expression of a Hepatitis B virus gene in a cell in a mammal. In another example, the present invention refers to use of a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for ameliorating one or more symptoms associated with Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In yet another example, the present invention refers to a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein for use in ameliorating one or more symptoms associated with a Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In another aspect, the present invention refers to Use of a nucleic acid-lipid particle as described herein or a

pharmaceutical composition as described herein in the manufacture of a medicament for treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In yet another aspect, the present invention refers to a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein for use in treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In a further example, the present invention refers to a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein for use in medical therapy. In yet another aspect, the present invention refers to use of a composition as disclosed herein in the manufacture of a medicament for silencing expression of a Hepatitis B virus gene in a cell. In one aspect, the present invention refers to a composition as described herein for use in silencing expression of a Hepatitis B virus gene in a cell in a mammal. In another aspect, the present invention refers to use of a composition as disclosed herein in the manufacture of a medicament for ameliorating one or more symptoms associated with Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In a further aspect, the present invention refers to a composition as disclosed herein for use in ameliorating one or more symptoms associated with a Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In yet another example, the present invention refers to use of a composition as disclosed herein in the manufacture of a medicament for treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In yet another aspect, the present invention refers to a composition as disclosed herein for use in treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal. In a further aspect, the present invention refers to a composition as disclosed herein for use in medical therapy. In another example, the present invention refers to use of a composition as disclosed herein, a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for inhibiting the replication of Hepatitis D virus and/or ameliorating one or more symptoms of Hepatitis D virus infection in a mammal. In a further aspect, the present invention refers to a composition as disclosed herein, a nucleic acid-lipid particle as disclosed herein or a pharmaceutical composition as disclosed herein for use in inhibiting the replication of Hepatitis D virus and/or ameliorating one or more symptoms of Hepatitis D virus infection in a mammal (e.g., a human), wherein the nucleic acid-lipid particle or composition inhibits the synthesis of Hepatitis B virus surface antigen. As described more fully herein, in one example there is disclosed isolated, double stranded, siRNA molecules, that each include a sense strand and an antisense strand that is hybridized to the sense strand. The siRNA of this example target one or more genes and/or transcripts of the HBV genome. Examples of siRNA molecules disclosed herein are the siRNA molecules set forth in Table A herein. The siRNA molecules are useful, for example, for the treatment of HBV infection and/or HDV infection when administered in a therapeutic amount to

a human subject infected with HBV or HBV/HDV. More generally, there are disclosed siRNA molecules that are capable of inhibiting or silencing HBV gene expression in vitro and in vivo.

[0010] In another example, there are disclosed isolated, single stranded, nucleic acid molecules, such as the isolated sense and antisense strands of the siRNA molecules set forth in Table A. The aforementioned isolated sense and antisense strands are set forth in Table B herein. As described more fully herein, the siRNA and single stranded nucleic acid molecules of the invention are modified and include one or more UNA moieties and/or one or more 2'O-methyl modifications (*see, e.g.,* Tables A and B).

[0011] The disclosure also describes compositions, such as pharmaceutical compositions, that include one or more siRNA molecules of the invention (*see, e.g.,* the siRNA molecules described in Table A). In one example, compositions that include two different siRNA molecules (*e.g.,* two different siRNA molecules selected from the siRNA molecules disclosed in Table A herein) are disclosed. In another example, compositions are disclosed that include three different siRNA molecules (*e.g.*, three different siRNA molecules selected from the siRNA molecules disclosed in Table A herein). All of the possible combinations of two different siRNAs ("two way combinations") selected from the siRNA molecules disclosed in Table A herein are set forth in Example 2 herein. All of the possible combinations of three different siRNAs ("three way combinations") selected from the siRNA molecules disclosed in Table A herein are set forth in Example 3 herein. Thus, in one example, there are disclosed compositions (*e.g.*, pharmaceutical compositions) that include one of the aforementioned two way or three way combinations of the siRNAs set forth in Table A.

[0012] The present disclosure also describes nucleic acid-lipid particles, and formulations thereof, wherein the lipid particles each include one or more (*e.g.,* a cocktail) of the siRNA described

herein, a cationic lipid, and a non-cationic lipid, and optionally a conjugated lipid that inhibits aggregation of particles. Examples of siRNA molecules that can be included in the lipid particles are the siRNA molecules set forth in Table A, and combinations of the foregoing siRNA (*e.g.*, the two way, and three way combinations described herein). Typically, the siRNA is fully encapsulated within the lipid particle. The lipid particles of the invention are useful, for example, for delivering a therapeutically effective amount of siRNA into cells (*e.g.*, liver cells) of a human body infected with HBV or HBV/HDV, thereby treating the HBV infection and/or HDV infection and/or ameliorating one or more symptoms of HBV infection and/or HDV infection.

[0013] The present disclosure also described a pharmaceutical composition comprising one or a cocktail of siRNA molecules that target HBV gene expression, and a pharmaceutically acceptable carrier. For example, the present disclosure describes pharmaceutical compositions that each include one, two, or three of the siRNA molecules set forth in Table A that target HBV gene expression. With respect to formulations that include a cocktail of siRNAs encapsulated within lipid particles, the different siRNA molecules may be co-encapsulated in the same lipid particle, or each type of siRNA species present in the cocktail may be encapsulated in its own particle, or some siRNA species may be coencapsulated in the same particle while other siRNA species are encapsulated in different particles within the formulation. Typically, the siRNA molecules are fully encapsulated in the lipid particle.

[0014] The nucleic acid-lipid particles disclosed herein are useful for the prophylactic or therapeutic delivery, into a human infected with HBV or HBV/HDV, of siRNA molecules that silence the expression of one or more HBV genes, thereby ameliorating at least one symptom of HBV infection and/or HDV infection in the human. In some examples, one or more of the siRNA molecules described herein are formulated into nucleic acid-lipid particles, and the particles are administered to a mammal (*e.g.*, a human) requiring such treatment. In certain examples, a therapeutically effective amount of the nucleic acid-lipid particle can be administered to the mammal, (*e.g.*, for treating HBV and/or HDV infection in a human being). The nucleic acid-lipid particles of the invention are particularly useful for targeting liver cells in humans which is the site of most HBV gene expression. Administration of the nucleic acid-lipid particle can be by any route known in the art, such as, *e.g.*, oral, intranasal, intravenous, intraperitoneal, intramuscular, intra-articular, intralesional, intratracheal, subcutaneous, or intradermal. In particular examples, the nucleic acid-lipid particle is administered systemically, *e.g.*, via enteral or parenteral routes of administration.

[0015] In some examples, downregulation of HBV gene expression is determined by detecting HBV RNA or protein levels in a biological sample from a mammal after nucleic acid-lipid particle administration. In other examples, downregulation of HBV gene expression is determined by detecting HBV mRNA or protein levels in a biological sample from a mammal after nucleic acid-lipid particle administration. In certain examples, downregulation of HBV gene expression is detected by monitoring symptoms associated with HBV infection in a mammal after particle administration.

[0016] In another example, methods for introducing an siRNA that silences HBV gene expression into a living cell are disclosed, the method comprising the step of contacting the cell with a nucleic acid-lipid particle of the invention, wherein the nucleic acid-lipid particle includes an siRNA that targets HBV, under conditions whereby the siRNA enters the cell and silences the expression of a Hepatitis B virus gene within the cell.

[0017] In another example, a method for ameliorating one or more symptoms associated with Hepatitis B virus and/or Hepatitis D virus infection in a human is disclosed, the method including the step of administering to the human a therapeutically effective amount of a nucleic acid-lipid particle of the present invention. In some examples, the nucleic acid-lipid particles used in the methods of this example include one, two or three or more different siRNA independently selected from the siRNAs set forth in Table A.

[0018] In another example, methods for silencing HBV gene expression in a mammal (*e.g.*, a human) in need thereof are disclosed, wherein the methods each include the step of administering to the mammal a nucleic acid-lipid particle of the present invention.

[0019] In another example, methods for treating and/or ameliorating one or more symptoms associated with HBV and/or HDV infection in a human are disclosed, wherein the methods each include the step of administering to the human a therapeutically effective amount of a nucleic acid-lipid particle of the present invention.

[0020] Certain examples describe compositions and methods for inhibiting the replication of HDV, and/or ameliorating one or more symptoms of HDV infection, by administering to an individual infected with HDV a therapeutically effective amount of one or more compositions or nucleic acid-particles of the present invention that inhibit the synthesis of HBV surface antigen.

[0021] In another example, methods for inhibiting the expression of HBV in a mammal in need thereof (*e.g.*, a human infected with HBV or HBV/HDV) are disclosed, wherein the methods each include the step of administering to the mammal a therapeutically effective amount of a nucleic acid-lipid particle.

[0022] In a further example, methods for treating HBV and/or HDV infection in a human are disclosed, wherein the methods each include the step of administering to the human a therapeutically effective amount of a nucleic acid-lipid particle.

[0023] In a further example, a siRNA molecule is disclosed for inhibiting Hepatitis B virus gene expression in a living cell.

[0024] In a further example, use of a pharmaceutical composition is disclosed for inhibiting Hepatitis B virus gene expression in a living cell.

[0025] The compositions of disclosed herein (*e.g.*, siRNA molecules and isolated sense and antisense strands thereof, and nucleic acid-lipid particles) are also useful, for example, in biological assays (*e.g.*, in vivo or in vitro assays) for inhibiting the expression of one or more HBV genes and/or transcripts to investigate HBV and/or HDV replication and biology, and/or to investigate or modulate the function of one or more HBV genes or transcripts. For example, the siRNA molecules of the invention can be screened using a biological assay to identify siRNA molecules that inhibit replication of HBV and/or HDV and that are candidate therapeutic agents for the treatment of HBV and/or HDV infection in humans, and/or the amelioration of at least one symptom associated with HBV and/or HDV infection in a human.

[0026] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0027] The siRNA drug therapy described herein advantageously provides significant new compositions and methods for treating HBV and/or HDV infection in human beings and the symptoms associated therewith. Embodiments of the present invention can be administered, for

NO:21 and 22), 12m (SEQ ID NO:23 and 24), 13m (SEQ ID NO:25 and 26), 14m (SEQ ID NO:27 and 28) and 15m (SEQ ID NO:29 and 30).

35. The nucleic acid-lipid particle of claim 34, wherein a combination of the three different double stranded siRNA molecules is selected from any one of the combinations described in Example 3.

36. A pharmaceutical composition comprising a nucleic acid-lipid particle of any one of claims 11-35 and a pharmaceutically acceptable carrier.

37. Use of a nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 in the manufacture of a medicament for silencing expression of a Hepatitis B virus gene in a cell.

38. The use of claim 37, wherein the cell is in a mammal.

39. The use of claim 38, wherein the cell is to be contacted to the particle, wherein the particle is to be administered to the mammal via a systemic route.

40. The use of claim 38 or 39, wherein the mammal is a human.

41. The use of claim 40, wherein the human has been diagnosed with liver disease caused by Hepatitis B virus infection or Hepatitis B virus/Hepatitis D virus infection.

42. The use of claim any one of claims 38-41, wherein silencing of the Hepatitis B virus gene expression reduces Hepatitis B virus and/or Hepatitis D virus particle load in the mammal by at least about 50% relative to Hepatitis B virus and/or Hepatitis D virus particle load in the absence of the nucleic acid-lipid particle.

43. A nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 for use in silencing expression of a Hepatitis B virus gene in a cell in a mammal.

44. Use of a nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 in the manufacture of a medicament for ameliorating one or more symptoms associated with Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

45. The use of claim 44, wherein the particle is to be administered via a systemic route.

46. The use of any one of claims 44-45, wherein the siRNA of the nucleic acid-lipid particle inhibits expression of a Hepatitis B virus gene in the mammal.

47. The use of any one of claims 44-46, wherein the mammal is a human.

48. The use of claim 47, wherein the human has liver disease.

49. A nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 for use in ameliorating one or more symptoms associated with a Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

50. Use of a nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 in the manufacture of a medicament for treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

51. A nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 for use in treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

52. A nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 for use in medical therapy.

53. Use of a composition of any one of claims 4-10 in the manufacture of a medicament for silencing expression of a Hepatitis B virus gene in a cell.

54. The use of claim 53, wherein the cell is in a mammal.

55. The use of claim 54, wherein the cell is to be contacted to the composition, wherein the composition is to be administered to the mammal via a systemic route.

56. The use of claim 54 or 55, wherein the mammal is a human.

57. The use of claim 56, wherein the human has been diagnosed with liver disease caused by Hepatitis B virus infection or Hepatitis B virus/Hepatitis D virus infection.

58. The use of claim any one of claims 54-57, wherein silencing of the Hepatitis B virus gene expression reduces Hepatitis B virus and/or Hepatitis D virus particle load in the mammal by at least about 50% relative to Hepatitis B virus and/or Hepatitis D virus particle load in the absence of the nucleic acid-lipid particle.

59. A composition of any one of claims 4-10 for use in silencing expression of a Hepatitis B virus gene in a cell in a mammal.

60. Use of a composition of any one of claims 4-10 in the manufacture of a medicament for ameliorating one or more symptoms associated with Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

61. The use of claim 60, wherein the composition is to be administered via a systemic route.

62. The use of any one of claims 60-61, wherein the siRNA of the composition inhibits expression of a Hepatitis B virus gene in the mammal.

63. The use of any one of claims 60-62, wherein the mammal is a human.

64. The use of claim 63, wherein the human has liver disease.

65. A composition of any one of claims 4-10 for use in ameliorating one or more symptoms associated with a Hepatitis B virus and/or Hepatitis D virus infection in a mammal (e.g., a human).

66. Use of a composition of any one of claims 4-10 in the manufacture of a medicament for treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

67. A composition of any one of claims 4-10 for use in treating a Hepatitis B virus and/or Hepatitis D virus infection in a mammal.

68. A composition of any one of claims 4-10 for use in medical therapy.

69. Use of a composition of any one of claims 4-10, a nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 to the mammal in the manufacture of a medicament for inhibiting the replication of Hepatitis D virus and/or ameliorating one or more symptoms of Hepatitis D virus infection in a mammal wherein the nucleic acid-lipid particle or composition inhibits the synthesis of Hepatitis B virus surface antigen.

70. A composition of any one of claims 4-10, a nucleic acid-lipid particle of any one of claims 11-35 or a pharmaceutical composition of claim 36 for use in inhibiting the replication of Hepatitis D virus and/or ameliorating one or more symptoms of Hepatitis D virus infection in

a mammal (e.g., a human), wherein the nucleic acid-lipid particle or composition inhibits the synthesis of Hepatitis B virus surface antigen.