HEPATITIS D VIRUS (HDV) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional Application, 62/077,672, filed on November 10, 2014, the entire contents of which are incorporated herein by reference.

This application also claims priority to U.S. Provisional Application, 62/077,799, filed on November 10, 2014 and U.S. Provisional Application, 62/137,464, filed on March 24, 2015. The entire contents of each of the foregoing patent applications are incorporated herein by reference.

This application is related to International Patent Application, PCT/US2015/XXXXX, entitled "Hepatitis B Viris (HBV) iRNA Compositions and Methods of Use Thereof," filed on November 10, 2015 (Attorney Docket No.: 121301-02820) ,the entire contents of which are incorporated herein by reference.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 6, 2015, is named 121301-02820_SL.txt and is 774,780 bytes in size.

Background of the Invention

Hepatitis D virus or hepatitis delta virus (HDV) is a human pathogen. Indeed HDV infection is highly endemic to several African countries, the Amazonian region, and the Middle East, while its prevalence is low in industrialized countries, except in the Mediterranean. However, the virus is defective and depends on obligatory helper functions provided by the hepatitis B virus (HBV) for transmission; indeed, HDV requires a simultaneous infection with HBV (co-infection) or superimposition on a pre-existing HBV infection (superinfection) to become infectious and thrive. In particular, HDV requires the HBV viral envelope containing the surface antigen of hepatitis B.

HDV is unique in human virology; it has a circular RNA genome of about 1,700 bases (see, *e.g.*, Genbank Accession No. M21012.1 (GI:329989)) and is

therefore the smallest infectious agent in man, and is similar to viroids and satellite RNAs of plants. HDV replicates by a rolling circle mechanism unknown to animal cells, possesses a self-cleaving ribozyme and is transcribed by host RNA polymerases that normally accept only DNA templates (Ciancio and Rizzetto, *Nat. Rev.* 11:68-71, 2014).

HDV is a circular, single stranded RNA virus that ranges from 1,672 (strain dFr45, Genbank accession number AX741144) to 1,697 nucleotides (dFr47, GenBank accession number AX741149). A unique open reading frame encodes the small and large hepatitis delta (sHD and lHD, respectively) antigens by way of an editing step in the hepatocyte nucleus.

The genetic diversity of HDV is related to the geographic origin of the isolates and there are at least eight genotypes that are referred to as HDV-1 through HDV-8. Apart from HDV-1, which is ubiquitous, HDV-2 (previously labeled HDV-IIa) is found in Japan, Taiwan, and Yakoutia, Russia; HDV-4 (previously labeled HDV-IIb) in Taiwan and Japan; HDV-3 which causes epidemics of severe and fulminant hepatitis in the Amazonian region (9); and HDV-5, HDV-6, HDV- 7, and HDV-8 in Africa (LeGal *et al.*, *Emerg. Infect. Dis.* 12:1447-1450, 2006).

Worldwide more than 400 million people are chronically infected with HBV. Furthermore, at the end of the 1980s, at least 5% of hepatitis B surface antigen (HBsAg) carriers throughout the globe (~15 million individuals) were estimated to also be infected with HDV. Subjects infected with HBV are at increased risk of developing serious liver disease, such as chronic hepatitis, cirrhosis, liver failure and hepatocellular carcinoma (HCC) resulting in an estimated 600,000 deaths each year, HDV infection in such subjects (either co-infection or superinfection) can lead to severe acute and chronic forms of liver disease in association with HBV. In fact, both superinfection and coinfection with HDV results in more severe complications compared to infection with HBV alone. These complications include resistance to treatment with standard therapies and a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased chance of developing liver cancer in chronic infections. In combination with hepatitis B virus, hepatitis D has the highest fatality rate of all the hepatitis infections, at 20%.

Accordingly, there is a need in the art for alternative therapies and combination therapies for subjects infected with HDV and/or having an HDV-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 D virus (HDV) gene. The HDV gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

The present invention also provides methods and therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an HDV gene, *e.g.,* an HDV infection and/or an HDV-associated disease, such as hepatitis B virus infection, chronic hepatitis B infection (CHB), chronic Hepatitis D infection (CHD), 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 HDV gene for inhibiting the expression of an HDV gene. As HDV depends on obligatory helper functions provided by HBV for transmission, the methods for treatment of HDV can comprise agents and therapies useful for treating an HBV infection and/or an HBV-associated disorder, such as hepatitis D virus infection, chronic hepatitis D infection (CHD), chronic Hepatitis B infection (CHB), cirrhosis, liver failure, and hepatocellular carcinoma (HCC).

In certain embodiments, the RNAi agents of the invention have been designed to target regions in the HDV genomes that are conserved across at least two, preferably 3, or more of the 8 serotypes of HDV (HDV-1, HDV-2, HDV-3, HDV-4, HDV-5, HDV-6, HDV-7, and HDV-8).

Accordingly, in one aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:29, and the 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.

In another aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:31, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:32, 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.

In one aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:33, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:34, 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.

In one aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:35, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:36, 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.

In another aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:37, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 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 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.

In yet another aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:39, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 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.

In one aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:41, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:42, 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.

In another aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:43, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:44, 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.

In one aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2551, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 2552, 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. In certain embodiments, the sense strand of the double stranded RNAi agents comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 1451-1484, nucleotides 1455-1480, nucleotides 1455-1474, or nucleotides 1417-1443 of the nucleotide sequence of SEQ ID NO:2551.

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

In one embodiment, 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 11, 12, 31, and 32.

In some embodiments, 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 the nucleotide sequences of the sense strand and the antisense strand sequences of any one of duplexes AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, and AD-70275.1. In other embodiments, 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 the nucleotide sequences of the sense strand and the antisense strand sequences of any one of duplexes AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, and AD-70295.1.

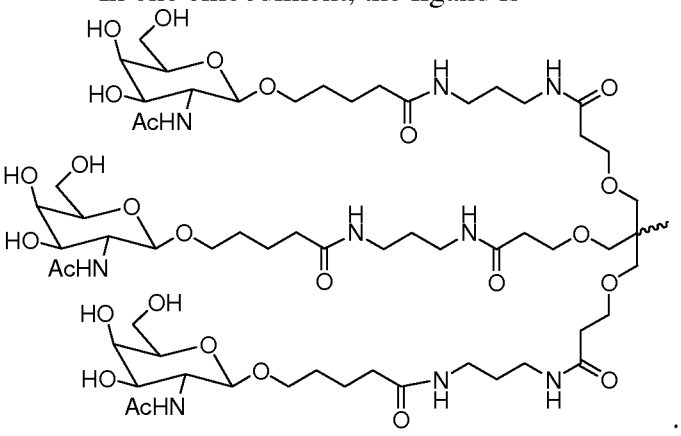
In one embodiment, the at least one of the modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally 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 embodiment, the at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, the at least one strand comprises a 3' overhang of at least 2 nucleotides.

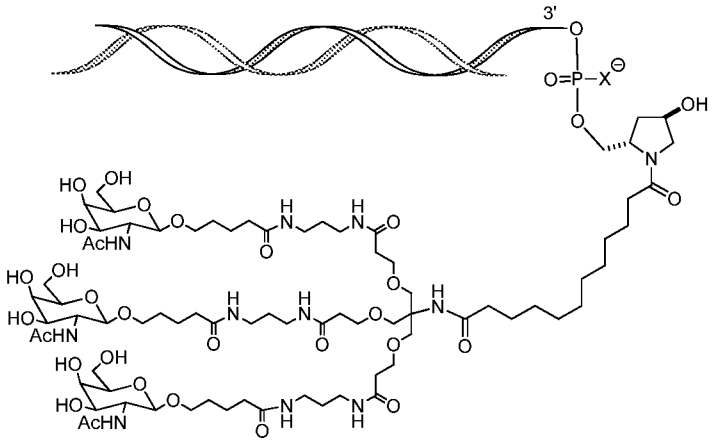
In one embodiment, the double-stranded region is 15-30 nucleotide pairs in length. In another embodiment, the double-stranded region is 17-23 nucleotide pairs in length. In yet another embodiment, the double-stranded region is 17-25 nucleotide pairs in length. In one embodiment, the e double-stranded region is 23-27 nucleotide pairs in length. In another embodiment, the double-stranded region is 19-21 nucleotide pairs in length. In yet another embodiment, the double-stranded region is 21-23 nucleotide pairs in length.

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

In one embodiment, the ligand is



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



wherein X is O or S.

In one embodiment, the RNAi agent is selected from the group of RNAi agents listed in any one of Tables 11, 12, 31, and 32.

In one embodiment the agent is selected from the group consisting of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-

67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, and AD-70275.1.

In one aspect, the present invention provides double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 any one of the sense sequences from Tables 11, 12, 31, and 32, and the antisense strand comprises any one of the antisense sequences from Tables 11, 12, 31, and 32, 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.

In one embodiment, the sense strand is selected from the group consisting of the sense strand sequences of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

In another embodiment, the antisense strand is selected from the group consisting of the antisense strand sequences of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

The present invention 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

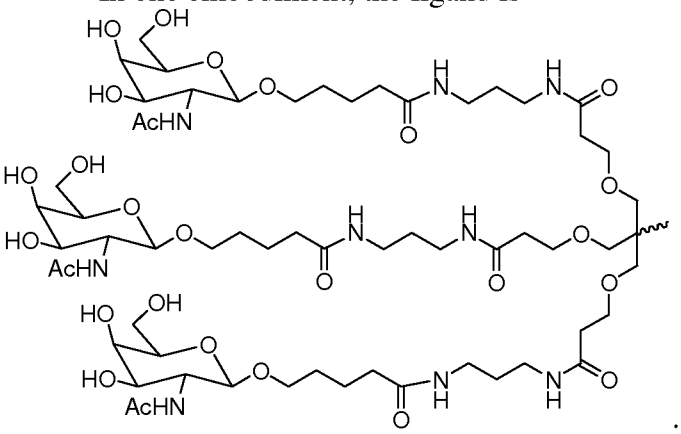
In one embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, at least one of the 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,

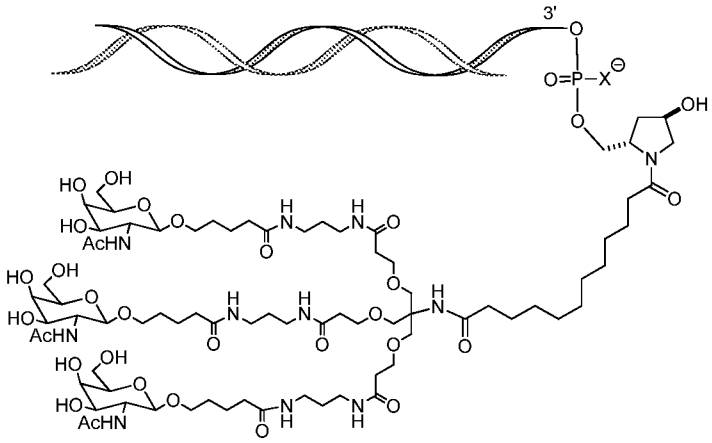
a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol 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 embodiment, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

In one embodiment, the ligand is



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



wherein X is O or S.

In one aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides

differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:29, and each of the antisense strands independently 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 each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 another aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:31, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:32, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 another aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:33, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:34, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 yet another aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:35, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:36, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:37, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:38, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 another aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID

NO:39, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:40, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 another aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:41, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:42, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:43, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:44, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 another aspect, the present invention provides compositions comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) 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 the sense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2551, and each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2552, wherein substantially all of the nucleotides of each of the sense strands and substantially all of the nucleotides of each of the antisense strands are independently modified nucleotides, wherein each of the 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 certain embodiments, the sense strand of one or both of the first and second double stranded RNAi agents comprises at least 15 contiguous nucleotides from nucleotides 1451-1484, nucleotides 1455-1480, nucleotides 1455-1474, or nucleotides 1417-1443 of the nucleotide sequence of SEQ ID NO:2551.

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

In one embodiment, 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 11, 12, 31, and 32.

In some embodiments, 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 the nucleotide sequences of the sense strand and the antisense strand sequences of any one of duplexes AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-

67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

In other embodiments, 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 the nucleotide sequences of the sense strand and antisense strand sequences of any one of duplexes AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, or AD-70295.1.

In one embodiment, the at least one of the 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 another aspect, the present invention provides compositions for inhibiting expression of hepatitis D virus (HDV) 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 any one of the sense sequences from Tables 11, 12, 31, and 32 (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 any one of the antisense sequences from Tables 11, 12, 31, and 32 (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 embodiment, the first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 another embodiment, the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 embodiment, the first and second sense strand and/or all of the nucleotides of the first and second antisense strand comprise a modification.

In one embodiment, the at least one of the 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 embodiment, the first and second RNAi agent are selected from the group consisting of any one of the the sequences provided in Tables 11, 12, 31, and 32.

In one embodiment, the first and second RNAi agent are selected from the group consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

In one embodiment, the first and second RNAi agent are selected from the group consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, or AD-70295.1.

In one aspect, the present invention provides a double stranded RNAi agent comprising any one of the RNAi agents listed in any one of Tables 11, 12, 31, 32,

In one embodiment the agent is selected from the group consisting of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1. In another embodiment, the agent is selected from the group consisting of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, or AD-70295.1.

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

In another aspect, the present invention provides pharmaceutical compositions comprising the double stranded RNAi agents of the invention, or the compositions of the invention, or the vectors of the invention.

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

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

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

In one aspect, the present invention provides methods of inhibiting Hepatitis D virus (HDV) gene expression in a cell. The methods include contacting the cell with the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention; and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of an HDV gene, thereby inhibiting expression of the HDV gene in the cell.

In one aspect, the present invention provides methods of inhibiting replication of a Hepatitis D virus (HDV) in a cell. The methods include contacting the cell with the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention; and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of an HDV gene, thereby inhibiting replication of the HDV in the cell.

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

In one embodiment, the subject suffers from an HDV-associated disease.

In one embodiment, HDV 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 embodiment, replication of HDV 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%.

In one aspect, the present invention provides methods of reducing the level of Hepatitis D virus (HDV) DNA in a subject infected with HDV. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention, thereby reducing the level of HDV DNA in the subject.

In another aspect, the present invention 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 the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention, thereby reducing the level of the HDV antigen in the subject. The level of the large HDV

antigen may be reduced, the level of the small HDV antigen may be reduced, or the level of both the large and the small HDV antigens may be reduced.

In another aspect, the present invention 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 the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention, thereby reducing the viral load of HDV in the subject.

In one aspect, the present invention 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 the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention, thereby treating the subject.

In another aspect, the present invention provides methods of treating a subject having a Hepatitis D virus (HDV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agent of the invention, or the composition of the invention, or the vector of the invention, or the pharmaceutical composition of the invention, thereby treating the subject.

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

In one aspect, the present invention 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 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 any one sense sequences from any one of Tables 11, 12, 31, and 32 (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 any one of the antisense sequences from any one of Tables 11, 12, 31, and 32 (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 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 one embodiment, the sense strand comprises a sequence selected from the group consisting of any one of the sense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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).

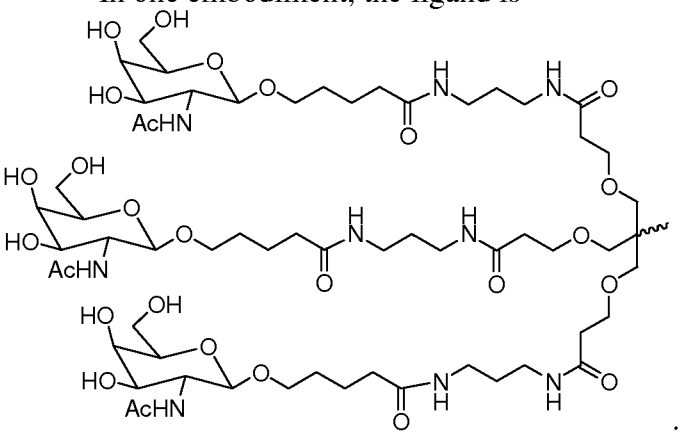
In another embodiment, the antisense strand comprises a sequence selected from the group consisting of any one of the antisense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

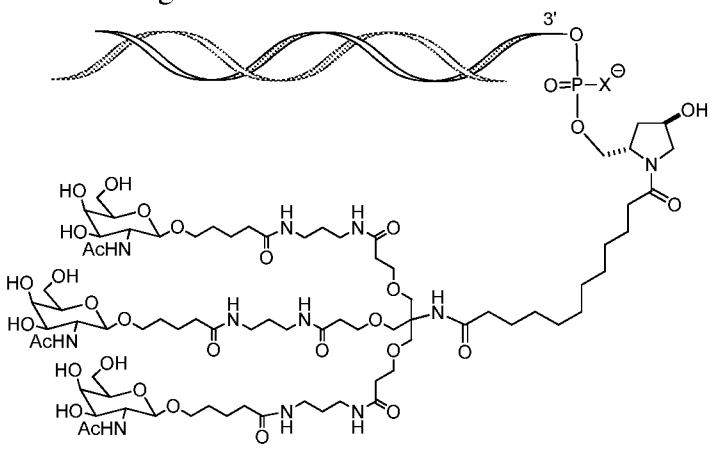
In one embodiment, the at least one of the modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally 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 embodiment, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

In one embodiment, the ligand is



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



wherein X is O or S.

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

In one aspect, the present invention 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 D virus (HDV) 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 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 any one of the sense sequences in any one of Tables 11, 12, 31, and 32 (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 any one of the antisense sequences from any one of Tables 11, 12, 31, and 32 (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 embodiment, the first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 another embodiment, the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 another aspect, the present invention provides methods of treating a subject having a Hepatitis D virus (HDV)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis D virus (HDV) 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 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 any one of the sense sequences from any one of Tables 11, 12, 31, and 32 (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 any one of the antisense sequences from any one of Tables 11, 12, 31, and 32 (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.

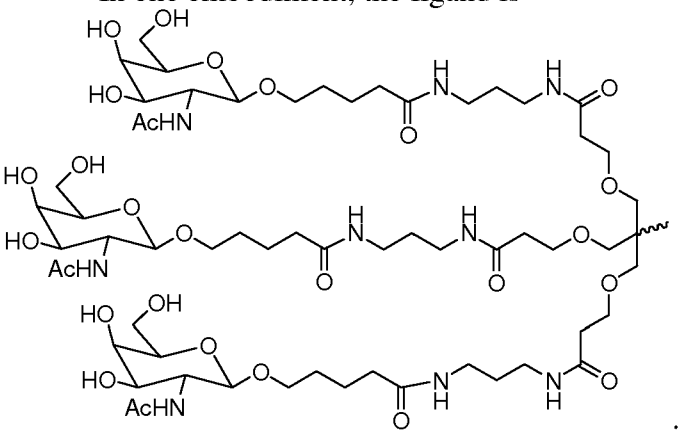
In one embodiment, the first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 another embodiment, the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 embodiment, 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.

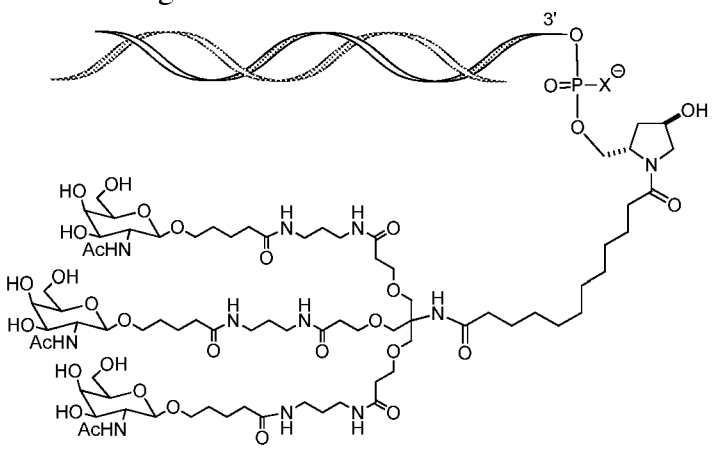
In one embodiment, the at least one of the modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally 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 embodiment, the ligand is



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



wherein X is O or S.

In one embodiment, the subject is a human.

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

In some embodiments, the methods of the invention further comprise treatment of hepatitis B virus (HBV) in the subject. Methods of treatment can include any methods of treatment known in the art. In certain embodiments, HBV is treated in the subject using one of more if the iRNA agents provided herein.

In some embodiments, the methods of the invention further include methods to modulate, preferably 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, which is hereby incorporated by reference.

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

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

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

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

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

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

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

In one embodiment, 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, a double-stranded iRNA agent targeting HBV, and a combination of any of the foregoing.

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

In one embodiment, 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 one embodiment, 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.

In one embodiment, the double-stranded iRNA agent targeting HBV is any one of the agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28. In another embodiment, the agent is AD-65403, AD-66810, or a combination thereof.

Detailed Description 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 D virus (HDV) gene. The gene may be within a cell, *e.g.*, a cell within a

subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the correponding gene (HDV gene) in mammals.

The RNAi agents of the invention have been designed to target regions in the HDV across various clades of HDV.

Also provided herein are HBV targeted RNAi agents of that 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 invention 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 invention 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 invention have been designed to inhibit HBV viral replication by targeting HBV structural genes, and the HBV X gene thereby permiting a subject's immune sytem to detect and respond to the presence of HBsAg to produce anti-HBV antibodies to clear an HBV infection.

HDV infection requires the presence of an HBV infection. Using in vitro assays, the present inventors have demonstrated that iRNA targeting HDV can potently mediate RNAi, resulting in significant inhibition of expression of the HDV gene. Moreover, using *in vitro* and *in vivo* assays, it is demonstrated herein that iRNAs targeting an HBV gene can potently mediate RNAi, resulting in significant inhibition of expression of more than one HBV gene,is effective in treating HDV infection as well. Thus, methods and compositions including these iRNAs are useful for treating a subject having an HDV infection and/or an HDV-associated disease, by administration of a iRNA targeted to one or both of HBV and HDV. Further, the compositions provided herein can be used in a subject with an HBV infection to prevent the development of an HDV infection.

Accordingly, the present invention also provides methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an HBV gene, *e.g.*, HDV infection, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an HBV gene.

Very low dosages of the iRNAs of the invention, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of the correponding gene (HDV gene).

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an HBV gene.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an angiotensinogen gene as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of an HDV gene.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

As used herein, “Hepatitis D virus,” used interchangeably with the term “HDV” refers to the well-known noncytopathic, liver-tropic DNA virus belonging to the Hepadnaviridae family. See, e.g., Ciancio and Rizzetto, Nat. Rev. 11:68-71, 2014; Le Gal et al., Emerg. Infect. Dis. 12:1447-1450, 2006; and Abbas and afzal, World J. Hep., 5:666-675, 2013, all of which are incorporated by reference. Unless otherwise indictate, HDV refers to all clades and variants of HDV.

HDV produces one protein, namely HDAg. It comes in two forms; a 27kDa large-HDAg (also referred to herein as lHD and large HDV antigen), and a small-

HDAg of 24kDa (also referred to herein as sHD and small HDV antigen). The N-terminals of the two forms are identical, they differ by 19 more amino acids in the C-terminal of the large HDAg. Both isoforms are produced from the same reading frame which contains an UAG stop codon at codon 196, which normally produces only the small-HDAg. However, editing by cellular enzyme adenosine deaminase-1 changes the stop codon to UCG, allowing the large-HDAg to be produced. Despite having 90% identical sequences, these two proteins play diverging roles during the course of an infection. HDAg-S is produced in the early stages of an infection and enters the nucleus and supports viral replication. HDAg-L, in contrast, is produced during the later stages of an infection, acts as an inhibitor of viral replication, and is required for assembly of viral particles.

Additional examples of HDV mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

The term"HDV," as used herein, also refers to naturally occurring DNA sequence variations of the HDV genome.

As used herein, "Hepatitis B virus," used interchangeably with the term "HBV" refers to the well-known noncytopathic, liver-tropic DNA virus belonging to the Hepadnaviridae family.

The HBV genome is partially double-stranded, circular DNA with overlapping reading frames.

There are four known genes encoded by the HBC genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg). Hepatitis B antigen (HBeAg) is produced by proteolytic processing of the pre-core (pre-C) protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced. The function of the non-structural protein coded for by gene X is not fully understood but it is associated with the development of liver cancer and encodes a decoy protein which permits HBsAg in the blood to sequester anti-HBsAg antibodies and allow infectious viral particles to escape immune detection.

The proteins encoded by the HBV genome include: envelope proteins - i) small, Hepatitis B surface antigen (HBsAg); ii) middle - preS2 plus HBsAg; iii) large - preSl plus preS2 plus HBsAg; nucleocapsid protein, hepatitis B core antigen (HBcAg). Hepatitis B e antigen (HBeAg) is a non-structural protein produced during the HBV

replication which shares 90% amino acids with the nucleocapsid HBcAg; and the X protein is a nonstructural protein (HBx) which functions in the cytoplasm to activate various signaling pathways, many of which are controlled by modulation of cytosolic calcium and in the nucleus to regulate transcription through a direct interaction with different transcription factors and, in some cases, enhance their binding to specific transcription elements.

HBV is one of the few DNA viruses that utilize reverse transcriptase in the replication process which involves multiple stages including entry, uncoating and transport of the virus genome to the nucleus. Initially, replication of the HBV genome involves the generation of an RNA intermediate that is then reverse transcribed to produce the DNA viral genome.

Upon infection of a cell with HBV, the viral genomic relaxed circular DNA (rcDNA) is transported into the cell nucleus and converted into episomal covalently closed circular DNA (cccDNA), which serves as the transcription template for the viral mRNAs. After transcription and nuclear export, cytoplasmic viral pregenomic RNA (pgRNA) is assembled with HBV polymerase and capsid proteins to form the nucleocapsid, inside which polymerase-catalyzed reverse transcription yields minus-strand DNA, which is subsequently copied into plus-strand DNA to form the progeny rcDNA genome. The mature nucleocapsids are then either packaged with viral envelope proteins to egress as virion particles or shuttled to the nucleus to amplify the cccDNA reservoir through the intracellular cccDNA amplification pathway. cccDNA is an essential component of the HBV replication cycle and is responsible for the establishment of infection and viral persistence.

HBV infection results in the production of two different particles: 1) the HBV virus itself (or Dane particle) which includes a viral capsid assembled from the HBcAg and is covered by the HBsAg and is capable of reinfecting cells and 2) subviral particles (or SVPs) which are high density lipoprotein-like particles comprised of lipids, cholesterol, cholesterol esters and the small and medium forms of the hepatitis B surface antigen HBsAg which are non-infectious. For each viral particle produced, 1,000-10,000 SVPs are released into the blood. As such SVPs (and the HBsAg protein they carry) represent the overwhelming majority of viral protein in the blood. HBV infected cells also secrete a soluble proteolytic product of the pre-core protein called the HBV e-antigen (HBeAg).

Eight genotypes of HBV, designated A to H, have been determined, each having a distinct geographical distribution. The virus is non-cytopathic, with virus-specific cellular immunity being the main determinant for the outcome of exposure to HBV -

acute infection with resolution of liver diseases with 6 months, or chronic HBV infection that is frequently associated with progressive liver injury.

The term "HBV" includes any of the eight genotypes of HBV (A to H). The amino acid and complete coding sequence of the reference sequence of the HBV genome may be found in for example, GenBank Accession Nos. GI:21326584 (SEQ ID NO:1) and GI:3582357 (SEQ ID NO:3).

Additional examples of HBV mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

The term"HBV," as used herein, also refers to naturally occurring DNA sequence variations of the HBV genome.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an HDV gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an HDV gene.

The target sequence may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.,* Table 2). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such

replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms "iRNA", "RNAi agent," "iRNA agent,", "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.,* inhibits, the expression of an HDV gene (*e.g.,* one or more HDV genes) in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.,* an HDV target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* an HDV gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded siRNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target

mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded siRNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In another embodiment, an "iRNA" for use in the compositions, uses, and methods of the invention is a double-stranded RNA and is referred to herein as a "double stranded RNAi agent," "double-stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* an HDV gene. In some embodiments of the invention, a double-stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides. As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, and/or modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.*, about 15-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-

24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

In one embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, an HDV target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more

endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188).

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of an iRNA, *e.g.*, a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or *vice versa*, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNAi agent, *i.e.*, no nucleotide overhang. A "blunt ended" RNAi agent is a dsRNA that is double-stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with nucleotide overhangs at one end (*i.e.*, agents with one overhang and one blunt end) or with nucleotide overhangs at both ends.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a HDV mRNA. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* an HDV nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, 2, or 1 nucleotides of the 5'- and/or 3'-terminus of the iRNA. In one embodiment, a double-stranded RNAi agent of the invention includea a nucleotide mismatch in the antisense strand. In another embodiment, a double-stranded RNAi

agent of the invention includea a nucleotide mismatch in the sense strand. In one embodiment, the nucleotide mismatch is, for example, within 5, 4, 3, 2, or 1 nucleotides from the 3'-terminus of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA.

The term "sense strand," or "passenger strand" as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, *e.g.,* "Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be

fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of an iRNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding an HDV gene). For example, a polynucleotide is complementary to at least a part of an HDV mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding an HDV gene.

Accordingly, in some embodiments, the sense strand polynucleotides and the antisense polynucleotides disclosed herein are fully complementary to the target HDV sequence. In other embodiments, the sense strand polynucleotides and/or the antisense polynucleotides disclosed herein are substantially complementary to the target HDV sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the sequences in Tables 11, 12, 31, and 32, or a fragment of any one of the sequences in Tables 11, 12, 31, and 32, such as about 85%,

about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to the target HDV sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the sequences in Tables 11, 12, 31, and 32, or a fragment of any one of the sequences in Tables 11, 12, 31, and 32, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In another embodiment, an RNAi agent of the invention includes an antisense strand that is substantially complementary to the target HDV sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the sequences in Tables 11, 12, 31, and 32, or a fragment of any one of the sequences in Tables 11, 12, 31, and 32, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the RNAi agents for use in the invention target HBV. In some embodiment, such agents are fully complementary to the target HBV sequence. In other embodiments, the sense strand polynucleotides and/or the antisense polynucleotides disclosed herein are substantially complementary to the target HBV sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the nucleotide sequences of the agents targeting HBV provided herein, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent targeting HBV for use in the present invention includes a sense strand that is substantially complementary to the target HBV sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the nucleotide sequences of the agents targeting HBV provided herein, such as about 85%, about 86%, about 87%, about 88%, about 89%,

about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In another embodiment, an RNAi agent targeting HBV for use in the present invention includes an antisense strand that is substantially complementary to the target HBV sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the nucleotide sequences of the agents targeting HBV provided herein, or a fragment of any one of the nucleotide sequences of the agents targeting HBV provided herein, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, an "iRNA" may include ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in an iRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In one aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense RNA molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense RNA molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense RNA molecule may be about 15 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose). In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from

reduction in HDV gene expression and/or replication; a human at risk for a disease, disorder or condition that would benefit from reduction in HDV gene expression and/or replication; a human having a disease, disorder or condition that would benefit from reduction in HDV gene expression and/or replication; and/or human being treated for a disease, disorder or condition that would benefit from reduction in HDV gene expression and/or replication, as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with unwanted HDV gene expression and/or HDV replication. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of HDV gene expression and/or HDV replication in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more and is preferably down to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, the expression of the target is normalized, *i.e.*, decreased to a level accepted as within the range of normal for an individual without such disorder, *e.g.*, the level of a disease marker, such as, ALT or AST, is decreased to a level accepted as within the range of normal for an individual without such disorder.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of an HDV gene and/or replication, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom of unwanted HDV infection, such as the presence of serum and/or liver HDV ccc DNA, the presence of serum HDV DNA, the presence of serum and/or liver HDV antigen.

As used herein, the term "Hepatitis D virus-associated disease" or "HDV-associated disease," is a disease or disorder that is caused by, or associated with HDV infection and/or replication. The term "HDV-associated disease" includes a disease, disorder or condition that would benefit from reduction in HDV gene expression and/or replication. Non-limiting examples of HDV-associated diseases include, for example, hepatitis B virus infection, acute hepatits B, acute hepatitis D; acute

fulminant hepatitis D; chronic hepatitis D; liver fibrosis; end-stage liver disease; hepatocellular carcinoma.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a patient for treating a subject having an HDV infection and/or HDV-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by HDV gene expression, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject who does not yet experience or display symptoms of an HDV infection and/or HDV-associated disease, but who may be predisposed, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. RNAi agents employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver,

such as, *e.g.*, hepatocytes), the retina or parts of the retina (*e.g.*, retinal pigment epithelium), the central nervous system or parts of the central nervous system (*e.g.*, ventricles or choroid plexus), or the pancreas or certain cells or parts of the pancreas. In some embodiments, a "sample derived from a subject" refers tocerebrospinal fluid obtained from the subject. In preferred embodiments, a "sample derived from a subject" refers to blood or plasma drawn from the subject. In further embodiments, a "sample derived from a subject" refers to liver tissue (or subcomponents thereof) or retinal tissue (or subcomponents thereof) derived from the subject.

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of one or more HDV genes. In one embodiment, the iRNA agent includes double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an HDV gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human having an HDV-associated disease, *e.g.,* chronic hepatitis D. The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an HDV gene. The region of complementarity is about 30 nucleotides or less in length (*e.g.,* about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the HDV gene, the iRNA inhibits the expression of the HDV gene (*e.g.,* a human, a primate, a non-primate, or a bird HDV gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western Blotting or flowcytometric techniques.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of an HDV gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is between 15 and 30 base pairs in length, *e.g.,* between, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence is between 15 and 30 nucleotides in length, *e.g.,* between 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, the dsRNA is between about 15 and about 20 nucleotides in length, or between about 25 and about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22

base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target HDV gene expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.,* 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double-stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In one aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 11, 12, 31, and 32 and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 11, 12, 31, and 32. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of an HDV gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any

one of Tables 11, 12, 31, and 32 and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 11, 12, 31, and 32. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although some of the sequences in Tables 12 and 32 are described as modified and/or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 11, 12, 31, and 32 that is un-modified, un-conjugated, and/or modified and/or conjugated differently than described therein.

The skilled person is well aware that dsRNAs having a duplex structure of between about 20 and 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 11, 12, 31, and 32, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of any one of Tables 11, 12, 31, and 32 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of any one of Tables 11, 12, 31, and 32, and differing in their ability to inhibit the expression of a HDV gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in any one of Tables 11, 12, 31, and 32 identify a site(s) in a HDV transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 15 contiguous nucleotides from one of the sequences provided inany one of Tables 11, 12, 31, and 32 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a HDV gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified, for example, in any one of Tables 11, 12, 31, and 32 represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, *e.g.*, inany one of Tables 11, 12, 31, and 32, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

An iRNA as described herein can contain one or more mismatches to the target sequence. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of an HDV gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of an HDV gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of an HDV gene is important, especially if the particular region of complementarity in an HDV gene is known to have polymorphic sequence variation within the population.

III. Modified iRNAs of the Invention

In one embodiment, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications and/or conjugations known in the art and described herein. In another embodiment, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA of the invention are modified iRNAs of the invention in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated

bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and

thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

In other embodiments, suitable RNA mimetics are contemplated for use in iRNAs, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2-, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-

position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$.

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxy-thymine (dT), 5-methylcytosine (5-

me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

The RNA of an iRNA can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A"bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety

comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4′ to 2′ bridge. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH2)—O-2′ (LNA); 4′-(CH2)—S-2′; 4′-(CH2)2—O-2′ (ENA); 4′-CH(CH3)—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-CH(CH2OCH3)—O-2′ (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4′-C(CH3)(CH3)—O-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4′-CH2—N(OCH3)-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4′-CH2—O—N(CH3)-2′ (see, e.g.,U.S. Patent Publication No. 2004/0171570); 4′-CH2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4′-CH2—C(H)(CH3)-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH2—C(=CH2)-2′ (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133;7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH3)-0-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2'and C4' carbons of ribose or the C3 and -C5′ carbons of ribose . CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

One or more of the nucleotides of an iRNA of the invention may also include a hydroxymethyl substituted nucleotide. A "hydroxymethyl substituted nucleotide" is an acyclic 2'-3'-seco-nucleotide, also referred to as an "unlocked nucleic acid" ("UNA") modification

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US Patent No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N- (acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"- phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of an RNAi agent. Suitable phosphate mimics are disclosed

in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double-stranded RNAi agents of the invention include agents with chemical modifications as disclosed, for example, in WO 2013/075035, filed on November 16, 2012, the entire contents of which are incorporated herein by reference. As shown herein and in PCT Publication No. WO 2013/075035, a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand and/or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense and/or antisense strand. The RNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand. The resulting RNAi agents present superior gene silencing activity.

More specifically, it has been surprisingly discovered that when the sense strand and antisense strand of the double-stranded RNAi agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of an RNAi agent, the gene silencing acitivity of the RNAi agent was superiorly enhanced.

Accordingly, the invention provides double-stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, HDV gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may range from 12-30 nucleotides in length. For example, each strand may be between 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent may be 12-30 nucleotide pairs in length. For example, the duplex region can be between 14-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17 - 23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in

length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In one embodiment, the RNAi agent may contain one or more overhang regions and/or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In one embodiment, the nucleotides in the overhang region of the RNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2-F, 2'-Omethyl, thymidine (T), 2`-O-methoxyethyl-5-methyluridine (Teo), 2`-O-methoxyethyladenosine (Aeo), 2`-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof. For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand or both strands of the RNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

The RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'-terminal end of the sense strand or, alternatively, at the 3'-terminal end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the RNAi has a

nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In one embodiment, the RNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In another embodiment, the RNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In yet another embodiment, the RNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In one embodiment each residue is

independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.,* in an alternating motif. Optionally, the RNAi agent further comprises a ligand (preferably $GalNAc_3$).

In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby

reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

In one embodiment, the sense strand of the RNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In one embodiment, the antisense strand of the RNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand

For an RNAi agent having a duplex region of 17-23 nucleotide in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the antisense strand, the count starting from the 1st nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the RNAi from the 5'-end.

The sense strand of the RNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In one embodiment, the sense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adajacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistry of the motifs are distinct from

each other and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the RNAi agent may contain more than one motifs of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In one embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end or both ends of the strand.

In another embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end or both ends of the strand.

When the sense strand and the antisense strand of the RNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two or three nucleotides.

When the sense strand and the antisense strand of the RNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

In one embodiment, every nucleotide in the sense strand and antisense strand of the RNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2′ hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or 5' terminal position, may only occur in a terminal region, *e.g.,* at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of a RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5' end or ends can be phosphorylated.

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5' or 3' overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, , 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase , and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In one embodiment, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'- O-methyl or 2'-fluoro modifications, or others.

In one embodiment, the N_a and/or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB…," "AABBAABBAABB…," "AABAABAABAAB…," "AAABAAABAAAB…," "AAABBBAAABBB…," or "ABCABCABCABC…," *etc.*

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB…", "ACACAC…" "BDBDBD…" or "CDCDCD…," *etc.*

In one embodiment, the RNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5'-3'of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisenese strand may start with "BBAABBAA" from 5'-3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one embodiment, the RNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, *i.e.*, the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the

2'-F modification, and the 1 position of the antisense strand may start with the 2'- O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand and/or antisense strand interrupts the initial modification pattern present in the sense strand and/or antisense strand. This interruption of the modification pattern of the sense and/or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense and/or antisense strand surprisingly enhances the gene silencing acitivty to the target gene.

In one embodiment, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "…N_aYYYN_b…," where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Altnernatively, N_a and/or N_b may be present or absent when there is a wing modification present.

The RNAi agent may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand and/or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand and/or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, a double-standed RNAi agent comprises 6-8phosphorothioate internucleotide linkages. In one embodiment, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide

linkages at the 3'-terminus, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-terminus or the 3'-terminus.

In one embodiment, the RNAi comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within the duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, and/or the 5'end of the antisense strand.

In one embodiment, the 2 nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the RNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In one embodiment, the RNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'- end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair.

In another embodiment, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT). In another embodiment, the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). In one embodiment, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense and/or antisense strand.

In one embodiment, the sense strand sequence may be represented by formula (I):

$5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$ (I)

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In one embodiment, the N_a and/or N_b comprise modifications of alternating pattern.

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' n_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib);

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic); or

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id).

When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6 Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

5' n_p-N_a-YYY- N_a-n_q 3' (Ia).

When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

5' $n_{q'}$-N_a′-(Z'Z'Z')$_k$-N_b′-Y′Y′Y′-N_b′-(X′X′X′)$_l$-N'_a-n_p′ 3' (II)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a′ independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b′ independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p′ and n_q′ independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification; and

X′X′X′, Y′Y′Y′ and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, the N_a' and/or N_b' comprise modifications of alternating pattern.

The Y′Y′Y′ motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23nucleotidein length, the Y′Y′Y′ motif can occur at positions 9, 10, 11;10, 11, 12; 11, 12, 13; 12, 13, 14 ; or 13, 14, 15 of the antisense strand, with the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. Preferably, the Y′Y′Y′ motif occurs at positions 11, 12, 13.

In one embodiment, Y′Y′Y′ motif is all 2'-OMe modified nucleotides.

In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:

5' $n_{q'}$-N_a′-Z′Z′Z′-N_b′-Y′Y′Y′-N_a′-$n_{p'}$ 3' (IIb);

5' $n_{q'}$-N_a′-Y′Y′Y′-N_b′-X′X′X′-$n_{p'}$ 3' (IIc); or

5' $n_{q'}$-N_a′- Z′Z′Z′-N_b′-Y′Y′Y′-N_b′- X′X′X′-N_a′-$n_{p'}$ 3' (IId).

When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

5' $n_{p'}$-$N_{a'}$-Y'Y'Y'- $N_{a'}$-$n_{q'}$ 3' (Ia).

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X′, Y′ and Z′ may be the same or different from each other.

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X′, Y′ and Z′, in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In one embodiment, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In one embodiment the antisense strand may contain Y′Y′Y′ motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y′ represents 2'-O-methyl modification. The antisense strand may additionally contain X′X′X′ motif or Z′Z′Z′ motifs as wing modifications

at the opposite end of the duplex region; and X′X′X′ and Z′Z′Z′ each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the RNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):

sense: 5' n_p -N_a-(X X X)$_i$ -N_b- Y Y Y -N_b -(Z Z Z)$_j$-N_a-n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'-n_q' 5'

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p′, q, and q′ are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

5' n_p - N_a -Y Y Y -N_a-n_q 3'

3' n_p'-N_a'-Y′Y′Y′ -N_a'n_q' 5'

(IIIa)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y′Y′Y′-N_b'-Z′Z′Z′-N_a'n_q' 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_b'-Z'Z'Z'-N_a-n_q' 5'

(IIId)

When the RNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b and N_b' independently comprises modifications of alternating pattern.

Each of X, Y and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the RNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), at least one of the Y nucleotides may form a base pair with one of the Y′ nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y′ nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y′ nucleotides.

When the RNAi agent is represented by formula (IIIb) or (IIId), at least one of the Z nucleotides may form a base pair with one of the Z′ nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z′ nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z′ nucleotides.

When the RNAi agent is represented as formula (IIIc) or (IIId), at least one of the X nucleotides may form a base pair with one of the X′ nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X′

nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X′ nucleotides.

In one embodiment, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, and/or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In one embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, when the RNAi agent is represented by formula (IIIa), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-

cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, two RNAi agents represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

Various publications describe multimeric RNAi agents that can be used in the methods of the invention. Such publications include WO2007/091269, US Patent No. 7858769, WO2010/141511, WO2007/117686, WO2009/014887 and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to a RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose sugar of one or more ribonucleotide subunits of a dsRNA agent can be replaced with another moiety, *e.g.*, a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide *via* a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point),

that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

The RNAi agents may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

In certain specific embodiments, the RNAi agent for use in the methods of the invention is an agent selected from the group of agents listed in any one of Tables 11, 12, 31, and 32. These agents may further comprise a ligand. In one embodiment, the agent is selected from the group consisting of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1. In another embodiment, the agent is selected from the group consisting of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, or AD-70295.1.

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-

1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. *Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In one embodiment, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucoseamine multivalent

mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG,

vitamins *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc*. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in

addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In a preferred embodiment, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 1831). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 1832) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO: 1833) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 1834) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

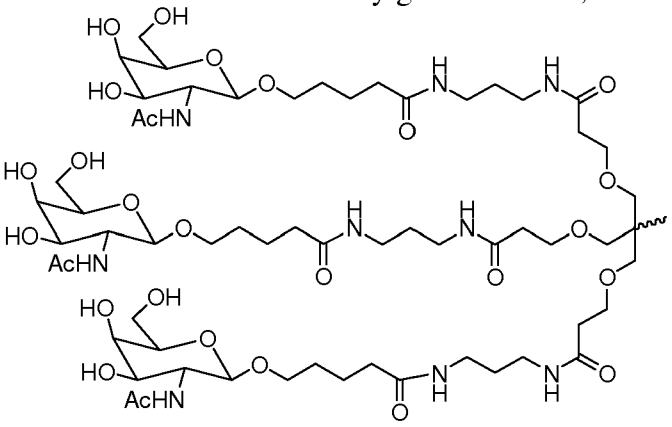
An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.,* glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

A "cell permeation peptide" is capable of permeating a cell, *e.g.,* a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, Nucl. Acids Res. 31:2717-2724, 2003).

C. Carbohydrate Conjugates

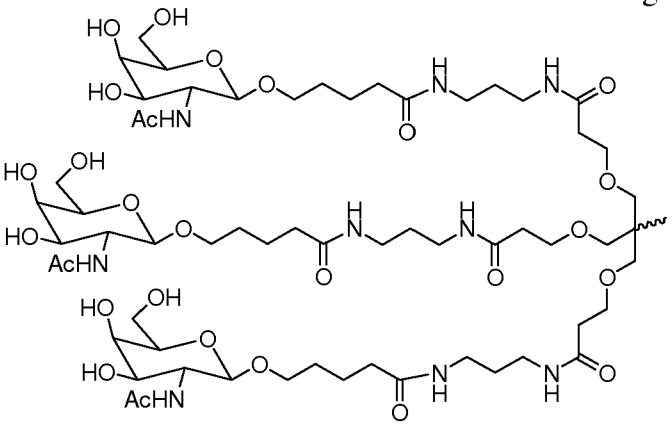
In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include HDV and above (*e.g.,* C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.,* C6, C7, or C8).

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as

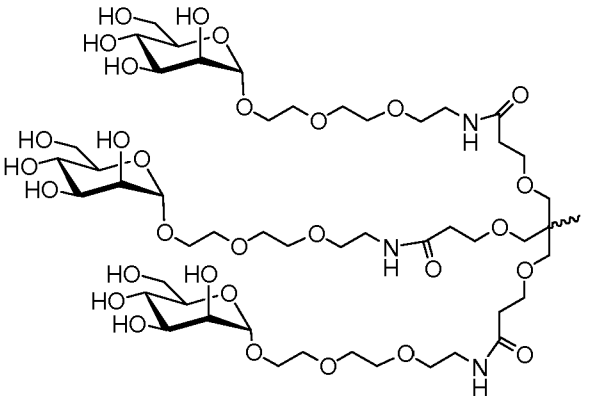


Formula II.

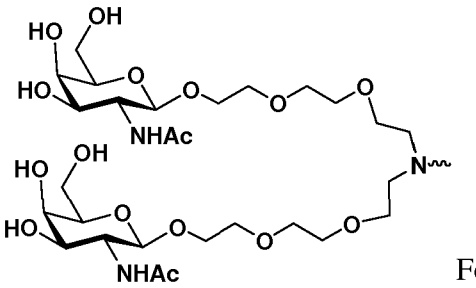
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



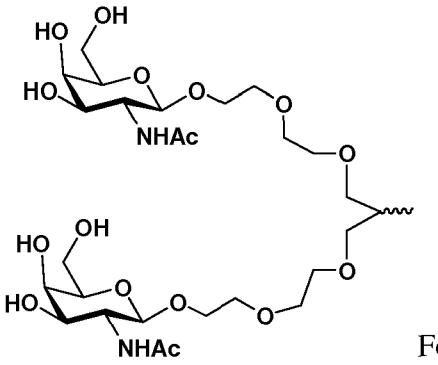
Formula II,



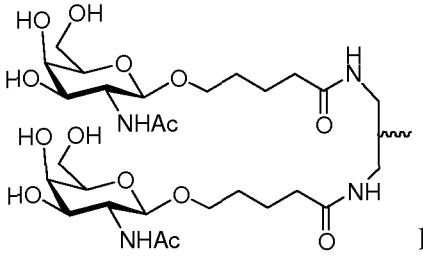
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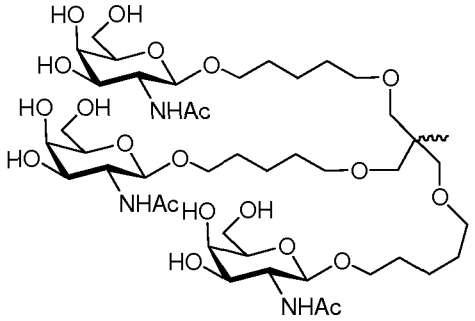
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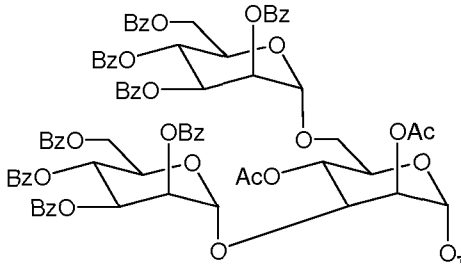
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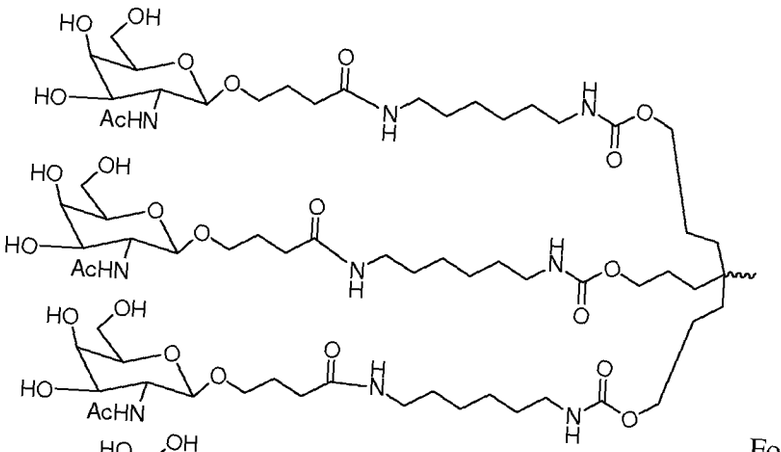
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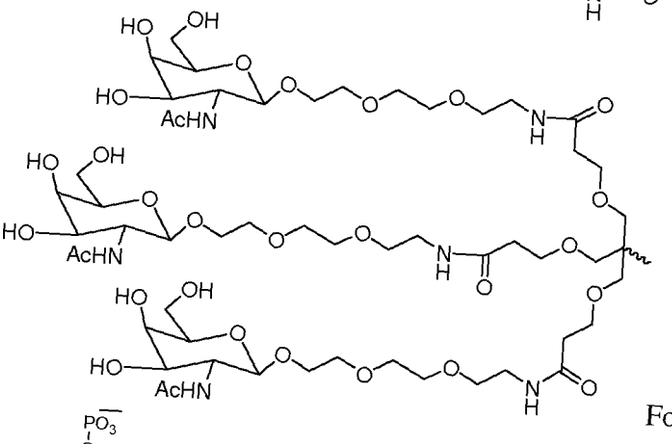
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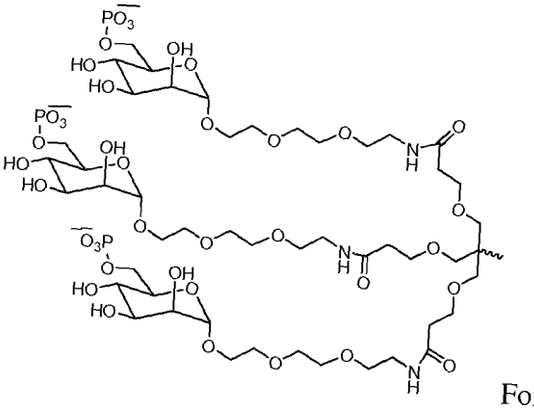
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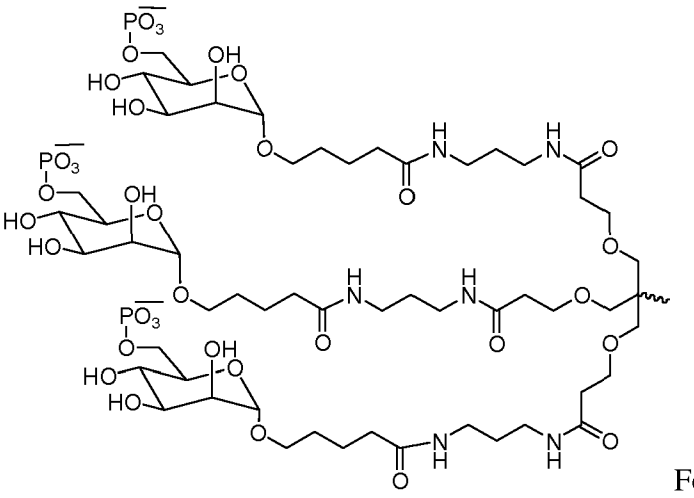
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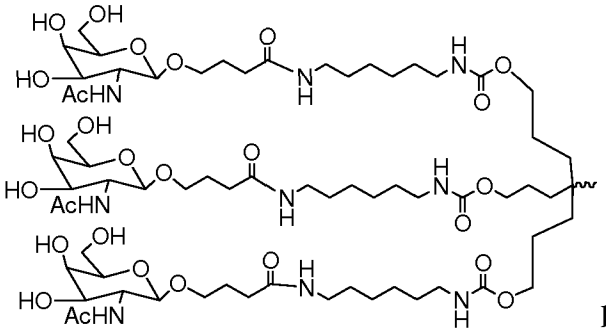
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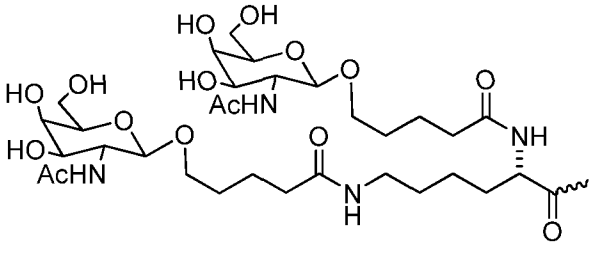
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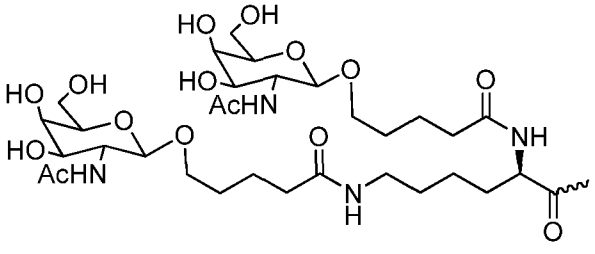
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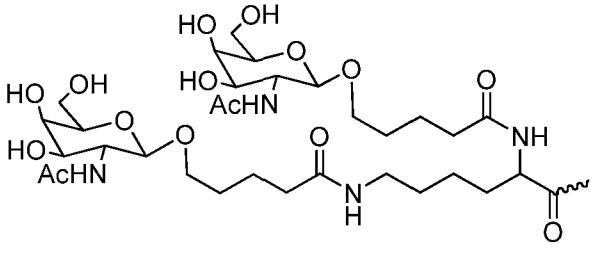
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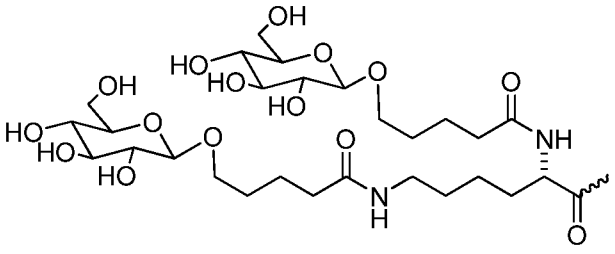
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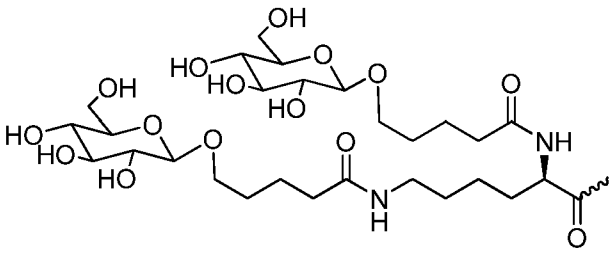
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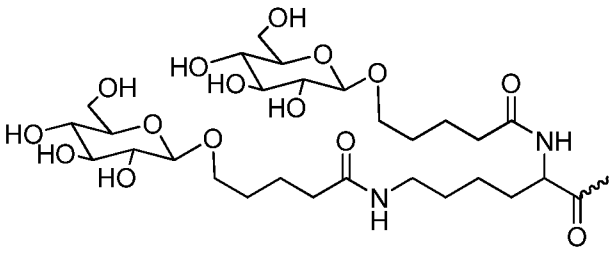
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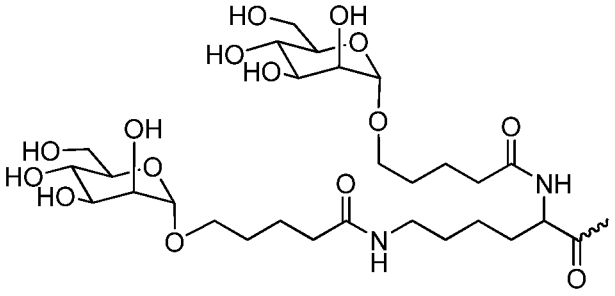
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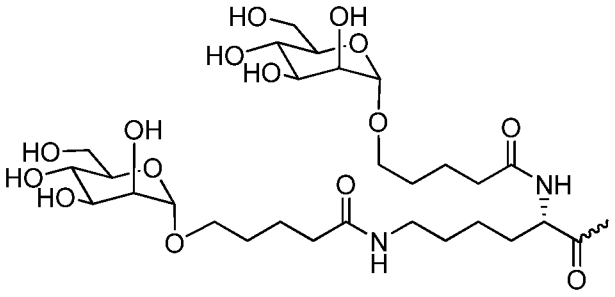
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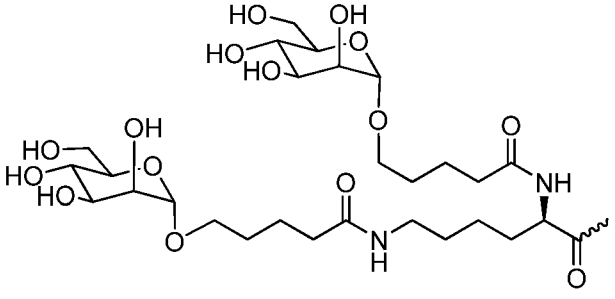
Formula XIX,



Formula XX,

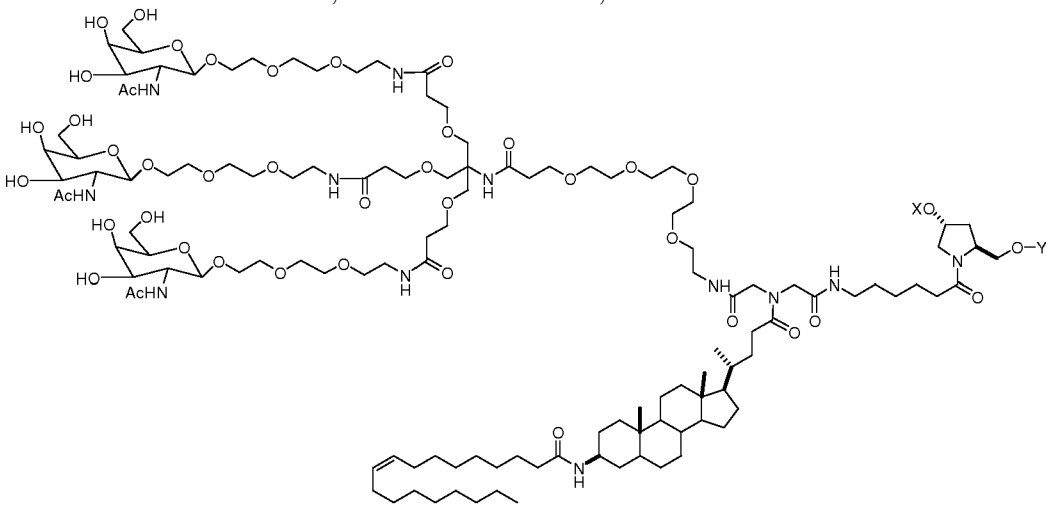


Formula XXI,



Formula XXII.

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a cell permeation peptide.

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or “linking group” means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl,

alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a

cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under

conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In another embodiment, a cleavable linker comprises an acid cleavable linking group. An *a*cid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as

dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

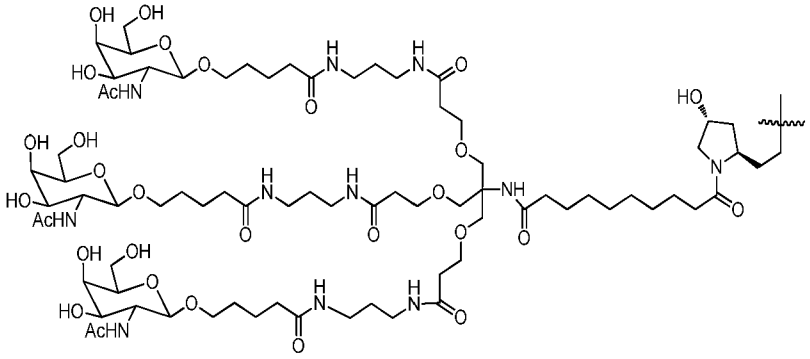
iv. Ester-based linking groups

In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated using methods analogous to those described above.

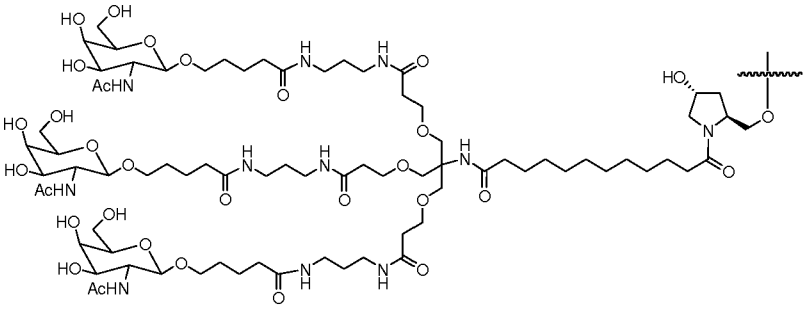
v. Peptide-based cleaving groups

In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

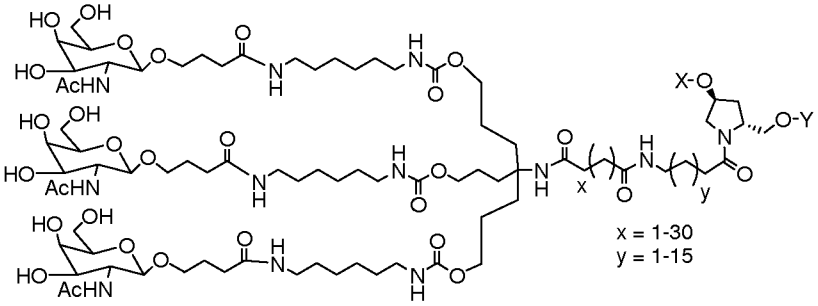
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



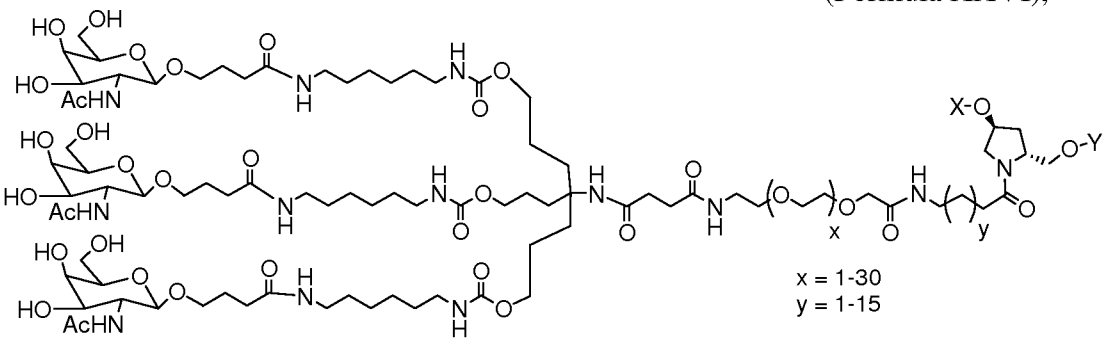
(Formula XXIV),



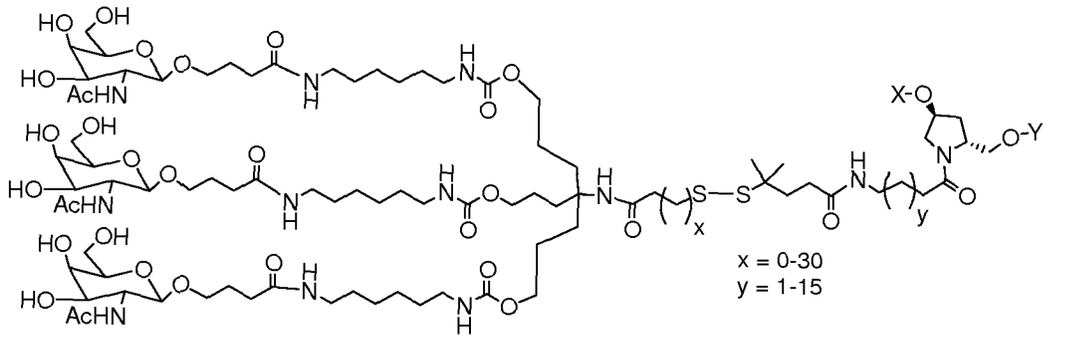
(Formula XXV),



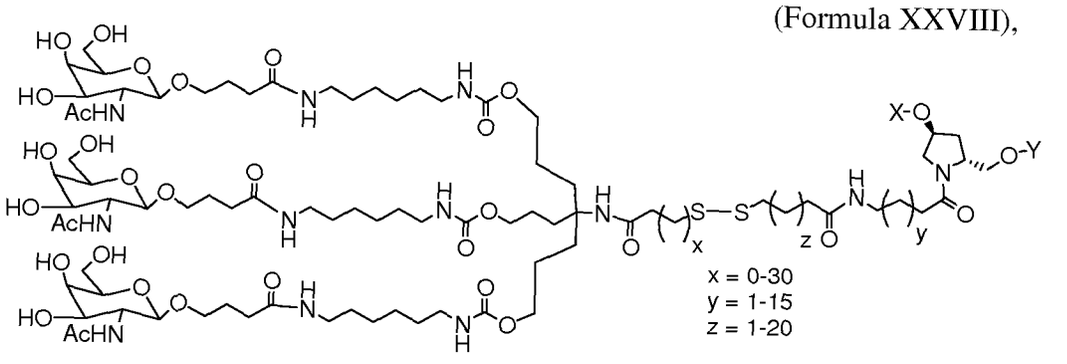
(Formula XXVI),



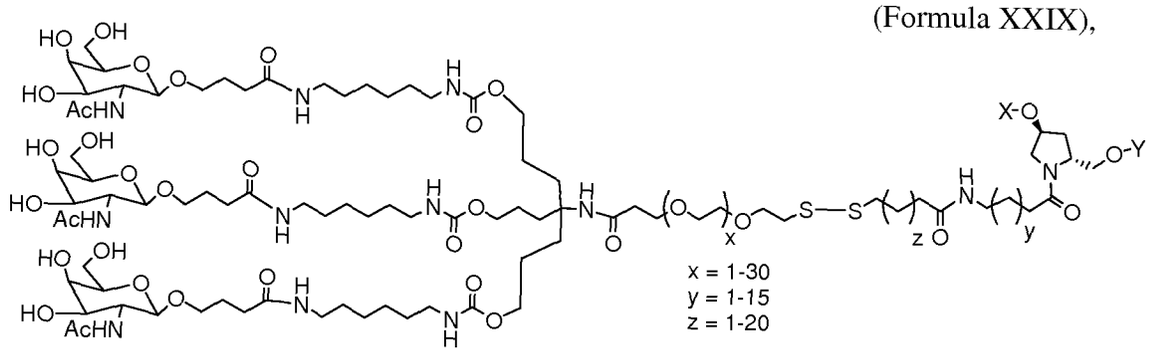
(Formula XXVII),



(Formula XXVIII),

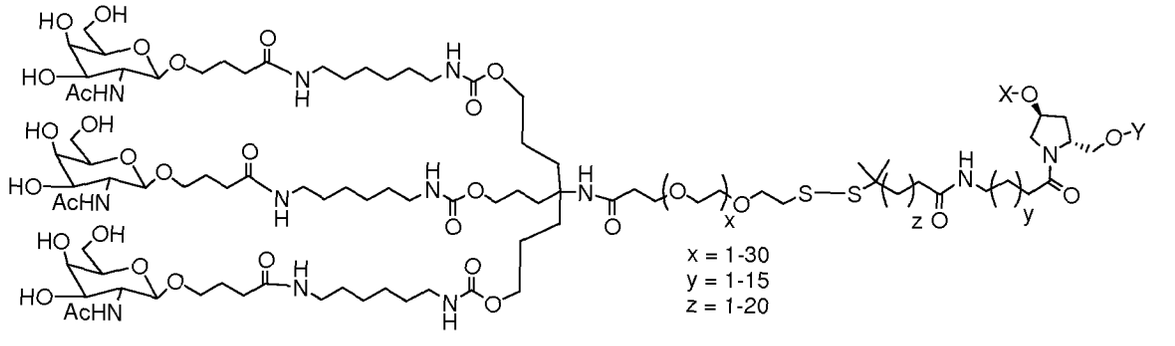


(Formula XXIX),



(Formula XXX),

and



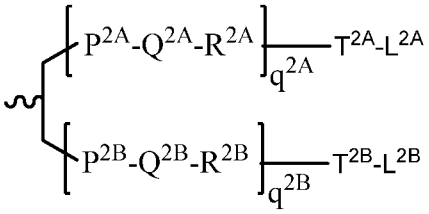
(Formula XXXI),

when one of X or Y is an oligonucleotide, the other is a hydrogen.

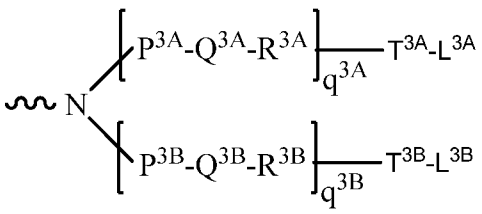
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII) – (XXXV):

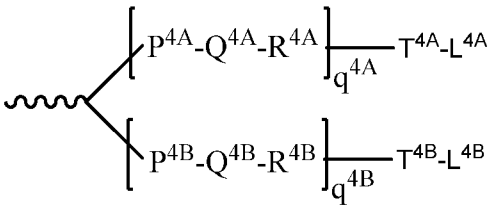
Formula XXXII



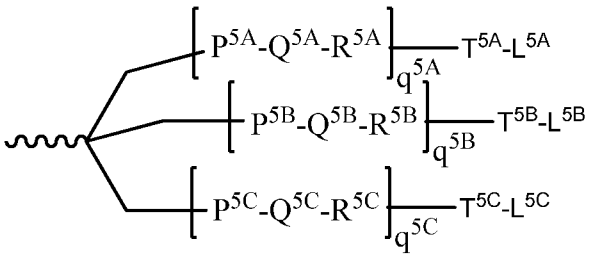
Formula XXXIII



,



,



;

Formula XXXIV Formula XXXV

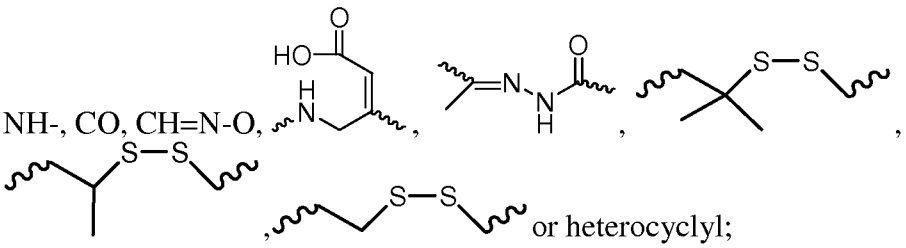
wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

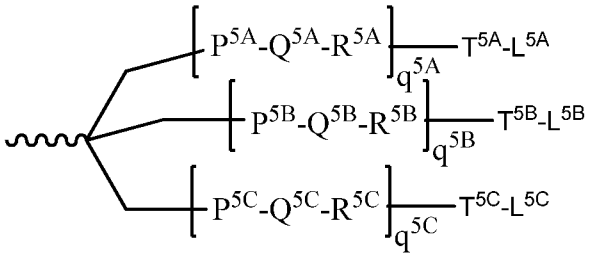
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, $-C(O)-CH(R^a)-$



or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; andR^a is H or amino acid side chain.Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XXXV):

Formula XXXV



,

wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds or "chimeras," in the context of this invention, are iRNA compounds, preferably dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al., Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides &*

Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of an RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject having a disease, disorder or condition associated with HDV infection) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular

delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ., *et al* (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ., *et al* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J., *et al* (2005) *Mol. Ther.*11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ., *et al* (2006) *Mol. Ther.* 14:343-350; Li, S., *et al* (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA., *et al* (2006) *Mol. Ther.* 14:476-484; Zhang, X., *et al* (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., *et al* (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al* (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO., *et al* (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., *et al* (2008) J*ournal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered

systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN., *et al* (2003) Clin. *Cancer Res.* 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN., *et al* (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., *et al* (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting the HDV gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

The individual strand or strands of an iRNA can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are

located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

iRNA expression plasmids can be transfected into target cells as a complex with cationic lipid carriers (*e.g.,* Oligofectamine) or non-cationic lipid-based carriers (*e.g.,* Transit-TKO™). Multiple lipid transfections for iRNA-mediated knockdowns targeting different regions of a target RNA over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements,

e.g., promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are further described below.

Vectors useful for the delivery of an iRNA will include regulatory elements (promoter, enhancer, *etc.*) sufficient for expression of the iRNA in the desired target cell or tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression.

Expression of the iRNA can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, *FASEB J.* 8:20-24). Such inducible expression systems, suitable for the control of dsRNA expression in cells or in mammals include, for example, regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 -thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the iRNA transgene.

Viral vectors that contain nucleic acid sequences encoding an iRNA can be used. For example, a retroviral vector can be used (see Miller *et al.*, *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding an iRNA are cloned into one or more vectors, which facilitate delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen *et al.*, *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, *J. Clin. Invest.* 93:644-651 (1994); Kiem *et al.*, *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference.

Adenoviruses are also contemplated for use in delivery of iRNAs of the invention. Adenoviruses are especially attractive vehicles, *e.g.*, for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the

advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, *Science* 252:431-434 (1991); Rosenfeld *et al.*, *Cell* 68:143-155 (1992); Mastrangeli *et al.*, *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, *et al.*, *Gene Therapy* 2:775-783 (1995). A suitable AV vector for expressing an iRNA featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

Adeno-associated virus (AAV) vectors may also be used to delivery an iRNA of the invention (Walsh *et al.*, Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Pat. No. 5,436,146). In one embodiment, the iRNA can be expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter. Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), *J. Virol.* 61: 3096-3101; Fisher K J *et al.* (1996), *J. Virol*, 70: 520-532; Samulski R *et al.* (1989), *J. Virol.* 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

Another viral vector suitable for delivery of an iRNA of the inevtion is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate. For example, lentiviral vectors can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors can be made to target different cells by engineering the vectors to express different capsid protein serotypes; see, *e.g.*, Rabinowitz J E *et al.* (2002), *J Virol* 76:791-801, the entire disclosure of which is herein incorporated by reference.

The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

VI. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of an HDV gene. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous, intramuscularly (IM), (SC) or intravenous (IV) delivery. Another example is compositions that are formulated for direct delivery into the brain parenchyma, *e.g.*, by infusion into the brain, such as by continuous pump infusion. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an HDV gene.

In one embodiment, an iRNA agent of the invention is administered to a subject as a weight-based dose. A "weight-based dose" (*e.g.,* a dose in mg/kg) is a dose of the iRNA agent that will change depending on the subject's weight. In another embodiement, an iRNA agent is administered to a subject as a fixed dose. A "fixed dose" (*e.g.,* a dose in mg) means that one dose of an iRNA agent is used for all subjects regardless of any specific subject-related factors, such as weight. In one particular embodiment, a fixed dose of an iRNA agent of the invention is based on a predetermined weight or age.

In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. For example, the dsRNA can be administered at about 0.01 mg/kg, about 0.05 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 3 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, or about 50 mg/kg per single dose.

For example, the dsRNA may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the dsRNA is administered at a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/kg, about 1.5 to about 30 mg/kg, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about

4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/kg, about 1.5 to about 20 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the dsRNA is administered at a dose of about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to

about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/kg, about 1.5 to about 30 mg/kg, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/kg, about 1.5 to about 20 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. In one embodiment, the dsRNA is administered at a dose of about 10mg/kg to about 30 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, subjects can be administered, *e.g.,* subcutaneously, intramuscularly, or intravenously, a single therapeutic amount of iRNA, such as about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In some embodiments, subjects are administered, *e.g.,* subcutaneously, intramuscularly, or intravenously, multiple doses of a therapeutic amount of iRNA, such as a dose about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7,

0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. A multi-dose regimine may include administration of a therapeutic amount of iRNA daily, such as for two days, three days, four days, five days, six days, seven days, or longer.

In other embodiments, subjects are administered, *e.g.,* subcutaneously, intramuscularly, or intravenously, a repeat dose of a therapeutic amount of iRNA, such as a dose about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. A repeat-dose regimine may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day, every third day, every fourth day, twice a week, once a week, every other week, or once a month.

In certain embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and a lipid, subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 5 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1

mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 10 mg/kg, about 4.5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5.5 mg/kg to about 10 mg/kg, about 6 mg/kg to about 10 mg/kg, about 6.5 mg/kg to about 10 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8 mg/kg to about 10 mg/kg, about 8.5 mg/kg to about 10 mg/kg, about 9 mg/kg to about 10 mg/kg, or about 9.5 mg/kg to about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In certain embodiments of the invention, for example, when a double-stranded RNAi agent includes a modification (*e.g.,* one or more motifs of three identical modifications on three consecutive nucleotides), including one such motif at or near the cleavage site of the agent, six phosphorothioate linkages, and a ligand, such an agent is administered at a dose of about 0.01 to about 0.5 mg/kg, about 0.01 to about 0.4 mg/kg, about 0.01 to about 0.3 mg/kg, about 0.01 to about 0.2 mg/kg, about 0.01 to about 0.1 mg/kg, about 0.01 mg/kg to about 0.09 mg/kg, about 0.01 mg/kg to about 0.08 mg/kg, about 0.01 mg/kg to about 0.07 mg/kg, about 0.01 mg/kg to about 0.06 mg/kg, about 0.01 mg/kg to about 0.05 mg/kg, about 0.02 to about 0.5 mg/kg, about 0.02 to about 0.4 mg/kg, about 0.02 to about 0.3 mg/kg, about 0.02 to about 0.2 mg/kg, about 0.02 to about 0.1 mg/kg, about 0.02 mg/kg to about 0.09 mg/kg, about 0.02 mg/kg to about 0.08 mg/kg, about 0.02 mg/kg to about 0.07 mg/kg, about 0.02 mg/kg to about 0.06 mg/kg, about 0.02 mg/kg to about 0.05 mg/kg, about 0.03 to about 0.5 mg/kg, about 0.03 to about 0.4 mg/kg, about 0.03 to about 0.3 mg/kg, about 0.03 to about 0.2 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.03 mg/kg to about 0.09 mg/kg, about 0.03 mg/kg to about 0.08 mg/kg, about 0.03 mg/kg to about 0.07 mg/kg, about 0.03 mg/kg to about 0.06 mg/kg, about 0.03 mg/kg to about 0.05 mg/kg,

about 0.04 to about 0.5 mg/kg, about 0.04 to about 0.4 mg/kg, about 0.04 to about 0.3 mg/kg, about 0.04 to about 0.2 mg/kg, about 0.04 to about 0.1 mg/kg, about 0.04 mg/kg to about 0.09 mg/kg, about 0.04 mg/kg to about 0.08 mg/kg, about 0.04 mg/kg to about 0.07 mg/kg, about 0.04 mg/kg to about 0.06 mg/kg, about 0.05 to about 0.5 mg/kg, about 0.05 to about 0.4 mg/kg, about 0.05 to about 0.3 mg/kg, about 0.05 to about 0.2 mg/kg, about 0.05 to about 0.1 mg/kg, about 0.05 mg/kg to about 0.09 mg/kg, about 0.05 mg/kg to about 0.08 mg/kg, or about 0.05 mg/kg to about 0.07 mg/kg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention, *e.g.*, the RNAi agent may be administered to the subject at a dose of about 0.015 mg/kg to about 0.45 mg/kg.

For example, the RNAi agent, *e.g.*, RNAi agent in a pharmaceutical composition, may be administered at a dose of about 0.01 mg/kg, 0.0125 mg/kg, 0.015 mg/kg, 0.0175 mg/kg, 0.02 mg/kg, 0.0225 mg/kg, 0.025 mg/kg, 0.0275 mg/kg, 0.03 mg/kg, 0.0325 mg/kg, 0.035 mg/kg, 0.0375 mg/kg, 0.04 mg/kg, 0.0425 mg/kg, 0.045 mg/kg, 0.0475 mg/kg, 0.05 mg/kg, 0.0525 mg/kg, 0.055 mg/kg, 0.0575 mg/kg, 0.06 mg/kg, 0.0625 mg/kg, 0.065 mg/kg, 0.0675 mg/kg, 0.07 mg/kg, 0.0725 mg/kg, 0.075 mg/kg, 0.0775 mg/kg, 0.08 mg/kg, 0.0825 mg/kg, 0.085 mg/kg, 0.0875 mg/kg, 0.09 mg/kg, 0.0925 mg/kg, 0.095 mg/kg, 0.0975 mg/kg, 0.1 mg/kg, 0.125 mg/kg, 0.15 mg/kg, 0.175 mg/kg, 0.2 mg/kg, 0.225 mg/kg, 0.25 mg/kg, 0.275 mg/kg, 0.3 mg/kg, 0.325 mg/kg, 0.35 mg/kg, 0.375 mg/kg, 0.4 mg/kg, 0.425 mg/kg, 0.45 mg/kg, 0.475 mg/kg, or about 0.5 mg/kg. Values intermediate to the foregoing recited values are also intended to be part of this invention.

In some embodiments, the RNAi agent is administered as a fixed dose of between about 100 mg to about 900 mg, e.g., between about 100 mg to about 850 mg, between about 100 mg to about 800 mg, between about 100 mg to about 750 mg, between about 100 mg to about 700 mg, between about 100 mg to about 650 mg, between about 100 mg to about 600 mg, between about 100 mg to about 550 mg, between about 100 mg to about 500 mg, between about 200 mg to about 850 mg, between about 200 mg to about 800 mg, between about 200 mg to about 750 mg, between about 200 mg to about 700 mg, between about 200 mg to about 650 mg, between about 200 mg to about 600 mg, between about 200 mg to about 550 mg, between about 200 mg to about 500 mg, between about 300 mg to about 850 mg, between about 300 mg to about 800 mg, between about 300 mg to about 750 mg, between about 300 mg to about 700 mg, between about 300 mg to about 650 mg, between about 300 mg to about 600 mg, between about 300 mg to about 550 mg, between about 300 mg to about 500 mg, between about 400 mg to about 850 mg,

between about 400 mg to about 800 mg, between about 400 mg to about 750 mg, between about 400 mg to about 700 mg, between about 400 mg to about 650 mg, between about 400 mg to about 600 mg, between about 400 mg to about 550 mg, or between about 400 mg to about 500 mg.

In some embodiments, the RNAi agent is administered as a fixed dose of about 100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about 275 mg, about 300 mg, about 325 mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, about 700 mg, about 725 mg, about 750 mg, about 775 mg, about 800 mg, about 825 mg, about 850 mg, about 875 mg, or about 900 mg.

The pharmaceutical composition can be administered by intravenous infusion over a period of time, such as over a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21, 22, 23, 24, or about a 25 minute period. The administration may be repeated, for example, on a regular basis, such as weekly, biweekly (*i.e.*, every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

The pharmaceutical composition can be administered once daily, or the iRNA can be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the iRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the iRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered once per week. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered bi-monthly. In

some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered once per month, once every other month, or once quarterly (*i.e.*, every three months).

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual iRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

The pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (*e.g.*, by a transdermal patch), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The iRNA can be delivered in a manner to target a particular tissue, such as the liver (*e.g.*, the hepatocytes of the liver).

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the iRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic

liposomes. Alternatively, iRNAs can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof). Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

A. iRNA Formulations Comprising Membranous Molecular Assemblies

An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the iRNA composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate iRNA. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the iRNA to particular cell types.

A liposome containing an iRNA agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The iRNA agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the iRNA agent and condense around the iRNA agent to form a liposome. After condensation,

the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of iRNA agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). pH can also adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al., Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; Bangham, *et al. M. Mol. Biol.* 23:238, 1965; Olson, *et al. Biochim. Biophys. Acta* 557:9, 1979; Szoka, *et al. Proc. Natl. Acad. Sci.* 75: 4194, 1978; Mayhew, e*t al. Biochim. Biophys. Acta* 775:169, 1984; Kim, *et al. Biochim. Biophys. Acta* 728:339, 1983; and Fukunaga, *et al. Endocrinol.* 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.,* Mayer, *et al. Biochim. Biophys. Acta* 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, *et al. Biochim. Biophys. Acta* 775:169, 1984). These methods are readily adapted to packaging iRNA agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.*, *Biochem. Biophys. Res. Commun.*, 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap nucleic acids rather than complex with it. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, *Journal of Controlled Release*, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, *J. Biol. Chem.* 269:2550, 1994; Nabel, *Proc. Natl. Acad. Sci.* 90:11307, 1993; Nabel, *Human Gene Ther.* 3:649, 1992; Gershon, *Biochem.* 32:7143, 1993; and Strauss *EMBO J.* 11:417, 1992.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu *et al. S.T.P.Pharma. Sci.*, 1994, 4(6) 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, *FEBS Letters*, 1987, 223, 42; Wu *et al.*, *Cancer Research*, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al*).

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages *in vivo* and can be used to deliver iRNA agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated iRNA agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of iRNA agent (see, e.g., Felgner, P. L. et al., Proc. Natl. Acad. Sci., USA 8:7413-7417, 1987 and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way

spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Commun.* 179:280, 1991). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., *Biochim. Biophys. Acta* 1065:8, 1991). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer iRNA agent into the skin. In some implementations, liposomes are used for delivering iRNA agent to epidermal cells and also to enhance the penetration of iRNA agent into dermal tissues, *e.g.*, into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner *et al., Journal of Drug Targeting,* 1992, vol. 2,405-410 and du Plessis *et al., Antiviral Research*, 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682-690, 1988; Itani, T. *et al. Gene* 56:267-276. 1987; Nicolau, C. *et al. Meth. Enz.* 149:157-176,

1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, L., P*roc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with iRNA agent are useful for treating a dermatological disorder.

Liposomes that include iRNA can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes.

Transferosomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include iRNA agent can be delivered, for example, subcutaneously by infection in order to deliver iRNA agent to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transferosomes can be self-optimizing (adaptive to the shape of pores, *e.g.*, in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present invention are described in, for example, PCT Publication No. WO 2008/042973, the entire contents of which are incorporated herein by reference.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, *e.g.,* they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been

shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The iRNA for use in the methods of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C_8 to C_{22} alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the siRNA composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the siRNA composition, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol and/or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol and/or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one,

i.e., there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.,* through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, *e.g.,* at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

B. *Lipid particles*

iRNAs, *e.g.,* dsRNAs of in the invention may be fully encapsulated in a lipid formulation, *e.g.,* a LNP, or other nucleic acid-lipid particle.

As used herein, the term "LNP" refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3- dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3- dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In another embodiment, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

In one embodiment, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC),

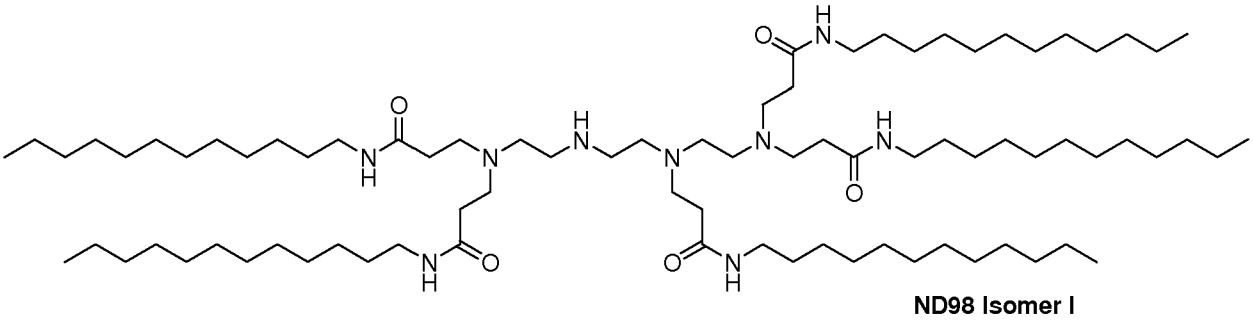
dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG- distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*i.e.,* LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous dsRNA (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion

step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are described in Table 1.

Table 1

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
SNALP-1	l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5,

		lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5

		Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described, *e.g.*, in PCT Publication No. WO 2010/088537, the entire contents of which are incorporated herein by reference.

MC3 comprising formulations are described, *e.g.*, in U.S. Publication No. 2010/0324120, filed June 10, 2010, the entire contents of which are incorporated herein by reference.

ALNY-100 comprising formulations are described, *e.g.*, PCT Publication No. WO 2010/054406, the entire contents of which are incorporated herein by reference.

C12-200 comprising formulations are described in PCT Publication No. WO 2010/129709, the entire contents of which are incorporated herein by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene

P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

C. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm in diameter (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid

or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and

Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y.,

volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see e.g., U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides et al., Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see e.g., U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides et al., Pharmaceutical Research, 1994, 11, 1385; Ho et al., J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly

advantageous when formulating thermolabile drugs, peptides or iRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of iRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of iRNAs and nucleic acids.

Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the iRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention can be classified as belonging to one of five broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

iii. Microparticles

An iRNA agent of the invention may be incorporated into a particle, e.g., a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee et al., Critical Reviews in

Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of iRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C1-20 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (see e.g., Touitou, E., et al. Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium

chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579-583).

Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of iRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(see e.g., Katdare, A. et al., Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al., J. Control Rel., 1990, 14, 43-51).

As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of iRNAs through the alimentary mucosa (see e.g., Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621-626).

Agents that enhance uptake of iRNAs at the cellular level can also be added to the pharmaceutical and other compositions of the present invention. For example,

cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), iRNAMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass[a] D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

Other agents can be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., DsRNA Res. Dev., 1995, 5, 115-121; Takakura et al., DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

vi. Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt

solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

vii. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more agents which function by a non- iRNA mechanism and which are useful in treating an HDV infection. Examples of such agents include, but are not lmited to antiviral agents aimed at suppressing or destroying HDV and/or HBV by interfering with viral replication; and

immune modulators aimed at helping the human immune system mount a defence against the virus. In contrast, immune modulators, such as corticosteroids, which induce an enhanced expression of virus and viral antigens, and a suppression of T-lymphocyte function, or adenine arabinoside, acyclovir, or dideoxyinosine, are not beneficial for the treatment of chronic hepatitis B and/or chronic hepatitis D. Suitable agents are discussed in more detail below.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.*e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by HDV expression. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VII. Methods of the Invention

The present invention provides therapeutic and prophylactic methods which include administering to a subject having an HDV infection and/or HDV-associated disease, disorder, and/or condition, or prone to developing, an HDV-associated disease, disorder, and/or condition (*e.g.*, CHB), compositions comprising an iRNA agent, or pharmaceutical compositions comprising an iRNA agent, or vectors comprising an iRNA of the invention.

The methods of the invention are useful for treating a subject having an HDV infection, *e.g.,* a subject that would benefit from reduction in HDV gene expression and/or HDV replication. In one aspect, the present invention provides methods of reducing the level of Hepatis B virus ccc DNA in a subject infected with HDV. In another aspect, the present invention provides methods of reducing the level of HDV antigen, *e.g.,* HBsAg and/or HBeAg, in a subject infected with HDV. In another aspect, the present invention provides methods of reducing the viral load of HDV in a subject infected with HDV. The present invention also provides methods of reducing the level of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) in a subject infected with HDV. In one aspect, the present invention provides methods for increasing the level of anti-HDV antibodies in a subject infected with HDV. In another aspect, the present invention provides methods of treating a subject having an HDV infection. In one aspect, the present invention provides methods of treating a subject having an HDV-associated disease, *e.g.,* acute hepatitis D; hepatits B virus infection, acute hepatits B, acute fulminant hepatitis D; chronic hepatitis D; liver fibrosis; end-stage liver disease; hepatocellular carcinoma. The treatment methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting an HDV gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an HDV gene or a vector of the invention comprising an iRNA agent targeting an HDV gene.

In one aspect, the invention provides methods of preventing at least one symptom in a subject having an HDV infection,

In another aspect, the present invention provides uses of a therapeutically effective amount of an iRNA agent of the invention for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of HDV gene expression.

In a further aspect, the present invention provides uses of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting an HDV gene or pharmaceutical composition

comprising an iRNA agent targeting an HDV gene in the manufacture of a medicament for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of HDV gene expression and/or HDV replication, such as a subject having a disorder that would benefit from reduction in HDV gene expression, *e.g.*, a HDV-associated disease.

In another aspect, the invention provides uses of an iRNA, *e.g.,* a dsRNA, of the invention for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of HDV gene expression and/or HDV replication.

In a further aspect, the present invention provides uses of an iRNA agent of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of HDV gene expression and/or HDV replication, such as a HDV-associated disease.

In one embodiment, an iRNA agent targeting HDV is administered to a subject having an HDV infection and/or an HDV-associated disease such that the expression of one or more HDV genes, HDV antigen levels, and/or HDV viral load levels, *e.g.*, in a cell, tissue, blood or other tissue or fluid of the subject are reduced by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more when the dsRNA agent is administered to the subject.

In one embodiment, an iRNA agent targeting HDV is administered to a subject having an HDV infection and/or an HDV-associated disease such that the level of anti-HDV antibodies, *e.g.*, in a cell, tissue, blood or other tissue or fluid of the subject are increased by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more when the dsRNA agent is administered to the subject.

The methods and uses of the invention include administering a composition described herein such that expression of the target HDV gene is decreased, such as for about 1, 2, 3, 4 5, 6, 7, 8, 12, 16, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, or about 80 hours. In one embodiment, expression of the target HDV gene is decreased for an extended duration, *e.g.*, at least about two, three, four, five, six, seven days or more, *e.g.*, about one week, two weeks, three weeks, or about four weeks or longer.

Administration of the dsRNA according to the methods and uses of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with an HDV infection and/or HDV-associated disease. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of CHB may be assessed, for example, by periodic monitoring of viral load and transaminase levels. Comparison of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an iRNA targeting HDV or pharmaceutical composition thereof, "effective against" an HDV-associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating HDV infection and/or an HDV-associated disease and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given iRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.3 mg/kg, 0.35 mg/kg, 0.4 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.65 mg/kg, 0.7 mg/kg, 0.75 mg/kg, 0.8 mg/kg, 0.85 mg/kg, 0.9 mg/kg, 0.95 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg dsRNA, 2.6 mg/kg dsRNA, 2.7 mg/kg dsRNA, 2.8 mg/kg dsRNA, 2.9 mg/kg dsRNA, 3.0 mg/kg dsRNA, 3.1 mg/kg dsRNA, 3.2 mg/kg dsRNA, 3.3 mg/kg dsRNA, 3.4 mg/kg dsRNA, 3.5 mg/kg dsRNA, 3.6 mg/kg dsRNA, 3.7 mg/kg dsRNA, 3.8 mg/kg dsRNA, 3.9 mg/kg dsRNA, 4.0 mg/kg dsRNA, 4.1 mg/kg dsRNA, 4.2 mg/kg dsRNA, 4.3 mg/kg dsRNA, 4.4 mg/kg dsRNA, 4.5 mg/kg dsRNA, 4.6 mg/kg dsRNA, 4.7 mg/kg dsRNA, 4.8 mg/kg dsRNA, 4.9 mg/kg dsRNA, 5.0 mg/kg dsRNA, 5.1 mg/kg dsRNA, 5.2 mg/kg dsRNA, 5.3 mg/kg dsRNA, 5.4 mg/kg dsRNA, 5.5 mg/kg dsRNA, 5.6 mg/kg dsRNA, 5.7 mg/kg dsRNA, 5.8 mg/kg dsRNA, 5.9 mg/kg dsRNA, 6.0 mg/kg dsRNA, 6.1 mg/kg dsRNA, 6.2 mg/kg dsRNA, 6.3 mg/kg dsRNA, 6.4 mg/kg dsRNA, 6.5 mg/kg dsRNA, 6.6 mg/kg dsRNA, 6.7 mg/kg dsRNA, 6.8 mg/kg dsRNA, 6.9 mg/kg dsRNA, 7.0 mg/kg dsRNA, 7.1 mg/kg dsRNA, 7.2 mg/kg dsRNA, 7.3 mg/kg dsRNA, 7.4 mg/kg dsRNA, 7.5 mg/kg dsRNA, 7.6 mg/kg dsRNA, 7.7 mg/kg dsRNA, 7.8 mg/kg dsRNA, 7.9 mg/kg dsRNA, 8.0 mg/kg dsRNA, 8.1 mg/kg dsRNA, 8.2 mg/kg dsRNA, 8.3 mg/kg dsRNA, 8.4 mg/kg dsRNA, 8.5 mg/kg dsRNA, 8.6 mg/kg dsRNA, 8.7 mg/kg dsRNA, 8.8 mg/kg dsRNA, 8.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 9.1 mg/kg dsRNA, 9.2 mg/kg dsRNA, 9.3 mg/kg dsRNA, 9.4 mg/kg dsRNA, 9.5 mg/kg dsRNA, 9.6 mg/kg dsRNA, 9.7 mg/kg dsRNA, 9.8 mg/kg dsRNA, 9.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 10 mg/kg dsRNA, 15 mg/kg dsRNA, 20 mg/kg dsRNA, 25 mg/kg dsRNA, 30 mg/kg dsRNA, 35 mg/kg dsRNA, 40 mg/kg dsRNA, 45 mg/kg dsRNA, or about 50 mg/kg

dsRNA. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In certain embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and a lipid, subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 5 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 10 mg/kg, about 4.5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5.5 mg/kg to about 10 mg/kg, about 6 mg/kg to about 10 mg/kg, about 6.5 mg/kg to about 10 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8 mg/kg to about 10 mg/kg, about 8.5 mg/kg to about 10 mg/kg, about 9 mg/kg to about 10 mg/kg, or about 9.5 mg/kg to about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In other embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of iRNA, such as a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to

about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/kg, about 1.5 to about 30 mg/kg, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/kg, about 1.5 to about 20 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. In one embodiment, when a composition of the invention comprises a dsRNA as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of about 10 to about 30 mg/kg of dsRNA. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, subjects can be administered a therapeutic amount of iRNA, such as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In certain embodiments of the invention, for example, when a double-stranded RNAi agent includes a modification (*e.g.,* one or more motifs of three identical modifications on three consecutive nucleotides), including one such motif at or near the cleavage site of the agent, six phosphorothioate linkages, and a ligand, such an agent is administered at a dose of about 0.01 to about 0.5 mg/kg, about 0.01 to about 0.4 mg/kg, about 0.01 to about 0.3 mg/kg, about 0.01 to about 0.2 mg/kg, about 0.01 to about 0.1 mg/kg, about 0.01 mg/kg to about 0.09 mg/kg, about 0.01 mg/kg to about 0.08 mg/kg, about 0.01 mg/kg to about 0.07 mg/kg, about 0.01 mg/kg to about 0.06 mg/kg, about 0.01 mg/kg to about 0.05 mg/kg, about 0.02 to about 0.5 mg/kg, about 0.02 to about 0.4 mg/kg, about 0.02 to about 0.3 mg/kg, about 0.02 to about 0.2 mg/kg, about 0.02 to about 0.1 mg/kg, about 0.02 mg/kg to about 0.09 mg/kg, about 0.02 mg/kg to about 0.08 mg/kg, about 0.02 mg/kg to about 0.07 mg/kg, about 0.02 mg/kg to about 0.06 mg/kg, about 0.02 mg/kg to about 0.05 mg/kg, about 0.03 to about 0.5 mg/kg, about 0.03 to about 0.4 mg/kg, about 0.03 to about 0.3 mg/kg, about 0.03 to about 0.2 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.03 mg/kg to about 0.09 mg/kg, about 0.03 mg/kg to about 0.08 mg/kg, about 0.03 mg/kg to about 0.07 mg/kg, about 0.03 mg/kg to about 0.06 mg/kg, about 0.03 mg/kg to about 0.05 mg/kg, about 0.04 to about 0.5 mg/kg, about 0.04 to about 0.4 mg/kg, about 0.04 to about 0.3 mg/kg, about 0.04 to about 0.2 mg/kg, about 0.04 to about 0.1 mg/kg, about 0.04 mg/kg to about 0.09 mg/kg, about 0.04 mg/kg to about 0.08 mg/kg, about 0.04 mg/kg to about 0.07 mg/kg, about 0.04 mg/kg to about 0.06 mg/kg, about 0.05 to about 0.5 mg/kg, about 0.05 to about 0.4 mg/kg, about 0.05 to about 0.3 mg/kg, about 0.05 to about 0.2 mg/kg, about 0.05 to about 0.1 mg/kg, about 0.05 mg/kg to about 0.09 mg/kg, about 0.05 mg/kg to about 0.08 mg/kg, or about 0.05 mg/kg to about 0.07 mg/kg. Values and ranges intermediate to the foregoing recited values are also

intended to be part of this invention, *e.g.*, the RNAi agent may be administered to the subject at a dose of about 0.015 mg/kg to about 0.45 mg/kg.

For example, the RNAi agent, *e.g.*, RNAi agent in a pharmaceutical composition, may be administered at a dose of about 0.01 mg/kg, 0.0125 mg/kg, 0.015 mg/kg, 0.0175 mg/kg, 0.02 mg/kg, 0.0225 mg/kg, 0.025 mg/kg, 0.0275 mg/kg, 0.03 mg/kg, 0.0325 mg/kg, 0.035 mg/kg, 0.0375 mg/kg, 0.04 mg/kg, 0.0425 mg/kg, 0.045 mg/kg, 0.0475 mg/kg, 0.05 mg/kg, 0.0525 mg/kg, 0.055 mg/kg, 0.0575 mg/kg, 0.06 mg/kg, 0.0625 mg/kg, 0.065 mg/kg, 0.0675 mg/kg, 0.07 mg/kg, 0.0725 mg/kg, 0.075 mg/kg, 0.0775 mg/kg, 0.08 mg/kg, 0.0825 mg/kg, 0.085 mg/kg, 0.0875 mg/kg, 0.09 mg/kg, 0.0925 mg/kg, 0.095 mg/kg, 0.0975 mg/kg, 0.1 mg/kg, 0.125 mg/kg, 0.15 mg/kg, 0.175 mg/kg, 0.2 mg/kg, 0.225 mg/kg, 0.25 mg/kg, 0.275 mg/kg, 0.3 mg/kg, 0.325 mg/kg, 0.35 mg/kg, 0.375 mg/kg, 0.4 mg/kg, 0.425 mg/kg, 0.45 mg/kg, 0.475 mg/kg, or about 0.5 mg/kg. Values intermediate to the foregoing recited values are also intended to be part of this invention.

In some embodiments, the RNAi agent is administered as a fixed dose of between about 100 mg to about 900 mg, e.g., between about 100 mg to about 850 mg, between about 100 mg to about 800 mg, between about 100 mg to about 750 mg, between about 100 mg to about 700 mg, between about 100 mg to about 650 mg, between about 100 mg to about 600 mg, between about 100 mg to about 550 mg, between about 100 mg to about 500 mg, between about 200 mg to about 850 mg, between about 200 mg to about 800 mg, between about 200 mg to about 750 mg, between about 200 mg to about 700 mg, between about 200 mg to about 650 mg, between about 200 mg to about 600 mg, between about 200 mg to about 550 mg, between about 200 mg to about 500 mg, between about 300 mg to about 850 mg, between about 300 mg to about 800 mg, between about 300 mg to about 750 mg, between about 300 mg to about 700 mg, between about 300 mg to about 650 mg, between about 300 mg to about 600 mg, between about 300 mg to about 550 mg, between about 300 mg to about 500 mg, between about 400 mg to about 850 mg, between about 400 mg to about 800 mg, between about 400 mg to about 750 mg, between about 400 mg to about 700 mg, between about 400 mg to about 650 mg, between about 400 mg to about 600 mg, between about 400 mg to about 550 mg, or between about 400 mg to about 500 mg.

In some embodiments, the RNAi agent is administered as a fixed dose of about 100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about 275 mg, about 300 mg, about 325 mg, about 350 mg, about 375

mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, about 700 mg, about 725 mg, about 750 mg, about 775 mg, about 800 mg, about 825 mg, about 850 mg, about 875 mg, or about 900 mg.

The iRNA can be administered by intravenous infusion over a period of time, such as over a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or about a 25 minute period. The administration may be repeated, for example, on a regular basis, such as weekly, biweekly (*i.e.*, every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

Administration of the iRNA can reduce the presence of serum and/or liver HDV DNA, the presence of serum and/or liver HDV antigen*e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more, *e.g.*, to below the level of detection of the assay.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

The methods of treatment of HDV can further comprise treatment of HBV using any method known in the art. In certain embodiments, methods of treatment of HBV comprise administration of an iRNA compound, *e.g.*, an iRNA agent targeting an HBV gene as provided herein.

Owing to the inhibitory effects on HDV expression, a composition according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

An iRNA of the invention may be administered in "naked" form, where the modified or unmodified iRNA agent is directly suspended in aqueous or suitable buffer solvent, as a "free iRNA." A free iRNA is administered in the absence of a pharmaceutical composition. The free iRNA may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from a reduction and/or inhibition of HDV gene expression are those having an HDV infection and/or an HDV-associated disease or disorder as described herein.

Treatment of a subject that would benefit from a reduction and/or inhibition of HDV gene expression includes therapeutic and prophylactic treatment.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of HDV gene expression, *e.g.,* a subject having a HDV-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

For example, a subject administered a first RNAi agent or a first and second RNAi agent targeting HDV may further be administered one or more iRNA agents targeting HBV and/or one or more agents which function by a non- iRNA mechanism and which are useful in treating an HBV and/or an HDV infection. Exemplary agents include immune modulators which stimulate the immune system by, for example, enhancing T-cell helper activity, maturation of B lymphocytes, inhibiting T-cell suppressors, and enhancing HLA type I expression. Suitable immune modulators include interferons which have a variety of properties that include antiviral, immunomodulatory, and antiproliferative effects.

For example, the current treatment for chronic hepatitis B is interferon therapy. Interferon therapy is administered to subjects who have a documented HBV infection for at least six months, elevated liver enzymes (AST and ALT) and an actively dividing virus in their blood (HBeAg, and/or HBV DNA positive tests).

Interferon-α therapy produces a long-term, sustained remission of the disease in about 35% of those with chronic hepatitis B, with normalization of liver enzymes and loss of the three markers for an active infection (HBeAg, HBV DNA, and HBsAg). Subjects with an acute HBV infection, end stage cirrhosis or other major medical problems are typically not treated with interferon.

In addition, interferon therapy for patients with HBV-related cirrhosis decreases significantly the hepatocellular carcinoma (HCC) rate, particularly in patients with a larger amount of serum HBV DNA. In patients with HBeAg-positive compensated cirrhosis, virological and biochemical remission following interferon therapy is associated with improved survival. In patients with chronic HBV infection, the clearance of HBeAg after treatment with interferon-α is associated with improved clinical outcomes.

The standard duration of therapy is considered 16 weeks. Patients who exhibit a low level of viral replication at the end of the standard regimen benefit most from prolonged treatment.

Thus, in some embodiments, an iRNA targeting one or more HDV genes is administered in combination with, *e.g.*, an agent useful in treating an HDV-associated disease as described elsewhere herein.

For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reduction in HDV expression, *e.g.,* a subject having a HDV-associated disease, include an iRNA agent targeting a different portion of the HDV genome, an antiviral agent, a nucleotide analog, a nucleoside analog, a reverse transcriptase inhibitor (*e.g.,* Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide, Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, AGX-1009, emtricitabine, clevudine, ritonavir, dipivoxil, lobucavir, famvir, FTC, N-Acetyl-Cysteine (NAC), PC1323, theradigm-HBV, thymosin-alpha, and ganciclovir), an immune stimulator (*e.g.,* pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and aToll-like receptor 7 (TLR7) agonist), a therapeutic vaccine (*e.g.,* GS-4774, DV-601, and TG1050), a viral entry inhibitor (*e.g.,* Myrcludex), an oligonucleotide that inhibits the secretion or release of HbsAg (*e.g.,* REP 9AC), a capsid inhibitor (*e.g.,* Bay41-4109 and NVR-1221), a cccDNA inhibitor (*e.g.,* IHVR-25), or other therapeutic agents and/or procedures, *e.g.,* liver transplant, chemotherapy, for treating a HBV-associated disease, a combination of any of the foregoing.

In certain embodiments, a first iRNA agent targeting one or more HDV genes is administered in combination with a second iRNA agent targeting a different portion

of the HDV genome. For example, the first RNAi agent comprises 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 the second RNAi agent comprises 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 a sequences selected from the group consisting of any one of the sense sequences in any one of Tables 11, 12, 31, and 32 (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 antisense strand comprises a sequence selected from the group consisting of any one of the antisense sequences in any one of Tables 11, 12, 31, and 32 (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); wherein the second sense strand comprises any one of the sense sequences in any one of Tables 11, 12, 31, and 32 (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 wherein the second antisense strand comprises any one of the antisense sequences in any one of Tables 11, 12, 31, and 32 (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).

In one embodiment, the first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 another embodiment, the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1 (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 embodiment, all of the nucleotides of the first and second sense strand and/or all of the nucleotides of the first and second antisense strand comprise a modification.

In one embodiment, the at least one of the 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.

A subject administered a first or a first and second RNAi agent may further be administered one or more iRNA agents targeting HBV antiviral agent, a reverse transcriptase inhibitor (*e.g.,* Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide, Lamivudine, Adefovir dipivoxil, Entecavir (ETV), Telbivudine, and AGX-1009), an immune stimulator (*e.g.,* pegylated interferon alfa 2a (PEG-IFN-α2a), Interferon alfa-2b, a recombinant human interleukin-7, and aToll-like receptor 7 (TLR7) agonist), a therapeutic vaccine (*e.g.,* GS-4774, DV-601, and TG1050), a viral entry inhibitor (*e.g.,* Myrcludex), an oligonucleotide that inhibits the secretion or release of HbsAg (*e.g.,* REP 9AC), a capsid inhibitor (*e.g.,* Bay41-4109 and NVR-1221), a cccDNA inhibitor (*e.g.,* IHVR-25), or other therapeutic agents and/or

procedures, *e.g.,* liver transplant, chemotherapy, for treating a HDV-associated disease, a combination of any of the foregoing.

In one embodiment, the methods of the invention include administering to a subject having an HDV infection and/or HDV-associated disease a reverse transcriptase inhibitor. In another embodiment, the methods of the invention include administering to a subject having an HDV infection and/or HDV-associate disease a reverse transcriptase inhibitor and an immune stimulator.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

The present invention also provides methods of using an iRNA agent of the invention and/or a composition containing an iRNA agent of the invention to reduce and/or inhibit HDV expression in a cell. In other aspects, the present invention provides an iRNA of the invention and/or a composition comprising an iRNA of the invention for use in reducing and/or inhibiting HDV gene expression in a cell. In yet other aspects, use of an iRNA of the invention and/or a composition comprising an iRNA of the invention for the manufacture of a medicament for reducing and/or inhibiting HDV gene expression in a cell are provided. In still other aspect, the the present invention provides an iRNA of the invention and/or a composition comprising an iRNA of the invention for use in reducing and/or inhibiting HDV replication in a cell. In yet other aspects, use of an iRNA of the invention and/or a composition comprising an iRNA of the invention for the manufacture of a medicament for reducing and/or inhibiting HDV replication in a cell are provided. The methods and uses include contacting the cell with an iRNA, *e.g.,* a dsRNA, of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an HDV gene, thereby inhibiting expression of the HDV gene or inhibiting HDV replication in the cell.

Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of HDV may be determined by determining the mRNA expression level of HDV using methods routine to one of ordinary skill in the art, *e.g.,* Northern blotting, qRT-PCR, by determining the protein level of HDV using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques, flow cytometry methods, ELISA, and/or by determining a biological activity of HDV.

In the methods and uses of the invention the cell may be contacted *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses an HDV gene, *e.g.,* a cell infected with HDV or a cell comprising an expression vector comprising an HDV genome or portion of an HDV gene. A cell suitable for use in the methods and uses of the invention may be a mammalian cell, *e.g.,* a primate cell (such as a human cell or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger cell, a bear cell, or a buffalo cell), a bird cell (*e.g.*, a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell, *e.g.,* a human liver cell.

HDV gene expression may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%, *i.e.*, to below the level of detection of the assay.

HDV replication may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%, *i.e.*, to below the level of detection of the assay.

The *in vivo* methods and uses of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the HDV gene of the mammal to be treated. When the organism to be treated is a human, the composition can be administered by any means known in the art including, but not limited to subcutaneous, intravenous, oral, intraperitoneal, or parenteral routes,

including intracranial (*e.g.*, intraventricular, intraparenchymal and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by subcutaneous injection. In some embodiments, the compositions are administered by intravenous infusion or injection. In other embodiments, the compositions are administered by intramuscular injection.

In some embodiments, the administration is *via* a depot injection. A depot injection may release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of HDV, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is *via* a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the iRNA to the liver.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present invention also provides methods for inhibiting the expression of an HDV gene in a mammal, *e.g.,* a human. The present invention also provides a composition comprising an iRNA, *e.g.,* a dsRNA, that targets an HDV gene in a cell of a mammal for use in inhibiting expression of the HDV gene in the mammal. In another aspect, the present invention provides use of an iRNA, *e.g.,* a dsRNA, that targets an HDV gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the HDV gene in the mammal.

The methods and uses include administering to the mammal, *e.g.,* a human, a composition comprising an iRNA, *e.g.,* a dsRNA, that targets an HDV gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the HDV gene, thereby inhibiting expression of the HDV gene in the mammal.

Reduction in gene expression can be assessed in peripheral blood sample of the iRNA-administered subject by any methods known it the art, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, *e.g.*, ELISA or western blotting, described herein. In one embodiment, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in HDV gene and/or protein expression. In another embodiment, a blood sample serves as the tissue material for monitoring the reduction in HDV gene and/or protein expression.

In one embodiment, verification of RISC medicated cleavage of target *in vivo* following administration of iRNA agent is done by performing 5'-RACE or modifications of the protocol as known in the art (Lasham A *et al.*, (2010) *Nucleic Acid Res.*, 38 (3) p-e19) (Zimmermann *et al.* (2006) *Nature* 441: 111-4).

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Transcripts

siRNA Design

The selection of siRNA designs targeting HBV was driven by two primary factors: a) potency and b), the desire to employ siRNA with near-perfect matches with greater than 90% fractional coverage of the large number of public HBV sequences of all known serotypes (A through H). The coordinates for the siRNA selection were determined relative to the NCBI HBV reference genome sequence NC_003977.1 (GenBank Accession No. GI:21326584 (SEQ ID NO:1). The reverse complement of

SEQ ID NO:1 is provided in SEQ ID NO:2. A first set of siRNAs containing structure-activity modifications, including various 2'-O-methyl and 2'-fluoro substitution patterns, centered on two adjacent regions of the HBV genome coding for surface antigen (HbSAg) and the HBV polymerase, were designed, synthesized and screened *in-vitro*. A second set of siRNAs were designed, synthesized and screened targeting additional target regions with particular attention to positions 1581-1599 of SEQ ID NO:1 that code, in addition to the HbSAg and polymerase, the X gene.

The sequence of Hepatitis B virus genomic DNA, complete sequence, isolate 22Y04HCC (GenBank Accession No., GI: 3582357) and its reverse complement are provided in SEQ ID NOs. 3 and 4, respectively.

A detailed list of the unmodified HBV sense and antisense strand sequences is shown in Tables 3, 14, 24, and 27.

A detailed list of the modified HBV sense and antisense strand sequences is shown in Tables 4, 6, 15, 25, and 28.

siRNA Synthesis

HBV siRNA sequences were synthesized at 1 μmol scale on Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500 A) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites were obtained from Thermo-Fisher (Milwaukee, WI) and Hongene (China). 2'F 2'-O-Methyl, GNA (glycol nucleic acids), 5'phosphate and abasic modifications were introduced employing the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands was performed on a GalNAc modified CPG support. Custom CPG universal solid support was used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) was 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as activator (0.6 M in acetonitrile). Phosphorothioate linkages were generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time was 3 minutes. All sequences were synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, oligoribonucleotides were cleaved from the solid support and deprotected in sealed 96 deep well plates using 200 μL Aqueous Methylamine reagents at 60°C for 20 minutes. At the end of

cleavage and deprotection step, the synthesis plate was allowed to come to room temperature and was precipitated by addition of 1mL of acetontile: ethanol mixture (9:1). The plates were cooled at -80 C for 2 hrs, superanatant decanted carefully with the aid of a multi channel pipette. The oligonucleotide pellet was re-suspended in 20mM NaOAc buffer and were desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction collector. Desalted samples were collected in 96-well plates. Samples from each sequence were analyzed by LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of HBV single strands was performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands were combined and annealed in 96 well plates. After combining the complementary single strands, the 96-well plate was sealed tightly and heated in an oven at 100°C for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex was normalized to 10μM in 1X PBS.

Example 2. *In vitro* screening of siRNA duplexes

Cell culture and transfections

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Dual-Glo® Luciferaseconstructs generated in the psiCHECK2 plasmid containing approximately 1.1 kb of HBV genomic sequences were transfected into approximately $15x10^4$ cells using Lipofectamine 2000 (Invitrogen, Carlsbad CA. cat # 11668-019). For each well of a 96 well plate, 0.2 μl of Lipofectamine was added to 10 ng of plasmid vector in 14.8 μl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells which were resuspended in 80 μl of fresh complete media. After approximately 24 hours, the media were removed and the cells re-transfected with siRNA. Each siRNA was transfected into cells that had previously been transfected with the psiCHECK2-HBV vector that had a perfect match for the siRNA. siRNA transfection was carried out by adding 14.8 μl of Opti-MEM plus 0.2 μ l of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5μl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. The mixture was then added to the cells previously

transfected with the psiCHECK2-HBV plasmid that had a perfect match to the siRNA sequence. Cells were incubated for 24 hours before luciferase was measured.

Single dose experiments were performed at 10nM and 0.01nM final duplex concentration.

Dual-Glo® Luciferase assay

Twenty-four hours after the siRNAs were transfected, Firefly (transfection control) and Rinella (fused to HBV target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding75 µl of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before lunimescense (500 nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 75 µl of room temperature of Dual-Glo® Stop & Glo® Reagent was added to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent , quench the firefl y luciferase signal and sustain luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (HBV) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done at n=2 or greater.

Essentially the same methods were used to screen siRNA targeted to HDV.

Table 5 shows the results of a single dose screen in Cos7 cells transfected with the indicated HBV iRNAs. Data are expressed as percent of mRNA remaining relative to negative control.

Table 2. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	**Nucleotide(s)**
A	Adenosine-3'-phosphate
Af	2'-fluoroadenosine-3'-phosphate

Afs	2’-fluoroadenosine-3’-phosphorothioate
As	adenosine-3’-phosphorothioate
C	cytidine-3’-phosphate
Cf	2’-fluorocytidine-3’-phosphate
Cfs	2’-fluorocytidine-3’-phosphorothioate
Cs	cytidine-3’-phosphorothioate
G	guanosine-3’-phosphate
Gf	2’-fluoroguanosine-3’-phosphate
Gfs	2’-fluoroguanosine-3’-phosphorothioate
Gs	guanosine-3’-phosphorothioate
T	5’-methyluridine-3’-phosphate
Tf	2’-fluoro-5-methyluridine-3’-phosphate
Tfs	2’-fluoro-5-methyluridine-3’-phosphorothioate
Ts	5-methyluridine-3’-phosphorothioate
U	Uridine-3’-phosphate
Uf	2’-fluorouridine-3’-phosphate
Ufs	2’-fluorouridine -3’-phosphorothioate
Us	uridine -3’-phosphorothioate
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3’-phosphate
as	2'-O-methyladenosine-3’- phosphorothioate
c	2'-O-methylcytidine-3’-phosphate
cs	2'-O-methylcytidine-3’- phosphorothioate
g	2'-O-methylguanosine-3’-phosphate
gs	2'-O-methylguanosine-3’- phosphorothioate
t	2’-O-methyl-5-methyluridine-3’-phosphate
ts	2’-O-methyl-5-methyluridine-3’-phosphorothioate
u	2'-O-methyluridine-3’-phosphate
us	2'-O-methyluridine-3’-phosphorothioate
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(dt)	deoxy-thymine
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)

Y44	2-hydroxymethyl-tetrahydrofurane-5-phosphate
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA)
P	Phosphate
VP	Vinyl-phosphate

Table 3. Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:	Position in NC_003977.1
AD-61522.2	A-123463.2	AGUUAUAUGGAUGAUGUGGUA	121	A-123464.2	UACCACAUCAUCCAUAUAACUGA	556	731_753
AD-61547.2	A-123487.2	GGAUGUGUCUGCGGCGUUUUA	122	A-123488.2	UAAAACGCCGCAGACACAUCCAG	557	373_395
AD-63938.2	A-127896.1	ACUCGUGGUGGACUUCUCUCA	123	A-127897.1	UGAGAGAAGUCCACCACGAGUCU	558	250_272
AD-63939.2	A-127909.1	ACUCGUGGUGGACUUCUCUCA	124	A-127906.3	UGAGAGAAGUCCACCACGAGUCU	559	250_272
AD-63940.2	A-127917.1	ACUCGUGGUGGACUUCTCUCA	125	A-127906.11	UGAGAGAAGUCCACCACGAGUCU	560	250_272
AD-63940.3	A-127917.4	ACUCGUGGUGGACUUCTCUCA	126	A-127906.19	UGAGAGAAGUCCACCACGAGUCU	561	250_272
AD-63941.2	A-127905.8	ACUCGUGGUGGACUUCUCUCA	127	A-127925.1	UGAGAGAAGUCCACCACGAGUCU	562	250_272
AD-63942.2	A-127933.1	UCGUGGUGGACUUCUCUCA	128	A-127934.1	UGAGAGAAGUCCACCACGAGU	563	252_274
AD-63943.2	A-127944.2	ACUCGUGGUGGACUUCUCUCA	129	A-127942.2	UGAGAGAAGUCCACCACGAGUCU	564	250_272
AD-63945.2	A-127910.1	ACUCGUGGUGGACUUCUCUCA	130	A-127906.4	UGAGAGAAGUCCACCACGAGUCU	565	250_272
AD-63946.2	A-127918.1	ACUCGUGGUGGACUUCUCUCA	131	A-127906.12	UGAGAGAAGUCCACCACGAGUCU	566	250_272
AD-63947.2	A-127905.9	ACUCGUGGUGGACUUCUCUCA	132	A-127926.1	UGAGAGAAGUCCACCACGAGUCU	567	250_272
AD-63948.2	A-127935.1	GUGGUGGACUUCUCUCA	133	A-127936.1	UGAGAGAAGUCCACCACGA	568	254_276
AD-63949.2	A-127944.3	ACUCGUGGUGGACUUCUCUCA	134	A-127906.14	UGAGAGAAGUCCACCACGAGUCU	569	250_272
AD-63950.2	A-127900.1	UCGUGGUGGACUUCUCUCAUU	135	A-127901.1	UGAGAGAAGUCCACCACGAUU	570	252_274
AD-63951.2	A-127911.1	ACUCGUGGUGGACUUCUCUCA	136	A-127906.5	UGAGAGAAGUCCACCACGAGUCU	571	250_272
AD-63952.2	A-127905.2	ACUCGUGGUGGACUUCUCUCA	137	A-127919.1	UGAGAGAAGUCCACCACGAGUCU	572	250_272
AD-63953.2	A-127905.10	ACUCGUGGUGGACUUCUCUCA	138	A-127927.1	UGAGAGAAGUCCACCACGAGUCU	573	250_272
AD-63955.2	A-127945.1	ACUCGUGGUGGACUUCUCUCA	139	A-127940.3	UGAGAGAAGUCCACCACGAGUCU	574	250_272
AD-63956.2	A-127902.1	UCGUGGUGGACUUCUCUCA	140	A-127903.1	UGAGAGAAGUCCACCACGAUU	575	252_274

AD-63957.2	A-127912.1	ACUCGUGGUGGACUUCUCUCA	141	A-127906.6	UGAGAGAAGUCCACCACGAGUCU	576	250_272
AD-63958.2	A-127905.3	ACUCGUGGUGGACUUCUCUCA	142	A-127920.1	UGAGAGAAGUCCACCACGAGUCU	577	250_272
AD-63959.2	A-127905.11	ACUCGUGGUGGACUUCUCUCA	143	A-127928.1	UGAGAGAAGUCCACCACGAGUCU	578	250_272
AD-63960.2	A-126619.2	UAUUUUCCUAGGGUACAA	144	A-127938.1	UGAGAGAAGUCCACCACGA	579	254_276
AD-63961.2	A-127945.2	ACUCGUGGUGGACUUCUCUCA	145	A-127942.3	UGAGAGAAGUCCACCACGAGUCU	580	250_272
AD-63962.2	A-127902.2	UCGUGGUGGACUUCUCUCA	146	A-127904.1	UGAGAGAAGUCCACCACGAUU	581	252_274
AD-63963.2	A-127913.1	ACUCGUGGUGGACUUCUCUCA	147	A-127906.7	UGAGAGAAGUCCACCACGAGUCU	582	250_272
AD-63964.2	A-127905.4	ACUCGUGGUGGACUUCUCUCA	148	A-127921.1	UGAGAGAAGUCCACCACGAGUCU	583	250_272
AD-63965.2	A-127905.12	ACUCGUGGUGGACUUCUCUCA	149	A-127929.1	UGAGAGAAGUCCACCACGAGUCU	584	250_272
AD-63966.2	A-127939.1	ACUCGUGGUGGACUUCUCUCA	150	A-127940.1	UGAGAGAAGUCCACCACGAGUCU	585	250_272
AD-63967.2	A-127945.3	ACUCGUGGUGGACUUCUCUCA	151	A-127906.15	UGAGAGAAGUCCACCACGAGUCU	586	250_272
AD-63968.2	A-127905.1	ACUCGUGGUGGACUUCUCUCA	152	A-127906.1	UGAGAGAAGUCCACCACGAGUCU	587	250_272
AD-63968.2	A-127905.1	ACUCGUGGUGGACUUCUCUCA	153	A-127906.1	UGAGAGAAGUCCACCACGAGUCU	588	250_272
AD-63968.4	A-127905.15	ACUCGUGGUGGACUUCUCUCA	154	A-127906.17	UGAGAGAAGUCCACCACGAGUCU	589	250_272
AD-63968.5	A-127905.17	ACUCGUGGUGGACUUCUCUCA	155	A-127906.18	UGAGAGAAGUCCACCACGAGUCU	590	250_272
AD-63969.2	A-127914.1	ACUCGUGGUGGACUUCUCUCA	156	A-127906.8	UGAGAGAAGUCCACCACGAGUCU	591	250_272
AD-63970.2	A-127905.5	ACUCGUGGUGGACUUCUCUCA	157	A-127922.1	UGAGAGAAGUCCACCACGAGUCU	592	250_272
AD-63971.2	A-127905.13	ACUCGUGGUGGACUUCUCUCA	158	A-127930.1	UGAGAGAAGUCCACCACGAGUCU	593	250_272
AD-63972.2	A-127941.1	ACUCGUGGUGGACUUCUCUCA	159	A-127942.1	UGAGAGAAGUCCACCACGAGUCU	594	250_272
AD-63973.2	A-127946.1	ACUCGUGGUGGACUUCUCUCA	160	A-127947.1	UGAGAGAAGTCCACCACGAGUCU	595	250_272
AD-63975.2	A-127915.1	ACUCGUGGUGGACUUCTCUCA	161	A-127906.9	UGAGAGAAGUCCACCACGAGUCU	596	250_272
AD-63976.2	A-127905.6	ACUCGUGGUGGACUUCUCUCA	162	A-127923.1	UGAGAGAAGUCCACCACGAGUCU	597	250_272
AD-63977.2	A-127917.2	ACUCGUGGUGGACUUCTCUCA	163	A-127931.1	UGAGAGAAGUCCACCACGAGUCU	598	250_272
AD-63978.2	A-127943.1	ACUCGUGGUGGACUUCUCUCA	164	A-127906.13	UGAGAGAAGUCCACCACGAGUCU	599	250_272

AD-63979.2	A-127908.1	ACUCGUGGUGGGACUUCUCUCA	165	A-127906.2	UGAGAGAAGUCCACCACGAGUCU	600	250_272
AD-63980.2	A-127916.1	ACUCGUGGUGGGACUUCUCUCA	166	A-127906.10	UGAGAGAAGUCCACCACGAGUCU	601	250_272
AD-63981.2	A-127905.7	ACUCGUGGUGGGACUUCUCUCA	167	A-127924.1	UGAGAGAAGUCCACCACGAGUCU	602	250_272
AD-63982.2	A-127917.3	ACUCGUGGUGGGACUUCUCUCA	168	A-127932.1	UGAGAGAAGUCCACCACGAGUCU	603	250_272
AD-63983.2	A-127944.1	ACUCGUGGUGGGACUUCUCUCA	169	A-127940.2	UGAGAGAAGUCCACCACGAGUCU	604	250_272
AD-63985.2	A-127961.1	GUGGUGGACUUCUCUCUCAAUUU	170	A-127956.4	AAAUUGAGAGAAGUCCACCACGA	605	254_276
AD-63986.2	A-127969.1	GUGGUGGACUUCUCUCUCAAUUU	171	A-127956.12	AAAUUGAGAGAAGUCCACCACGA	606	254_276
AD-63987.2	A-127955.9	GUGGUGGACUUCUCUCUCAAUUU	172	A-127977.1	AAAUUGAGAGAAGUCCACCACGA	607	254_276
AD-63988.2	A-127986.1	UGGACUUCUCUCUCAAUUU	173	A-127987.1	AAAUUGAGAGAAGUCCACC	608	258_280
AD-63989.2	A-127996.1	GUGGUGGACUUCUCUCUCAAUUU	174	A-127992.2	AAAUUGAGAGAAGUCCACCACGA	609	254_276
AD-63990.2	A-127950.1	GGUGGACUUCUCUCUCAAUUUUU	175	A-127951.1	AAAUUGAGAGAAGUCCACCUU	610	256_278
AD-63991.2	A-127962.1	GUGGUGGACUUCUCUCUCAAUUU	176	A-127956.5	AAAUUGAGAGAAGUCCACCACGA	611	254_276
AD-63992.2	A-127955.2	GUGGUGGACUUCUCUCUCAAUUU	177	A-127970.1	AAAUUGAGAGAAGUCCACCACGA	612	254_276
AD-63993.2	A-127955.10	GUGGUGGACUUCUCUCUCAAUUU	178	A-127978.1	AAAUUGAGAGAAGUCCACCACGA	613	254_276
AD-63994.2	A-127984.2	GGUGGACUUCUCUCUCAAUUU	179	A-127988.1	AAAUUGAGAGAAGUCCACCAC	614	256_278
AD-63995.2	A-127996.2	GUGGUGGACUUCUCUCUCAAUUU	180	A-127993.2	AAAUUGAGAGAAGUCCACCACGA	615	254_276
AD-63996.2	A-127952.1	GGUGGACUUCUCUCUCAAUUU	181	A-127953.1	AAAUUGAGAGAAGUCCACCUU	616	256_278
AD-63997.2	A-127963.1	GUGGUGGACUUCUCUCUCAAUUU	182	A-127956.6	AAAUUGAGAGAAGUCCACCACGA	617	254_276
AD-63999.2	A-127955.11	GUGGUGGACUUCUCUCUCAAUUU	183	A-127979.1	AAAUUGAGAGAAGUCCACCACGA	618	254_276
AD-64000.2	A-127986.2	UGGACUUCUCUCUCAAUUU	184	A-127989.1	AAAUUGAGAGAAGUCCACC	619	258_280
AD-64001.2	A-127996.3	GUGGUGGACUUCUCUCUCAAUUU	185	A-127994.2	AAAUUGAGAGAAGUCCACCACGA	620	254_276
AD-64002.2	A-127952.2	GGUGGACUUCUCUCUCAAUUU	186	A-127954.1	AAAUUGAGAGAAGUCCACCUU	621	256_278
AD-64003.2	A-127964.1	GUGGUGGACUUCUCUCUCAAUUU	187	A-127956.7	AAAUUGAGAGAAGUCCACCACGA	622	254_276
AD-64004.2	A-127955.4	GUGGUGGACUUCUCUCUCAAUUU	188	A-127972.1	AAAUUGAGAGAAGUCCACCACGA	623	254_276

AD-64005.2	A-127955.12	GUGGUGGACUUCUCUCAAUUU	189	A-127980.1	AAAUUGAGAGAAGUCCACCACGA	624	254_276
AD-64006.2	A-127990.1	GUGGUGGACUUCUCUCAAUUU	190	A-127991.1	AAAUUGAGAGAAGUCCACCACGA	625	254_276
AD-64007.2	A-127996.4	GUGGUGGACUUCUCUCAAUUU	191	A-127995.2	AAAUUGAGAGAAGUCCACCACGA	626	254_276
AD-64008.2	A-127955.1	GUGGUGGACUUCUCUCAAUUU	192	A-127956.1	AAAUUGAGAGAAGUCCACCACGA	627	254_276
AD-64008.2	A-127955.1	GUGGUGGACUUCUCUCAAUUU	193	A-127956.1	AAAUUGAGAGAAGUCCACCACGA	628	254_276
AD-64008.4	A-127955.15	GUGGUGGACUUCUCUCAAUUU	194	A-127956.14	AAAUUGAGAGAAGUCCACCACGA	629	254_276
AD-64009.2	A-127965.1	GUGGUGGACUUCUCUCAAUUU	195	A-127956.8	AAAUUGAGAGAAGUCCACCACGA	630	254_276
AD-64010.2	A-127955.5	GUGGUGGACUUCUCUCAAUUU	196	A-127973.1	AAAUUGAGAGAAGUCCACCACGA	631	254_276
AD-64011.2	A-127955.13	GUGGUGGACUUCUCUCAAUUU	197	A-127981.1	AAAUUGAGAGAAGUCCACCACGA	632	254_276
AD-64012.2	A-127990.2	GUGGUGGACUUCUCUCAAUUU	198	A-127992.1	AAAUUGAGAGAAGUCCACCACGA	633	254_276
AD-64013.2	A-127997.1	GUGGUGGACTTCUCUCAAUUU	199	A-127998.1	AAAUUGAGAGAAGTCCACCACGA	634	254_276
AD-64014.2	A-127957.1	GUGGUGGACUUCUCUCAAUUU	200	A-127958.1	AAAUUGAGAGAAGUCCACCACGA	635	254_276
AD-64015.2	A-127966.1	GUGGUGGACUUCUCUCAAUUU	201	A-127956.9	AAAUUGAGAGAAGUCCACCACGA	636	254_276
AD-64016.2	A-127955.6	GUGGUGGACUUCUCUCAAUUU	202	A-127974.1	AAAUUGAGAGAAGUCCACCACGA	637	254_276
AD-64017.2	A-127968.2	GUGGUGGACUTCUCUCAAUUU	203	A-127982.1	AAAUUGAGAGAAGTCCACCACGA	638	254_276
AD-64018.2	A-127990.3	GUGGUGGACUUCUCUCAAUUU	204	A-127993.1	AAAUUGAGAGAAGUCCACCACGA	639	254_276
AD-64019.2	A-127959.1	GUGGUGGACUUCUCUCAAUUU	205	A-127956.2	AAAUUGAGAGAAGUCCACCACGA	640	254_276
AD-64020.2	A-127967.1	GUGGUGGACUUCUCUCAAUUU	206	A-127956.10	AAAUUGAGAGAAGUCCACCACGA	641	254_276
AD-64021.2	A-127955.7	GUGGUGGACUUCUCUCAAUUU	207	A-127975.1	AAAUUGAGAGAAGUCCACCACGA	642	254_276
AD-64022.2	A-127968.3	GUGGUGGACUTCUCUCAAUUU	208	A-127983.1	AAAUUGAGAGAAGTCCACCACGA	643	254_276
AD-64023.2	A-127990.4	GUGGUGGACUUCUCUCAAUUU	209	A-127994.1	AAAUUGAGAGAAGUCCACCACGA	644	254_276
AD-64024.2	A-127960.1	GUGGUGGACUUCUCUCAAUUU	210	A-127956.3	AAAUUGAGAGAAGUCCACCACGA	645	254_276
AD-64025.2	A-127968.1	GUGGUGGACUTCUCUCAAUUU	211	A-127956.11	AAAUUGAGAGAAGUCCACCACGA	646	254_276
AD-64026.2	A-127955.8	GUGGUGGACUUCUCUCAAUUU	212	A-127976.1	AAAUUGAGAGAAGUCCACCACGA	647	254_276

AD-64027.2	A-127984.1	GGUGGACUUCUCUCAAUUU	213	A-127985.1	AAAUUGAGAGAAGUCCACCAC	648	256_278
AD-64028.2	A-127990.5	GUGGUGGACUUCUCUCAAUUU	214	A-127995.1	AAAUUGAGAGAAGUCCACCACGA	649	254_276
AD-64272.2	A-128001.2	GUGCACUUCGCUUCACCUCUG	215	A-128002.2	CAGAGGUGAAGCGAAGUGCACAC	650	1577_1599
AD-64274.1	A-128363.1	GUUGACAAAAAUCCUCACAAU	216	A-128364.1	AUUGUGAGGAUUUUUGUCAACAA	651	215_237
AD-64275.1	A-128377.1	UGUUGACAAAAAUCCUCACAA	217	A-128378.1	UUGUGAGGAUUUUUGUCAACAAG	652	214_236
AD-64276.1	A-128393.1	GGUGGACUUCUCUCAAUUUUA	218	A-128394.1	UAAAAUUGAGAGAAGUCCACCAC	653	256_278
AD-64277.1	A-128407.1	UCUUUUUGGAGUGUGGAUUCGA	219	A-128408.1	UCGAAUCCACACUCCAAAAGACA	654	2259_2281
AD-64277.1	A-128407.1	UCUUUUUGGAGUGUGGAUUCGA	220	A-128408.1	UCGAAUCCACACUCCAAAAGACA	655	2259_2281
AD-64278.1	A-128423.1	ACUGUUCAAGCCUCCAAGCUA	221	A-128424.1	UAGCUUGGAGGCUUGAACAAGAC	656	1857_1879
AD-64279.1	A-128435.1	UCUGCCGAUCCAUACUGCGGA	222	A-128436.1	UCCGCAGUAUGGAUCGGCAGAGG	657	1255_1277
AD-64280.1	A-128379.1	AUGUGUCUGCGGCGUUUUAUA	223	A-128380.1	UAUAAAACGCCCGCAGACACAUCC	658	375_397
AD-64281.1	A-128395.1	CCCCGUCUGUGCCUUCUCAUA	224	A-128396.1	UAUGAGAAGGCACAGACGGGGAG	659	1545_1567
AD-64282.1	A-128409.1	GCCUAAUCAUCUCUUGUUCAU	225	A-128410.1	AUGAACAAGAGAUGAUUAGCGAG	660	1831_1853
AD-64283.1	A-128425.1	UCUAGACUCGUGGUGGACUUC	226	A-128426.1	GAAGUCCACCACGAGUCUAGACU	661	245_267
AD-64284.1	A-128437.1	CUGCCGAUCCAUACUGCGGAA	227	A-128438.1	UUCCGCAGUAUGGAUCGGCAGAG	662	1256_1278
AD-64285.1	A-128365.1	UUUUUCUUGUUUGACAAAAAUA	228	A-128366.1	UAUUUUUGUCAACAAGAAAAAACC	663	207_229
AD-64286.1	A-128381.1	AUCUUCUUGUUGGUUCUUCUA	229	A-128382.1	UAGAAGAACCAACAAGAAGAUGA	664	426_448
AD-64289.1	A-128367.1	GUUUUUCUUGUUGACAAAAAAU	230	A-128368.1	AUUUUUGUCAACAAGAAAAAACCC	665	206_228
AD-64290.1	A-128383.1	CUGCCUAAUCAUCUCUUGUUA	231	A-128384.1	UAACAAGAGAUGAUUAGGCAGAG	666	1829_1851
AD-64291.1	A-128399.1	UCCUCACAAUACCACACAGAGUA	232	A-128400.1	UACUCUGUGGUAUUGUGAGGAUU	667	226_248
AD-64292.1	A-128413.1	CUUGUUGACAAAAAAUCCUCAA	233	A-128414.1	UUGAGGAUUUUUGUCAACAAGAA	668	212_234
AD-64293.1	A-128439.1	GCAACUUUUUCACCUCUGCCU	234	A-128440.1	AGGCAGAGGUGAAAAAGUUGCAU	669	1814_1836
AD-64294.1	A-128369.1	GGGAACAAGAGCUACAGCAUA	235	A-128370.1	UAUGCUGUAGCUCUUGUUCCCAA	670	2828_2850
AD-64295.1	A-128385.1	CGUGGUGGACUUCUCUCAAUU	236	A-128386.1	AAUUGAGAGAAGUCCACCAGCAG	671	253_275

AD-64297.1	A-128415.1	CUGCUGCUAUGCCUCAUCUUA	237	A-128416.1	UAAGAUGAGGCAUAGCAGCAGGA	672	411_433
AD-64298.1	A-128427.1	GUUGGAUGUGUCUGCGGCGUU	238	A-128428.1	AACGCCGCAGACACAUCCAACGA	673	370_392
AD-64299.1	A-128441.1	UUCAUCCUGCUGCUAUGCCUA	239	A-128442.1	UAGGCAUAGCAGCAGGAUGAAGA	674	405_427
AD-64300.1	A-128371.1	UUCUUGUUGACAAAAAUCCUA	240	A-128372.1	UAGGAUUUUUGUCAACAAGAAAA	675	210_232
AD-64302.1	A-128417.1	UAUAUGGAUGAUGUGGUAUUA	241	A-128418.1	UAAUACCACAUCAUCCAUAUAAC	676	734_756
AD-64303.1	A-128429.1	UUCAUCCUGCUGCUAUGCCUC	242	A-128430.1	GAGGCAUAGCAGCAGGAUGAAGA	677	405_427
AD-64304.1	A-128443.1	GUGCACUUCGCUUCACCUCUA	243	A-128444.1	UAGAGGUGAAGCGAAGUGCACAC	678	1577_1599
AD-64305.1	A-128373.1	UUGACAAAAAAUCCUCACAAUA	244	A-128374.1	UAUUGUGAGGAUUUUUGUCAACA	679	216_238
AD-64307.1	A-128403.1	AAGCCUCCAAGCUGUGCCUUA	245	A-128404.1	UAAGGCACAGCUUGGAGGCUUGA	680	1864_1886
AD-64308.1	A-128419.1	CCUCUUCAUCCUGCUGCUAUA	246	A-128420.1	UAUAGCAGCAGGAUGAAGAGGAA	681	401_423
AD-64309.1	A-128431.1	CCUGCUGCUAUGCCUCAUCUU	247	A-128432.1	AAGAUGAGGCAUAGCAGCAGGAU	682	410_432
AD-64310.1	A-128375.1	CAUCUUCUUGUUGGUUCUUCU	248	A-128376.1	AGAAGAACCAACAAGAAGAUGAG	683	425_447
AD-64311.1	A-128391.1	CCGUCUGUGCCUUCUCAUCUA	249	A-128392.1	UAGAUGAGAAGGCACAGACGGGG	684	1547_1569
AD-64312.1	A-128405.1	CCUCAUCUUCUUGUUGGUUCU	250	A-128406.1	AGAACCAACAAGAAGAUGAGGCA	685	422_444
AD-64313.1	A-128421.1	CCACCAAAUGCCCCUAUCUUA	251	A-128422.1	UAAGAUAGGGGCAUUUGGUGGUC	686	2298_2320
AD-64314.1	A-128433.1	GCUCCUCUGCCGAUCCAUACU	252	A-128434.1	AGUAUGGAUCGGCAGAGGAGCCA	687	1250_1272
AD-64315.1	A-128363.2	GUUGACAAAAAAUCCUCACAAU	253	A-128445.1	AUUGUGAGGAUUUUUGUCAACAA	688	215_237
AD-64316.1	A-128377.2	UGUUGACAAAAAAUCCUCACAA	254	A-128453.1	UUGUGAGGAUUUUUGUCAACAAG	689	214_236
AD-64317.1	A-128393.2	GGUGGACUUCUCUCAAUUUUA	255	A-128461.1	UAAAAUUGAGAGAAGUCCACCAC	690	256_278
AD-64318.1	A-128407.2	UCUUUUGGAGUGUGGAUUCGA	256	A-128469.1	UCGAAUCCACACUCCAAAAGACA	691	2259_2281
AD-64318.1	A-128407.2	UCUUUUGGAGUGUGGAUUCGA	257	A-128469.1	UCGAAUCCACACUCCAAAAGACA	692	2259_2281
AD-64319.1	A-128423.2	ACUGUUCAAGCCUCCAAGCUA	258	A-128477.1	UAGCUUGGAGGCUUGAACAAGAC	693	1857_1879
AD-64320.1	A-128435.2	UCUGCCGAUCCAUACUGCGGA	259	A-128483.1	UCCGCAGUAUGGAUCGGCAGAGG	694	1255_1277
AD-64321.1	A-123463.3	AGUUAUAUGGAUGAUGUGGUA	260	A-128446.1	UACCACAUCAUCCAUAUAACUGA	695	731_753

AD-64322.1	A-128379.2	AUGUGUCUGCGGCGUUUUAUA	261	A-128454.1	UAUAAAACGCCGCAGACACAUCC	696	375_397
AD-64323.1	A-128395.2	CCCCGUCUGUGCCUUCUCAUA	262	A-128462.1	UAUGAGAAGGCACAGACGGGGAG	697	1545_1567
AD-64324.1	A-128409.2	GCCUAAUCAUCUCUUGUUCAU	263	A-128470.1	AUGAACAAGAGAUGAUUAGCGAG	698	1831_1853
AD-64325.1	A-128425.2	UCUAGACUCGUGGUGGACUUC	264	A-128478.1	GAAGUCCACCACGAGUCUAGACU	699	245_267
AD-64326.1	A-128437.2	CUGCCGAUCCAUACUGCGGAA	265	A-128484.1	UUCCGCAGUAUGGAUCGGCAGAG	700	1256_1278
AD-64328.1	A-128381.2	AUCUUCUUGUUGGUUCUUCUA	266	A-128455.1	UAGAAGAACCAACAAGAAGAUGA	701	426_448
AD-64330.1	A-128411.2	UUCUCUCAAUUUUUCUAGGGA	267	A-128471.1	UCCCCUAGAAAAUUGAGAGAAGU	702	263_285
AD-64331.1	A-127905.16	ACUCGUGGUGGACUUCUCUCA	268	A-127907.2	UGAGAGAAGUCCACCACGAGUCU	703	250_272
AD-64332.1	A-128001.3	GUGCACUUCGCUUCACCUCUG	269	A-128485.1	CAGAGGUGAAGCGAAGUGCACAC	704	1577_1599
AD-64333.1	A-128367.2	GUUUUUCUUGUUUGACAAAAAU	270	A-128448.1	AUUUUUGUCAACAAGAAAAACCC	705	206_228
AD-64334.1	A-128383.2	CUGCCUAAUCAUCUCUUGUUA	271	A-128456.1	UAACAAGAGAUGAUUAGGCAGAG	706	1829_1851
AD-64335.1	A-128399.2	UCCUCACAAUACCACACAGAGUA	272	A-128464.1	UACUCUGUGGUAUUGUGAGGAUU	707	226_248
AD-64336.1	A-128413.2	CUUGUUGACAAAAAUCCUCAA	273	A-128472.1	UUGAGGAUUUUUUGUCAACAAGAA	708	212_234
AD-64337.1	A-127955.16	GUGGUGGACUUCUCUCAAUUU	274	A-127958.2	AAAUUGAGAGAAGUCCACCACGA	709	254_276
AD-64338.1	A-128439.2	GCAACUUUUUUCACCUCUGCCU	275	A-128486.1	AGGCAGAGGUGAAAAAGUUUGCAU	710	1814_1836
AD-64339.1	A-128369.2	GGGAACAAGAGCUACAGCAUA	276	A-128449.1	UAUGCUGUAGCUCUUGUUCCCAA	711	2828_2850
AD-64341.1	A-128401.2	UCAUCUUCUUGUUGGUUCUUA	277	A-128465.1	UAAGAACCAACAAGAAGAUGAGG	712	424_446
AD-64342.1	A-128415.2	CUGCUGCUAUGCCUCAUCUUA	278	A-128473.1	UAAGAUGAGGCAUAGCAGCAGGA	713	411_433
AD-64343.1	A-128427.2	GUUGGAUGUGUCUGCGGCGUU	279	A-128479.1	AACGCCGCAGACACAUCCAACGA	714	370_392
AD-64344.1	A-128441.2	UUCAUCCUGCUGCUAUGCCUA	280	A-128487.1	UAGGCAUAGCAGCAGGAUGAAGA	715	405_427
AD-64345.1	A-128371.2	UUCUUGUUGACAAAAAAUCCUA	281	A-128450.1	UAGGAUUUUUUGUCAACAAGAAAA	716	210_232
AD-64347.1	A-123487.3	GGAUGUGUCUGCGGCGUUUUA	282	A-128466.1	UAAAACGCCGCAGACACAUCCAG	717	373_395
AD-64348.1	A-128417.2	UAUAUGGAUGAUGUGGUAUUA	283	A-128474.1	UAAUACCACAUCAUCCAUAUAAC	718	734_756
AD-64349.1	A-128429.2	UUCAUCCUGCUGCUAUGCCUC	284	A-128480.1	GAGGCAUAGCAGCAGGAUGAAGA	719	405_427

AD-64350.1	A-128443.2	GUGCACUUCGCUUCACCUCUA	285	A-128488.1	UAGAGGUGAAGCGAAGUGCACAC	720	1577_1599
AD-64351.1	A-128373.2	UUGACAAAAAAUCCUCACAAUA	286	A-128451.1	UAUUGUGAGGAUUUUUGUCAACA	721	216_238
AD-64352.1	A-128389.2	CCAAGUGUUUGCUGACGCAAA	287	A-128459.1	UUUGCGUCAGCAAACACACUUGGCA	722	1174_1196
AD-64352.1	A-128389.2	CCAAGUGUUUGCUGACGCAAA	288	A-128459.1	UUUGCGUCAGCAAACACACUUGGCA	723	1174_1196
AD-64353.1	A-128403.2	AAGCCUCCAAGCUGUGCCUUA	289	A-128467.1	UAAGGCACAGCUUGGAGGCUUGA	724	1864_1886
AD-64354.1	A-128419.2	CCUCUUCAUCCUGCUGCUAUA	290	A-128475.1	UAUAGCAGCAGGAUGAAGAGGAA	725	401_423
AD-64355.1	A-128431.2	CCUGCUGCUAUGCCUCAUCUU	291	A-128481.1	AAGAUGAGGCAUAGCAGCAGGAU	726	410_432
AD-64356.1	A-128375.2	CAUCUUCUUGUUGGUUCUUCU	292	A-128452.1	AGAAGAACCAACAAGAAGAUGAG	727	425_447
AD-64357.1	A-128391.2	CCGUCUGUGCCUUCUCAUCUA	293	A-128460.1	UAGAUGAGAAGGCACAGACGGGG	728	1547_1569
AD-64358.1	A-128405.2	CCUCAUCUUCUUGUUGGUUCU	294	A-128468.1	AGAACCAACAAGAAGAUGAGGCA	729	422_444
AD-64359.1	A-128421.2	CCACCAAAUGCCCCUAUCUUA	295	A-128476.1	UAAGAUAGGGGCAUUUGGUGGUC	730	2298_2320
AD-64360.1	A-128433.2	GCUCCUCUGCCGAUCCAUACU	296	A-128482.1	AGUAUGGAUCGGCAGAGGAGCCA	731	1250_1272
AD-64700.1	A-129379.1	ACUCGUGGUGTACUUCUCUCA	297	A-127906.26	UGAGAGAAGUCCACCACGAGUCU	732	250_272
AD-64701.1	A-127905.20	ACUCGUGGUGGACUUCUCUCA	298	A-129387.1	UGAGAGAAGTCCACCACGAGUCU	733	250_272
AD-64702.1	A-127905.28	ACUCGUGGUGGACUUCUCUCA	299	A-129395.1	UGAGAGAAGUCCACCACGAGUCU	734	250_272
AD-64703.1	A-129376.2	ACUCGUGGUGGACUUCACUCA	300	A-129385.5	UGAGAGAAGTCCACCACGAGUCU	735	250_272
AD-64704.1	A-129381.3	ACUCGUGGTGTACUUCACUCA	301	A-129389.6	UGAGAGAAGUCCACCACGAGUCU	736	250_272
AD-64705.1	A-129380.1	ACUCGUGGUGTACUUCACUCA	302	A-127906.27	UGAGAGAAGUCCACCACGAGUCU	737	250_272
AD-64706.1	A-127905.21	ACUCGUGGUGGACUUCUCUCA	303	A-129388.1	UGAGAGAAGUCCACCACGAGUCU	738	250_272
AD-64707.1	A-127905.29	ACUCGUGGUGGACUUCUCUCA	304	A-129396.1	UGAGAGAAGTCCACCACGAGUCU	739	250_272
AD-64708.1	A-129382.2	ACUCGUGGTGGACUUCTCUCA	305	A-129385.6	UGAGAGAAGTCCACCACGAGUCU	740	250_272
AD-64709.1	A-129373.4	ACUCGUGGUGGACUUCUCUCA	306	A-129391.2	UGAGAGAAGTCCACCACGAGUCU	741	250_272
AD-64710.1	A-129373.1	ACUCGUGGUGGACUUCUCUCA	307	A-127906.20	UGAGAGAAGUCCACCACGAGUCU	742	250_272
AD-64711.1	A-129381.1	ACUCGUGGTGTACUUCACUCA	308	A-127906.28	UGAGAGAAGUCCACCACGAGUCU	743	250_272

AD-64712.1	A-127905.22	ACUCGUGGUGGUGGACUUCUCUCUCA	309	A-129389.1	UGAGAGAAGUCCACCACGAGUCU	744	250_272
AD-64713.1	A-127905.30	ACUCGUGGUGGUGGACUUCUCUCUCA	310	A-129397.1	UGAGAGAAGTCCACCACGAGUCU	745	250_272
AD-64714.1	A-129384.2	ACUCGUGGTGGACUUCACUCA	311	A-129385.7	UGAGAGAAGTCCACCACGAGUCU	746	250_272
AD-64715.1	A-129376.4	ACUCGUGGUGGUGGACUUCACUCA	312	A-129391.3	UGAGAGAAGTCCACCACGAGUCU	747	250_272
AD-64716.1	A-129374.1	ACUCGUGGUGGUGGACUUCUCUCUCA	313	A-127906.21	UGAGAGAAGUCCACCACGAGUCU	748	250_272
AD-64717.1	A-129382.1	ACUCGUGGTGGACUUCTCUCA	314	A-127906.29	UGAGAGAAGUCCACCACGAGUCU	749	250_272
AD-64718.1	A-127905.23	ACUCGUGGUGGUGGACUUCUCUCUCA	315	A-129390.1	UGAGAGAAGUCCACCACGAGUCU	750	250_272
AD-64719.1	A-127917.5	ACUCGUGGUGGUGGACUUCTCUCA	316	A-129385.2	UGAGAGAAGTCCACCACGAGUCU	751	250_272
AD-64720.1	A-129381.2	ACUCGUGGTGTACUUCACUCA	317	A-129385.8	UGAGAGAAGTCCACCACGAGUCU	752	250_272
AD-64721.1	A-129382.4	ACUCGUGGTGGACUUCTCUCA	318	A-129391.4	UGAGAGAAGTCCACCACGAGUCU	753	250_272
AD-64722.1	A-129375.1	ACUCGUGGUGGACUUCCUCA	319	A-127906.22	UGAGAGAAGUCCACCACGAGUCU	754	250_272
AD-64723.1	A-129383.1	ACUCGUGGUGGACUUCTCUCA	320	A-127906.30	UGAGAGAAGUCCACCACGAGUCU	755	250_272
AD-64725.1	A-127917.6	ACUCGUGGUGGACUUCTCUCA	321	A-129398.1	UGAGAGAAGTCCACCACGAGUCU	756	250_272
AD-64726.1	A-129373.3	ACUCGUGGUGGACUUCUCUCA	322	A-129389.2	UGAGAGAAGUCCACCACGAGUCU	757	250_272
AD-64727.1	A-129384.4	ACUCGUGGTGGACUUCACUCA	323	A-129391.5	UGAGAGAAGTCCACCACGAGUCU	758	250_272
AD-64728.1	A-129376.1	ACUCGUGGUGGACUUCACUCA	324	A-127906.23	UGAGAGAAGUCCACCACGAGUCU	759	250_272
AD-64729.1	A-129384.1	ACUCGUGGTGGACUUCACUCA	325	A-127906.31	UGAGAGAAGUCCACCACGAGUCU	760	250_272
AD-64730.1	A-127905.25	ACUCGUGGUGGACUUCUCUCA	326	A-129392.1	UGAGAGAAGTCCACCACGAGUCU	761	250_272
AD-64731.1	A-129399.1	ACUCGUGGUGGACUUCTCUCA	327	A-129385.3	UGAGAGAAGTCCACCACGAGUCU	762	250_272
AD-64732.1	A-129376.3	ACUCGUGGUGGACUUCACUCA	328	A-129389.3	UGAGAGAAGUCCACCACGAGUCU	763	250_272
AD-64733.1	A-129381.4	ACUCGUGGTGTACUUCACUCA	329	A-129391.6	UGAGAGAAGTCCACCACGAGUCU	764	250_272
AD-64734.1	A-129377.1	ACUCGUGGUGGACUUCCCUCA	330	A-127906.24	UGAGAGAAGUCCACCACGAGUCU	765	250_272
AD-64735.1	A-127905.18	ACUCGUGGUGGACUUCUCUCA	331	A-129385.1	UGAGAGAAGTCCACCACGAGUCU	766	250_272
AD-64736.1	A-127905.26	ACUCGUGGUGGACUUCUCUCA	332	A-129393.1	UGAGAGAAGTCCACCACGAGUCU	767	250_272

AD-64737.1	A-129399.2	ACUCGUGGUGGACUUCUCUCA	333	A-129398.2	UGAGAGAAGUCCACCACGAGUCU	768	250_272
AD-64738.1	A-129382.3	ACUCGUGGUGGACUUCUCUCA	334	A-129389.4	UGAGAGAAGUCCACCACGAGUCU	769	250_272
AD-64739.1	A-129378.1	ACUCGUGGUGGACUUCUCUCA	335	A-127906.25	UGAGAGAAGUCCACCACGAGUCU	770	250_272
AD-64740.1	A-127905.19	ACUCGUGGUGGACUUCUCUCA	336	A-129386.1	UGAGAGAAGUCCACCACGAGUCU	771	250_272
AD-64741.1	A-127905.27	ACUCGUGGUGGACUUCUCUCA	337	A-129394.1	UGAGAGAAGUCCACCACGAGUCU	772	250_272
AD-64742.1	A-129373.2	ACUCGUGGUGGACUUCUCUCA	338	A-129385.4	UGAGAGAAGUCCACCACGAGUCU	773	250_272
AD-64743.1	A-129384.3	ACUCGUGGUGGACUUCUCUCA	339	A-129389.5	UGAGAGAAGUCCACCACGAGUCU	774	250_272

AD-61522.2	A-123463.2	AGUUAUAUGGAUGAUGUGGUA	340	A-123464.2	UACCACAUCAUCCAUAUAACUGA	775	731_753
AD-61547.2	A-123487.2	GGAUGUGUCUGCGGCGUUUUA	341	A-123488.2	UAAAACGCCGCAGACACAUCCAG	776	373_395
AD-63938.2	A-127896.1	ACUCGUGGUGGACUUCUCUCA	342	A-127897.1	UGAGAGAAGUCCACCACGAGUCU	777	250_272
AD-63939.2	A-127909.1	ACUCGUGGUGGACUUCUCUCA	343	A-127906.3	UGAGAGAAGUCCACCACGAGUCU	778	250_272
AD-63940.2	A-127917.1	ACUCGUGGUGGACUUCUCUCA	344	A-127906.11	UGAGAGAAGUCCACCACGAGUCU	779	250_272
AD-63941.2	A-127905.8	ACUCGUGGUGGACUUCUCUCA	345	A-127925.1	UGAGAGAAGUCCACCACGAGUCU	780	250_272
AD-63942.2	A-127933.1	UCGUGGUGGACUUCUCUCA	346	A-127934.1	UGAGAGAAGUCCACCACGAGU	781	252_274
AD-63943.2	A-127944.2	ACUCGUGGUGGACUUCUCUCA	347	A-127942.2	UGAGAGAAGUCCACCACGAGUCU	782	250_272
AD-63945.2	A-127910.1	ACUCGUGGUGGACUUCUCUCA	348	A-127906.4	UGAGAGAAGUCCACCACGAGUCU	783	250_272
AD-63946.2	A-127918.1	ACUCGUGGUGGACUUCUCUCA	349	A-127906.12	UGAGAGAAGUCCACCACGAGUCU	784	250_272
AD-63947.2	A-127905.9	ACUCGUGGUGGACUUCUCUCA	350	A-127926.1	UGAGAGAAGUCCACCACGAGUCU	785	250_272
AD-63948.2	A-127935.1	GUGGUGGACUUCUCUCA	351	A-127936.1	UGAGAGAAGUCCACCACGA	786	254_276
AD-63949.2	A-127944.3	ACUCGUGGUGGACUUCUCUCA	352	A-127906.14	UGAGAGAAGUCCACCACGAGUCU	787	250_272
AD-63950.2	A-127900.1	UCGUGGUGGACUUCUCUCAUU	353	A-127901.1	UGAGAGAAGUCCACCACGAUU	788	252_274

AD-63951.2	A-127911.1	ACUCGUGGUGGACUUCUCUCA	354	A-127906.5	UGAGAGAAGUCCACCACGAGUCU	789	250_272
AD-63952.2	A-127905.2	ACUCGUGGUGGACUUCUCUCA	355	A-127919.1	UGAGAGAAGUCCACCACGAGUCU	790	250_272
AD-63953.2	A-127905.10	ACUCGUGGUGGACUUCUCUCA	356	A-127927.1	UGAGAGAAGUCCACCACGAGUCU	791	250_272
AD-63955.2	A-127945.1	ACUCGUGGUGGACUUCUCUCA	357	A-127940.3	UGAGAGAAGUCCACCACGAGUCU	792	250_272
AD-63956.2	A-127902.1	UCGUGGUGGACUUCUCUCA	358	A-127903.1	UGAGAGAAGUCCACCACGAUU	793	252_274
AD-63957.2	A-127912.1	ACUCGUGGUGGACUUCUCUCA	359	A-127906.6	UGAGAGAAGUCCACCACGAGUCU	794	250_272
AD-63958.2	A-127905.3	ACUCGUGGUGGACUUCUCUCA	360	A-127920.1	UGAGAGAAGUCCACCACGAGUCU	795	250_272
AD-63959.2	A-127905.11	ACUCGUGGUGGACUUCUCUCA	361	A-127928.1	UGAGAGAAGUCCACCACGAGUCU	796	250_272
AD-63960.2	A-126619.2	UAUUUCCUAGGGUACAA	362	A-127938.1	UGAGAGAAGUCCACCACGA	797	254_276
AD-63961.2	A-127945.2	ACUCGUGGUGGACUUCUCUCA	363	A-127942.3	UGAGAGAAGUCCACCACGAGUCU	798	250_272
AD-63962.2	A-127902.2	UCGUGGUGGACUUCUCUCA	364	A-127904.1	UGAGAGAAGUCCACCACGAUU	799	252_274
AD-63963.2	A-127913.1	ACUCGUGGUGGACUUCUCUCA	365	A-127906.7	UGAGAGAAGUCCACCACGAGUCU	800	250_272
AD-63964.2	A-127905.4	ACUCGUGGUGGACUUCUCUCA	366	A-127921.1	UGAGAGAAGUCCACCACGAGUCU	801	250_272
AD-63965.2	A-127905.12	ACUCGUGGUGGACUUCUCUCA	367	A-127929.1	UGAGAGAAGUCCACCACGAGUCU	802	250_272
AD-63966.2	A-127939.1	ACUCGUGGUGGACUUCUCUCA	368	A-127940.1	UGAGAGAAGUCCACCACGAGUCU	803	250_272
AD-63967.2	A-127945.3	ACUCGUGGUGGACUUCUCUCA	369	A-127906.15	UGAGAGAAGUCCACCACGAGUCU	804	250_272
AD-63968.2	A-127905.1	ACUCGUGGUGGACUUCUCUCA	370	A-127906.1	UGAGAGAAGUCCACCACGAGUCU	805	250_272
AD-63968.4	A-127905.15	ACUCGUGGUGGACUUCUCUCA	371	A-127906.17	UGAGAGAAGUCCACCACGAGUCU	806	250_272
AD-63968.5	A-127905.17	ACUCGUGGUGGACUUCUCUCA	372	A-127906.18	UGAGAGAAGUCCACCACGAGUCU	807	250_272
AD-63969.2	A-127914.1	ACUCGUGGUGGACUUCUCUCA	373	A-127906.8	UGAGAGAAGUCCACCACGAGUCU	808	250_272
AD-63970.2	A-127905.5	ACUCGUGGUGGACUUCUCUCA	374	A-127922.1	UGAGAGAAGUCCACCACGAGUCU	809	250_272
AD-63971.2	A-127905.13	ACUCGUGGUGGACUUCUCUCA	375	A-127930.1	UGAGAGAAGUCCACCACGAGUCU	810	250_272
AD-63972.2	A-127941.1	ACUCGUGGUGGACUUCUCUCA	376	A-127942.1	UGAGAGAAGUCCACCACGAGUCU	811	250_272
AD-63973.2	A-127946.1	ACUCGUGGUGGACUUCUCUCA	377	A-127947.1	UGAGAGAAGTCCACCACGAGUCU	812	250_272

AD-63975.2	A-127915.1	ACUCGUGGUGGACUUCUCUCA	378	A-127906.9	UGAGAGAAGUCCACCACGAGUCU	813	250_272
AD-63976.2	A-127905.6	ACUCGUGGUGGACUUCUCUCA	379	A-127923.1	UGAGAGAAGUCCACCACGAGUCU	814	250_272
AD-63977.2	A-127917.2	ACUCGUGGUGGACUUCUCUCA	380	A-127931.1	UGAGAGAAGUCCACCACGAGUCU	815	250_272
AD-63978.2	A-127943.1	ACUCGUGGUGGACUUCUCUCA	381	A-127906.13	UGAGAGAAGUCCACCACGAGUCU	816	250_272
AD-63979.2	A-127908.1	ACUCGUGGUGGACUUCUCUCA	382	A-127906.2	UGAGAGAAGUCCACCACGAGUCU	817	250_272
AD-63980.2	A-127916.1	ACUCGUGGUGGACUUCUCUCA	383	A-127906.10	UGAGAGAAGUCCACCACGAGUCU	818	250_272
AD-63981.2	A-127905.7	ACUCGUGGUGGACUUCUCUCA	384	A-127924.1	UGAGAGAAGUCCACCACGAGUCU	819	250_272
AD-63982.2	A-127917.3	ACUCGUGGUGGACUUCUCUCA	385	A-127932.1	UGAGAGAAGUCCACCACGAGUCU	820	250_272
AD-63983.2	A-127944.1	ACUCGUGGUGGACUUCUCUCA	386	A-127940.2	UGAGAGAAGUCCACCACGAGUCU	821	250_272
AD-63985.2	A-127961.1	GUGGUGGACUUCUCUCUCAAUUU	387	A-127956.4	AAAUUGAGAGAAGUCCACCACGA	822	254_276
AD-63986.2	A-127969.1	GUGGUGGACUUCUCUCUCAAUUU	388	A-127956.12	AAAUUGAGAGAAGUCCACCACGA	823	254_276
AD-63987.2	A-127955.9	GUGGUGGACUUCUCUCUCAAUUU	389	A-127977.1	AAAUUGAGAGAAGUCCACCACGA	824	254_276
AD-63988.2	A-127986.1	UGGACUUCUCUCUCAAUUU	390	A-127987.1	AAAUUGAGAGAAGUCCACC	825	258_280
AD-63989.2	A-127996.1	GUGGUGGACUUCUCUCUCAAUUU	391	A-127992.2	AAAUUGAGAGAAGUCCACCACGA	826	254_276
AD-63990.2	A-127950.1	GGUGGACUUCUCUCUCAAUUUUUU	392	A-127951.1	AAAUUGAGAGAAGUCCACCUU	827	256_278
AD-63991.2	A-127962.1	GUGGUGGACUUCUCUCUCAAUUU	393	A-127956.5	AAAUUGAGAGAAGUCCACCACGA	828	254_276
AD-63992.2	A-127955.2	GUGGUGGACUUCUCUCUCAAUUU	394	A-127970.1	AAAUUGAGAGAAGUCCACCACGA	829	254_276
AD-63993.2	A-127955.10	GUGGUGGACUUCUCUCUCAAUUU	395	A-127978.1	AAAUUGAGAGAAGUCCACCACGA	830	254_276
AD-63994.2	A-127984.2	GGUGGACUUCUCUCUCAAUUU	396	A-127988.1	AAAUUGAGAGAAGUCCACCAC	831	256_278
AD-63995.2	A-127996.2	GUGGUGGACUUCUCUCUCAAUUU	397	A-127993.2	AAAUUGAGAGAAGUCCACCACGA	832	254_276
AD-63996.2	A-127952.1	GGUGGACUUCUCUCUCAAUUU	398	A-127953.1	AAAUUGAGAGAAGUCCACCUU	833	256_278
AD-63997.2	A-127963.1	GUGGUGGACUUCUCUCUCAAUUU	399	A-127956.6	AAAUUGAGAGAAGUCCACCACGA	834	254_276
AD-63999.2	A-127955.11	GUGGUGGACUUCUCUCUCAAUUU	400	A-127979.1	AAAUUGAGAGAAGUCCACCACGA	835	254_276
AD-64000.2	A-127986.2	UGGACUUCUCUCUCAAUUU	401	A-127989.1	AAAUUGAGAGAAGUCCACC	836	258_280

AD-64001.2	A-127996.3	GUGGUGGACUUCUCUCAAUUU	402	A-127994.2	AAAUUGAGAGAAGUCCACCACGA	837	254_276
AD-64002.2	A-127952.2	GGUGGACUUCUCUCAAUUU	403	A-127954.1	AAAUUGAGAGAAGUCCACCUU	838	256_278
AD-64003.2	A-127964.1	GUGGUGGACUUCUCUCAAUUU	404	A-127956.7	AAAUUGAGAGAAGUCCACCACGA	839	254_276
AD-64004.2	A-127955.4	GUGGUGGACUUCUCUCAAUUU	405	A-127972.1	AAAUUGAGAGAAGUCCACCACGA	840	254_276
AD-64005.2	A-127955.12	GUGGUGGACUUCUCUCAAUUU	406	A-127980.1	AAAUUGAGAGAAGUCCACCACGA	841	254_276
AD-64006.2	A-127990.1	GUGGUGGACUUCUCUCAAUUU	407	A-127991.1	AAAUUGAGAGAAGUCCACCACGA	842	254_276
AD-64007.2	A-127996.4	GUGGUGGACUUCUCUCAAUUU	408	A-127995.2	AAAUUGAGAGAAGUCCACCACGA	843	254_276
AD-64008.2	A-127955.1	GUGGUGGACUUCUCUCAAUUU	409	A-127956.1	AAAUUGAGAGAAGUCCACCACGA	844	254_276
AD-64008.4	A-127955.15	GUGGUGGACUUCUCUCAAUUU	410	A-127956.14	AAAUUGAGAGAAGUCCACCACGA	845	254_276
AD-64009.2	A-127965.1	GUGGUGGACUUCUCUCAAUUU	411	A-127956.8	AAAUUGAGAGAAGUCCACCACGA	846	254_276
AD-64010.2	A-127955.5	GUGGUGGACUUCUCUCAAUUU	412	A-127973.1	AAAUUGAGAGAAGUCCACCACGA	847	254_276
AD-64011.2	A-127955.13	GUGGUGGACUUCUCUCAAUUU	413	A-127981.1	AAAUUGAGAGAAGUCCACCACGA	848	254_276
AD-64012.2	A-127990.2	GUGGUGGACUUCUCUCAAUUU	414	A-127992.1	AAAUUGAGAGAAGUCCACCACGA	849	254_276
AD-64013.2	A-127997.1	GUGGUGGACTTCUCUCAAUUU	415	A-127998.1	AAAUUGAGAGAAGTCCACCACGA	850	254_276
AD-64014.2	A-127957.1	GUGGUGGACUUCUCUCAAUUU	416	A-127958.1	AAAUUGAGAGAAGUCCACCACGA	851	254_276
AD-64015.2	A-127966.1	GUGGUGGACUUCUCUCAAUUU	417	A-127956.9	AAAUUGAGAGAAGUCCACCACGA	852	254_276
AD-64016.2	A-127955.6	GUGGUGGACUUCUCUCAAUUU	418	A-127974.1	AAAUUGAGAGAAGUCCACCACGA	853	254_276
AD-64017.2	A-127968.2	GUGGUGGACUTCUCUCAAUUU	419	A-127982.1	AAAUUGAGAGAAGTCCACCACGA	854	254_276
AD-64018.2	A-127990.3	GUGGUGGACUUCUCUCAAUUU	420	A-127993.1	AAAUUGAGAGAAGUCCACCACGA	855	254_276
AD-64019.2	A-127959.1	GUGGUGGACUUCUCUCAAUUU	421	A-127956.2	AAAUUGAGAGAAGUCCACCACGA	856	254_276
AD-64020.2	A-127967.1	GUGGUGGACUUCUCUCAAUUU	422	A-127956.10	AAAUUGAGAGAAGUCCACCACGA	857	254_276
AD-64021.2	A-127955.7	GUGGUGGACUUCUCUCAAUUU	423	A-127975.1	AAAUUGAGAGAAGUCCACCACGA	858	254_276
AD-64022.2	A-127968.3	GUGGUGGACUTCUCUCAAUUU	424	A-127983.1	AAAUUGAGAGAAGTCCACCACGA	859	254_276
AD-64023.2	A-127990.4	GUGGUGGACUUCUCUCAAUUU	425	A-127994.1	AAAUUGAGAGAAGUCCACCACGA	860	254_276

AD-64024.2	A-127960.1	GUGGUGGACUUCUCUCAAUUU	426	A-127956.3	AAAUUGAGAGAAGUCCACCACGA	861	254_276
AD-64025.2	A-127968.1	GUGGUGGACUTCUCUCAAUUU	427	A-127956.11	AAAUUGAGAGAAGUCCACCACGA	862	254_276
AD-64026.2	A-127955.8	GUGGUGGACUUCUCUCAAUUU	428	A-127976.1	AAAUUGAGAGAAGUCCACCACGA	863	254_276
AD-64027.2	A-127984.1	GGUGGACUUCUCUCAAUUU	429	A-127985.1	AAAUUGAGAGAAGUCCACCAC	864	256_278
AD-64028.2	A-127990.5	GUGGUGGACUUCUCUCAAUUU	430	A-127995.1	AAAUUGAGAGAAGUCCACCACGA	865	254_276
AD-64272.2	A-128001.2	GUGCACUUCGCUUCACCUCUG	431	A-128002.2	CAGAGGUGAAGCGAAGUGCACAC	866	1577_1599
AD-64274.1	A-128363.1	GUUGACAAAAAAUCCUCACAAU	432	A-128364.1	AUUGUGAGGAUUUUUUGUCAACAA	867	215_237
AD-64275.1	A-128377.1	UGUUGACAAAAAAUCCUCACAA	433	A-128378.1	UUGUGAGGAUUUUUUGUCAACAAG	868	214_236
AD-64276.1	A-128393.1	GGUGGACUUCUCUCAAUUUUA	434	A-128394.1	UAAAAUUGAGAGAAGUCCACCAC	869	256_278
AD-64277.1	A-128407.1	UCUUUUUGGAGUGUGGAUUCGA	435	A-128408.1	UCGAAUCCACACUCCAAAAGACA	870	2259_2281
AD-64277.1	A-128407.1	UCUUUUUGGAGUGUGGAUUCGA	436	A-128408.1	UCGAAUCCACACUCCAAAAGACA	871	2259_2281
AD-64278.1	A-128423.1	ACUGUUCAAGCCUCCAAGCUA	437	A-128424.1	UAGCUUGGAGGCUUGAACAAGAC	872	1857_1879
AD-64279.1	A-128435.1	UCUGCCGAUCCAUACUGCGGA	438	A-128436.1	UCCGCAGUAUGGAUCGGCAGAGG	873	1255_1277
AD-64280.1	A-128379.1	AUGUGUCUGCGGCGUUUUAUA	439	A-128380.1	UAUAAAACGCCGCAGACACAUCC	874	375_397
AD-64281.1	A-128395.1	CCCCGUCUGUGCCUUCUCAUA	440	A-128396.1	UAUGAGAAGGCACAGACGGGGAG	875	1545_1567
AD-64282.1	A-128409.1	GCCUAAUCAUCUCUUGUUCAU	441	A-128410.1	AUGAACAAGAGAUGAUUAGCGAG	876	1831_1853
AD-64283.1	A-128425.1	UCUAGACUCGUGGUGGACUUC	442	A-128426.1	GAAGUCCACCACGAGUCUAGACU	877	245_267
AD-64284.1	A-128437.1	CUGCCGAUCCAUACUGCGGAA	443	A-128438.1	UUCCGCAGUAUGGAUCGGCAGAG	878	1256_1278
AD-64285.1	A-128365.1	UUUUUCUUGUUUGACAAAAAUA	444	A-128366.1	UAUUUUUGUCAACAAGAAAAAACC	879	207_229
AD-64286.1	A-128381.1	AUCUUCUUGUUGGUUCUUCUA	445	A-128382.1	UAGAAGAACCAACAAGAAGAUGA	880	426_448
AD-64289.1	A-128367.1	GUUUUUCUUGUUUGACAAAAAU	446	A-128368.1	AUUUUUGUCAACAAGAAAAAACCC	881	206_228
AD-64290.1	A-128383.1	CUGCCUAAUCAUCUCUUGUUA	447	A-128384.1	UAACAAGAGAUGAUUAGGCAGAG	882	1829_1851
AD-64291.1	A-128399.1	UCCUCACAAUACCACAGAGUA	448	A-128400.1	UACUCUGUGGUAUUGUGAGGAUU	883	226_248
AD-64292.1	A-128413.1	CUUGUUGACAAAAAAUCCUCAA	449	A-128414.1	UUGAGGAUUUUUUGUCAACAAGAA	884	212_234

AD-64293.1	A-128439.1	GCAACUUUUUUCACCUCUGCCU	450	A-128440.1	AGGCAGAGGUGAAAAAGUUGCAU	885	1814_1836
AD-64294.1	A-128369.1	GGGAACAAGAGCUACAGCAUA	451	A-128370.1	UAUGCUGUAGCUCUUGUUCCCAA	886	2828_2850
AD-64295.1	A-128385.1	CGUGGUGGACUUUCUCUCAAUU	452	A-128386.1	AAUUGAGAGAAGUCCACCAGCAG	887	253_275
AD-64297.1	A-128415.1	CUGCUGCUAUGCCUCAUCUUA	453	A-128416.1	UAAGAUGAGGCAUAGCAGCAGGA	888	411_433
AD-64298.1	A-128427.1	GUUGGAUGUGUCUGCGGCGUU	454	A-128428.1	AACGCCGCAGACACAUCCAACGA	889	370_392
AD-64299.1	A-128441.1	UUCAUCCUGCUGCUAUGCCUA	455	A-128442.1	UAGGCAUAGCAGCAGGAUGAAGA	890	405_427
AD-64300.1	A-128371.1	UUCUUUGUUGACAAAAAAUCCUA	456	A-128372.1	UAGGAUUUUUUGUCAACAAGAAAA	891	210_232
AD-64302.1	A-128417.1	UAUAUGGAUGAUGUGGUAUUA	457	A-128418.1	UAAUACCACAUCAUCCAUAUAAC	892	734_756
AD-64303.1	A-128429.1	UUCAUCCUGCUGCUAUGCCUC	458	A-128430.1	GAGGCAUAGCAGCAGGAUGAAGA	893	405_427
AD-64304.1	A-128443.1	GUGCACUUUCGCUUCACCUCUA	459	A-128444.1	UAGAGGUGAAGCGAAGUGCACAC	894	1577_1599
AD-64305.1	A-128373.1	UUGACAAAAAAUCCUCACAAUA	460	A-128374.1	UAUUGUGAGGAUUUUUGUCAACA	895	216_238
AD-64307.1	A-128403.1	AAGCCUCCAAGCUGUGCCUUA	461	A-128404.1	UAAGGCACAGCUUGGAGGCUUGA	896	1864_1886
AD-64308.1	A-128419.1	CCUCUUCAUCCUGCUGCUAUA	462	A-128420.1	UAUAGCAGCAGGAUGAAGAGGAA	897	401_423
AD-64309.1	A-128431.1	CCUGCUGCUAUGCCUCAUCUU	463	A-128432.1	AAGAUGAGGCAUAGCAGCAGGAU	898	410_432
AD-64310.1	A-128375.1	CAUCUUCUUGUUGGUUCUUCU	464	A-128376.1	AGAAGAACCAACAAGAAGAUGAG	899	425_447
AD-64311.1	A-128391.1	CCGUCUGUGCCUUCUCAUCUA	465	A-128392.1	UAGAUGAGAAGGCACAGACGGGG	900	1547_1569
AD-64312.1	A-128405.1	CCUCAUCUUCUUGUUGGUUCU	466	A-128406.1	AGAACCAACAAGAAGAUGAGGCA	901	422_444
AD-64313.1	A-128421.1	CCACCAAAUGCCCCUAUCUUA	467	A-128422.1	UAAGAUAGGGGCAUUUGGUGGUC	902	2298_2320
AD-64314.1	A-128433.1	GCUCCUCUGCCGAUCCAUACU	468	A-128434.1	AGUAUGGAUCGGCAGAGGAGCCA	903	1250_1272
AD-64315.1	A-128363.2	GUUGACAAAAAAUCCUCACAAU	469	A-128445.1	AUUGUGAGGAUUUUUGUCAACAA	904	215_237
AD-64316.1	A-128377.2	UGUUGACAAAAAAUCCUCACAA	470	A-128453.1	UUGUGAGGAUUUUUGUCAACAAG	905	214_236
AD-64317.1	A-128393.2	GGUGGACUUCUCUCAAUUUUA	471	A-128461.1	UAAAAUUGAGAGAAGUCCACCAC	906	256_278
AD-64318.1	A-128407.2	UCUUUUUGGAGUGUGGAUUCGA	472	A-128469.1	UCGAAUCCACACUCCAAAAGACA	907	2259_2281
AD-64318.1	A-128407.2	UCUUUUUGGAGUGUGGAUUCGA	473	A-128469.1	UCGAAUCCACACUCCAAAAGACA	908	2259_2281

AD-64319.1	A-128423.2	ACUGUUCAAGCCUCCAAGCUA	474	A-128477.1	UAGCUUGGAGGCUUGAACAAGAC	909	1857_1879
AD-64320.1	A-128435.2	UCUGCCGAUCCAUACUGCGGA	475	A-128483.1	UCCGCAGUAUGGAUCGGCAGAGG	910	1255_1277
AD-64321.1	A-123463.3	AGUUAUAUGGAUGAUGUGGUA	476	A-128446.1	UACCACAUCAUCCAUAUAACUGA	911	731_753
AD-64322.1	A-128379.2	AUGUGUCUGCGGCGUUUUAUA	477	A-128454.1	UAUAAAACGCCGCAGACACAUCC	912	375_397
AD-64323.1	A-128395.2	CCCCGUCUGUGCCUUCUCAUA	478	A-128462.1	UAUGAGAAGGCACAGACGGGGAG	913	1545_1567
AD-64324.1	A-128409.2	GCCUAAUCAUCUCUUGUUCAU	479	A-128470.1	AUGAACAAGAGAUGAUUAGCGAG	914	1831_1853
AD-64325.1	A-128425.2	UCUAGACUCGUGGUGGACUUC	480	A-128478.1	GAAGUCCACCACGAGUCUAGACU	915	245_267
AD-64326.1	A-128437.2	CUGCCGAUCCAUACUGCGGAA	481	A-128484.1	UUCCGCAGUAUGGAUCGGCAGAG	916	1256_1278
AD-64328.1	A-128381.2	AUCUUCUUGUUGGUUCUUCUA	482	A-128455.1	UAGAAGAACCAACAAGAAGAUGA	917	426_448
AD-64330.1	A-128411.2	UUCUCUCAAUUUUCUAGGGGA	483	A-128471.1	UCCCCUAGAAAAUUGAGAGAAGU	918	263_285
AD-64331.1	A-127905.16	ACUCGUGGUGGACUUCUCUCA	484	A-127907.2	UGAGAGAAGUCCACCACGAGUCU	919	250_272
AD-64332.1	A-128001.3	GUGCACUUCGCUUCACCUCUG	485	A-128485.1	CAGAGGUGAAGCGAAGUGCACAC	920	1577_1599
AD-64333.1	A-128367.2	GUUUUUCUUGUUGACAAAAAU	486	A-128448.1	AUUUUUGUCAACAAGAAAAACCC	921	206_228
AD-64334.1	A-128383.2	CUGCCUAAUCAUCUCUUGUUA	487	A-128456.1	UAACAAGAGAUGAUUAGGCAGAG	922	1829_1851
AD-64335.1	A-128399.2	UCCUCACAAUACCACAGAGUA	488	A-128464.1	UACUCUGUGGUAUUGUGAGGAUU	923	226_248
AD-64336.1	A-128413.2	CUUGUUGACAAAAAAUCCUCAA	489	A-128472.1	UUGAGGAUUUUUUGUCAACAAGAA	924	212_234
AD-64337.1	A-127955.16	GUGGUGGACUUCUCUCAAUUU	490	A-127958.2	AAAUUGAGAGAAGUCCACCACGA	925	254_276
AD-64338.1	A-128439.2	GCAACUUUUUUCACCUCUGCCU	491	A-128486.1	AGGCAGAGGUGAAAAAAGUUGCAU	926	1814_1836
AD-64339.1	A-128369.2	GGGAACAAGAGCUACAGCAUA	492	A-128449.1	UAUGCUGUAGCUCUUGUUCCCAA	927	2828_2850
AD-64341.1	A-128401.2	UCAUCUUCUUGUUGGUUCUUA	493	A-128465.1	UAAGAACCAACAAGAAGAUGAGG	928	424_446
AD-64342.1	A-128415.2	CUGCUGCUAUGCCUCAUCUUA	494	A-128473.1	UAAGAUGAGGCAUAGCAGCAGGA	929	411_433
AD-64343.1	A-128427.2	GUUGGAUGUGUCUGCGGCGUU	495	A-128479.1	AACGCCGCAGACACAUCCAACGA	930	370_392
AD-64344.1	A-128441.2	UUCAUCCUGCUGCUAUGCCUA	496	A-128487.1	UAGGCAUAGCAGCAGGAUGAAGA	931	405_427
AD-64345.1	A-128371.2	UUCUUGUUGACAAAAAAUCCUA	497	A-128450.1	UAGGAUUUUUUGUCAACAAGAAAA	932	210_232

AD-64347.1	A-123487.3	GGAUGUGUCUGCGGCGUUUUA	498	A-128466.1	UAAAACGCCGCAGACACAUCCAG	933	373_395
AD-64348.1	A-128417.2	UAUAUGGAUGAUGUGGUAUUA	499	A-128474.1	UAAUACCACAUCAUCCAUAUAAC	934	734_756
AD-64349.1	A-128429.2	UUCAUCCUGCUGCUAUGCCUC	500	A-128480.1	GAGGCAUAGCAGCAGGAUGAAGA	935	405_427
AD-64350.1	A-128443.2	GUGCACUUUCGCUUUCACCUCUA	501	A-128488.1	UAGAGGUGAAGCGAAGUGCACAC	936	1577_1599
AD-64351.1	A-128373.2	UUGACAAAAAAUCCUCACAAUA	502	A-128451.1	UAUUGUGAGGAUUUUUUGUCAACA	937	216_238
AD-64352.1	A-128389.2	CCAAGUGUUUGCUGACGCAAA	503	A-128459.1	UUUGCGUCAGCAAACACUUGGCA	938	1174_1196
AD-64352.1	A-128389.2	CCAAGUGUUUGCUGACGCAAA	504	A-128459.1	UUUGCGUCAGCAAACACUUGGCA	939	1174_1196
AD-64353.1	A-128403.2	AAGCCUCCAAGCUGUGCCUUA	505	A-128467.1	UAAGGCACAGCUUGGAGGCUUGA	940	1864_1886
AD-64354.1	A-128419.2	CCUCUUCAUCCUGCUGCUAUA	506	A-128475.1	UAUAGCAGCAGGAUGAAGAGGAA	941	401_423
AD-64355.1	A-128431.2	CCUGCUGCUAUGCCUCAUCUU	507	A-128481.1	AAGAUGAGGCAUAGCAGCAGGAU	942	410_432
AD-64356.1	A-128375.2	CAUCUUCUUGUUGGUUUCUUCU	508	A-128452.1	AGAAGAACCAACAAGAAGAUGAG	943	425_447
AD-64357.1	A-128391.2	CCGUCUGUGCCUUCUCAUCUA	509	A-128460.1	UAGAUGAGAAGGCACAGACGGGG	944	1547_1569
AD-64358.1	A-128405.2	CCUCAUCUUCUUGUUGGUUCU	510	A-128468.1	AGAACCAACAAGAAGAUGAGGCA	945	422_444
AD-64359.1	A-128421.2	CCACCAAAUGCCCCUAUCUUA	511	A-128476.1	UAAGAUAGGGGCAUUUUGGUGGUC	946	2298_2320
AD-64360.1	A-128433.2	GCUCCUCUGCCGAUCCAUACU	512	A-128482.1	AGUAUGGAUCGGCAGAGGAGCCA	947	1250_1272
AD-64700.1	A-129379.1	ACUCGUGGUGTACUUCUCUCA	513	A-127906.26	UGAGAGAAGUCCACCACGAGUCU	948	250_272
AD-64701.1	A-127905.20	ACUCGUGGUGGACUUCUCUCA	514	A-129387.1	UGAGAGAAGTCCACCACGAGUCU	949	250_272
AD-64702.1	A-127905.28	ACUCGUGGUGGACUUCUCUCA	515	A-129395.1	UGAGAGAAGUCCACCACGAGUCU	950	250_272
AD-64703.1	A-129376.2	ACUCGUGGUGGACUUCACUCA	516	A-129385.5	UGAGAGAAGTCCACCACGAGUCU	951	250_272
AD-64704.1	A-129381.3	ACUCGUGGTGTACUUCACUCA	517	A-129389.6	UGAGAGAAGUCCACCACGAGUCU	952	250_272
AD-64705.1	A-129380.1	ACUCGUGGUGTACUUCACUCA	518	A-127906.27	UGAGAGAAGUCCACCACGAGUCU	953	250_272
AD-64706.1	A-127905.21	ACUCGUGGUGGACUUCUCUCA	519	A-129388.1	UGAGAGAAGUCCACCACGAGUCU	954	250_272
AD-64707.1	A-127905.29	ACUCGUGGUGGACUUCUCUCA	520	A-129396.1	UGAGAGAAGTCCACCACGAGUCU	955	250_272
AD-64708.1	A-129382.2	ACUCGUGGTGGACUUCTCUCA	521	A-129385.6	UGAGAGAAGTCCACCACGAGUCU	956	250_272

AD-64709.1	A-129373.4	ACUCGUGGUGGACUUCUCUCA	522	A-129391.2	UGAGAGAAGTCCACCACGAGUCU	957	250_272
AD-64710.1	A-129373.1	ACUCGUGGUGGACUUCUCUCA	523	A-127906.20	UGAGAGAAGUCCACCACGAGUCU	958	250_272
AD-64711.1	A-129381.1	ACUCGUGGUGTACUUCACUCA	524	A-127906.28	UGAGAGAAGUCCACCACGAGUCU	959	250_272
AD-64712.1	A-127905.22	ACUCGUGGUGGACUUCUCUCA	525	A-129389.1	UGAGAGAAGUCCACCACGAGUCU	960	250_272
AD-64713.1	A-127905.30	ACUCGUGGUGGACUUCUCUCA	526	A-129397.1	UGAGAGAAGTCCACCACGAGUCU	961	250_272
AD-64714.1	A-129384.2	ACUCGUGGTGGACUUCACUCA	527	A-129385.7	UGAGAGAAGTCCACCACGAGUCU	962	250_272
AD-64715.1	A-129376.4	ACUCGUGGUGGACUUCACUCA	528	A-129391.3	UGAGAGAAGTCCACCACGAGUCU	963	250_272
AD-64716.1	A-129374.1	ACUCGUGGUGGACUUCUCUCA	529	A-127906.21	UGAGAGAAGUCCACCACGAGUCU	964	250_272
AD-64717.1	A-129382.1	ACUCGUGGTGGACUUCTCUCA	530	A-127906.29	UGAGAGAAGUCCACCACGAGUCU	965	250_272
AD-64718.1	A-127905.23	ACUCGUGGUGGACUUCUCUCA	531	A-129390.1	UGAGAGAAGUCCACCACGAGUCU	966	250_272
AD-64719.1	A-127917.5	ACUCGUGGUGGACUUCTCUCA	532	A-129385.2	UGAGAGAAGTCCACCACGAGUCU	967	250_272
AD-64720.1	A-129381.2	ACUCGUGGTGTACUUCACUCA	533	A-129385.8	UGAGAGAAGTCCACCACGAGUCU	968	250_272
AD-64721.1	A-129382.4	ACUCGUGGTGGACUUCTCUCA	534	A-129391.4	UGAGAGAAGTCCACCACGAGUCU	969	250_272
AD-64722.1	A-129375.1	ACUCGUGGUGGACUUCCUCA	535	A-127906.22	UGAGAGAAGUCCACCACGAGUCU	970	250_272
AD-64723.1	A-129383.1	ACUCGUGGUGGACUUCTCUCA	536	A-127906.30	UGAGAGAAGUCCACCACGAGUCU	971	250_272
AD-64725.1	A-127917.6	ACUCGUGGUGGACUUCTCUCA	537	A-129398.1	UGAGAGAAGTCCACCACGAGUCU	972	250_272
AD-64726.1	A-129373.3	ACUCGUGGUGGACUUCUCUCA	538	A-129389.2	UGAGAGAAGUCCACCACGAGUCU	973	250_272
AD-64727.1	A-129384.4	ACUCGUGGTGGACUUCACUCA	539	A-129391.5	UGAGAGAAGTCCACCACGAGUCU	974	250_272
AD-64728.1	A-129376.1	ACUCGUGGUGGACUUCACUCA	540	A-127906.23	UGAGAGAAGUCCACCACGAGUCU	975	250_272
AD-64729.1	A-129384.1	ACUCGUGGTGGACUUCACUCA	541	A-127906.31	UGAGAGAAGUCCACCACGAGUCU	976	250_272
AD-64730.1	A-127905.25	ACUCGUGGUGGACUUCUCUCA	542	A-129392.1	UGAGAGAAGTCCACCACGAGUCU	977	250_272
AD-64731.1	A-129399.1	ACUCGUGGUGGACUUCTCUCA	543	A-129385.3	UGAGAGAAGTCCACCACGAGUCU	978	250_272
AD-64732.1	A-129376.3	ACUCGUGGUGGACUUCACUCA	544	A-129389.3	UGAGAGAAGUCCACCACGAGUCU	979	250_272
AD-64733.1	A-129381.4	ACUCGUGGTGTACUUCACUCA	545	A-129391.6	UGAGAGAAGTCCACCACGAGUCU	980	250_272

AD-64734.1	A-129377.1	ACUCGUGGUGGACUUCCCUCA	546	A-127906.24	UGAGAGAAGUCCACCACGAGUCU	981	250_272
AD-64735.1	A-127905.18	ACUCGUGGUGGACUUCUCUCA	547	A-129385.1	UGAGAGAAGTCCACCACGAGUCU	982	250_272
AD-64736.1	A-127905.26	ACUCGUGGUGGACUUCUCUCA	548	A-129393.1	UGAGAGAAGTCCACCACGAGUCU	983	250_272
AD-64737.1	A-129399.2	ACUCGUGGUGGACUUCTCUCA	549	A-129398.2	UGAGAGAAGTCCACCACGAGUCU	984	250_272
AD-64738.1	A-129382.3	ACUCGUGGTGGACUUCTCUCA	550	A-129389.4	UGAGAGAAGUCCACCACGAGUCU	985	250_272
AD-64739.1	A-129378.1	ACUCGUGGUGGACUUCGCUCA	551	A-127906.25	UGAGAGAAGUCCACCACGAGUCU	986	250_272
AD-64740.1	A-127905.19	ACUCGUGGUGGACUUCUCUCA	552	A-129386.1	UGAGAGAAGTCCACCACGAGUCU	987	250_272
AD-64741.1	A-127905.27	ACUCGUGGUGGACUUCUCUCA	553	A-129394.1	UGAGAGAAGTCCACCACGAGUCU	988	250_272
AD-64742.1	A-129373.2	ACUCGUGGUGGACUUCUCUCA	554	A-129385.4	UGAGAGAAGTCCACCACGAGUCU	989	250_272
AD-64743.1	A-129384.3	ACUCGUGGTGGACUUCACUCA	555	A-129389.5	UGAGAGAAGUCCACCACGAGUCU	990	250_272

Table 4. Modified Sense and Antisense Strand Sequences of HBV dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-61522.2	A-123463.2	AfsgsUfuAfuAfuGfGfAfuGfaUfgUfgGfuAfL96	991	A-123464.2	usAfscCfaCfaUfcAfuccAfuAfuAfaCfusgsa	1210
AD-61547.2	A-123487.2	GfsgsAfuGfuGfuCfUfGfcGfgCfgUfuUfuAfL96	992	A-123488.2	usAfsaAfaCfgCfcGfcagAfcAfcAfuCfcsasg	1211
AD-63938.2	A-127896.1	Y44ACUCGUGGUGGACUUCUCUCA	993	A-127897.1	UGAGAGAAGUCCACCACGAGUCU	1212
AD-63939.2	A-127909.1	ascsucGfuGfgUfGfGfaCfuucUfcucaL96	994	A-127906.3	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1213
AD-63940.2	A-127917.1	ascsucguggugdGacuuc(Tgn)cucaL96	995	A-127906.11	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1214
AD-63940.3	A-127917.4	ascsucguggugdGacuuc(Tgn)cucaL96	996	A-127906.19	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1215
AD-63941.2	A-127905.8	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	997	A-127925.1	usGfsaGfagaAfguccaCfcAfcgaGfuscsu	1216
AD-63942.2	A-127933.1	uscsGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	998	A-127934.1	usGfsaGfaGfaAfgUfccaCfcAfcGfasgsu	1217

AD-63943.2	A-127944.2	ascsucGfuGfguGfGfaCfuucucucaL96	999	A-127942.2	usGfsAfgaGfaAfgUfccaCfcAfcGfaguscsu	1218
AD-63945.2	A-127910.1	ascsucguGfgUfGfGfaCfuucUfcucaL96	1000	A-127906.4	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1219
AD-63946.2	A-127918.1	ascsucguGfgUfGfGfacuuCfucucaL96	1001	A-127906.12	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1220
AD-63947.2	A-127905.9	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1002	A-127926.1	usGfsaGfagaagUfccaCfcAfcgaGfuscsu	1221
AD-63948.2	A-127935.1	gsusGfgUfGfGfaCfuUfcUfcUfcAfL96	1003	A-127936.1	usGfsaGfaGfaAfgUfccaCfcAfcsgsa	1222
AD-63949.2	A-127944.3	ascsucGfuGfguGfGfaCfuucucucaL96	1004	A-127906.14	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1223
AD-63950.2	A-127900.1	Y44UfcGfuGfgUfgGfaCfuUfcUfcUfcAfusuY44	1005	A-127901.1	usGfsasGfaGfaAfgUfcCfaCfcAfcGfausu	1224
AD-63951.2	A-127911.1	ascsucguGfgUfGfGfaCfuucucucaL96	1006	A-127906.5	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1225
AD-63952.2	A-127905.2	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1007	A-127919.1	usGfsaGfaGfaagUfccaCfcAfcGfaGfuscsu	1226
AD-63953.2	A-127905.10	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1008	A-127927.1	usGfsagagaAfgUfccaCfcAfcgaguscsu	1227
AD-63955.2	A-127945.1	ascsucgugguGfGfacuucucucaL96	1009	A-127940.3	usGfsAfgAfgAfaGfuccaCfCfaCfgAfguscsu	1228
AD-63956.2	A-127902.1	Y44uscsGfuGfgUfgGfaCfuUfcUfcUfcAfY44	1010	A-127903.1	usGfsaGfaGfaAfgUfcCfaCfcAfcGfasusu	1229
AD-63957.2	A-127912.1	ascsucguGfgUfGfGfacuucucucaL96	1011	A-127906.6	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1230
AD-63958.2	A-127905.3	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1012	A-127920.1	usGfsagaGfaAfgUfccaCfcAfcgaGfuscsu	1231
AD-63959.2	A-127905.11	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1013	A-127928.1	usGfsaGfagaAfguccaCfcAfcgaguscsu	1232
AD-63960.2	A-126619.2	usasUfuUfCfCfuAfgGfgUfaCfaAfL96	1014	A-127938.1	PusGfsaGfaGfaAfgUfccaCfcAfcsgsa	1233
AD-63961.2	A-127945.2	ascsucgugguGfGfacuucucucaL96	1015	A-127942.3	usGfsAfgaGfaAfgUfccaCfcAfcGfaguscsu	1234
AD-63962.2	A-127902.2	Y44uscsGfuGfgUfgGfaCfuUfcUfcUfcAfY44	1016	A-127904.1	PusGfsaGfaGfaAfgUfcCfaCfcAfcGfasusu	1235
AD-63963.2	A-127913.1	ascsucguggUfgGfacuucucucaL96	1017	A-127906.7	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1236
AD-63964.2	A-127905.4	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1018	A-127921.1	usGfsaGfaGfaAfgUfccaCfcAfcgaguscsu	1237
AD-63965.2	A-127905.12	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1019	A-127929.1	usGfsagaGfaaGfuccaCfcAfcgaguscsu	1238
AD-63966.2	A-127939.1	ascsUfcGfugguGfGfaCfuuCfuCfucaL96	1020	A-127940.1	usGfsAfgAfgAfaGfuccaCfCfaCfgAfguscsu	1239
AD-63967.2	A-127945.3	ascsucgugguGfGfacuucucucaL96	1021	A-127906.15	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1240
AD-63968.2	A-127905.1	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1022	A-127906.1	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1241

AD-63968.2	A-127905.1	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1023	A-127906.1	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1242
AD-63968.4	A-127905.15	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1024	A-127906.17	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1243
AD-63968.5	A-127905.17	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1025	A-127906.18	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1244
AD-63969.2	A-127914.1	ascsucguggugGfacuucucucaL96	1026	A-127906.8	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1245
AD-63970.2	A-127905.5	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1027	A-127922.1	usGfsagaGfaagUfccaCfcAfcgaGfuscsu	1246
AD-63971.2	A-127905.13	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1028	A-127930.1	usGfsagaGfaaguccaCfcAfcgaguscsu	1247
AD-63972.2	A-127941.1	ascsUfcGfuGfguGfGfaCfuuCfuCfucaL96	1029	A-127942.1	usGfsAfgaGfaAfgUfccaCfcAfcGfaguscsu	1248
AD-63973.2	A-127946.1	ascsucguggudGdGacuucucucaL96	1030	A-127947.1	usdGsaGfaGfaAfgdTccadCcAfcGfaguscsu	1249
AD-63975.2	A-127915.1	ascsucguggUfgGfacuuc(Tgn)cucaL96	1031	A-127906.9	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1250
AD-63976.2	A-127905.6	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1032	A-127923.1	usGfsagaGfaAfgUfccaCfcAfcgaguscsu	1251
AD-63977.2	A-127917.2	ascsucguggugdGacuuc(Tgn)cucaL96	1033	A-127931.1	usdGsagagaaguccadCcacgaguscsu	1252
AD-63978.2	A-127943.1	ascsUfcGfuGfguGfGfaCfuUfcUfcUfcaL96	1034	A-127906.13	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1253
AD-63979.2	A-127908.1	ascsucGfuGfgUfGfGfaCfuucUfcucAfL96	1035	A-127906.2	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1254
AD-63980.2	A-127916.1	ascsucguggugGfacuuc(Tgn)cucaL96	1036	A-127906.10	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1255
AD-63981.2	A-127905.7	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1037	A-127924.1	usGfsaGfagaAfgUfccaCfcAfcgaGfuscsu	1256
AD-63982.2	A-127917.3	ascsucguggugdGacuuc(Tgn)cucaL96	1038	A-127932.1	PusdGsagagaaguccadCcacgaguscsu	1257
AD-63983.2	A-127944.1	ascsucGfuGfguGfGfaCfuucucucaL96	1039	A-127940.2	usGfsAfgAfgAfaGfuccaCfCfaCfgAfguscsu	1258
AD-63985.2	A-127961.1	gsusggugGfaCfUfUfcUfcucAfauuuL96	1040	A-127956.4	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1259
AD-63986.2	A-127969.1	gsusggugGfaCfUfUfcucuCfaauuuL96	1041	A-127956.12	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1260
AD-63987.2	A-127955.9	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1042	A-127977.1	asAfsaUfugagaGfaagUfcCfaccAfcsgsa	1261
AD-63988.2	A-127986.1	usgsGfaCfUfUfcUfcUfcAfaUfuUfL96	1043	A-127987.1	asAfsaUfuGfaGfaGfaagUfcCfascsc	1262
AD-63989.2	A-127996.1	gsusgguggacUfUfcucucaauuuL96	1044	A-127992.2	asAfsAfUfuGfaGfaGfaagUfcCfaCfcacsgsa	1263
AD-63990.2	A-127950.1	Y44GfgUfgGfaCfuUfcUfcUfcAfaUfuUfusuY44	1045	A-127951.1	asAfsasUfuGfaGfaGfaAfgUfcCfaCfcusu	1264
AD-63991.2	A-127962.1	gsusggugGfaCfUfUfcUfcucaauuuL96	1046	A-127956.5	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1265

AD-63992.2	A-127955.2	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1047	A-127970.1	asAfsaUfuGfagaGfaagUfcCfaCfcAfcsgsa	1266
AD-63993.2	A-127955.10	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1048	A-127978.1	asAfsauugaGfaGfaagUfcCfaccacsgsa	1267
AD-63994.2	A-127984.2	gsgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1049	A-127988.1	PasAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	1268
AD-63995.2	A-127996.2	gsusgguggacUfUfcucucaauuuL96	1050	A-127993.2	asAfsAfuuGfaGfaGfaagUfCfcaCfcacsgsa	1269
AD-63996.2	A-127952.1	Y44gsgsUfgGfaCfuUfcUfcUfcAfaUfuUfY44	1051	A-127953.1	asAfsaUfuGfaGfaGfaAfgUfcCfaCfcsusu	1270
AD-63997.2	A-127963.1	gsusggugGfaCfUfUfcucucaauuuL96	1052	A-127956.6	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1271
AD-63999.2	A-127955.11	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1053	A-127979.1	asAfsaUfugaGfagaagUfcCfaccacsgsa	1272
AD-64000.2	A-127986.2	usgsGfaCfUfUfcUfcUfcAfaUfuUfL96	1054	A-127989.1	PasAfsaUfuGfaGfaGfaagUfcCfascsc	1273
AD-64001.2	A-127996.3	gsusgguggacUfUfcucucaauuuL96	1055	A-127994.2	asAfsAfUfuGfaGfaGfaagUfCfcaCfcacsgsa	1274
AD-64002.2	A-127952.2	Y44gsgsUfgGfaCfuUfcUfcUfcAfaUfuUfY44	1056	A-127954.1	PasAfsaUfuGfaGfaGfaAfgUfcCfaCfcsusu	1275
AD-64003.2	A-127964.1	gsusgguggaCfuUfcucucaauuuL96	1057	A-127956.7	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1276
AD-64004.2	A-127955.4	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1058	A-127972.1	asAfsaUfuGfaGfaGfaagUfcCfaccacsgsa	1277
AD-64005.2	A-127955.12	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1059	A-127980.1	asAfsauuGfagAfgaagUfcCfaccacsgsa	1278
AD-64006.2	A-127990.1	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	1060	A-127991.1	asAfsaUfuGfaGfaGfaagUfcCfaCfcacsgsa	1279
AD-64007.2	A-127996.4	gsusgguggacUfUfcucucaauuuL96	1061	A-127995.2	asAfsAfUfugaGfaGfaagUfCfcaCfcacsgsa	1280
AD-64008.2	A-127955.1	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1062	A-127956.1	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1281
AD-64008.2	A-127955.1	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1063	A-127956.1	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1282
AD-64008.4	A-127955.15	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1064	A-127956.14	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1283
AD-64009.2	A-127965.1	gsusgguggacuUfcucucaauuuL96	1065	A-127956.8	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1284
AD-64010.2	A-127955.5	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1066	A-127973.1	asAfsauuGfagaGfaagUfcCfaccAfcsgsa	1285
AD-64011.2	A-127955.13	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1067	A-127981.1	asAfsauuGfagagaagUfcCfaccacsgsa	1286
AD-64012.2	A-127990.2	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	1068	A-127992.1	asAfsAfUfuGfaGfaGfaagUfcCfaCfcacsgsa	1287
AD-64013.2	A-127997.1	gsusgguggacdTdTcucucaauuuL96	1069	A-127998.1	asdAsAfuugaGfaGfaagdTdCcaCfcacsgsa	1288
AD-64014.2	A-127957.1	Y44GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1070	A-127958.1	PasAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1289

AD-64015.2	A-127966.1	gsusgguggaCfuUfcucuc(Agn)auuuL96	1071	A-127956.9	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1290
AD-64016.2	A-127955.6	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1072	A-127974.1	asAfsauuGfaGfaGfaagUfcCfaccacsgsa	1291
AD-64017.2	A-127968.2	gsusgguggacudTcucuc(Agn)auuuL96	1073	A-127982.1	asdAsauugagagaagdTccaccacsgsa	1292
AD-64018.2	A-127990.3	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	1074	A-127993.1	asAfsAfuuGfaGfaGfaagUfCfcaCfcacsgsa	1293
AD-64019.2	A-127959.1	gsusggUfgGfaCfUfUfcUfcucAfauuUfL96	1075	A-127956.2	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1294
AD-64020.2	A-127967.1	gsusgguggacuUfcucuc(Agn)auuuL96	1076	A-127956.10	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1295
AD-64021.2	A-127955.7	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1077	A-127975.1	asAfsaUfugaGfaGfaagUfcCfaccAfcsgsa	1296
AD-64022.2	A-127968.3	gsusgguggacudTcucuc(Agn)auuuL96	1078	A-127983.1	PasdAsauugagagaagdTccaccacsgsa	1297
AD-64023.2	A-127990.4	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	1079	A-127994.1	asAfsAfUfuGfaGfaGfaagUfCfcaCfcacsgsa	1298
AD-64024.2	A-127960.1	gsusggUfgGfaCfUfUfcUfcucAfauuuL96	1080	A-127956.3	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1299
AD-64025.2	A-127968.1	gsusgguggacudTcucuc(Agn)auuuL96	1081	A-127956.11	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1300
AD-64026.2	A-127955.8	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1082	A-127976.1	asAfsaUfugaGfagaagUfcCfaccAfcsgsa	1301
AD-64027.2	A-127984.1	gsgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1083	A-127985.1	asAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	1302
AD-64028.2	A-127990.5	gsusGfgugGfaCfUfUfcUfcUfcAfaUfuuL96	1084	A-127995.1	asAfsAfUfugaGfaGfaagUfCfcaCfcacsgsa	1303
AD-64272.2	A-128001.2	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	1085	A-128002.2	csAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1304
AD-64274.1	A-128363.1	GfsusUfgAfcAfaAfAfAfuCfcUfcAfcAfaUfL96	1086	A-128364.1	asUfsuGfuGfaGfgAfuuuUfuGfuCfaAfcsasa	1305
AD-64275.1	A-128377.1	UfsgsUfuGfaCfaAfAfAfaUfcCfuCfaCfaAfL96	1087	A-128378.1	usUfsgUfgAfgGfaUfuuuUfgUfcAfaCfasasg	1306
AD-64276.1	A-128393.1	GfsgsUfgGfaCfuUfCfUfcUfcAfaUfuUfuAfL96	1088	A-128394.1	usAfsaAfaUfuGfaGfagaAfgUfcCfaCfcsasc	1307
AD-64277.1	A-128407.1	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	1089	A-128408.1	usCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	1308
AD-64277.1	A-128407.1	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	1090	A-128408.1	usCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	1309
AD-64278.1	A-128423.1	AfscsUfgUfuCfaAfGfCfcUfcCfaAfgCfuAfL96	1091	A-128424.1	usAfsgCfuUfgGfaGfgcuUfgAfaCfaAfgsasc	1310
AD-64279.1	A-128435.1	UfscsUfgCfcGfaUfCfCfaUfaCfuGfcGfgAfL96	1092	A-128436.1	usCfscGfcAfgUfaUfggaUfcGfgCfaGfasgsg	1311
AD-64280.1	A-128379.1	AfsusGfuGfuCfuGfCfGfgCfgUfuUfuAfuAfL96	1093	A-128380.1	usAfsuAfaAfaCfgCfcgcAfgAfcAfcAfuscsc	1312
AD-64281.1	A-128395.1	CfscsCfcGfuCfuGfUfGfcCfuUfcUfcAfuAfL96	1094	A-128396.1	usAfsuGfaGfaAfgGfcacAfgAfcGfgGfgsasg	1313

AD-64282.1	A-128409.1	GfscsCfuAfaUfcAfUfCfuCfuUfgUfuCfaUfL96	1095	A-128410.1	asUfsgAfaCfaAfgAfgauGfaUfuAfgCfgsasg	1314
AD-64283.1	A-128425.1	UfscsUfaGfaCfuCfGfUfgGfuGfgAfcUfuCfL96	1096	A-128426.1	gsAfsaGfuCfcAfcCfacgAfgUfcUfaGfascsu	1315
AD-64284.1	A-128437.1	CfsusGfcCfgAfuCfCfAfuAfcUfgCfgGfaAfL96	1097	A-128438.1	usUfscCfgCfaGfuAfuggAfuCfgGfcAfgsasg	1316
AD-64285.1	A-128365.1	UfsusUfuUfcUfuGfUfUfgAfcAfaAfaAfuAfL96	1098	A-128366.1	usAfsuUfuUfuGfuCfaacAfaGfaAfaAfascsc	1317
AD-64286.1	A-128381.1	AfsusCfuUfcUfuGfUfUfgGfuUfcUfuCfuAfL96	1099	A-128382.1	usAfsgAfaGfaAfcCfaacAfaGfaAfgAfusgsa	1318
AD-64289.1	A-128367.1	GfsusUfuUfuCfuUfGfUfuGfaCfaAfaAfaUfL96	1100	A-128368.1	asUfsuUfuUfgUfcAfacaAfgAfaAfaAfcscsc	1319
AD-64290.1	A-128383.1	CfsusGfcCfuAfaUfCfAfuCfuCfuUfgUfuAfL96	1101	A-128384.1	usAfsaCfaAfgAfgAfugaUfuAfgGfcAfgsasg	1320
AD-64291.1	A-128399.1	UfscsCfuCfaCfaAfUfAfcCfaCfaGfaGfuAfL96	1102	A-128400.1	usAfscUfcUfgUfgGfuauUfgUfgAfgGfasusu	1321
AD-64292.1	A-128413.1	CfsusUfgUfuGfaCfAfAfaAfaUfcCfuCfaAfL96	1103	A-128414.1	usUfsgAfgGfaUfuUfuugUfcAfaCfaAfgsasa	1322
AD-64293.1	A-128439.1	GfscsAfaCfuUfuUfUfCfaCfcUfcUfgCfcUfL96	1104	A-128440.1	asGfsgCfaGfaGfgUfgaaAfaAfgUfuGfcsasu	1323
AD-64294.1	A-128369.1	GfsgsGfaAfcAfaGfAfGfcUfaCfaGfcAfuAfL96	1105	A-128370.1	usAfsuGfcUfgUfaGfcucUfuGfuUfcCfcsasa	1324
AD-64295.1	A-128385.1	CfsgsUfgGfuGfgAfCfUfuCfuCfuCfaAfuUfL96	1106	A-128386.1	asAfsuUfgAfgAfgAfaguCfcAfcCfaGfcsasg	1325
AD-64297.1	A-128415.1	CfsusGfcUfgCfuAfUfGfcCfuCfaUfcUfuAfL96	1107	A-128416.1	usAfsaGfaUfgAfgGfcauAfgCfaGfcAfgsgsa	1326
AD-64298.1	A-128427.1	GfsusUfgGfaUfgUfGfUfcUfgCfgGfcGfuUfL96	1108	A-128428.1	asAfscGfcCfgCfaGfacaCfaUfcCfaAfcsgsa	1327
AD-64299.1	A-128441.1	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuAfL96	1109	A-128442.1	usAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	1328
AD-64300.1	A-128371.1	UfsusCfuUfgUfuGfAfCfaAfaAfaUfcCfuAfL96	1110	A-128372.1	usAfsgGfaUfuUfuUfgucAfaCfaAfgAfasasa	1329
AD-64302.1	A-128417.1	UfsasUfaUfgGfaUfGfAfuGfuGfgUfaUfuAfL96	1111	A-128418.1	usAfsaUfaCfcAfcAfucaUfcCfaUfaUfasasc	1330
AD-64303.1	A-128429.1	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuCfL96	1112	A-128430.1	gsAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	1331
AD-64304.1	A-128443.1	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuAfL96	1113	A-128444.1	usAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1332
AD-64305.1	A-128373.1	UfsusGfaCfaAfaAfAfUfcCfuCfaCfaAfuAfL96	1114	A-128374.1	usAfsuUfgUfgAfgGfauuUfuUfgUfcAfascsa	1333
AD-64307.1	A-128403.1	AfsasGfcCfuCfcAfAfGfcUfgUfgCfcUfuAfL96	1115	A-128404.1	usAfsaGfgCfaCfaGfcuuGfgAfgGfcUfusgsa	1334
AD-64308.1	A-128419.1	CfscsUfcUfuCfaUfCfCfuGfcUfgCfuAfuAfL96	1116	A-128420.1	usAfsuAfgCfaGfcAfggaUfgAfaGfaGfgsasa	1335
AD-64309.1	A-128431.1	CfscsUfgCfuGfcUfAfUfgCfcUfcAfuCfuUfL96	1117	A-128432.1	asAfsgAfuGfaGfgCfauaGfcAfgCfaGfgsasu	1336
AD-64310.1	A-128375.1	CfsasUfcUfuCfuUfGfUfuGfgUfuCfuUfcUfL96	1118	A-128376.1	asGfsaAfgAfaCfcAfacaAfgAfaGfaUfgsasg	1337

AD-64311.1	A-128391.1	CfscsGfuCfuGfuGfCfCfuUfcUfcAfuCfuAfL96	1119	A-128392.1	usAfsgAfuGfaGfaAfggcAfcAfgAfcGfgsgsg	1338
AD-64312.1	A-128405.1	CfscsUfcAfuCfuUfCfUfuGfuUfgGfuUfcUfL96	1120	A-128406.1	asGfsaAfcCfaAfcAfagaAfgAfuGfaGfgscsa	1339
AD-64313.1	A-128421.1	CfscsAfcCfaAfaUfGfCfcCfcUfaUfcUfuAfL96	1121	A-128422.1	usAfsaGfaUfaGfgGfgcaUfuUfgGfuGfgsusc	1340
AD-64314.1	A-128433.1	GfscsUfcCfuCfuGfCfCfgAfuCfcAfuAfcUfL96	1122	A-128434.1	asGfsuAfuGfgAfuCfggcAfgAfgGfaGfcscsa	1341
AD-64315.1	A-128363.2	GfsusUfgAfcAfaAfAfAfuCfcUfcAfcAfaUfL96	1123	A-128445.1	PasUfsuGfuGfaGfgAfuuuUfuGfuCfaAfcsasa	1342
AD-64316.1	A-128377.2	UfsgsUfuGfaCfaAfAfAfaUfcCfuCfaCfaAfL96	1124	A-128453.1	PusUfsgUfgAfgGfaUfuuuUfgUfcAfaCfasasg	1343
AD-64317.1	A-128393.2	GfsgsUfgGfaCfuUfCfUfcUfcAfaUfuUfuAfL96	1125	A-128461.1	PusAfsaAfaUfuGfaGfagaAfgUfcCfaCfcsasc	1344
AD-64318.1	A-128407.2	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	1126	A-128469.1	PusCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	1345
AD-64318.1	A-128407.2	UfscsUfuUfuGfgAfGfUfgUfgGfaUfuCfgAfL96	1127	A-128469.1	PusCfsgAfaUfcCfaCfacuCfcAfaAfaGfascsa	1346
AD-64319.1	A-128423.2	AfscsUfgUfuCfaAfGfCfcUfcCfaAfgCfuAfL96	1128	A-128477.1	PusAfsgCfuUfgGfaGfgcuUfgAfaCfaAfgsasc	1347
AD-64320.1	A-128435.2	UfscsUfgCfcGfaUfCfCfaUfaCfuGfcGfgAfL96	1129	A-128483.1	PusCfscGfcAfgUfaUfggaUfcGfgCfaGfasgsg	1348
AD-64321.1	A-123463.3	AfsgsUfuAfuAfuGfGfAfuGfaUfgUfgGfuAfL96	1130	A-128446.1	PusAfscCfaCfaUfcAfuccAfuAfuAfaCfusgsa	1349
AD-64322.1	A-128379.2	AfsusGfuGfuCfuGfCfGfgCfgUfuUfuAfuAfL96	1131	A-128454.1	PusAfsuAfaAfaCfgCfcgcAfgAfcAfcAfuscsc	1350
AD-64323.1	A-128395.2	CfscsCfcGfuCfuGfUfGfcCfuUfcUfcAfuAfL96	1132	A-128462.1	PusAfsuGfaGfaAfgGfcacAfgAfcGfgGfgsasg	1351
AD-64324.1	A-128409.2	GfscsCfuAfaUfcAfUfCfuCfuUfgUfuCfaUfL96	1133	A-128470.1	PasUfsgAfaCfaAfgAfgauGfaUfuAfgCfgsasg	1352
AD-64325.1	A-128425.2	UfscsUfaGfaCfuCfGfUfgGfuGfgAfcUfuCfL96	1134	A-128478.1	PgsAfsaGfuCfcAfcCfacgAfgUfcUfaGfascsu	1353
AD-64326.1	A-128437.2	CfsusGfcCfgAfuCfCfAfuAfcUfgCfgGfaAfL96	1135	A-128484.1	PusUfscCfgCfaGfuAfuggAfuCfgGfcAfgsasg	1354
AD-64328.1	A-128381.2	AfsusCfuUfcUfuGfUfUfgGfuUfcUfuCfuAfL96	1136	A-128455.1	PusAfsgAfaGfaAfcCfaacAfaGfaAfgAfusgsa	1355
AD-64330.1	A-128411.2	UfsusCfuCfuCfaAfUfUfuUfcUfaGfgGfgAfL96	1137	A-128471.1	PusCfscCfcUfaGfaAfaauUfgAfgAfgAfasgsu	1356
AD-64331.1	A-127905.16	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1138	A-127907.2	PusGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1357
AD-64332.1	A-128001.3	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	1139	A-128485.1	PcsAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1358
AD-64333.1	A-128367.2	GfsusUfuUfuCfuUfGfUfuGfaCfaAfaAfaUfL96	1140	A-128448.1	PasUfsuUfuUfgUfcAfacaAfgAfaAfaAfcscsc	1359
AD-64334.1	A-128383.2	CfsusGfcCfuAfaUfCfAfuCfuCfuUfgUfuAfL96	1141	A-128456.1	PusAfsaCfaAfgAfgAfugaUfuAfgGfcAfgsasg	1360
AD-64335.1	A-128399.2	UfscsCfuCfaCfaAfUfAfcCfaCfaGfaGfuAfL96	1142	A-128464.1	PusAfscUfcUfgUfgGfuauUfgUfgAfgGfasusu	1361

AD-64336.1	A-128413.2	CfsusUfgUfuGfaCfAfAfaAfaUfcCfuCfaAfL96	1143	A-128472.1	PusUfsgAfgGfaUfuUfuugUfcAfaCfaAfgsasa	1362
AD-64337.1	A-127955.16	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1144	A-127958.2	PasAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1363
AD-64338.1	A-128439.2	GfscsAfaCfuUfuUfUfCfaCfcUfcUfgCfcUfL96	1145	A-128486.1	PasGfsgCfaGfaGfgUfgaaAfaAfgUfuGfcsasu	1364
AD-64339.1	A-128369.2	GfsgsGfaAfcAfaGfAfGfcUfaCfaGfcAfuAfL96	1146	A-128449.1	PusAfsuGfcUfgUfaGfcucUfuGfuUfcCfcsasa	1365
AD-64341.1	A-128401.2	UfscsAfuCfuUfcUfUfGfuUfgGfuUfcUfuAfL96	1147	A-128465.1	PusAfsaGfaAfcCfaAfcaaGfaAfgAfuGfasgsg	1366
AD-64342.1	A-128415.2	CfsusGfcUfgCfuAfUfGfcCfuCfaUfcUfuAfL96	1148	A-128473.1	PusAfsaGfaUfgAfgGfcauAfgCfaGfcAfgsgsa	1367
AD-64343.1	A-128427.2	GfsusUfgGfaUfgUfGfUfcUfgCfgGfcGfuUfL96	1149	A-128479.1	PasAfscGfcCfgCfaGfacaCfaUfcCfaAfcsgsa	1368
AD-64344.1	A-128441.2	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuAfL96	1150	A-128487.1	PusAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	1369
AD-64345.1	A-128371.2	UfsusCfuUfgUfuGfAfCfaAfaAfaUfcCfuAfL96	1151	A-128450.1	PusAfsgGfaUfuUfuUfgucAfaCfaAfgAfasasa	1370
AD-64347.1	A-123487.3	GfsgsAfuGfuGfuCfUfGfcGfgCfgUfuUfuAfL96	1152	A-128466.1	PusAfsaAfaCfgCfcGfcagAfcAfcAfuCfcsasg	1371
AD-64348.1	A-128417.2	UfsasUfaUfgGfaUfGfAfuGfuGfgUfaUfuAfL96	1153	A-128474.1	PusAfsaUfaCfcAfcAfucaUfcCfaUfaUfasasc	1372
AD-64349.1	A-128429.2	UfsusCfaUfcCfuGfCfUfgCfuAfuGfcCfuCfL96	1154	A-128480.1	PgsAfsgGfcAfuAfgCfagcAfgGfaUfgAfasgsa	1373
AD-64350.1	A-128443.2	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuAfL96	1155	A-128488.1	PusAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1374
AD-64351.1	A-128373.2	UfsusGfaCfaAfaAfAfUfcCfuCfaCfaAfuAfL96	1156	A-128451.1	PusAfsuUfgUfgAfgGfauuUfuUfgUfcAfascsa	1375
AD-64352.1	A-128389.2	CfscsAfaGfuGfuUfUfGfcUfgAfcGfcAfaAfL96	1157	A-128459.1	PusUfsuGfcGfuCfaGfcaaAfcAfcUfuGfgscsa	1376
AD-64352.1	A-128389.2	CfscsAfaGfuGfuUfUfGfcUfgAfcGfcAfaAfL96	1158	A-128459.1	PusUfsuGfcGfuCfaGfcaaAfcAfcUfuGfgscsa	1377
AD-64353.1	A-128403.2	AfsasGfcCfuCfcAfAfGfcUfgUfgCfcUfuAfL96	1159	A-128467.1	PusAfsaGfgCfaCfaGfcuuGfgAfgGfcUfusgsa	1378
AD-64354.1	A-128419.2	CfscsUfcUfuCfaUfCfCfuGfcUfgCfuAfuAfL96	1160	A-128475.1	PusAfsuAfgCfaGfcAfggaUfgAfaGfaGfgsasa	1379
AD-64355.1	A-128431.2	CfscsUfgCfuGfcUfAfUfgCfcUfcAfuCfuUfL96	1161	A-128481.1	PasAfsgAfuGfaGfgCfauaGfcAfgCfaGfgsasu	1380
AD-64356.1	A-128375.2	CfsasUfcUfuCfuUfGfUfuGfgUfuCfuUfcUfL96	1162	A-128452.1	PasGfsaAfgAfaCfcAfacaAfgAfaGfaUfgsasg	1381
AD-64357.1	A-128391.2	CfscsGfuCfuGfuGfCfCfuUfcUfcAfuCfuAfL96	1163	A-128460.1	PusAfsgAfuGfaGfaAfggcAfcAfgAfcGfgsgsg	1382
AD-64358.1	A-128405.2	CfscsUfcAfuCfuUfCfUfuGfuUfgGfuUfcUfL96	1164	A-128468.1	PasGfsaAfcCfaAfcAfagaAfgAfuGfaGfgscsa	1383
AD-64359.1	A-128421.2	CfscsAfcCfaAfaUfGfCfcCfcUfaUfcUfuAfL96	1165	A-128476.1	PusAfsaGfaUfaGfgGfgcaUfuUfgGfuGfgsusc	1384
AD-64360.1	A-128433.2	GfscsUfcCfuCfuGfCfCfgAfuCfcAfuAfcUfL96	1166	A-128482.1	PasGfsuAfuGfgAfuCfggcAfgAfgGfaGfcscsa	1385

AD-64700.1	A-129379.1	ascsucguggugdTacuu(Cgn)ucucaL96	1167	A-127906.26	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1386
AD-64701.1	A-127905.20	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1168	A-129387.1	PusgsagagaagdTccadCcacgaguscsu	1387
AD-64702.1	A-127905.28	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1169	A-129395.1	usGsagadGaaguccaCcacgaguscsu	1388
AD-64703.1	A-129376.2	ascsucguggugdGacuucdAcucaL96	1170	A-129385.5	usdGsagagaagdTccadCcacgaguscsu	1389
AD-64704.1	A-129381.3	ascsucguggdTgdTacuucdAcucaL96	1171	A-129389.6	usdGsagadGaaguccadCcacgaguscsu	1390
AD-64705.1	A-129380.1	ascsucguggugdTacuucdAcucaL96	1172	A-127906.27	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1391
AD-64706.1	A-127905.21	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1173	A-129388.1	usdGsadGagaaguccadCcacgaguscsu	1392
AD-64707.1	A-127905.29	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1174	A-129396.1	usgsagadGaagdTccadCcacgaguscsu	1393
AD-64708.1	A-129382.2	ascsucguggdTgdGacuuc(Tgn)cucaL96	1175	A-129385.6	usdGsagagaagdTccadCcacgaguscsu	1394
AD-64709.1	A-129373.4	ascsucguggugdGacuu(Cgn)ucucaL96	1176	A-129391.2	usdGsagadGaagdTccadCcacgaguscsu	1395
AD-64710.1	A-129373.1	ascsucguggugdGacuu(Cgn)ucucaL96	1177	A-127906.20	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1396
AD-64711.1	A-129381.1	ascsucguggdTgdTacuucdAcucaL96	1178	A-127906.28	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1397
AD-64712.1	A-127905.22	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1179	A-129389.1	usdGsagadGaaguccadCcacgaguscsu	1398
AD-64713.1	A-127905.30	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1180	A-129397.1	PusgsagadGaagdTccadCcacgaguscsu	1399
AD-64714.1	A-129384.2	ascsucguggdTgdGacuucdAcucaL96	1181	A-129385.7	usdGsagagaagdTccadCcacgaguscsu	1400
AD-64715.1	A-129376.4	ascsucguggugdGacuucdAcucaL96	1182	A-129391.3	usdGsagadGaagdTccadCcacgaguscsu	1401
AD-64716.1	A-129374.1	ascsucguggugdGacuucu(Cgn)ucaL96	1183	A-127906.21	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1402
AD-64717.1	A-129382.1	ascsucguggdTgdGacuuc(Tgn)cucaL96	1184	A-127906.29	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1403
AD-64718.1	A-127905.23	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1185	A-129390.1	usdGsagagadAguccadCcacgaguscsu	1404
AD-64719.1	A-127917.5	ascsucguggugdGacuuc(Tgn)cucaL96	1186	A-129385.2	usdGsagagaagdTccadCcacgaguscsu	1405
AD-64720.1	A-129381.2	ascsucguggdTgdTacuucdAcucaL96	1187	A-129385.8	usdGsagagaagdTccadCcacgaguscsu	1406
AD-64721.1	A-129382.4	ascsucguggdTgdGacuuc(Tgn)cucaL96	1188	A-129391.4	usdGsagadGaagdTccadCcacgaguscsu	1407
AD-64722.1	A-129375.1	ascsucguggugdGacuucY34cucaL96	1189	A-127906.22	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1408
AD-64723.1	A-129383.1	ascsucguggugdGdAcuuc(Tgn)cucaL96	1190	A-127906.30	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1409

AD-64725.1	A-127917.6	ascsucguggugdGacuuc(Tgn)cucaL96	1191	A-129398.1	PusdGsagagaagdTccadCcacgaguscsu	1410
AD-64726.1	A-129373.3	ascsucguggugdGacuu(Cgn)ucucaL96	1192	A-129389.2	usdGsagadGaaguccadCcacgaguscsu	1411
AD-64727.1	A-129384.4	ascsucguggdTgdGacuucdAcucaL96	1193	A-129391.5	usdGsagadGaagdTccadCcacgaguscsu	1412
AD-64728.1	A-129376.1	ascsucguggugdGacuucdAcucaL96	1194	A-127906.23	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1413
AD-64729.1	A-129384.1	ascsucguggdTgdGacuucdAcucaL96	1195	A-127906.31	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1414
AD-64730.1	A-127905.25	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1196	A-129392.1	usGsagagaagdTccadCcacgaguscsu	1415
AD-64731.1	A-129399.1	Y34ascsucguggugdGacuuc(Tgn)cucaL96	1197	A-129385.3	usdGsagagaagdTccadCcacgaguscsu	1416
AD-64732.1	A-129376.3	ascsucguggugdGacuucdAcucaL96	1198	A-129389.3	usdGsagadGaaguccadCcacgaguscsu	1417
AD-64733.1	A-129381.4	ascsucguggdTgdTacuucdAcucaL96	1199	A-129391.6	usdGsagadGaagdTccadCcacgaguscsu	1418
AD-64734.1	A-129377.1	ascsucguggugdGacuucdCcucaL96	1200	A-127906.24	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1419
AD-64735.1	A-127905.18	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1201	A-129385.1	usdGsagagaagdTccadCcacgaguscsu	1420
AD-64736.1	A-127905.26	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1202	A-129393.1	usdGsagagaagdTccaCcacgaguscsu	1421
AD-64737.1	A-129399.2	Y34ascsucguggugdGacuuc(Tgn)cucaL96	1203	A-129398.2	PusdGsagagaagdTccadCcacgaguscsu	1422
AD-64738.1	A-129382.3	ascsucguggdTgdGacuuc(Tgn)cucaL96	1204	A-129389.4	usdGsagadGaaguccadCcacgaguscsu	1423
AD-64739.1	A-129378.1	ascsucguggugdGacuucdGcucaL96	1205	A-127906.25	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1424
AD-64740.1	A-127905.19	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1206	A-129386.1	usgsagagaagdTccadCcacgaguscsu	1425
AD-64741.1	A-127905.27	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1207	A-129394.1	usGsagagaagdTccaCcacgaguscsu	1426
AD-64742.1	A-129373.2	ascsucguggugdGacuu(Cgn)ucucaL96	1208	A-129385.4	usdGsagagaagdTccadCcacgaguscsu	1427
AD-64743.1	A-129384.3	ascsucguggdTgdGacuucdAcucaL96	1209	A-129389.5	usdGsagadGaaguccadCcacgaguscsu	1428

Table 5. HBV single dose screen using Dual-Glo Luciferase® Assay

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-63938.2	0.12	ND	0.01	ND
AD-63950.2	0.38	ND	0.04	ND
AD-63956.2	0.31	ND	0.02	ND
AD-63962.2	0.16	ND	0.03	ND
AD-63968.2	0.56	ND	0.10	ND
AD-63968.2	0.79	ND	0.09	ND
AD-63979.2	0.54	ND	0.02	ND
AD-63939.2	0.51	ND	0.01	ND
AD-63945.2	0.54	ND	0.08	ND
AD-63951.2	0.60	ND	0.03	ND
AD-63957.2	0.57	ND	0.02	ND
AD-63963.2	0.91	ND	0.06	ND
AD-63969.2	0.92	ND	0.02	ND
AD-63975.2	0.83	ND	0.01	ND
AD-63980.2	0.77	ND	0.01	ND
AD-63940.2	0.77	ND	0.06	ND
AD-63946.2	0.60	ND	0.10	ND
AD-63952.2	0.48	ND	0.04	ND
AD-63958.2	0.51	ND	0.01	ND
AD-63964.2	0.58	ND	0.04	ND
AD-63970.2	0.69	ND	0.07	ND
AD-63976.2	0.63	ND	0.04	ND
AD-63981.2	0.60	ND	0.04	ND
AD-63941.2	0.56	ND	0.09	ND
AD-63947.2	0.55	ND	0.08	ND
AD-63953.2	0.56	ND	0.06	ND
AD-63959.2	0.51	ND	0.03	ND
AD-63965.2	0.55	ND	0.03	ND
AD-63971.2	0.65	ND	0.02	ND
AD-63977.2	0.88	ND	0.01	ND
AD-63982.2	0.73	ND	0.07	ND
AD-63942.2	0.32	ND	0.09	ND
AD-63948.2	0.57	ND	0.09	ND
AD-63960.2	0.92	ND	0.05	ND
AD-63966.2	0.85	ND	0.06	ND
AD-63972.2	0.82	ND	0.06	ND
AD-63978.2	0.83	ND	0.02	ND
AD-63983.2	0.89	ND	0.02	ND

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-63943.2	0.86	ND	0.04	ND
AD-63949.2	0.76	ND	0.02	ND
AD-63955.2	0.82	ND	0.02	ND
AD-63961.2	0.83	ND	0.07	ND
AD-63967.2	0.86	ND	0.03	ND
AD-63973.2	0.86	ND	0.03	ND
AD-63990.2	0.27	ND	0.07	ND
AD-63996.2	0.29	ND	0.06	ND
AD-64002.2	0.30	ND	0.11	ND
AD-64008.2	0.28	ND	0.05	ND
AD-64008.2	0.34	ND	0.07	ND
AD-64014.2	0.30	ND	0.03	ND
AD-64019.2	0.36	ND	0.04	ND
AD-64024.2	0.27	ND	0.03	ND
AD-63985.2	0.28	ND	0.06	ND
AD-63991.2	0.33	ND	0.02	ND
AD-63997.2	0.47	ND	0.07	ND
AD-64003.2	0.69	ND	0.06	ND
AD-64009.2	0.91	ND	0.03	ND
AD-64015.2	0.69	ND	0.09	ND
AD-64020.2	0.81	ND	0.06	ND
AD-64025.2	0.77	ND	0.06	ND
AD-63986.2	0.28	ND	0.05	ND
AD-63992.2	0.44	ND	0.04	ND
AD-64004.2	0.45	ND	0.04	ND
AD-64010.2	0.37	ND	0.05	ND
AD-64016.2	0.48	ND	0.05	ND
AD-64021.2	0.39	ND	0.03	ND
AD-64026.2	0.30	ND	0.02	ND
AD-63987.2	0.20	ND	0.02	ND
AD-63993.2	0.33	ND	0.02	ND
AD-63999.2	0.36	ND	0.05	ND
AD-64005.2	0.45	ND	0.11	ND
AD-64011.2	0.39	ND	0.08	ND
AD-64017.2	0.84	ND	0.06	ND
AD-64022.2	0.81	ND	0.03	ND
AD-64027.2	0.38	ND	0.05	ND
AD-63988.2	0.37	ND	0.04	ND
AD-63994.2	0.23	ND	0.01	ND
AD-64000.2	0.29	ND	0.00	ND

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-64006.2	0.40	ND	0.04	ND
AD-64012.2	0.45	ND	0.17	ND
AD-64018.2	0.65	ND	0.07	ND
AD-64023.2	0.53	ND	0.07	ND
AD-64028.2	0.52	ND	0.07	ND
AD-63989.2	0.47	ND	0.04	ND
AD-63995.2	0.81	ND	0.03	ND
AD-64001.2	0.83	ND	0.04	ND
AD-64007.2	0.87	ND	0.04	ND
AD-64013.2	0.88	ND	0.03	ND
AD-64289.1	0.276	ND	0.009	ND
AD-64333.1	0.208	ND	0.015	ND
AD-64285.1	0.324	ND	0.034	ND
AD-64300.1	0.225	ND	0.005	ND
AD-64345.1	0.102	ND	0.090	ND
AD-64292.1	0.288	ND	0.232	ND
AD-64336.1	0.199	ND	0.056	ND
AD-64275.1	0.287	ND	0.185	ND
AD-64316.1	0.297	ND	0.024	ND
AD-64274.1	0.209	ND	0.033	ND
AD-64315.1	0.199	ND	0.002	ND
AD-64305.1	0.360	ND	0.035	ND
AD-64351.1	0.281	ND	0.014	ND
AD-64291.1	0.725	ND	0.005	ND
AD-64335.1	0.478	ND	0.020	ND
AD-64283.1	0.917	ND	0.018	ND
AD-64304.1	0.937	ND	0.050	ND
AD-64325.1	0.446	ND	0.223	ND
AD-64350.1	0.934	ND	0.055	ND
AD-63968.4	0.748	ND	0.008	ND
AD-64331.1	0.294	ND	0.038	ND
AD-64008.4	0.416	ND	0.028	ND
AD-64337.1	0.318	ND	0.049	ND
AD-64295.1	0.415	ND	0.034	ND
AD-64276.1	0.453	ND	0.073	ND
AD-64317.1	0.203	ND	0.040	ND
AD-64330.1	0.313	ND	0.030	ND
AD-64298.1	0.797	ND	0.007	ND
AD-64343.1	0.667	ND	0.020	ND
AD-61547.2	0.637	ND	0.019	ND

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-64347.1	0.418	ND	0.066	ND
AD-64280.1	0.754	ND	0.092	ND
AD-64322.1	0.407	ND	0.013	ND
AD-64308.1	0.720	ND	0.055	ND
AD-64354.1	0.315	ND	0.034	ND
AD-64303.1	0.815	ND	0.150	ND
AD-64349.1	0.447	ND	0.030	ND
AD-64299.1	0.831	ND	0.007	ND
AD-64344.1	0.404	ND	0.009	ND
AD-64309.1	0.856	ND	0.005	ND
AD-64355.1	0.498	ND	0.040	ND
AD-64297.1	0.895	ND	0.024	ND
AD-64342.1	0.508	ND	0.006	ND
AD-64312.1	0.590	ND	0.034	ND
AD-64358.1	0.425	ND	0.044	ND
AD-64341.1	0.223	ND	0.119	ND
AD-64310.1	0.301	ND	0.064	ND
AD-64356.1	0.336	ND	0.024	ND
AD-64286.1	0.611	ND	0.012	ND
AD-64328.1	0.317	ND	0.043	ND
AD-61522.2	0.447	ND	0.008	ND
AD-64321.1	0.237	ND	0.009	ND
AD-64302.1	0.523	ND	0.020	ND
AD-64348.1	0.208	ND	0.003	ND
AD-64352.1	0.343	ND	0.224	ND
AD-64352.1	0.567	ND	0.015	ND
AD-64314.1	0.920	ND	0.044	ND
AD-64360.1	0.778	ND	0.029	ND
AD-64279.1	0.882	ND	0.034	ND
AD-64320.1	0.589	ND	0.017	ND
AD-64284.1	0.696	ND	0.119	ND
AD-64326.1	0.552	ND	0.009	ND
AD-64281.1	0.921	ND	0.019	ND
AD-64323.1	0.715	ND	0.097	ND
AD-64311.1	0.815	ND	0.030	ND
AD-64357.1	0.549	ND	0.001	ND
AD-64272.2	0.965	ND	0.024	ND
AD-64332.1	0.548	ND	0.013	ND
AD-64293.1	0.837	ND	0.013	ND
AD-64338.1	0.597	ND	0.031	ND

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-64290.1	0.489	ND	0.026	ND
AD-64334.1	0.368	ND	0.003	ND
AD-64282.1	0.767	ND	0.009	ND
AD-64324.1	0.726	ND	0.077	ND
AD-64278.1	0.951	ND	0.077	ND
AD-64319.1	0.895	ND	0.029	ND
AD-64307.1	0.890	ND	0.065	ND
AD-64353.1	0.567	ND	0.500	ND
AD-64277.1	0.416	ND	0.019	ND
AD-64277.1	0.839	ND	0.058	ND
AD-64318.1	0.613	ND	0.042	ND
AD-64318.1	0.768	ND	0.042	ND
AD-64313.1	0.698	ND	0.062	ND
AD-64359.1	0.441	ND	0.081	ND
AD-64294.1	0.563	ND	0.066	ND
AD-64339.1	0.486	ND	0.044	ND
AD-63968.5	0.57	0.72	0.07	0.03
AD-63940.3	0.81	0.83	0.11	0.03
AD-64710.1	0.79	0.85	0.12	0.04
AD-64716.1	0.73	0.85	0.08	0.01
AD-64722.1	0.67	0.80	0.06	0.02
AD-64728.1	0.74	0.87	0.06	0.05
AD-64734.1	0.78	0.83	0.08	0.05
AD-64739.1	0.73	0.85	0.07	0.02
AD-64700.1	0.54	0.75	0.13	0.02
AD-64705.1	0.67	0.79	0.15	0.04
AD-64711.1	0.57	0.83	0.13	0.04
AD-64717.1	0.72	0.83	0.13	0.02
AD-64723.1	0.83	0.87	0.12	0.01
AD-64729.1	0.74	0.87	0.08	0.07
AD-64735.1	0.73	0.89	0.05	0.04
AD-64740.1	0.89	0.88	0.05	0.07
AD-64701.1	0.88	0.84	0.07	0.05
AD-64706.1	0.71	0.88	0.12	0.05
AD-64712.1	0.81	0.86	0.13	0.07
AD-64718.1	0.84	0.89	0.16	0.01
AD-64730.1	0.88	0.89	0.02	0.04
AD-64736.1	0.80	0.88	0.10	0.05
AD-64741.1	0.85	0.83	0.06	0.05
AD-64702.1	0.87	0.93	0.02	0.06

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-64707.1	0.95	0.88	0.05	0.08
AD-64713.1	0.90	0.85	0.08	0.03
AD-64719.1	0.80	0.89	0.09	0.09
AD-64725.1	0.70	0.84	0.09	0.03
AD-64731.1	0.82	0.87	0.04	0.08
AD-64737.1	0.76	0.84	0.09	0.08
AD-64742.1	0.76	0.85	0.09	0.03
AD-64703.1	0.79	0.88	0.05	0.02
AD-64708.1	0.83	0.82	0.08	0.06
AD-64714.1	0.75	0.85	0.12	0.03
AD-64720.1	0.61	0.81	0.17	0.04
AD-64726.1	0.75	0.83	0.07	0.02
AD-64732.1	0.86	0.84	0.14	0.10
AD-64738.1	0.80	0.90	0.04	0.02
AD-64743.1	0.75	0.85	0.12	0.04
AD-64704.1	0.67	0.78	0.16	0.02
AD-64709.1	0.83	0.86	0.16	0.03
AD-64715.1	0.87	0.88	0.09	0.04
AD-64721.1	0.77	0.82	0.12	0.06
AD-64727.1	0.75	0.85	0.14	0.02
AD-64733.1	0.67	0.81	0.14	0.03

Example 3. Synthesis and *In vitro* Screening of Additional iRNA Duplexes

Additional iRNA molecules targeting the HBV genome were synthesized as described above. A detailed list of the additional modified HBV sense and antisense strand sequences is shown in Table 6.

A single dose screen of these duplexes was performed by transfecting the duplexes into HepG2.215 and Hep3B cells and measuring Firefly (transfection control) and Rinella (fused to HBV target sequence) luciferase, as described above. The results of the assays in HepG2.2.15 cells are shown in Table 7 and the results of the assays in Hep3B cells are provided in Table 8.

Table 6. Modified Sense and Antisense Strand Sequences of HBV dsRNAs

DuplexID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65369	uscsguGfgUfGfGfacuuCfUfcucaL96	1429	PusGfsagaGfaAfGfuccaCfcAfcgasusu	1458
AD-65381	uscsguGfgUfGfGfacuucucucaL96	1430	PusGfsagaGfaAfGfuccaCfcAfcgasusu	1459
AD-63962	Y44uscsGfuGfgUfgGfaCfuUfcUfcUfcAfY44	1431	PusGfsaGfaGfaAfgUfcCfaCfcAfcGfasusu	1460
AD-63938	Y44ACUCGUGGUGGACUUCUCUCA	1432	UGAGAGAAGUCCACCACGAGUCU	1461
AD-65561	uscsguGfgUfGfGfacuuCfUfcucaL96	1433	UfsGfsagaGfaAfGfuccaCfcAfcgasusu	1462
AD-65566	uscsguGfgUfGfGfacuucucucaL96	1434	UfsGfsagaGfaAfGfuccaCfcAfcgasusu	1463
AD-63944	Y44ucGuGGuGGAcuucucucAusuY44	1435	UfGfagAfgAfAfGUfccaCfCAfcgAusu	1464
AD-63968	AfscsUfcGfuGfgUfGfGfaCfuUfcUfcUfcAfL96	1436	usGfsaGfaGfaAfgUfccaCfcAfcGfaGfuscsu	1465
AD-65406	uscsguGfgUfGfGfacuuCfUfcucaL96	1437	usGfsagaGfaAfGfuccaCfcAfcgasusu	1466
AD-65396	ascsucguGfgUfGfGfacuucucucaL96	1438	usGfsagaGfaaguccaCfcAfcgagususu	1467
AD-65427	gsusgcacUfuCfGfCfuucaccucuaL96	1439	PusAfsgagGfugaagcgAfaGfugcacsusu	1468
AD-65573	gsusgcacUfuCfGfCfuucaCfCfucuaL96	1440	UfsAfsgagGfuGfAfagcgAfaGfugcacsasc	1469
AD-65432	gscsacUfucGfCfuucacCfucuaL96	1441	PusAfsgagGfuGfAfagcgAfaGfugcsasc	1470
AD-64332	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	1442	PcsAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1471
AD-64322	AfsusGfuGfuCfuGfCfGfgCfgUfuUfuAfuAfL96	1443	PusAfsuAfaAfaCfgCfcgcAfgAfcAfcAfuscsc	1472
AD-64272	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuGfL96	1444	csAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1473
AD-65583	gscsacuucgdCuucac(Cgn)ucuaL96	1445	usdAsgagdGugaagcgdAagugcsusu	1474
AD-63994	gsgsUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1446	PasAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	1475
AD-65370	csgsugguGfgAfCfUfucucUfCfaauuL96	1447	asAfsuugAfgAfGfaaguCfcAfccagcsasg	1476
AD-65265	gsusggugGfaCfUfUfcUfcucaauuuL96	1448	asAfsaUfugagaGfaagUfcCfaccAfcsgsa	1477
AD-65407	csgsugguGfgAfCfUfucucUfCfaauuL96	1449	asAfsuugAfgAfgAfaguCfcAfccagcsasg	1478

DuplexID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-64027	gsgsUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1450	asAfsaUfuGfaGfaGfaagUfcCfaCfcsasc	1479
AD-65266	gsusgguGfaCfUfUfcucuCfaauuuL96	1451	asAfsaUfugagaGfaagUfcCfaccAfcsgsa	1480
AD-65389	usgsgudGgucdTucucuaaaauuL96	1452	asdAsuugagagdAagudCcaccasusu	1481
AD-64008	GfsusGfgUfgGfaCfUfUfcUfcUfcAfaUfuUfL96	1453	asAfsaUfuGfaGfaGfaagUfcCfaCfcAfcsgsa	1482
AD-65377	csgsuggudGgucdTucucuaaaauuL96	1454	asdAsuugagagdAagudCcaccagcsusu	1483
AD-65409	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1455	PusAfsaaaUfuGfAfgagaAfgUfccaccsasc	1484
AD-65403	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1456	usAfsaaaUfuGfAfgagaAfgUfccaccsasc	1485
AD-65385	usgsgacuacdTcucaaauuuaL96	1457	usdAsaaauugadGagadAguccasusu	1486

Table 7. HBV single dose screen In HepG2.2.15 cells using Dual-Glo Luciferase® Assay

Duplex ID	PORF-1_A	PORF-1_B	SORF-2_A	SORF-2_B
AD-65369	0.1875	0.042	0.0446	0.3018
AD-65381	0.086	0.249	0.1008	0.553
AD-63962	0.4838	0.3475	0.2237	0.5258
AD-63938	0.3587	2.1213	0.0501	1.1434
AD-65561	0.1076	0.3801	0.0718	0.6897
AD-65566	0.4127	0.3211	0.185	11.1161
AD-63944	0.9489	0.7098	0.393	0.2771
AD-63968	NoIC50	NoIC50	1.8788	NoIC50
AD-65406	3.3749	18.8396	3.8204	2.2662
AD-65396	NoIC50	6.8758	3.7382	4.2157
AD-65427	0.0089	0.0181	0.0066	0.015
AD-65573	0.0174	0.0332	0.0029	0.0227
AD-65432	0.0211	0.0593	0.0112	0.0366
AD-64332	0.0268	0.0329	0.0624	0.0217
AD-64322	0.0963	0.1077	0.0992	0.0963
AD-64272	0.0773	0.1199	0.0763	0.093
AD-65583	0.1624	0.2228	0.1568	0.1496
AD-63994	0.7019	0.1467	0.0832	0.0385
AD-65370	0.2404	0.7916	0.3952	0.1964
AD-65265	0.2255	0.5008	0.2893	0.318
AD-65407	0.9533	0.261	0.4254	0.1121
AD-64027	0.7692	0.5887	0.5208	0.5697
AD-65266	3.4109	0.5055	0.8532	0.3658
AD-65389	0.9172	0.6514	0.4915	0.2872
AD-64008	1.2738	0.7865	1.9519	0.808
AD-65377	0.6052	1.6	24.9403	0.6065
AD-65409	1.8304	1.6479	0.104	0.0557
AD-65403	12.1516	0.667	1.006	0.233
AD-65385	NoIC50	NoIC50	NoIC50	NoIC50

Table 8. HBV single dose screen In Hep3B cells using Dual-Glo Luciferase® Assay

Duplex ID	PORF-1_A	PORF-1_B
AD-65369	0.0982	0.0508
AD-65381	0.2392	0.1097
AD-63962	0.0769	0.0706
AD-63938	0.039	0.0111
AD-65561	0.6316	0.6931
AD-65566	0.2747	0.5331
AD-63944	0.1317	0.0566
AD-63968	0.4374	0.8811
AD-65406	1.4961	1.2573
AD-65396	1.9971	0.9952
AD-65427	0.0234	0.006
AD-65573	0.0346	0.0334
AD-65432	0.0352	0.2664
AD-64332	0.0221	0.4541
AD-64322	0.1743	0.1616
AD-64272	0.1885	0.6699
AD-65583	0.1241	8.1611
AD-63994	3.3623	5.2897
AD-65370	0.2281	NoIC50
AD-65265	NoIC50	7.3426
AD-65407	0.1404	1.3833
AD-64027	27.1417	1.1832
AD-65266	NoIC50	NoIC50
AD-65389	NoIC50	NoIC50
AD-64008	NoIC50	NoIC50
AD-65377	NoIC50	NoIC50
AD-65409	1.8065	3.436
AD-65403	0.5113	18.0359
AD-65385	NoIC50	NoIC50

A subset of these duplexes were also assayed for *in vitro* stability using two assays, a tritosome stability assay and a cytosol stability assay.

For the tritosome stability assays, rat liver tritosomes (Xenotech custom product PR14044) were thawed to room temperature and diluted to 0.5units/mL Acid Phosphatase in 20mM Sodium Citrate pH 5.0 Buffer. Twenty-four hour samples were prepared by mixing 100µL of 0.5units/mL Acid Phosphatase Tritosomes with 25µL of 0.4mg/mL siRNA sample in a microcentrifuge tube and incubating for twenty-four hours in an eppendorf Thermomixer set to 37°C and 300rpm. After twenty-four hours of incubation 300µL of Phenomenex Lysis Loading Buffer (Cat.# ALO-8498) and 12.5µL of a 0.4mg/mL internal standard siRNA were added to each sample. Time 0 hour samples were prepared by by mixing 100µL of 0.5units/mL Acid Phosphatase Tritosomes with 25µL of 0.4mg/mL siRNA sample, 300µL of Phenomenex Lysis Loading Buffer, and 12.5µL of a 0.4mg/mL internal standard siRNA. siRNA was extracted from twenty-four hour samples and 0 hour samples using a Phenomenex Clarity OTX Starter Kit (Cat.# KSO-8494). After the samples were extracted they were transferred to a microcentrifuge tube and dried down using a Labconco CentriVap Concentrator (Cat.# 7810010). The samples were then resuspended with 500µL of nuclease free water. Fifty µL of each sample was run on an Agilent Technologies 1260 Infinity Binary LC with Agilent Technologies 6130 Quadrupole LC/MS. The Quaternary pump method was run for 12.20 minutes at 0.400mL/min with the following timetable:

Time Function	Parameter
0.20	5% Buffer A(16mM TEA 200mM HFIP), 95% Buffer B (100% Methanol)
2.50	5% Buffer A(16mM TEA 200mM HFIP), 95% Buffer B (100% Methanol)
3.00	100% Buffer A(16mM TEA 200mM HFIP)

The Binary Pump method was run for 12.20min at 0.700mL/min with the following timetable:

Time Function	Parameter
0.00	100% Buffer A(16mM TEA 200mM HFIP)
0.40	100% Buffer A(16mM TEA 200mM HFIP)
10.00	60% Buffer A(16mM TEA 200mM HFIP), 40% Buffer B (100% ACN)
10.10	100% Buffer A(16mM TEA 200mM HFIP)
12.20	100% Buffer A(16mM TEA 200mM HFIP)

Both the left and right column was set at 75.00°C. The UV signal was measured at 260nm wavelength. The percent remaining of each strand was calculated using the following equation:

% Strand remaining =100*(Peak $Area_{Strand\ 24h}$/Peak $Area_{Strand\ 0h}$*(Peak $Area_{Standard\ 24h}$/Peak $Area_{Standard\ 0h}$)).

For the cytosol stability assay, female rat liver cytosol (Xenotech Cat. # R1500.C) were thawed to room temperature and diluted to 1mg/mL in 50mM Tris buffer: HCl pH 7.4, 5mM MgCl2. 24 hour samples were prepared by mixing 100uL of 1mg/mL Cytosol with 25uL of 0.4mg/mL siRNA sample in a microcentrifuge tube and incubating for 24 hours in an eppendorf Thermomixer set to 37°C and 300rpm. After 24 hours of incubation 300uL of Phenomenex Lysis Loading Buffer (Cat.# ALO-8498) and 12.5uL of a 0.4mg/mL internal standard siRNA were added to each sample. 0 hour samples were prepared by by mixing 100uL of 1mg/mL Cytosol with 25uL of 0.4mg/mL siRNA sample, 300uL of Phenomenex Lysis Loading Buffer, and 12.5uL of a 0.4mg/mL internal standard siRNA. siRNA was extracted from 24 hour samples and 0 hour samples using a Phenomenex Clarity OTX Starter Kit (Cat.# KSO-8494). After the samples were extracted they were transferred to a microcentrifuge tube and dried down using a Labconco CentriVap Concentrator (Cat.# 7810010). The samples were then resuspended with 500uL of nuclease free water. 50uL of each sample was run on an Agilent Technologies 1260 Infinity Binary LC with Agilent Technologies 6130 Quadrupole LC/MS. The Quaternary pump method was run for 12.20 minutes at 0.400mL/min with the following timetable:

Time Function	Parameter
0.20	5% Buffer A(16mM TEA 200mM HFIP), 95% Buffer B (100% Methanol)
2.50	5% Buffer A(16mM TEA 200mM HFIP), 95% Buffer B (100% Methanol)
3.00	100% Buffer A(16mM TEA 200mM HFIP)

The Binary Pump method was run for 12.20min at 0.700mL/min with the following timetable:

Time Function Parameter

Time Function	Parameter
0.00	100% Buffer A(16mM TEA 200mM HFIP)
0.40	100% Buffer A(16mM TEA 200mM HFIP)
10.00	60% Buffer A(16mM TEA 200mM HFIP), 40% Buffer B (100% ACN)
10.10	100% Buffer A(16mM TEA 200mM HFIP)
12.20	100% Buffer A(16mM TEA 200mM HFIP)

Both the left and right column was set at 75.00°C. The UV signal was measured at 260nm wavelength. The percent remaining of each strand was calculated using the following equation:

% Strand remaining =100*(Peak $Area_{Strand\ 24h}$/Peak $Area_{Strand\ 0h}$*(Peak $Area_{Standard\ 24h}$/Peak $Area_{Standard\ 0h}$)).

The results of the twenty-four hour tritosome stability assays are provided in Table 9 and the results of the twenty-four hour cytosol stability assays are provided in Table 10.

Table 9. Twenty-four hour tritosome stability assays.

% Antisense Remaining	% Sense Remaining	DuplexID
		AD-65369
87.59	72.43	AD-65381
		AD-63962
		AD-63938
		AD-65561
67.59	82.48	AD-65566
		AD-63944
30.52	34.98	AD-63968
		AD-65406
		AD-65396
115.17	79.61	AD-65427
43.00	76.84	AD-65573
		AD-65432
		AD-64332
		AD-64322
129.69	128.59	AD-64272
		AD-65583
		AD-63994
		AD-65370
		AD-65265
100.30	119.85	AD-65407
		AD-64027
		AD-65266
		AD-65389
94.06	110.90	AD-64008
98.63	127.48	AD-65377
105.06	119.88	AD-65409
117.55	104.30	AD-65403
		AD-65385

Table 10. Twenty-four hour cytosol stability assays.

% Antisense Remaining	% Sense Remaining	DuplexID
		AD-65369
67.78	22.42	AD-65381
		AD-63962
		AD-63938
		AD-65561
55.89	15.26	AD-65566
		AD-63944
88.39	46.94	AD-63968
		AD-65406
		AD-65396
89.50	66.35	AD-65427
69.01	41.47	AD-65573
		AD-65432
		AD-64332
		AD-64322
96.77	78.00	AD-64272
		AD-65583
		AD-63994
		AD-65370
		AD-65265
64.46	24.10	AD-65407
		AD-64027
		AD-65266
		AD-65389
35.39	26.39	AD-64008
79.98	66.50	AD-65377
86.24	74.25	AD-65409
60.45	62.41	AD-65403
		AD-65385

Example 4. Synthesis and *In vitro* Screening of iRNA Duplexes targeting HDV

The selection of iRNA designs targeting HDV was driven by two primary factors: a) potency and b), the desire to employ iRNA with near-perfect matches with greater than 90% fractional coverage of the large number of public HDV sequences of all known clades (I-VIII or 1-8). However, due to the low level of conservation between members of the various HDV clades and the small genome, selection of sequences that effectively target multiple HDV clades was difficult. Exemplary sequences for the various HDV clades 1-8 are provided in SEQ ID NOs: 29, 31, 33, 35,37, 39, 41, and 43. Their reverse complements are provided in SEQ ID NOs: 30, 32, 34, 36, 38, 40, 42, and 44.

iRNA molecules targeting the HDV genome were synthesized as described above. A detailed list of the unmodified HDV sense and antisense strand sequences is shown in Table 11 and a detailed list of the modified HDV sense and antisense strand sequences is shown in Table 12. The results of a single dose screen of these agents using the Dual-Glo Luciferase® Assay described above are shown in Table 13.

Table 11. Unmodified Sense and Antisense Strand Sequences of HDV dsRNAs

Duplex Name	Sense Strand	Antisense Strand	Clade	Sense Seq	Antisense Seq	SEQ ID NO Sense /Antisense
AD-45013.1	A-94349.1	A-94350.1	HDV1	ACGAAGGAAGGCCCUCGAGdTdT	CUCGAGGGCCUUCCUUCGUdTdT	45/46
AD-45019.1	A-94351.1	A-94352.1	HDV1	CGAAGGAAGGCCCUCGAGAdTdT	UCUCGAGGGCCUUCCUUCGdTdT	47/48
AD-45025.1	A-94353.1	A-94354.1	HDV1	GAAGGAAGGCCCUCGAGAAdTdT	UUCUCGAGGGCCUUCCUUCdTdT	49/50
AD-45036.1	A-94357.1	A-94358.1	HDV1	AGGAAGGCCCUCGAGAACAdTdT	UGUUCUCGAGGGCCUUCCUdTdT	51/52
AD-45041.1	A-94359.1	A-94360.1	HDV1	GGAAGGCCCUCGAGAACAAdTdT	UUGUUCUCGAGGGCCUUCCdTdT	53/54
AD-45046.1	A-94361.1	A-94362.1	HDV1	GAAGGCCCUCGAGAACAAGdTdT	CUUGUUCUCGAGGGCCUUCdTdT	55/56
AD-45051.1	A-94363.1	A-94364.1	HDV1	GGGGUGUGAACCCCCUCGAdTdT	UCGAGGGGGUUCACACCCCdTdT	57/58
AD-45014.1	A-94365.1	A-94366.1	HDV1	GGGUGUGAACCCCCUCGAAdTdT	UUCGAGGGGGUUCACACCCdTdT	59/60
AD-45020.1	A-94367.1	A-94368.1	HDV1	GGUGUGAACCCCCUCGAAGdTdT	CUUCGAGGGGGUUCACACCdTdT	61/62
AD-45026.1	A-94369.1	A-94370.1	HDV1	UGUGAACCCCCUCGAAGGUdTdT	ACCUUCGAGGGGGUUCACAdTdT	63/64
AD-45032.1	A-94371.1	A-94372.1	HDV2	AAAAUCCCUGGCUGGGGAAdTdT	UUCCCCAGCCAGGGAUUUUdTdT	65/66
AD-45042.1	A-94375.1	A-94376.1	HDV2	AAUCCCUGGCUGGGGAACAdTdT	UGUUCCCCAGCCAGGGAUUdTdT	67/68
AD-45047.1	A-94377.1	A-94378.1	HDV2	AUCCCUGGCUGGGGAACAUdTdT	AUGUUCCCCAGCCAGGGAUdTdT	69/70
AD-45015.1	A-94381.1	A-94382.1	HDV2	AAGAGCGGGUUCACCGACAdTdT	UGUCGGUGAACCCGCUCUUdTdT	71/72
AD-45021.1	A-94383.1	A-94384.1	HDV2	AGAGCGGGUUCACCGACAAdTdT	UUGUCGGUGAACCCGCUCUdTdT	73/74
AD-45027.1	A-94385.1	A-94386.1	HDV2	GAGCGGGUUCACCGACAAGdTdT	CUUGUCGGUGAACCCGCUCdTdT	75/76
AD-45033.1	A-94387.1	A-94388.1	HDV2	GCGGGUUCACCGACAAGGAdTdT	UCCUUGUCGGUGAACCCGCdTdT	77/78
AD-45038.1	A-94389.1	A-94390.1	HDV2	CGGGUUCACCGACAAGGAGdTdT	CUCCUUGUCGGUGAACCCGdTdT	79/80
AD-45043.1	A-94391.1	A-94392.1	HDV2	GGGUUCACCGACAAGGAGAdTdT	UCUCCUUGUCGGUGAACCCdTdT	81/82

AD-45048.1	A-94393.1	A-94394.1	HDV2	GGUUCACCGACAAGGAGAGdTdT	CUCUCCUUGUCGGUGAACCdTdT	83/84
AD-45053.1	A-94395.1	A-94396.1	HDV2	GGGAGGGACUGGACAUCAGdTdT	CUGAUGUCCAGUCCCUCCCCdTdT	85/86
AD-45016.1	A-94397.1	A-94398.1	HDV3	GGGUAGAGGGAAAGGAAGAAdTdT	UUCUUCCUUUCCUCUACCCCdTdT	87/88
AD-45022.1	A-94399.1	A-94400.1	HDV3	GAGGCGGGACCACAGAAGAdTdT	UCUUCUGUGGUCCCGCCUCdTdT	89/90
AD-45028.1	A-94401.1	A-94402.1	HDV3	ACAGAAGAAGGAAGGCCCUdTdT	AGGGCCUUCCUUCUUCUGUdTdT	91/92
AD-45034.1	A-94403.1	A-94404.1	HDV3	GAAGAAGAGGAACUCCCGGAdTdT	UCCGGAGUUCCUCUUCUUCdTdT	93/94
AD-45039.1	A-94405.1	A-94406.1	HDV3	CAGGGAUGACGACGAAAGAdTdT	UCUUUCGUCGUCAUCCCUGdTdT	95/96
AD-45044.1	A-94407.1	A-94408.1	HDV3	GGGAUGACGACGAAAGAGAdTdT	UCUCUUUCGUCGUCAUCCCCdTdT	97/98
AD-45049.1	A-94409.1	A-94410.1	HDV3	GGAUGACGACGAAAGAGAAdTdT	UUCUCUUUCGUCGUCAUCCdTdT	99/100
AD-45054.1	A-94411.1	A-94412.1	HDV3	CCUGGGGGUGUGAACCCAAdTdT	UUGGGUUCACACCCCCAGGdTdT	101/102
AD-45017.1	A-94413.1	A-94414.1	HDV3	CUGGGGGUGUGAACCCAAUdTdT	AUUGGGUUCACACCCCCAGdTdT	103/104
AD-45023.1	A-94415.1	A-94416.1	HDV3	CCUUUUCCCCGAACGGGAGAdTdT	UCUCCCGUUCGGGAAAAGGdTdT	105/106
AD-45035.1	A-94419.1	A-94420.1	HDV3	GAACGGGAGAGGGGAUCGAdTdT	UCGAUCCCCUCUCCCGUUCdTdT	107/108
AD-45040.1	A-94421.1	A-94422.1	HDV3	ACGGGAGAGGGGAUCGACAdTdT	UGUCGAUCCCCUCUCCCGUdTdT	109/110
AD-45050.1	A-94425.1	A-94426.1	HDV3	AGAGGGGAUCGACAUCCGAdTdT	UCGGAUGUCGAUCCCCUCUdTdT	111/112
AD-45055.1	A-94427.1	A-94428.1	HDV3	GGGGAUCGACAUCCGAGGAdTdT	UCCUCGGAUGUCGAUCCCCdTdT	113/114
AD-45018.1	A-94429.1	A-94430.1	HDV3	GGGAUCGACAUCCGAGGAAdTdT	UUCCUCGGAUGUCGAUCCCdTdT	115/116
AD-45024.1	A-94431.1	A-94432.1	HDV3	ACAUCCGAGGAACCCAGCAdTdT	UGCUGGGUUCCUCGGAUGUdTdT	117/118
AD-45030.1	A-94433.1	A-94434.1	HDV3	GGAACCCAGCAGUUCCCAUdTdT	AUGGGAACUGCUGGGUUCCdTdT	119/120

Table 12. Modified Sense and Antisense Strand Sequences of HDV dsRNAs

Duplex Name	Sense Strand	Antisense Strand	Clade	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-45013.1	A-94349.1	A-94350.1	HDV1	AcGAAGGAAGGccccucGAGdTsdT	1487	CUCGAGGGCCUUCCUUCGUdTsdT	1525
AD-45019.1	A-94351.1	A-94352.1	HDV1	cGAAGGAAGGccccucGAGAdTsdT	1488	UCUCGAGGGCCUUCCUUCGdTsdT	1526
AD-45025.1	A-94353.1	A-94354.1	HDV1	GAAGGAAGGccccucGAGAAdTsdT	1489	UUCUCGAGGGCCUUCCUUCdTsdT	1527
AD-45036.1	A-94357.1	A-94358.1	HDV1	AGGAAGGccccucGAGAAcAdTsdT	1490	UGUUCUCGAGGGCCUUCCUdTsdT	1528
AD-45041.1	A-94359.1	A-94360.1	HDV1	GGAAGGccccucGAGAAcAAdTsdT	1491	UUGUUCUCGAGGGCCUUCCdTsdT	1529
AD-45046.1	A-94361.1	A-94362.1	HDV1	GAAGGccccucGAGAAcAAGdTsdT	1492	CUUGUUCUCGAGGGCCUUCdTsdT	1530
AD-45051.1	A-94363.1	A-94364.1	HDV1	GGGGuGuGAAcccccucGAdTsdT	1493	UCGAGGGGGUUcAcACCCCdTsdT	1531
AD-45014.1	A-94365.1	A-94366.1	HDV1	GGGuGuGAAcccccucGAAdTsdT	1494	UUCGAGGGGGUUcAcACCCdTsdT	1532
AD-45020.1	A-94367.1	A-94368.1	HDV1	GGuGuGAAcccccucGAAGdTsdT	1495	CUUCGAGGGGGUUcAcACCdTsdT	1533
AD-45026.1	A-94369.1	A-94370.1	HDV1	uGuGAAcccccucGAAGGudTsdT	1496	ACCUUCGAGGGGGUUcAcAdTsdT	1534
AD-45032.1	A-94371.1	A-94372.1	HDV2	AAAAucccuGGcuGGGGAAdTsdT	1497	UUCCCcAGCcAGGGAUUUUdTsdT	1535
AD-45042.1	A-94375.1	A-94376.1	HDV2	AAucccuGGcuGGGGAAcAdTsdT	1498	UGUUCCCcAGCcAGGGAUUdTsdT	1536
AD-45047.1	A-94377.1	A-94378.1	HDV2	AucccuGGcuGGGGAAcAudTsdT	1499	AUGUUCCCcAGCcAGGGAUdTsdT	1537
AD-45015.1	A-94381.1	A-94382.1	HDV2	AAGAGcGGGuucAccGAcAdTsdT	1500	UGUCGGUGAACCCGCUCUUdTsdT	1538
AD-45021.1	A-94383.1	A-94384.1	HDV2	AGAGcGGGuucAccGAcAAdTsdT	1501	UUGUCGGUGAACCCGCUCUdTsdT	1539
AD-45027.1	A-94385.1	A-94386.1	HDV2	GAGcGGGuucAccGAcAAGdTsdT	1502	CUUGUCGGUGAACCCGCUCdTsdT	1540
AD-45033.1	A-94387.1	A-94388.1	HDV2	GcGGGuucAccGAcAAGGAdTsdT	1503	UCCUUGUCGGUGAACCCGCdTsdT	1541
AD-45038.1	A-94389.1	A-94390.1	HDV2	cGGGuucAccGAcAAGGAGdTsdT	1504	CUCCUUGUCGGUGAACCCGdTsdT	1542
AD-45043.1	A-94391.1	A-94392.1	HDV2	GGGuucAccGAcAAGGAGAdTsdT	1505	UCUCCUUGUCGGUGAACCCdTsdT	1543

Duplex Name	Sense Strand	Antisense Strand	Clade	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-45048.1	A-94393.1	A-94394.1	HDV2	GGuucAccGAcAAGGAGAGdTsdT	1506	CUCUCCUUGUCGGUGAACCdTsdT	1544
AD-45053.1	A-94395.1	A-94396.1	HDV2	GGGAGGGAcuGGAcAucAGdTsdT	1507	CUGAUGUCcAGUCCCUCCCCdTsdT	1545
AD-45016.1	A-94397.1	A-94398.1	HDV3	GGGuAGAGGAAAGGAAGAAdTsdT	1508	UUCUUCCUUUCCUCuACCCCdTsdT	1546
AD-45022.1	A-94399.1	A-94400.1	HDV3	GAGGcGGGAccAcAGAAGAdTsdT	1509	UCUUCUGUGGUCCCGCCUCdTsdT	1547
AD-45028.1	A-94401.1	A-94402.1	HDV3	AcAGAAGAAGGAAGGcccudTsdT	1510	AGGGCCUUCCUUCUUCUGUdTsdT	1548
AD-45034.1	A-94403.1	A-94404.1	HDV3	GAAGAAGAGGAAcuccGGAdTsdT	1511	UCCGGAGUUCCUCUUCUUCdTsdT	1549
AD-45039.1	A-94405.1	A-94406.1	HDV3	cAGGGAuGAcGAcGAAAGAdTsdT	1512	UCUUUCGUCGUcAUCCCUGdTsdT	1550
AD-45044.1	A-94407.1	A-94408.1	HDV3	GGGAuGAcGAcGAAAGAGAdTsdT	1513	UCUCUUUCGUCGUcAUCCCdTsdT	1551
AD-45049.1	A-94409.1	A-94410.1	HDV3	GGAuGAcGAcGAAAGAGAAdTsdT	1514	UUCUCUUUCGUCGUcAUCCdTsdT	1552
AD-45054.1	A-94411.1	A-94412.1	HDV3	ccuGGGGGuGuGAAcccAAdTsdT	1515	UUGGGUUcAcACCCCcAGGdTsdT	1553
AD-45017.1	A-94413.1	A-94414.1	HDV3	cuGGGGGuGuGAAcccAAudTsdT	1516	AUUGGGUUcAcACCCCcAGdTsdT	1554
AD-45023.1	A-94415.1	A-94416.1	HDV3	ccuuuucccGAAcGGGAGAdTsdT	1517	UCUCCCGUUCGGGAAAAGGdTsdT	1555
AD-45035.1	A-94419.1	A-94420.1	HDV3	GAAcGGGAGAGGGGAucGAdTsdT	1518	UCGAUCCCCUCUCCCGUUCdTsdT	1556
AD-45040.1	A-94421.1	A-94422.1	HDV3	AcGGGAGAGGGGAucGAcAdTsdT	1519	UGUCGAUCCCCUCUCCCGUdTsdT	1557
AD-45050.1	A-94425.1	A-94426.1	HDV3	AGAGGGGAucGAcAuccGAdTsdT	1520	UCGGAUGUCGAUCCCCUCUdTsdT	1558
AD-45055.1	A-94427.1	A-94428.1	HDV3	GGGGAucGAcAuccGAGGAdTsdT	1521	UCCUCGGAUGUCGAUCCCCdTsdT	1559
AD-45018.1	A-94429.1	A-94430.1	HDV3	GGGAucGAcAuccGAGGAAdTsdT	1522	UUCCUCGGAUGUCGAUCCCCdTsdT	1560
AD-45024.1	A-94431.1	A-94432.1	HDV3	AcAuccGAGGAAcccAGcAdTsdT	1523	UGCUGGGUUCCUCGGAUGUdTsdT	1561
AD-45030.1	A-94433.1	A-94434.1	HDV3	GGAAcccAGcAGuucccAudTsdT	1524	AUGGGAACUGCUGGGUUCCdTsdT	1562

Table 13. HDV single dose screen using Dual-Glo Luciferase® Assay

Duplex Name	10nM mean KD	0.1nM mean KD	Sense Strand	Antisense Strand	Clade
AD-45013.1	0.564	0.885	A-94349.1	A-94350.1	HDV1
AD-45019.1	0.238	0.749	A-94351.1	A-94352.1	HDV1
AD-45025.1	0.203	0.747	A-94353.1	A-94354.1	HDV1
AD-45036.1	0.557	0.865	A-94357.1	A-94358.1	HDV1
AD-45041.1	0.710	0.865	A-94359.1	A-94360.1	HDV1
AD-45046.1	0.849	0.937	A-94361.1	A-94362.1	HDV1
AD-45051.1	0.863	0.909	A-94363.1	A-94364.1	HDV1
AD-45014.1	0.556	0.883	A-94365.1	A-94366.1	HDV1
AD-45020.1	0.860	0.949	A-94367.1	A-94368.1	HDV1
AD-45026.1	0.793	0.847	A-94369.1	A-94370.1	HDV1
AD-45032.1	0.569	0.878	A-94371.1	A-94372.1	HDV2
AD-45042.1	0.913	0.928	A-94375.1	A-94376.1	HDV2
AD-45047.1	0.593	0.858	A-94377.1	A-94378.1	HDV2
AD-45015.1	0.615	0.948	A-94381.1	A-94382.1	HDV2
AD-45021.1	0.856	0.903	A-94383.1	A-94384.1	HDV2
AD-45027.1	1.001	0.934	A-94385.1	A-94386.1	HDV2
AD-45033.1	0.926	0.939	A-94387.1	A-94388.1	HDV2
AD-45038.1	0.838	0.876	A-94389.1	A-94390.1	HDV2
AD-45043.1	0.721	0.949	A-94391.1	A-94392.1	HDV2
AD-45048.1	0.873	0.927	A-94393.1	A-94394.1	HDV2
AD-45053.1	0.415	0.826	A-94395.1	A-94396.1	HDV2
AD-45016.1	0.634	0.917	A-94397.1	A-94398.1	HDV3
AD-45022.1	0.943	0.900	A-94399.1	A-94400.1	HDV3
AD-45028.1	0.817	0.914	A-94401.1	A-94402.1	HDV3
AD-45034.1	0.601	0.890	A-94403.1	A-94404.1	HDV3
AD-45039.1	0.931	0.888	A-94405.1	A-94406.1	HDV3
AD-45044.1	0.912	0.905	A-94407.1	A-94408.1	HDV3
AD-45049.1	0.976	0.980	A-94409.1	A-94410.1	HDV3
AD-45054.1	0.929	0.896	A-94411.1	A-94412.1	HDV3
AD-45017.1	0.977	0.974	A-94413.1	A-94414.1	HDV3
AD-45023.1	0.887	0.878	A-94415.1	A-94416.1	HDV3
AD-45035.1	0.915	0.928	A-94419.1	A-94420.1	HDV3
AD-45040.1	1.671	0.934	A-94421.1	A-94422.1	HDV3
AD-45050.1	0.878	0.963	A-94425.1	A-94426.1	HDV3
AD-45055.1	0.926	0.873	A-94427.1	A-94428.1	HDV3
AD-45018.1	0.912	0.976	A-94429.1	A-94430.1	HDV3
AD-45024.1	0.873	0.884	A-94431.1	A-94432.1	HDV3
AD-45030.1	0.909	0.937	A-94433.1	A-94434.1	HDV3

Example 5. Synthesis and Screening of Additional siRNA Duplexes Targeted to HBV

Additional iRNA molecules targeting the HBV genome were designed and synthesized as described above. A detailed list of the additional unmodified HBV sense and antisense strand sequences is shown in Table 14 and a detailed list of the modified HBV sense and antisense strand sequences is shown in Table 15.

Table 14. Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs

Duplex ID	Sense ID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense ID	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65381	A-130366.9	UCGUGGUGGACUUCUCUCA	1563	A-131904.1	UGAGAGAAGUCCACCACGAUU	1574
AD-66019	A-130366.9	UCGUGGUGGACUUCUCUCA	1564	A-131904.1	UGAGAGAAGUCCACCACGAUU	1575
AD-65375	A-130366.9	UCGUGGUGGACUUCUCUCA	1565	A-130364.7	UGAGAGAAGUCCACCACGAUU	1576
AD-65427	A-130441.7	GUGCACUUCGCUUCACCUCUA	1566	A-131905.1	UAGAGGUGAAGCGAAGUGCACUU	1577
AD-66110	A-130441.7	GUGCACUUCGCUUCACCUCUA	1567	A-131905.1	UAGAGGUGAAGCGAAGUGCACUU	1578
AD-65421	A-130441.7	GUGCACUUCGCUUCACCUCUA	1568	A-130442.6	UAGAGGUGAAGCGAAGUGCACUU	1579
AD-65407	A-130371.12	CGUGGUGGACUUCUCUCAAUU	1569	A-130372.5	AAUUGAGAGAAGUCCACCAGCAG	1580
AD-65377	A-130384.4	CGUGGUGGUCTUCUCUAAAUU	1570	A-130748.3	AAUUGAGAGAAGUCCACCAGCUU	1581
AD-65409	A-130388.15	GGUGGACUUCUCUCAAUUUUA	1571	A-131906.1	UAAAAUUGAGAGAAGUCCACCAC	1582
AD-66111	A-130388.15	GGUGGACUUCUCUCAAUUUUA	1572	A-131906.1	UAAAAUUGAGAGAAGUCCACCAC	1583
AD-65403	A-130388.15	GGUGGACUUCUCUCAAUUUUA	1573	A-130389.4	UAAAAUUGAGAGAAGUCCACCAC	1584

Table 15. Modified Sense and Antisense Strand Sequences of HBV dsRNAs

Duplex ID	Sense ID	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense ID	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65381	A-130366.9	uscsguGfgUfGfGfacuucucucaL96	1585	A-131904.1	PusGfsagaGfaAfGfuccaCfcAfcgasusu	1596
AD-66019	A-130366.9	uscsguGfgUfGfGfacuucucucaL96	1586	A-131904.1	VPusGfsagaGfaAfGfuccaCfcAfcgasusu	1597
AD-65375	A-130366.9	uscsguGfgUfGfGfacuucucucaL96	1587	A-130364.7	usGfsagaGfaAfGfuccaCfcAfcgasusu	1598
AD-65427	A-130441.7	gsusgcacUfuCfGfCfuucaccucuaL96	1588	A-131905.1	PusAfsgagGfugaagcgAfaGfugcacsusu	1599
AD-66110	A-130441.7	gsusgcacUfuCfGfCfuucaccucuaL96	1589	A-131905.1	VPusAfsgagGfugaagcgAfaGfugcacsusu	1600
AD-65421	A-130441.7	gsusgcacUfuCfGfCfuucaccucuaL96	1590	A-130442.6	usAfsgagGfugaagcgAfaGfugcacsusu	1601
AD-65407	A-130371.12	csgsugguGfgAfCfUfucucUfCfaauuL96	1591	A-130372.5	asAfsuugAfgAfgAfaguCfcAfccagcsasg	1602
AD-65377	A-130384.4	csgsuggudGgucdTucucuaaauuL96	1592	A-130748.3	asdAsuugagagdAagudCcaccagcsusu	1603
AD-65409	A-130388.15	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1593	A-131906.1	PusAfsaaaUfuGfAfgagaAfgUfccaccsasc	1604
AD-66111	A-130388.15	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1594	A-131906.1	VPusAfsaaaUfuGfAfgagaAfgUfccaccsasc	1605
AD-65403	A-130388.15	gsgsuggaCfuUfCfUfcucaAfUfuuuaL96	1595	A-130389.4	usAfsaaaUfuGfAfgagaAfgUfccaccsasc	1606

A primary single dose screen of these iRNA duplexes was performed using the Dual-Glo® Luciferase assay, as described above. The results of this screen in Cos7 cells transfected with the indicated HBV iRNAs are shown in Table 16. Data are expressed as percent of mRNA remaining relative to negative control at 24 hours.

Table 16. HBV single dose primary screen In Cos7 cells using Dual-Glo Luciferase® Assay

	Dual luciferase primary screen				
	% Message remaining at 24 hr				DRC ED50
Duplex ID	at 50 nM	STDEV	at 1 nM	STDEV	(nM)
AD-65381	9.3	0.24	15.6	0.77	0.019
AD-66019	ND	ND	ND	ND	ND
AD-65375	24.2	0.36	71.4	0.69	No ED50
AD-65427	28.8	1.60	41.0	1.73	0.117
AD-66110	ND	ND	ND	ND	ND
AD-65421	47.6	3.49	85.5	4.76	No ED50
AD-65407	14.3	0.52	25.3	2.11	0.038
AD-65377	21.8	0.31	37.9	1.12	0.130
AD-65409	9.5	0.41	13.2	0.71	0.013
AD-66111	ND	ND	ND	ND	ND
AD-65403	12.6	0.50	37.2	2.31	0.069

ND – not done

These duplexes were also assayed for dose response for silencing viral RNA using the Dual-Glo® Luciferase assay, as described above. The doses of the duplexes used for these asaays were 50 nM, 8.333333333 nM, 1.388888889 nM, 0.231481481 nM, 0.038580247 nM, 0.006430041 nM, 0.001071674 nM, 0.000178612 nM, 2.97687 x 10^{-5} nM, 4.96145 x 10^{-6} nM, 8.26909 x 10^{-7} nM, and 1.37818E x 10^{-7} nM, which represent a 1 to 6 dilution of the duplexes starting at 50 nM over 12 doses. The results of this screen in Cos7 cells transfected with the indicated HBV iRNAs are shown in Table 17. Data are expressed as percent of mRNA remaining relative to negative control at 24 hours.

Table 17. Dose response screen In Cos7 cells using Dual-Glo Luciferase® Assay

Dual luciferase HBV reporter cells IC50 (nM) at 24 hr									
Duplex ID	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Assay 5**	**Assay 6**	**Assay 7**	**Average[1]**	**Stdev**
AD-65381	0.019	ND	ND	ND	ND	ND	ND	0.019	
AD-66019	ND	0.021	0.021	0.016	0.026	0.019	0.031	0.022	0.005
AD-65375	UD	0.215	0.149	0.081	0.246	0.138	0.276	0.184	0.074
AD-65407	0.038	0.045	0.051	0.021	0.050	0.056	0.068	0.047	0.015
AD-65377	0.130	0.029	0.046	0.087	0.096	0.146	0.090	0.089	0.042
AD-65409	0.013	ND	ND	ND	ND	ND	ND	0.013	
AD-66111	ND	0.018	0.013	0.012	0.018	0.021	0.033	0.019	0.007
AD-65403	0.069	0.044	0.033	0.039	0.042	0.046	0.062	0.048	0.013
AD-65427	0.017	ND	ND	ND	ND	ND	ND	0.117	
AD-66110	ND	0.238	0.296	0.145	0.157	0.161	ND	0.199	0.065
AD-65421	UD	1.219	1.385	2.254	0.799	2.906	ND	1.713	0.852

[1]Averages from 5-7 biological replicates run in triplicate
ND – not done

The *in vitro* efficacy and potency of these duplexes were also assayed. In particular, the dose response of the duplexes for silencing viral RNA in transfected HepG2.2.15 and Hep3B cell lysates and for silencing HBsAg in HepG2.2.15 cell supernatants were determined. Cells were transfected with 12 separate doses of the duplexes ranging from 50 nM to 1 x 10^{-7} nM and at sevently-two hours after transfection, the level of viral RNA was

determined using primer/probe pairs to detect the P ORF and/or the S ORF. The level of HBsAg was determined using an ELISA assay.

The results of the P ORF viral RNA silencing in HepG2.2.15 cells using the indicated duplexes are provided in Table 18. The results of the S ORF viral RNA silencing in HepG2.2.15 cells using the indicated duplexes are provided in Table 19. The results of HBsAg silencing in HepG2.2.15 cells are provided in Table 20.

The results of the P ORF viral RNA silencing in Hep3B cells using the indicated duplexes are provided in Table 21.

Table 18. Dose response screen In HepG2.2.15 cells

Viral RNA silencing in HepG2.2.15 cells **P-ORF primer/probe set** **IC50 (nM) at 72 hr**								
Duplex ID	Assay Development					Optimized Assay		
						Assay 1	Assay 2	Assay 3
AD-65381	0.079	0.208	ND	ND		ND	ND	ND
AD-66019	ND	ND	0.265	0.010		0.022	0.032	0.023
AD-65375	12.3	UD	UD	UD		0.172	0.257	0.672
AD-65407	0.247	1.0	0.365	0.109		0.069	0.103	0.095
AD-65377	1.3	UD	4.9	UD		0.842	0.838	0.615
AD-65409	0.436	1.0	ND	ND		ND	ND	ND
AD-66111	ND	ND	0.456	0.030		50	0.294	ND
AD-65403	9.2	10.4	3.4	UD		0.114	0.384	1.0
AD-65427	0.007	0.018	ND	ND		ND	ND	ND
AD-66110	ND	ND	0.012	0.053		0.016	0.010	0.021
AD-65421	0.069	0.091	0.034	0.006		0.002	0.003	0.007

ND – not done

Table 19. Dose response screen In HepG2.2.15 cells

Viral RNA silencing in HepG2.2.15 cells **S-ORF primer/probe set** **IC50 (nM) at 72 hr**								
Duplex ID	Assay Development					Optimized Assay		
						Assay 1	Assay 2	Assay 3
AD-65381	0.252	0.215	ND	ND		ND	ND	ND
AD-66019	ND	ND	0.245	0.011		0.009	0.016	0.005
AD-65375	45	UD	UD	UD		0.124	0.048	0.056
AD-65407	0.232	0.645	0.577	0.015		0.021	0.023	0.016
AD-65377	1.4	8.6	UD	UD		0.575	0.483	0.117
AD-65409	0.433	0.242	ND	ND		ND	ND	ND
AD-66111	ND	ND	2.1	0.455		ND	0.416	ND
AD-65403	0.997	0.670	0.668	UD		0.074	0.270	1.1
AD-65427	0.008	0.018	ND	ND		ND	ND	ND
AD-66110	ND	ND	0.022	0.050		0.035	0.038	0.020
AD-65421	0.083	0.097	0.046	0.003		0.003	0.005	0.001

ND – not done

Table 20. Dose response screen In HepG2.2.15 cells

HBsAg ELISA **IC50 (nM)**	
Duplex ID	Assay 1
AD-65381	ND
AD-66019	0.105
AD-65375	1.2
AD-65407	0.102
AD-65377	2.9
AD-65409	ND
AD-66111	0.018
AD-65403	0.064

AD-65427	ND
AD-66110	0.002
AD-65421	0.008

ND – not done

Table 21. Dose response screen In Hep3B cells

	Hep3B cells screen		
	DRC ED50 P-ORF primer/probe set		
Duplex ID	**P-ORF run 1**	**P-ORF run 2**	**Combined**
AD-65381	0.239	0.110	0.194
AD-66019	ND	ND	ND
AD-65375	ND	ND	ND
AD-65427	0.023	0.006	0.018
AD-66110	ND	ND	ND
AD-65421	ND	ND	ND
AD-65407	0.140	1.383	0.527
AD-65377	No ED50	No ED50	No ED50
AD-65409	1.807	3.436	2.905
AD-66111	ND	ND	ND
AD-65403	0.511	18.036	5.013

ND – not done

These duplexes were also assayed for *in vitro* stability using two assays, a tritosome stability assay and a cytosol stability assay, as described above. The results of these assays are provided in Table 22.

Table 22. Twenty-four hour tritosome and cytosol stability assays.

	In vitro metabolic stability % parent remaining at 24hr incubation			
	Endo-lysosome		Cytosol	
Duplex ID	% AS	% SS	% AS	% SS
AD-65381	88	72	68	22
AD-66019	ND	ND	ND	ND
AD-65375	ND	ND	ND	ND
AD-65407	100	120	64	24
AD-65377	99	127	80	67
AD-65409	105	120	86	74
AD-66111	ND	ND	ND	ND
AD-65403	ND	ND	ND	ND
AD-65427	115	80	89	66
AD-66110	ND	ND	ND	ND
AD-65421	ND	ND	ND	ND

Dose response screens of various combinations of these duplexes were also performed in HepG2.215 cells. The doses of the duplexes used for these assays were 50 nM, 8.333333333 nM, 1.388888889 nM, 0.231481481 nM, 0.038580247 nM, 0.006430041 nM, 0.001071674 nM, 0.000178612 nM, 2.97687 x 10^{-5} nM, 4.96145 x 10^{-6} nM, 8.26909 x 10^{-7} nM, and 1.37818E x 10^{-7} nM, which represent a 1 to 6 dilution of the duplexes starting at 50 nM over 12 doses. At seventy-two hours after transfection of these duplexes, the level of viral RNA (P ORF and S ORF) and the level of secreted HBsAg were determined, as described above. The results of these assays are provided in Table 23.

Table 23. Seventy-two hour HBV single dose screen In HepG2.2.15 cells

DuplexID	S-ORF2 IC50_A (nM)	S-ORF2 IC50_B (nM)	S-ORF2 IC50_Combine (nM)	P-ORF1 IC50_A (nM)	P-ORF1 IC50_B (nM)	P-ORF1 IC50_Combine (nM)	S Ag ELISA ED50 (nM)
AD-66019/AD-66110	0.0091	0.0017	0.0038	0.0213	0.002	0.0076	0.007482
AD-66019/AD-65421	0.0438	0.2371	0.0131	0.0367	0.0106	0.0204	0.026398
AD-65375/AD-66110	0.0832	1.0896	0.193	0.0377	0.2348	0.2022	0.004174
AD-65375/AD-65421	0.084	0.0475	0.0708	0.0566	0.0388	0.0371	0.030822
AD-65407/AD-66110	0.0387	0.001	0.0083	0.0402	0.0018	0.0116	0.010172
AD-65407/AD-65421	0.0686	0.0062	0.0225	0.0711	0.0177	0.0396	0.066556
AD-65377/AD-66110	0.0634	0.8267	0.6269	0.0477	0.073	0.0618	0.01435
AD-65377/AD-65421	0.1461	0.0468	0.1372	0.1207	0.0088	0.0451	0.03419
AD-66111/AD-66110	0.0382	0.0094	0.0161	0.0292	0.0027	0.0088	0.013155
AD-66111/AD-65421	0.1628	0.0919	0.1579	0.1297	0.0396	0.0722	0.026889
AD-65403/AD-66110	0.0499	0.0094	0.0444	0.0383	0.0164	0.0348	0.003783
AD-65403/AD-65421	0.1011	0.0007	0.0208	0.1118	0.0031	0.0297	0.014569

Example 6. Synthesis and *In vitro* Screening of Additional siRNA Duplexes Targeting HBV

Additional iRNA molecules targeting the X ORF of the HBV genome were designed and synthesized as described above. A detailed list of the additional unmodified HBV sense and antisense strand sequences is shown in Table 24. A detailed list of the additional modified HBV sense and antisense strand sequences is shown in Table 25.

Table 24. Unmodified Sense and Antisense Strand Sequences of HBV dsRNAs

Duplex ID	Sense OligoName	Sence Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65776	A-131859.1	UGUGCACUUCGCUUCACCUCU	1607	A-131860.1	AGAGGUGAAGCGAAGUGCACACG	1653
AD-65782	A-131877.1	UGCACUUCGCUUCACCUCUGA	1608	A-131878.1	UCAGAGGUGAAGCGAAGUGCACA	1654
AD-65792	A-131865.1	GUGUGCACUUCGCUUCACCUA	1609	A-131866.1	UAGGUGAAGCGAAGUGCACACGG	1655
AD-65781	A-131861.1	CGUGUGCACUUCGCUUCACCU	1610	A-131862.1	AGGUGAAGCGAAGUGCACACGGU	1656
AD-64304	A-128443.6	GUGCACUUCGCUUCACCUCUA	1611	A-128444.5	UAGAGGUGAAGCGAAGUGCACAC	1657
AD-65771	A-131857.1	CCGUGUGCACUUCGCUUCACA	1612	A-131858.1	UGUGAAGCGAAGUGCACACGGUC	1658
AD-65758	A-131867.1	CACUUCGCUUCACCUCUGCAA	1613	A-131868.1	UUGCAGAGGUGAAGCGAAGUGCA	1659
AD-65777	A-131875.1	ACUUCGCUUCACCUCUGCACA	1614	A-131876.1	UGUGCAGAGGUGAAGCGAAGUGC	1660
AD-61567	A-123525.2	GGCUGUAGGCAUAAAUUGGUA	1615	A-123526.2	UACCAAUUUAUGCCUACAGCCUC	1661
AD-65772	A-131873.1	UUCGCUUCACCUCUGCACGUA	1616	A-131874.1	UACGUGCAGAGGUGAAGCGAAGU	1662
AD-65767	A-131871.1	UCGCUUCACCUCUGCACGUCA	1617	A-131872.1	UGACGUGCAGAGGUGAAGCGAAG	1663
AD-65763	A-131869.1	CUUCGCUUCACCUCUGCACGU	1618	A-131870.1	ACGUGCAGAGGUGAAGCGAAGUG	1664
AD-64281	A-128395.3	CCCCGUCUGUGCCUUCUCAUA	1619	A-128396.2	UAUGAGAAGGCACACAGACGGGGAG	1665
AD-64311	A-128391.3	CCGUCUGUGCCUUCUCAUCUA	1620	A-128392.2	UAGAUGAGAAGGCACACAGACGGGG	1666
AD-65790	A-131837.1	CCAGCACCAUGCAACUUUUUA	1621	A-131838.1	UAAAAAGUUGCAUGGUGCUGGUG	1667
AD-65761	A-131841.1	CACCAGCACCAUGCAACUUUU	1622	A-131842.1	AAAAGUUGCAUGGUGCUGGUGCG	1668
AD-65786	A-131849.1	CACCAUGCAACUUUUUUCACCU	1623	A-131850.1	AGGUGAAAAAGUUGCAUGGUGCU	1669
AD-65785	A-131835.1	CAAUGUCAACGACCGACCUUA	1624	A-131836.1	UAAGGUCGGUCGUUGACAUUGCA	1670
AD-65787	A-131863.1	CGCUUCACCUCUGCACGUCGA	1625	A-131864.1	UCGACGUGCAGAGGUGAAGCGAA	1671

Duplex ID	Sense OligoName	Sence Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65770	A-131845.1	ACCUUGAGGCAUACUUCAAAG	1626	A-131846.1	CUUUGAAGUAUGCCUCAAGGUCG	1672
AD-65766	A-131843.1	CCGACCUUGAGGCAUACUUCA	1627	A-131844.1	UGAAGUAUGCCUCAAGGUCGGUC	1673
AD-61555	A-123521.2	GACCUUGAGGCAUACUUCAAA	1628	A-123522.2	UUUGAAGUAUGCCUCAAGGUCGG	1674
AD-65762	A-131855.1	ACCGACCUUGAGGCAUACUUA	1629	A-131856.1	UAAGUAUGCCUCAAGGUCGGUCG	1675
AD-65755	A-131827.1	UCGCAUGGAGACCACCGUGAA	1630	A-131828.1	UUCACGGUGGUCUCCAUGCGACG	1676
AD-65788	A-131811.1	UUACAUAAGAGGACUCUUGGA	1631	A-131812.1	UCCAAGAGUCCUCUUAUGUAAGA	1677
AD-65768	A-131803.1	UCUUACAUAAGAGGACUCUUA	1632	A-131804.1	UAAGAGUCCUCUUAUGUAAGACC	1678
AD-61561	A-123523.2	ACUUCAAAGACUGUUUGUUUA	1633	A-123524.2	UAAACAAACAGUCUUUGAAGUAU	1679
AD-65764	A-131801.1	UACUUCAAAGACUGUUUGUUU	1634	A-131802.1	AAACAAACAGUCUUUGAAGUAUG	1680
AD-65753	A-131799.1	AUACUUCAAAGACUGUUUGUU	1635	A-131800.1	AACAAACAGUCUUUGAAGUAUGC	1681
AD-65765	A-131817.1	UUGUUUAAAGACUGGGAGGAA	1636	A-131818.1	UUCCUCCCAGUCUUUAAACAAAC	1682
AD-65769	A-131819.1	GCAUACUUCAAAGACUGUUUA	1637	A-131820.1	UAAACAGUCUUUGAAGUAUGCCU	1683
AD-65759	A-131815.1	CAAAGACUGUUUGUUUAAAGA	1638	A-131816.1	UCUUUAAACAAACAGUCUUUGAA	1684
AD-65774	A-131831.1	AGACUGUUUGUUUAAAGACUA	1639	A-131832.1	UAGUCUUUAAACAAACAGUCUUU	1685
AD-65778	A-131807.1	GUUUGUUUAAAGACUGGGAGA	1640	A-131808.1	UCUCCCAGUCUUUAAACAAACAG	1686
AD-65773	A-131805.1	GGGGGAGGAGAUUAGAUUAAA	1641	A-131806.1	UUUAAUCUAAUCUCCUCCCCCAA	1687
AD-65789	A-131825.1	GGGGAGGAGAUUAGAUUAAAG	1642	A-131826.1	CUUUAAUCUAAUCUCCUCCCCCA	1688
AD-65783	A-131809.1	GUUGGGGGAGGAGAUUAGAUU	1643	A-131810.1	AAUCUAAUCUCCUCCCCCAACUC	1689
AD-65754	A-131813.1	UUGGGGGAGGAGAUUAGAUUA	1644	A-131814.1	UAAUCUAAUCUCCUCCCCCAACU	1690
AD-65779	A-131821.1	GGGAGGAGAUUAGAUUAAAGA	1645	A-131822.1	UCUUUAAUCUAAUCUCCUCCCCC	1691
AD-65791	A-131851.1	UUAGAUUAAAGGUCUUUGUAA	1646	A-131852.1	UUACAAAGACCUUUAAUCUAAUC	1692
AD-65760	A-131829.1	UAGAUUAAAGGUCUUUGUACU	1647	A-131830.1	AGUACAAAGACCUUUAAUCUAAU	1693
AD-65784	A-131823.1	AUUAGAUUAAAGGUCUUUGUA	1648	A-131824.1	UACAAAGACCUUUAAUCUAAUCU	1694

Duplex ID	Sense OligoName	Sence Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65757	A-131853.1	GAGGAGAUUAGAUUAAAGGUA	1649	A-131854.1	UACCUUUAAUCUAAUCUCCUCCC	1695
AD-65775	A-131847.1	GGACUCUUGGACUCUCUGCAA	1650	A-131848.1	UUGCAGAGAGUCCAAGAGUCCUC	1696
AD-65780	A-131833.1	ACUCUUGGACUCUCUGCAAUA	1651	A-131834.1	UAUUGCAGAGAGUCCAAGAGUCC	1697
AD-65756	A-131839.1	AGAUUAAAGGUCUUUGUACUA	1652	A-131840.1	UAGUACAAAGACCUUUAAUCUAA	1698

Table 25. Modified Sense and Antisense Strand Sequences of HBV dsRNAs

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65776	A-131859.1	UfsgsUfgCfaCfuUfCfGfcUfuCfaCfcUfcUfL96	1699	A-131860.1	asGfsaGfgUfgAfaGfcgaAfgUfgCfaCfascsg	1745
AD-65782	A-131877.1	UfsgsCfaCfuUfcGfCfUfuCfaCfcUfcUfgAfL96	1700	A-131878.1	usCfsaGfaGfgUfgAfagcGfaAfgUfgCfascsa	1746
AD-65792	A-131865.1	GfsusGfuGfcAfcUfUfCfgCfuUfcAfcCfuAfL96	1701	A-131866.1	usAfsgGfuGfaAfgCfgaaGfuGfcAfcAfcsgsg	1747
AD-65781	A-131861.1	CfsgsUfgUfgCfaCfUfUfcGfcUfuCfaCfcUfL96	1702	A-131862.1	asGfsgUfgAfaGfcGfaagUfgCfaCfaCfgsgsu	1748
AD-64304	A-128443.6	GfsusGfcAfcUfuCfGfCfuUfcAfcCfuCfuAfL96	1703	A-128444.5	usAfsgAfgGfuGfaAfgcgAfaGfuGfcAfcsasc	1749
AD-65771	A-131857.1	CfscsGfuGfuGfcAfCfUfuCfgCfuUfcAfcAfL96	1704	A-131858.1	usGfsuGfaAfgCfgAfaguGfcAfcAfcGfgsusc	1750
AD-65758	A-131867.1	CfsasCfuUfcGfcUfUfUfCfaCfcUfcUfgCfaAfL96	1705	A-131868.1	usUfsgCfaGfaGfgUfgaaGfcGfaAfgUfgscsa	1751
AD-65777	A-131875.1	AfscsUfuCfgCfuUfCfAfcCfuCfuGfcAfcAfL96	1706	A-131876.1	usGfsuGfcAfgAfgGfugaAfgCfgAfaGfusgsc	1752

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-61567	A-123525.2	GfsgsCfuGfuAfgGfCfAfuAfaAfuUfgGfuAfL96	1707	A-123526.2	usAfscCfaAfuUfuAfugcCfuAfcAfgCfcsusc	1753
AD-65772	A-131873.1	UfsusCfgCfuUfcAfCfCfuCfuGfcAfcGfuAfL96	1708	A-131874.1	usAfscGfuGfcAfgAfgguGfaAfgCfgAfasgsu	1754
AD-65767	A-131871.1	UfscsGfcUfuCfaCfCfUfcUfgCfaCfgUfcAfL96	1709	A-131872.1	usGfsaCfgUfgCfaGfaggUfgAfaGfcGfasasg	1755
AD-65763	A-131869.1	CfsusUfcGfcUfuCfAfCfcUfcUfgCfaCfgUfL96	1710	A-131870.1	asCfsgUfgCfaGfaGfgugAfaGfcGfaAfgsusg	1756
AD-64281	A-128395.3	CfscsCfcGfuCfuGfUfGfcCfuUfcUfcAfuAfL96	1711	A-128396.2	usAfsuGfaGfaAfgGfcacAfgAfcGfgGfgsasg	1757
AD-64311	A-128391.3	CfscsGfuCfuGfuGfCfCfuUfcUfcAfuCfuAfL96	1712	A-128392.2	usAfsgAfuGfaGfaAfggcAfcAfgAfcGfgsgsg	1758
AD-65790	A-131837.1	CfscsAfgCfaCfcAfUfGfcAfaCfuUfuUfuAfL96	1713	A-131838.1	usAfsaAfaAfgUfuGfcauGfgUfgCfuGfgsusg	1759
AD-65761	A-131841.1	CfsasCfcAfgCfaCfCfAfuGfcAfaCfuUfuUfL96	1714	A-131842.1	asAfsaAfgUfuGfcAfuggUfgCfuGfgUfgsCsg	1760
AD-65786	A-131849.1	CfsasCfcAfuGfcAfAfCfuUfuUfuCfaCfcUfL96	1715	A-131850.1	asGfsgUfgAfaAfaAfguuGfcAfuGfgUfgsCsu	1761
AD-65785	A-131835.1	CfsasAfuGfuCfaAfCfGfaCfcGfaCfcUfuAfL96	1716	A-131836.1	usAfsaGfgUfcGfgUfcguUfgAfcAfuUfgsCsa	1762
AD-65787	A-131863.1	CfsgsCfuUfcAfcCfUfCfuGfcAfcGfuCfgAfL96	1717	A-131864.1	usCfsgAfcGfuGfcAfgagGfuGfaAfgCfgsasa	1763
AD-65770	A-131845.1	AfscsCfuUfgAfgGfCfAfuAfcUfuCfaAfaGfL96	1718	A-131846.1	csUfsuUfgAfaGfuAfugcCfuCfaAfgGfuscsg	1764
AD-65766	A-131843.1	CfscsGfaCfcUfuGfAfGfgCfaUfaCfuUfcAfL96	1719	A-131844.1	usGfsaAfgUfaUfgCfcucAfaGfgUfcGfgsusc	1765
AD-61555	A-123521.2	GfsasCfcUfuGfaGfGfCfaUfaCfuUfcAfaAfL96	1720	A-123522.2	usUfsuGfaAfgUfaUfgccUfcAfaGfgUfcsgsg	1766

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65762	A-131855.1	AfscsCfgAfcCfuUfGfAfgGfcAfuAfcUfuAfL96	1721	A-131856.1	usAfsaGfuAfuGfcCfucaAfgGfuCfgGfuscsg	1767
AD-65755	A-131827.1	UfscsGfcAfuGfgAfGfAfcCfaCfcGfuGfaAfL96	1722	A-131828.1	usUfscAfcGfgUfgGfucuCfcAfuGfcGfascsg	1768
AD-65788	A-131811.1	UfsusAfcAfuAfaGfAfGfgAfcUfcUfuGfgAfL96	1723	A-131812.1	usCfscAfaGfaGfuCfcucUfuAfuGfuAfasgsa	1769
AD-65768	A-131803.1	UfscsUfuAfcAfuAfAfGfaGfgAfcUfcUfuAfL96	1724	A-131804.1	usAfsaGfaGfuCfcUfcuuAfuGfuAfaGfascsc	1770
AD-61561	A-123523.2	AfscsUfuCfaAfaGfAfCfuGfuUfuGfuUfuAfL96	1725	A-123524.2	usAfsaAfcAfaAfcAfgucUfuUfgAfaGfusasu	1771
AD-65764	A-131801.1	UfsasCfuUfcAfaAfGfAfcUfgUfuUfgUfuUfL96	1726	A-131802.1	asAfsaCfaAfaCfaGfucuUfuGfaAfgUfasusg	1772
AD-65753	A-131799.1	AfsusAfcUfuCfaAfAfGfaCfuGfuUfuGfuUfL96	1727	A-131800.1	asAfscAfaAfcAfgUfcuuUfgAfaGfuAfusgsc	1773
AD-65765	A-131817.1	UfsusGfuUfuAfaAfGfAfcUfgGfgAfgGfaAfL96	1728	A-131818.1	usUfscCfuCfcCfaGfucuUfuAfaAfcAfasasc	1774
AD-65769	A-131819.1	GfscsAfuAfcUfuCfAfAfaGfaCfuGfuUfuAfL96	1729	A-131820.1	usAfsaAfcAfgUfcUfuugAfaGfuAfuGfcscsu	1775
AD-65759	A-131815.1	CfsasAfaGfaCfuGfUfUfuGfuUfuAfaAfgAfL96	1730	A-131816.1	usCfsuUfuAfaAfcAfaacAfgUfcUfuUfgsasa	1776
AD-65774	A-131831.1	AfsgsAfcUfgUfuUfGfUfuUfaAfaGfaCfuAfL96	1731	A-131832.1	usAfsgUfcUfuUfaAfacaAfaCfaGfuCfususu	1777
AD-65778	A-131807.1	GfsusUfuGfuUfuAfAfAfgAfcUfgGfgAfgAfL96	1732	A-131808.1	usCfsuCfcCfaGfuCfuuuAfaAfcAfaAfcsasg	1778
AD-65773	A-131805.1	GfsgsGfgGfaGfgAfGfAfuUfaGfaUfuAfaAfL96	1733	A-131806.1	usUfsuAfaUfcUfaAfucuCfcUfcCfcCfcsasa	1779
AD-65789	A-131825.1	GfsgsGfgAfgGfaGfAfUfuAfgAfuUfaAfaGfL96	1734	A-131826.1	csUfsuUfaAfuCfuAfaucUfcCfuCfcCfcscsa	1780

Duplex ID	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense OligoName	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-65783	A-131809.1	GfsusUfgGfgGfgAfGfGfaGfaUfuAfgAfuUfL96	1735	A-131810.1	asAfsuCfuAfaUfcUfccuCfcCfcCfaAfcsusc	1781
AD-65754	A-131813.1	UfsusGfgGfgGfaGfGfAfgAfuUfaGfaUfuAfL96	1736	A-131814.1	usAfsaUfcUfaAfuCfuccUfcCfcCfcAfascsu	1782
AD-65779	A-131821.1	GfsgsGfaGfgAfgAfUfUfaGfaUfuAfaAfgAfL96	1737	A-131822.1	usCfsuUfuAfaUfcUfaauCfuCfcUfcCfcscsc	1783
AD-65791	A-131851.1	UfsusAfgAfuUfaAfAfGfgUfcUfuUfgUfaAfL96	1738	A-131852.1	usUfsaCfaAfaGfaCfcuuUfaAfuCfuAfasusc	1784
AD-65760	A-131829.1	UfsasGfaUfuAfaAfGfGfuCfuUfuGfuAfcUfL96	1739	A-131830.1	asGfsuAfcAfaAfgAfccuUfuAfaUfcUfasasu	1785
AD-65784	A-131823.1	AfsusUfaGfaUfuAfAfAfgGfuCfuUfuGfuAfL96	1740	A-131824.1	usAfscAfaAfgAfcCfuuuAfaUfcUfaAfuscsu	1786
AD-65757	A-131853.1	GfsasGfgAfgAfuUfAfGfaUfuAfaAfgGfuAfL96	1741	A-131854.1	usAfscCfuUfuAfaUfcuaAfuCfuCfcUfcscsc	1787
AD-65775	A-131847.1	GfsgsAfcUfcUfuGfGfAfcUfcUfgCfaAfL96	1742	A-131848.1	usUfsgCfaGfaGfaGfuccAfaGfaGfuCfcsusc	1788
AD-65780	A-131833.1	AfscsUfcUfuGfgAfCfUfcUfcUfgCfaAfuAfL96	1743	A-131834.1	usAfsuUfgCfaGfaGfaguCfcAfaGfaGfuscsc	1789
AD-65756	A-131839.1	AfsgsAfuUfaAfaGfGfUfcUfuUfgUfaCfuAfL96	1744	A-131840.1	usAfsgUfaCfaAfaGfaccUfuUfaAfuCfusasa	1790

A single dose screen of these duplexes was performed in Cos7 cells at 1 nm and 50 nm using the Dual-Glo® Luciferase assay described above. The results of the assays are provided in Table 26.

Table 26. HBV single dose screen using Dual-Glo Luciferase® Assay

Duplex ID	50 nM	STDEV	1 nM	STDEV
AD-65776	20.11	4.21	40.79	1.89
AD-65782	26.31	3.10	61.07	9.16
AD-65792	43.31	5.24	61.09	6.02
AD-65781	25.77	3.66	39.63	2.87
AD-64304	18.87	1.26	29.72	3.37
AD-65771	17.16	1.78	37.55	2.20
AD-65758	31.74	8.26	65.77	11.05
AD-65777	59.76	11.15	77.63	5.14
AD-61567	17.69	5.29	26.45	5.66
AD-65772	58.07	9.67	75.66	4.92
AD-65767	29.65	1.60	39.64	4.36
AD-65763	25.10	5.77	47.78	9.99
AD-64281	39.07	6.80	51.46	4.19
AD-64311	20.51	1.96	37.80	3.53
AD-65790	50.41	7.00	70.30	1.95
AD-65761	13.30	4.38	21.14	3.49
AD-65786	12.45	3.51	22.62	0.33
AD-65785	36.87	6.04	51.49	4.18
AD-65787	27.97	5.73	48.18	7.65
AD-65770	22.67	5.39	41.48	8.52
AD-65766	31.44	3.35	50.25	0.45
AD-61555	18.43	10.83	22.61	0.57
AD-65762	18.87	4.86	34.94	4.81
AD-65755	47.03	9.38	83.19	9.68
AD-65788	35.85	10.13	58.07	4.78
AD-65768	24.02	2.49	28.55	2.53
AD-61561	8.11	1.29	14.26	2.27
AD-65764	16.89	3.99	29.10	1.03
AD-65753	19.10	2.87	29.79	5.26
AD-65765	55.40	10.72	76.93	8.79
AD-65769	19.24	4.47	23.18	2.54

Duplex ID	50 nM	STDEV	1 nM	STDEV
AD-65759	48.86	4.81	87.31	13.75
AD-65774	102.27	12.33	100.79	3.24
AD-65778	64.39	2.60	80.67	2.59
AD-65773	72.64	7.87	80.80	4.83
AD-65789	73.59	4.35	94.72	3.32
AD-65783	54.41	7.15	84.46	4.32
AD-65754	62.51	4.12	102.63	21.42
AD-65779	47.40	7.51	76.20	2.05
AD-65791	12.09	0.70	19.19	3.46
AD-65760	13.50	4.84	25.37	2.09
AD-65784	19.84	1.27	31.04	3.49
AD-65757	22.66	3.97	24.50	5.81
AD-65775	47.78	3.30	58.81	3.05
AD-65780	29.10	2.87	42.85	2.73
AD-65756	10.49	1.62	19.95	2.58

Based on these assays, RNAi agents targeting five sites in the HBV X ORF (nucleotides 1551, 1577, 1580, 1806, and 1812 of GenBank Accession No. NC_003977.1) were selected for lead optimization and additional agents were designed and synthesized. These additional agents are evaluated in *in vitro* assays as described above. A detailed list of the additional unmodified sense and antisense strand sequences targeting the HBV X ORF is shown in Table 27. A detailed list of the additional modified sense and antisense strand sequences targeting the HBV X ORF is shown in Table 28.

These iRNA agents were also assessed for *in vivo* efficacy using an AAV-HBV mouse model (see, *e.g.,* Yang, *et al.* (2014) *Cell and Mol Immunol* 11:71). This mouse model exhibits sustained HBV viremia after infection with a recombinant adeno-associated virus (AAV) carrying a replicable HBV genome. Liver expression of the HBV gene in these mice mimics HBV infection in humans and these mice exhibit significant liver inflammation and liver damage, manifested by increased ALT levels, fibrosis and steatosis.

These AAV-HBV mice were subcutaneously administered a single 3 mg/kg dose of AD-66808, AD-66809, AD-66810, AD-66811, AD-66812, AD-66813, AD-

66814, AD-66815, AD-66816, and AD-66817 and the level of HBsAg was determined in the serum of the animals pre-dose, and at day 14/15 post-dose. The results of these experiments are provided in Table 29 and demonstrate that serum levels of HBsAg are decrease following a single administration of these agents. Table 29 also provides the results of a single dose screen in Cos7 cells transfected with the indicated HBV iRNAs using the Dual-Glo® Luciferase assay, as described above, for the same RNAi agents. Data are expressed as percent of mRNA remaining relative to negative control at 24 hours.

Table 27. Unmodified HBV X ORF Sense and Antisense Sequences.

DuplexID	SenseSequence (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-66808	GUCUGUGCCUUCUCAUCUA	1791	UAGAUGAGAAGGCACAGACUU	1801
AD-66809	GUCUGUGCCUUCUCAUCUA	1792	UAGAUGAGAAGGCACAGACUU	1802
AD-66810	GUGUGCACUUCGCUUCACA	1793	UGUGAAGCGAAGUGCACACUU	1803
AD-66811	GUGUGCACUUCGCUUCACA	1794	UGUGAAGCGAAGUGCACACUU	1804
AD-66812	UGUGCACUUCGCUUCACCUCU	1795	AGAGGUGAAGCGAAGUGCACAUU	1805
AD-66813	UGUGCACUUCGCUUCACCUCU	1796	AGAGGUGAAGCGAAGUGCACAUU	1806
AD-66814	CACCAGCACCAUGCAACUUUU	1797	AAAAGUUGCAUGGUGCUGGUGUU	1807
AD-66815	CACCAGCACCAUGCAACUUUU	1798	AAAAGUUGCAUGGUGCUGGUGUU	1808
AD-66816	CACCAUGCAACUUUUUCACCU	1799	AGGUGAAAAAGUUGCAUGGUGUU	1809
AD-66817	CACCAUGCAACUUUUUCACCU	1800	AGGUGAAAAAGUUGCAUGGUGUU	1810

Table 28. Modified HBV X ORF Sense and Antisense Sequences.

DuplexID	Sense Seque nce (5' to 3')	SEQ ID NO:	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-66808	gsuscuGfuGfCfCfuucucaucuaL96	1811	usAfsgauGfaGfAfaggcAfcAfgacsusu	1821
AD-66809	gsuscuGfuGfCfCfuucucaucuaL96	1812	UfsAfsgauGfaGfAfaggcAfcAfgacsusu	1822
AD-66810	gsusguGfcAfCfUfucgcuucacaL96	1813	usGfsugaAfgCfGfaaguGfcAfcacsusu	1823
AD-66811	gsusguGfcAfCfUfucgcuucacaL96	1814	UfsGfsugaAfgCfGfaaguGfcAfcacsusu	1824
AD-66812	usgsugcaCfuUfCfGfcuucaccucuL96	1815	asGfsaggUfgAfAfgcgaAfgUfgcacasusu	1825
AD-66813	usgsugcaCfuUfCfGfcuucaccucuL96	1816	AfsGfsaggUfgAfAfgcgaAfgUfgcacasusu	1826
AD-66814	csasccagCfaCfCfAfugcaacuuuuL96	1817	asAfsaagUfuGfCfauggUfgCfuggugsusu	1827
AD-66815	csasccagCfaCfCfAfugcaacuuuuL96	1818	AfsAfsaagUfuGfCfauggUfgCfuggugsusu	1828
AD-66816	csasccauGfcAfAfCfuuuuucaccuL96	1819	asGfsgugAfaAfAfaguuGfcAfuggugsusu	1829
AD-66817	csasccauGfcAfAfCfuuuuucaccuL96	1820	AfsGfsgugAfaAfAfaguuGfcAfuggugsusu	1830

Table 29.

Site (# vRNA[1])	Duplex ID	In vitro IC_{50} Luc HBV (nM)	Log_{10} HBsAg KD In Vivo @3 mg/kg
1551 (4)	AD-66808	**0.187**	**2.4**
	AD-66809	**0.014**	**1.46**
1577 (4)	AD-66810	**0.290**	**1.7**
	AD-66811	**0.029**	**1.3**
1580 (4)	AD-66812	**0.795**	**2.19**
	AD-66813	**0.074**	**>>1.14**
1806 (4)	AD-66814	**0.0002**	**1.5**
	AD-66815	**0.0001**	**>>1.56**
1812 (4)	AD-66816	**0.047**	**1.61**
	AD-66817	**0.0001**	**1.60**

[1]Number of viral RNAs silenced

Example 6. *In vivo* Screening of iRNA Duplexes

A subset of lead iRNA agents was assessed for *in vivo* efficacy using the AAV-HBV mouse model described above. AAV-HBV mice were administered a single 3 mg/kg dose of AD-66019, AD-65375, AD-65047, AD-65377, AD-66111, AD-65421, or AD-66110 and the level of HBsAg was determined in the serum of the animals pre-dose, and at days 5 and 10 post-dose. As a control, AAV-HBV mice were administered a 3 mg/kg dose of a dsRNA targeting mouse/rat transtherytin (mrTTR). The results of these experiments demonstrate that serum levels of HBsAg are decreased following a single administration of these agents.

The percent of pre-dose HBsAg remaining at days 5 and 10 was determined in these animals following administration of a single 3 mg/kg dose. A significant decrease in HBsA

at both days 5 and 10 after administration were observed in at least AD-65375, AD-65403, AD-65407, AD-65421, AD-66019, and AD-66111.

Based, at least in part, on the results of the *in vitro* and *in vivo* assays described above, AD-65403, which silences 3 HBV RNAs, and AD-66810, which silences the X gene, were selected for further analysis for use in a monotherapy or in a combination therapy of HBV.

In the AAV-HBV mouse model of HBV infection, a single 3 mg/kg dose of AD-65403 achieved potent and specific knockdown of HBsAg. In particular, a single 3 mg/kg subcutaneous dose of AD-65403 achieved up to a 3.9 $\log_{10}$ reduction in HBsAg levels, with a mean HBsAg reduction of 1.8 $\log_{10}$ 5-10 days after a single dose.

In the AAV-HBV mouse model of HBV infection, a single 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 9 mg/kg dose of AD-66810 achieved potent and specific knockdown of HBsAg, especially at the higher doses of AD-66810.

In the AAV-HBV mouse model of HBV infection, AD-66810 administered in three weekly 3 mg/kg doses, achieved potent and specific knockdown of HBsAg for a period of greater than 4 months.

Example 7. *In vitro* screening of additional iRNA duplexes targeting HDV

***In vitro* screening**:

Cell culture and transfections:

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Dual-Glo® Luciferase constructs generated in the psiCHECK2 plasmid containing HDV AY633627 derived sequences (Table 30) were transfected into approximately $4x10^3$ cells using Lipofectamine 2000 (Invitrogen, Carlsbad CA. cat # 11668-019). For each well of a 384 well plate, 0.15µl of Lipofectamine, 4ng of vector, and an appropriate amount of one of the modified siRNA agents provided in Table 32 were mixed in 15µl final volume of Opti-MEM and allowed to complex at room temperature for 15 minutes. Subsequently the mixture was then supplemented with the suspention of cells (35 ul in complete media). Cells were incubated for 48 hours before luciferase was measured.

The unmodified sequences of the agents provided in Table 32 are provided in Table 3, which were synthesized as described above.

Dual-Glo® Luciferase assay

Firefly (transfection control) and Renilla (fused to HBV target sequence) luciferases were measured using Dual-Glo® Luciferase Assay (Promega, Madison, Wisconsin, # E2980). First, media was removed from cells. Then Firefly luciferase activity was measured by adding a mixture of 10µl of the complete media and 10µl of Dual-Glo® Luciferase Reagent per well. The mixture was incubated at room temperature for 10- 15 minutes before lunimescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 10µl of room temperature Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenches the firefly luciferase signal and sustained luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (HDV) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA (mock). All transfections were done at n=2 or greater. siRNA concentration sufficient to inhibit relative Renilla luciferase expression by 50% (IC_{50}) was determined by the method described above at a range of concentrations (40 fM-10 nM).

The results of the single dose screen are provided in Table 33 and the results of the dose response screens are provided in Table 34.

Table 30. HDV sequences used in luciferase plasmids

Vector Name	HDV sequence
psiCHECK2-HDV-antigenome	GCGATCGCCTCGAACTTGGGCGGCGAGTCCAGCAGTCTCCTCTTTATCAGAAAAGAGTAAGA GCACTGAGGACTGCCGCCTCTTGTCGAGATGAGCCGGTCCGAGTTGAAGAAGAAGCGCGATG GAAGAGAAGATATTCTCGAGAAGTGGGTGAGTGGAAGAAAGAAAGCGGAGGAACTCGAGAGG GATCTCCGGAAGACAAAGAAGAAGATCAAGAAACTTGAGGTCGAAAATCCCTGGCTGGGAAA CATCAAAGGAATTCTCGGAAAGAAGGACAGGGATGGAGAGGGGGCTCCCCCGGCGAAGAGGG CCCGGACGGACCAGATGGAGATAGACTCCGGGCCTAGGAAGAGGCCTCTCAGGGGAGGATTC ACCGACAGGGAGAGGCAGGATCACCGACGAAGGAAGGCCCTCGAGAACAAGAAGAAGCAGCT AGCCGCGGGAGGGAAGAGCCTGAGCAAGGAGGAGGAAGAGGAACTCGGAAGGTTGACCCGGG AAGACGAGGAAAGGAAAAGAAGAGTAGCCGGCCCGCGGGTTGGGGGTGTGAACCCCCTCGAA GGCGGATCGAGGGGAGCGCCCGGGGGCGGCTTCGTCCCCAGCATGCAAGGAGTCCCGGAGTC CCCCTTCGCTCGGACGGGGGAGGGGCTGGACATAAGAGGAACCCGGGGATTCCCATAGGATA TACTCTTCCCATCCGATCCACCCTTCTCTCCCCAGAGTTGTCGTCCCCAGTGAATAAAGCGG GTTTCCACTCACAGGTTCACCGTCTCGCGTCCTTCTTTCCTCTTCGGGTCGGCATGGCATCT CCACCTCCTCGCGGTCCGACCTGGGCATCCGAAGGAGGACGCACGTCCACTCGGATGGCTAA GGGAGAGCCACTTTTCTCTCGATTCTCTATCGGAATCTAGAGAGATTTGTGGGTCCCATTCG CCATTACCGAGGGGACGGTCCCCTCGGAATGTTGCCCAGCCGGCGCCAGCGAGGAGGCTGGG ACCATGCCGGCCATCAGGTAAGAAAGGATGGAACGCGGACCCTGCAGAGTGGGGTCCCGCCA TTCCTGGGCGACCCTTGGGGGGGGAGTCGGAATCGAGCATCGGGAAGGGCATCCCATGGCTC CACTGGTCCCCGGTGTTCCCAGCACCCCCTCCGGTCACTTTCGAAGGGGGTCCGGGGTCCCG CTAGATGGGGACGATAAGTCGAGTTCCCCGGGATAAGCCTCACTCGTCCCCTCTCGGGGGGC GGAACACCCACCGGCTAGCCCCGTTGCTTTCTTTGCTTTCCTCCTCGCTTCGGTCTCCCCCT ACTCCTAGCATCTCCTCCTATCGCTATGGCCTTACTCCTACCGCTCGAAGCGCCTCTGTTCG CTGAAGGGGTCCTCTGGAGGTGATTTCTCTGCTCATCTCCGAGTGTGTTCCTCCCTCTGGTG TTCTCAACCCTTCGGCCGGAGTGCTCTCCAAACTTGGGCGTCGGGCCTTTCGGATCGGGGGG GCCCCCCCTTCTCTTCCATCTGTCCTCTTTCCCCTTCCGAGATGTCTCCAGCGTTATGGGGA AAGCTTCCGACTCTTGTATTCTCTTTTGGCCTTCTTGGGAGACATCTCCTCGGCGTTCCAAT ACTCTTTACCACTTTACCCCTCTCGGGCACTGATCCTTCCCCCGCGGACTCTTCGCTCGGAA TTGGCCCATGGCGGCCGC (SEQ ID NO: 1835)

psiCHECK2-HDV-genome	GCGATCGCCATGGGCCAATTCCGAGCGAAGAGTCCGCGGGGGAAGGATCAGTGCCCGAGAGG GGTAAAGTGGTAAAGAGTATTGGAACGCCGAGGAGATGTCTCCCAAGAAGGCCAAAAGAGAA TACAAGAGTCGGAAGCTTTCCCCATAACGCTGGAGACATCTCGGAAGGGGAAAGAGGACAGA TGGAAGAGAAGGGGGGGCCCCCCCGATCCGAAAGGCCCGACGCCCAAGTTTGGAGAGCACTC CGGCCGAAGGGTTGAGAACACCAGAGGGAGGAACACACTCGGAGATGAGCAGAGAAATCACC TCCAGAGGACCCCTTCAGCGAACAGAGGCGCTTCGAGCGGTAGGAGTAAGGCCATAGCGATA GGAGGAGATGCTAGGAGTAGGGGGAGACCGAAGCGAGGAGGAAAGCAAAGAAAGCAACGGGG CTAGCCGGTGGGTGTTCCGCCCCCCGAGAGGGGACGAGTGAGGCTTATCCCGGGGAACTCGA CTTATCGTCCCCATCTAGCGGGACCCCGGACCCCCTTCGAAAGTGACCGGAGGGGGTGCTGG GAACACCGGGGACCAGTGGAGCCATGGGATGCCCTTCCCGATGCTCGATTCCGACTCCCCCC CCAAGGGTCGCCCAGGAATGGCGGGACCCCACTCTGCAGGGTCCGCGTTCCATCCTTTCTTA CCTGATGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATTCCGAGGGGA CCGTCCCCTCGGTAATGGCGAATGGGACCCACAAATCTCTCTAGATTCCGATAGAGAATCGA GAGAAAAGTGGCTCTCCCTTAGCCATCCGAGTGGACGTGCGTCCTCCTTCGGATGCCCAGGT CGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGAAGAGGAAAGAAGGACGCGAGACGGT GAACCTGTGAGTGGAAACCCGCTTTATTCACTGGGGACGACAACTCTGGGGAGAGAAGGGTG GATCGGATGGGAAGAGTATATCCTATGGGAATCCCCGGGTTCCTCTTATGTCCAGCCCCTCC CCCGTCCGAGCGAAGGGGGACTCCGGGACTCCTTGCATGCTGGGGACGAAGCCGCCCCCGGG CGCTCCCCTCGATCCGCCTTCGAGGGGGTTCACACCCCCAACCCGCGGGCCGGCTACTCTTC TTTTCCTTTCCTCGTCTTCCCGGGTCAACCTTCCGAGTTCCTCTTCCTCCTCCTTGCTCAGG CTCTTCCCTCCCGCGGCTAGCTGCTTCTTCTTGTTCTCGAGGGCCTTCCTTCGTCGGTGATC CTGCCTCTCCCTGTCGGTGAATCCTCCCCTGAGAGGCCTCTTCCTAGGCCCGGAGTCTATCT CCATCTGGTCCGTCCGGGCCCTCTTCGCCGGGGGAGCCCCCTCTCCATCCCTGTCCTTCTTT CCGAGAATTCCTTTGATGTTTCCCAGCCAGGGATTTTCGACCTCAAGTTTCTTGATCTTCTT CTTTGTCTTCCGGAGATCCCTCTCGAGTTCCTCCGCTTTCTTTCTTCCACTCACCCACTTCT CGAGAATATCTTCTCTTCCATCGCGCTTCTTCTTCAACTCGGACCGGCTCATCTCGACAAGA GGCGGCAGTCCTCAGTGCTCTTACTCTTTTCTGATAAAGAGGAGACTGCTGGACTCGCCGCC CAAGTTCGAGGCGGCCGC (SEQ ID NO: 1836)
psiCHECK2-HDV-antigenome hot spot	GCGATCGCGAAGACAAAGAAGAAGATCAAGAAACTTGAGGTCGAAAATCCCTGGCTGGGAAA CATCAAAGGAATTCTCGGAAAGAAGGACAGGGATGGCGGCCGC (SEQ ID NO: 1837)
psiCHECK2-HDV-genome hot spot	GCGATCGCCATCCCTGTCCTTCTTTCCGAGAATTCCTTTGATGTTTCCCAGCCAGGGATTTT CGACCTCAAGTTTCTTGATCTTCTTCTTTGTCTTCGCGGCCGC (SEQ ID NO: 1838)

Table 31. Unmodified Sense and Antisense Strand Sequences of HDV dsRNAs

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-67176.1	A-134242.1	UCCCAAGAAGGCCAAAAGAGA	1839	A-134243.1	UCUCUUUUGGCCUUCUUGGGAGA	2017
AD-67177.1	A-134244.1	AGUUUCUUGAUCUUCUUCUUU	1840	A-134245.1	AAAGAAGAAGAUCAAGAAACUUG	2018
AD-67178.1	A-134246.1	UCUUUUCUGAUAAAGAGGAGA	1841	A-134247.1	UCUCCUCUUUAUCAGAAAAGAGU	2019
AD-67179.1	A-134248.1	AGUGGUAAAGAGUAUUGGAAA	1842	A-134249.1	UUUCCAAUACUCUUUACCACUUU	2020
AD-67180.1	A-134250.1	CUCAAGUUUCUUGAUCUUCUU	1843	A-134251.1	AAGAAGAUCAAGAAACUUGAGGU	2021
AD-67181.1	A-134252.1	AAGGCCAAAAGAGAAUACAAA	1844	A-134253.1	UUUGUAUUCUCUUUUGGCCUUCU	2022
AD-67182.1	A-134254.1	GACCUCAAGUUUCUUGAUCUU	1845	A-134255.1	AAGAUCAAGAAACUUGAGGUCGA	2023
AD-67183.1	A-134256.1	AGGCCAAAAGAGAAUACAAGA	1846	A-134257.1	UCUUGUAUUCUCUUUUGGCCUUC	2024
AD-67184.1	A-134258.1	CGAUAGAGAAUCGAGAGAAAA	1847	A-134259.1	UUUUCUCUCGAUUCUCUAUCGGA	2025
AD-67185.1	A-134260.1	GAUAGAGAAUCGAGAGAAAAG	1848	A-134261.1	CUUUUCUCUCGAUUCUCUAUCGG	2026
AD-67186.1	A-134262.1	ACCCACAAAUCUCUCUAGAUU	1849	A-134263.1	AAUCUAGAGAGAUUUGUGGGUCC	2027
AD-67187.1	A-134264.1	GGAUGGGAAGAGUAUAUCCUA	1850	A-134265.1	UAGGAUAUACUCUUCCCAUCCGA	2028
AD-67188.1	A-134266.1	CCGAUAGAGAAUCGAGAGAAA	1851	A-134267.1	UUUCUCUCGAUUCUCUAUCGGAA	2029
AD-67189.1	A-134268.1	UCCGAUAGAGAAUCGAGAGAA	1852	A-134269.1	UUCUCUCGAUUCUCUAUCGGAAU	2030
AD-67190.1	A-134270.1	UGAGUGGAAACCCGCUUUAUU	1853	A-134271.1	AAUAAAGCGGGUUUCCACUCACA	2031
AD-67191.1	A-134272.1	CCUUCUUUCCGAGAAUUCCUU	1854	A-134273.1	AAGGAAUUCUCGGAAAGAAGGAC	2032
AD-67192.1	A-134274.1	CUGUGAGUGGAAACCCGCUUU	1855	A-134275.1	AAAGCGGGUUUCCACUCACAGGU	2033
AD-67193.1	A-134276.1	GACCCACAAAUCUCUCUAGAU	1856	A-134277.1	AUCUAGAGAGAUUUGUGGGUCCC	2034
AD-67194.1	A-134278.1	GCUGGGAAACAUCAAAGGAAU	1857	A-134279.1	AUUCCUUUGAUGUUUCCCAGCCA	2035
AD-67195.1	A-134280.1	ACAGUUGGAAGGCUCAAGGAG	1858	A-134281.1	UGUCAACCUUCCGAGUUCCUCUU	2036

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-67196.1	A-134282.1	AAGGAGGAGGAACGAGUCCGA	1859	A-134283.1	UUCCUCCUCCUUGCUCAGGCUCU	2037
AD-67197.1	A-134284.1	UCUCCUCUUUAUCAGAAAAGA	1860	A-134285.1	UCUUUUUCUGAUAAAGAGGAGACU	2038
AD-67198.1	A-134286.1	UCCAGCAGUCUCCUCUUUAUA	1861	A-134287.1	UAUAAAGAGGAGACUGCUGGACU	2039
AD-67199.1	A-134288.1	AAAGAAGAAGAUCAAGAAAACU	1862	A-134289.1	AGUUUCUUGAUCUUCUUCUUUGU	2040
AD-67200.1	A-134290.1	AAGAAGAAGAUCAAGAAAACUU	1863	A-134291.1	AAGUUUCUUGAUCUUCUUCUUUG	2041
AD-67201.1	A-134292.1	GAGGUCGAAAAUCCCUGGCUA	1864	A-134293.1	UAGCCAGGGAUUUUCGACCUCAA	2042
AD-67202.1	A-134294.1	CCCUGGCUGGGAAACAUCAAA	1865	A-134295.1	UUUGAUGUUUCCCAGCCAGGGAU	2043
AD-67203.1	A-134296.1	CUGGCUGGGAAACAUCAAAGA	1866	A-134297.1	UCUUUGAUGUUUCCCAGCCAGGG	2044
AD-67204.1	A-134298.1	UGGCUGGGAAACAUCAAAGGA	1867	A-134299.1	UCCUUUGAUGUUUCCCAGCCAGG	2045
AD-67205.1	A-134300.1	GGCUGGGAAACAUCAAAGGAA	1868	A-134301.1	UUCCUUUGAUGUUUCCCAGCCAG	2046
AD-67206.1	A-134302.1	UCAAAGGAAUUCUCGGAAAGA	1869	A-134303.1	UCUUUCCGAGAAUUCCUUUGAUG	2047
AD-67207.1	A-134304.1	UGGGAAACAUCAAAGGAAUUA	1870	A-134305.1	UAAUUCCUUUGAUGUUUCCCAGC	2048
AD-67208.1	A-134306.1	GGAAACAUCAAAGGAAUUCUA	1871	A-134307.1	UAGAAUUCCUUUGAUGUUUCCCA	2049
AD-67209.1	A-134308.1	GAAACAUCAAAGGAAUUCUCA	1872	A-134309.1	UGAGAAUUCCUUUGAUGUUUCCC	2050
AD-67210.1	A-134310.1	AAACAUCAAAGGAAUUCUCGA	1873	A-134311.1	UCGAGAAUUCCUUUGAUGUUUCC	2051
AD-67211.1	A-134312.1	CAUCAAAGGAAUUCUCGGAAA	1874	A-134313.1	UUUCCGAGAAUUCCUUUGAUGUU	2052
AD-67212.1	A-134314.1	AAAGGAAUUCUCGGAAAGAAA	1875	A-134315.1	UUUCUUUCCGAGAAUUCCUUUGA	2053
AD-67213.1	A-134316.1	AAGGAAUUCUCGGAAAGAAGA	1876	A-134317.1	UCUUCUUUCCGAGAAUUCCUUUG	2054
AD-67214.1	A-134320.1	GGCCCUCGAGAACAAGAAGAA	1877	A-134321.1	UUCUUCUUGUUCUCGAGGGCCUU	2055
AD-67215.1	A-134322.1	GCCCUCGAGAACAAGAAGAAA	1878	A-134323.1	UUUCUUCUUGUUCUCGAGGGCCU	2056
AD-67216.1	A-134324.1	CCCUCGAGAACAAGAAGAAGA	1879	A-134325.1	UCUUCUUCUUGUUCUCGAGGGCC	2057

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-67217.1	A-134326.1	GGAAAGGAAAAGAAGAGUAGA	1880	A-134327.1	UCUACUCUUCUUUUCCUUUCCUC	2058
AD-67218.1	A-134328.1	AAGGAAAAGAAGAGUAGCCGA	1881	A-134329.1	UCGGCUACUCUUCUUUUCCUUUC	2059
AD-67219.1	A-134330.1	GGGGGUGUGAACCCCCUCGAA	1882	A-134331.1	UUCGAGGGGGUUCACACCCCCAA	2060
AD-67220.1	A-134332.1	GGGUGUGAACCCCCUCGAAGA	1883	A-134333.1	UCUUCGAGGGGGUUCACACCCCC	2061
AD-67250.1	A-134346.1	AGUUUCUUGAUCUUCUUCUUU	1884	A-134347.1	AAAGAAGAAGAUCAAGAAACUUG	2062
AD-67251.1	A-134348.1	UCUUUUCUGAUAAAGAGGAGA	1885	A-134349.1	UCUCCUCUUUAUCAGAAAAGAGU	2063
AD-67252.1	A-134350.1	AGUGGUAAAGAGUAUUGGAAA	1886	A-134351.1	UUUCCAAUACUCUUUACCACUUU	2064
AD-67253.1	A-134352.1	CUCAAGUUUCUUGAUCUUCUU	1887	A-134353.1	AAGAAGAUCAAGAAACUUGAGGU	2065
AD-67254.1	A-134354.1	AAGGCCAAAAGAGAAUACAAA	1888	A-134355.1	UUUGUAUUCUCUUUUGGCCUUCU	2066
AD-67255.1	A-134358.1	AGGCCAAAAGAGAAUACAAGA	1889	A-134359.1	UCUUGUAUUCUCUUUUGGCCUUC	2067
AD-67256.1	A-134360.1	CGAUAGAGAAUCGAGAGAAAA	1890	A-134361.1	UUUUCUCUCGAUUCUCUAUCGGA	2068
AD-67257.1	A-134362.1	GAUAGAGAAUCGAGAGAAAAG	1891	A-134363.1	CUUUUCUCUCGAUUCUCUAUCGG	2069
AD-67258.1	A-134364.1	ACCCACAAAUCUCUCUAGAUU	1892	A-134365.1	AAUCUAGAGAGAUUUGUGGGUCC	2070
AD-67259.1	A-134366.1	GGAUGGGAAGAGUAUAUCCUA	1893	A-134367.1	UAGGAUAUACUCUUCCCAUCCGA	2071
AD-67260.1	A-134368.1	CCGAUAGAGAAUCGAGAGAAA	1894	A-134369.1	UUUCUCUCGAUUCUCUAUCGGAA	2072
AD-67261.1	A-134370.1	UCCGAUAGAGAAUCGAGAGAA	1895	A-134371.1	UUCUCUCGAUUCUCUAUCGGAAU	2073
AD-67262.1	A-134372.1	UGAGUGGAAACCCGCUUUAUU	1896	A-134373.1	AAUAAAGCGGGUUUCCACUCACA	2074
AD-67263.1	A-134374.1	CCUUCUUUCCGAGAAUUCCUU	1897	A-134375.1	AAGGAAUUCUCGGAAAGAAGGAC	2075
AD-67264.1	A-134376.1	CUGUGAGUGGAAACCCGCUUU	1898	A-134377.1	AAAGCGGGUUUCCACUCACAGGU	2076
AD-67265.1	A-134378.1	GACCCACAAAUCUCUCUAGAU	1899	A-134379.1	AUCUAGAGAGAUUUGUGGGUCCC	2077
AD-67266.1	A-134380.1	GCUGGGAAACAUCAAAGGAAU	1900	A-134381.1	AUUCCUUUGAUGUUUCCCAGCCA	2078

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-67267.1	A-134382.1	ACAGUUGGAAGGCUCAAGGAG	1901	A-134383.1	UGUCAACCUUCCGAGUUCCUCUU	2079
AD-67268.1	A-134384.1	AAGGAGGAGGAACGAGUCCGA	1902	A-134385.1	UUCCUCCUCCUUGCUCAGGCUCU	2080
AD-67269.1	A-134386.1	UCUCCUCUUUAUCAGAAAAGA	1903	A-134387.1	UCUUUUCUGAUAAAGAGGAGACU	2081
AD-67270.1	A-134388.1	UCCAGCAGUCUCCUCUUUAUA	1904	A-134389.1	UAUAAAGAGGAGACUGCUGGACU	2082
AD-67271.1	A-134390.1	AAAGAAGAAGAUCAAGAAACU	1905	A-134391.1	AGUUUCUUGAUCUUCUUCUUUGU	2083
AD-67272.1	A-134392.1	AAGAAGAAGAUCAAGAAACUU	1906	A-134393.1	AAGUUUCUUGAUCUUCUUCUUUG	2084
AD-67273.1	A-134394.1	GAGGUCGAAAAUCCCCUGGCUA	1907	A-134395.1	UAGCCAGGGAUUUUUCGACCUCAA	2085
AD-67274.1	A-134396.1	CCCUGGCUGGGAAACAUCAAA	1908	A-134397.1	UUUGAUGUUUCCCAGCCAGGGAU	2086
AD-67275.1	A-134398.1	CUGGCUGGGAAACAUCAAAGA	1909	A-134399.1	UCUUUGAUGUUUCCCAGCCAGGG	2087
AD-67276.1	A-134400.1	UGGCUGGGAAACAUCAAAGGA	1910	A-134401.1	UCCUUUGAUGUUUCCCAGCCAGG	2088
AD-67277.1	A-134402.1	GGCUGGGAAACAUCAAAGGAA	1911	A-134403.1	UUCCUUUGAUGUUUCCCAGCCAG	2089
AD-67278.1	A-134404.1	UCAAAGGAAUUCUCGGAAAGA	1912	A-134405.1	UCUUUCCGAGAAUUCCUUUGAUG	2090
AD-67279.1	A-134406.1	UGGGAAACAUCAAAGGAAUUA	1913	A-134407.1	UAAUUCCUUUGAUGUUUCCCAGC	2091
AD-67280.1	A-134408.1	GGAAACAUCAAAGGAAUUCUA	1914	A-134409.1	UAGAAUUCCUUUGAUGUUUCCCA	2092
AD-67281.1	A-134410.1	GAAACAUCAAAGGAAUUCUCA	1915	A-134411.1	UGAGAAUUCCUUUGAUGUUUCCC	2093
AD-67282.1	A-134414.1	CAUCAAAGGAAUUCUCGGAAA	1916	A-134415.1	UUUCCGAGAAUUCCUUUGAUGUU	2094
AD-67283.1	A-134416.1	AAAGGAAUUCUCGGAAAGAAA	1917	A-134417.1	UUUCUUUCCGAGAAUUCCUUUGA	2095
AD-67284.1	A-134418.1	AAGGAAUUCUCGGAAAGAAGA	1918	A-134419.1	UCUUCUUUCCGAGAAUUCCUUUG	2096
AD-67285.1	A-134420.1	ACGAAGGAAGGCCCUCGAGAA	1919	A-134421.1	UUCUCGAGGGCCUUCCUUCGUCG	2097
AD-67286.1	A-134422.1	GGCCCUCGAGAACAAGAAGAA	1920	A-134423.1	UUCUUCUUGUUCUCGAGGGCCUU	2098
AD-67287.1	A-134424.1	GCCCUCGAGAACAAGAAGAAA	1921	A-134425.1	UUUCUUCUUGUUCUCGAGGGCCU	2099

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-67288.1	A-134426.1	CCCUCGAGAACAAGAAGAAGA	1922	A-134427.1	UCUUCUUCUUGUUCUCGAGGGCC	2100
AD-67289.1	A-134428.1	GGAAAGGAAAAGAAGAGUAGA	1923	A-134429.1	UCUACUCUUCUUUUCCUUUCCUC	2101
AD-67290.1	A-134430.1	AAGGAAAAGAAGAGUAGCCGA	1924	A-134431.1	UCGGCUACUCUUCUUUUCCUUUC	2102
AD-67291.1	A-134432.1	GGGGGUGUGAACCCCCUCGAA	1925	A-134433.1	UUCGAGGGGGUUCACACCCCCAA	2103
AD-67292.1	A-134434.1	GGGUGUGAACCCCCUCGAAGA	1926	A-134435.1	UCUUCGAGGGGGUUCACACCCCC	2104
AD-70224.1	A-141079.1	AAACUUGAGGUCGAAAAUA	1927	A-141080.1	UAUUUUCGACCUCAAGUUUUU	2105
AD-70225.1	A-141081.1	AAACUUGAGGUCGAAAAUA	1928	A-141082.1	UAUUUUCGACCUCAAGUUUUU	2106
AD-70226.1	A-141083.1	AAACUUGAGGUCGAAAAUA	1929	A-141084.1	UAUUUUCGACCUCAAGUUUUU	2107
AD-70227.1	A-141085.1	AAACUUGAGGUCGAAAAUA	1930	A-141084.1	UAUUUUCGACCUCAAGUUUUU	2108
AD-70228.1	A-141086.1	AGAAACUUGAGGUCGAAAAUA	1931	A-141087.1	UAUUUUCGACCUCAAGUUUCU	2109
AD-70229.1	A-141090.1	GAUCAAGAAACUUGAGGUA	1932	A-141091.1	UACCUCAAGUUUCUUGAUCUU	2110
AD-70230.1	A-141092.1	GAUCAAGAAACUUGAGGUA	1933	A-141093.1	UACCUCAAGUUUTCUUGAUCUU	2111
AD-70231.1	A-141094.1	GAUCAAGAAACUUGAGGUA	1934	A-141093.1	UACCUCAAGUUUTCUUGAUCUU	2112
AD-70232.1	A-141095.1	AAGAUCAAGAAACUUGAGGUA	1935	A-141096.1	UACCUCAAGUUUCUUGAUCUU	2113
AD-70233.1	A-141097.1	UCAAGAAACUUGAGGUCGA	1936	A-141098.1	UCGACCUCAAGUUUCUUGAUU	2114
AD-70234.1	A-141097.1	UCAAGAAACUUGAGGUCGA	1937	A-141099.1	UCGACCUCAAGUUUCUUGAUU	2115
AD-70235.1	A-141100.1	UCAAGAAACUUGAGGUCGA	1938	A-141101.1	UCGACCUCAAGUUUCUUGAUU	2116
AD-70236.1	A-141102.1	UCAAGAAACUUGAGGUCGA	1939	A-141103.1	UCGACCUCAAGTUUCUUGAUU	2117
AD-70237.1	A-141104.1	UCAAGAAACUUGAGCUCGA	1940	A-141103.1	UCGACCUCAAGTUUCUUGAUU	2118
AD-70238.1	A-141105.1	UCAAGAAACUUGAGGUCGA	1941	A-141106.1	UCGACCUCAAGUUUCUUGA	2119
AD-70239.1	A-141107.1	AAGAUCAAGAAACUUGAGA	1942	A-141108.1	UCUCAAGUUUCUUGAUCUUUU	2120

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-70240.1	A-141107.1	AAGAUCAAGAAACUUGAGA	1943	A-141109.1	UCUCAAGUUUCUUGAUCUUUU	2121
AD-70241.1	A-141110.1	AAGAUCAAGAAACUUGAGA	1944	A-141111.1	UCUCAAGUUUCUUGAUCUUUU	2122
AD-70242.1	A-141112.1	AAGAUCAAGAAACUUGAGA	1945	A-141113.1	UCUCAAGUUUCUUGAUCUUUU	2123
AD-70243.1	A-141114.1	AAGAUCAAGAAACUAGAGA	1946	A-141113.1	UCUCAAGUUUCUUGAUCUUUU	2124
AD-70244.1	A-141115.1	AGAAGAUCAAGAAACUUGAGA	1947	A-141116.1	UCUCAAGUUUCUUGAUCUUCU	2125
AD-70245.1	A-141117.1	CAUCAAAGGAAUUCUCGGA	1948	A-141118.1	UCCGAGAAUUCCUUUGAUGUU	2126
AD-70246.1	A-141119.1	CAUCAAAGGAAUUCUCGGA	1949	A-141120.1	UCCGAGAAUUCCUUUGAUGUU	2127
AD-70247.1	A-141121.1	CAUCAAAGGAAUUCUCGGA	1950	A-141122.1	UCCGAGAAUUCCUUUGAUGUU	2128
AD-70248.1	A-141123.1	CAUCAAAGGAAUUCUCGGA	1951	A-141122.1	UCCGAGAAUUCCUUUGAUGUU	2129
AD-70249.1	A-141124.1	AACAUCAAAGGAAUUCUCGGA	1952	A-141125.1	UCCGAGAAUUCCUUUGAUGUU	2130
AD-70250.1	A-141126.1	AUCAAGAAACUUGAGGUCA	1953	A-141127.1	UGACCUCAAGUUUCUUGAUUU	2131
AD-70251.1	A-141126.1	AUCAAGAAACUUGAGGUCA	1954	A-141128.1	UGACCUCAAGUUUCUUGAUUU	2132
AD-70252.1	A-141129.1	AUCAAGAAACUUGAGGUCA	1955	A-141130.1	UGACCUCAAGUUUCUUGAUUU	2133
AD-70253.1	A-141131.1	AUCAAGAAACUUGAGGUCA	1956	A-141132.1	UGACCUCAAGUUUCUUGAUUU	2134
AD-70254.1	A-141133.1	AUCAAGAAACUUGACGUCA	1957	A-141132.1	UGACCUCAAGUUUCUUGAUUU	2135
AD-70255.1	A-141134.1	AGAUCAAGAAACUUGAGGUCA	1958	A-141135.1	UGACCUCAAGUUUCUUGAUCU	2136
AD-70256.1	A-141136.1	GAAGAUCAAGAAACUUGAA	1959	A-141137.1	UUCAAGUUUCUUGAUCUUCUU	2137
AD-70257.1	A-141138.1	GAAGAUCAAGAAACUUGAA	1960	A-141139.1	UUCAAGUUUCUUGAUCUUCUU	2138
AD-70258.1	A-141140.1	GAAGAUCAAGAAACUUGAA	1961	A-141141.1	UUCAAGUUUCUTGAUCUUCUU	2139
AD-70259.1	A-141142.1	GAAGAUCAAGAAACTUGAA	1962	A-141141.1	UUCAAGUUUCUTGAUCUUCUU	2140
AD-70260.1	A-141143.1	AAGAAGAUCAAGAAACUUGAA	1963	A-141144.1	UUCAAGUUUCUUGAUCUUCUU	2141

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-70261.1	A-141145.1	CAAAGGAAUUCUCGGAAAG	1964	A-141146.1	CUUUCCGAGAAUUCCUUUGAU	2142
AD-70262.1	A-141145.1	CAAAGGAAUUCUCGGAAAG	1965	A-141147.1	CUUUCCGAGAAUUCCUUUGAU	2143
AD-70263.1	A-141148.1	CAAAGGAAUUCUCGGAAAG	1966	A-141149.1	CUUUCCGAGAAUUCCUUUGAU	2144
AD-70264.1	A-141150.1	CAAAGGAAUUCUCGGAAAG	1967	A-141151.1	CUUUCCGAGAAUUCCUUUGAU	2145
AD-70265.1	A-141152.1	CAAAGGAAUUCUCGCAAAG	1968	A-141151.1	CUUUCCGAGAAUUCCUUUGAU	2146
AD-70266.1	A-141153.1	AUCAAAGGAAUUCUCGGAAAG	1969	A-141154.1	CUUUCCGAGAAUUCCUUUGAU	2147
AD-70267.1	A-141155.1	CAAGAAACUUGAGGUCGAA	1970	A-141156.1	UUCGACCUCAAGUUUCUUGAU	2148
AD-70268.1	A-141155.1	CAAGAAACUUGAGGUCGAA	1971	A-141157.1	UUCGACCUCAAGUUUCUUGAU	2149
AD-70269.1	A-141158.1	CAAGAAACUUGAGGUCGAA	1972	A-141159.1	UUCGACCUCAAGUUUCUUGAU	2150
AD-70270.1	A-141160.1	CAAGAAACUUGAGGUCGAA	1973	A-141161.1	UUCGACCUCAAGUUUCUUGAU	2151
AD-70271.1	A-141162.1	CAAGAAACUUGAGGTCGAA	1974	A-141161.1	UUCGACCUCAAGUUUCUUGAU	2152
AD-70272.1	A-141163.1	AUCAAGAAACUUGAGGUCGAA	1975	A-141164.1	UUCGACCUCAAGUUUCUUGAU	2153
AD-70273.1	A-141165.1	GAAGAAGAUCAAGAAACUU	1976	A-141166.1	AAGUUUCUUGAUCUUCUUCUU	2154
AD-70274.1	A-141165.1	GAAGAAGAUCAAGAAACUU	1977	A-141167.1	AAGUUUCUUGAUCUUCUUCUU	2155
AD-70275.1	A-141168.1	GAAGAAGAUCAAGAAACUU	1978	A-141169.1	AAGUUUCUUGAUCUUCUUCUU	2156
AD-70276.1	A-141170.1	GAAGAAGAUCAAGAAACUU	1979	A-141171.1	AAGUUUCUUGATCUUCUUCUU	2157
AD-70277.1	A-141172.1	GAAGAAGAUCAAGAAACUU	1980	A-141171.1	AAGUUUCUUGATCUUCUUCUU	2158
AD-70278.1	A-141173.1	AAGAAGAAGAUCAAGAAACUU	1981	A-141174.1	AAGUUUCUUGAUCUUCUUCUU	2159
AD-70279.1	A-141175.1	GGAAACAUCAAAGGAAUUA	1982	A-141176.1	UAAUUCCUUUGAUGUUUCCUU	2160
AD-70280.1	A-141175.1	GGAAACAUCAAAGGAAUUA	1983	A-141177.1	UAAUUCCUUUGAUGUUUCCUU	2161
AD-70281.1	A-141178.1	GGAAACAUCAAAGGAAUUA	1984	A-141179.1	UAAUUCCUUUGAUGUUUCCUU	2162

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-70282.1	A-141180.1	GGAAACAUCAAAGGAAUUA	1985	A-141181.1	UAAUUCCUUUGAUGUUUCUU	2163
AD-70283.1	A-141182.1	GGAAACAUCAAAGGAAUUA	1986	A-141183.1	UAAUUCCUUUGAUGUUUCCUU	2164
AD-70284.1	A-141184.1	UGGGAAACAUCAAAGGAAUUA	1987	A-141185.1	UAAUUCCUUUGAUGUUUCCCA	2165
AD-70285.1	A-134288.1	AAAGAAGAAGAUCAAGAAACU	1988	A-141186.1	AGUUUCUUGAUCUUCUUCUUUGU	2166
AD-70286.1	A-141187.1	AAAGAAGAAGAUCAAGAAACU	1989	A-141188.1	AGUUUCUUGAUCUUCUUCUUUGU	2167
AD-70287.1	A-141189.1	AAAGAAGAAGAUCAAGAAACU	1990	A-141190.1	AGUUUCUUGAUCUUCUUCUUUGU	2168
AD-70288.1	A-141191.1	AAAGAAGAAGAUCAAGAAACU	1991	A-141190.1	AGUUUCUUGAUCUUCUUCUUUGU	2169
AD-70289.1	A-141192.1	AAAGAAGAAGAUCAAGAAACU	1992	A-141193.1	AGUUUCUUGAUCUUCUUCUUU	2170
AD-70290.1	A-141194.1	AGAAGAAGAUCAAGAAACU	1993	A-141195.1	AGUUUCUUGAUCUUCUUCUUU	2171
AD-70291.1	A-141194.1	AGAAGAAGAUCAAGAAACU	1994	A-141196.1	AGUUUCUUGAUCUUCUUCUUU	2172
AD-70292.1	A-141197.1	AGAAGAAGAUCAAGAAACU	1995	A-141198.1	AGUUUCUUGAUCUUCUUCUUU	2173
AD-70293.1	A-141199.1	AGAAGAAGAUCAAGAAACU	1996	A-141200.1	AGUUUCUUGAUCUUCUUCUUU	2174
AD-70294.1	A-141201.1	AGAAGAAGAUCAAGAAACU	1997	A-141200.1	AGUUUCUUGAUCUUCUUCUUU	2175
AD-70295.1	A-141202.1	AGAAGAAGAUCAAGAAACU	1998	A-141203.1	AGUUUCUUGAUCUUCUUCU	2176
AD-70296.1	A-141204.1	AACAUCAAAGGAAUUCUCA	1999	A-141205.1	UGAGAAUUCCUUUGAUGUUUU	2177
AD-70297.1	A-141204.1	AACAUCAAAGGAAUUCUCA	2000	A-141206.1	UGAGAAUUCCUUUGAUGUUUU	2178
AD-70298.1	A-141207.1	AACAUCAAAGGAAUUCUCA	2001	A-141208.1	UGAGAAUUCCUUUGAUGUUUU	2179
AD-70299.1	A-141209.1	AACAUCAAAGGAAUUCUCA	2002	A-141210.1	UGAGAAUUCCUUUGAUGUUUU	2180
AD-70300.1	A-141211.1	AACAUCAAAGGAAUUCUCA	2003	A-141210.1	UGAGAAUUCCUUUGAUGUUUU	2181
AD-70301.1	A-141212.1	AACAUCAAAGGAAUUCUCA	2004	A-141213.1	UGAGAAUUCCUUUGAUGUU	2182
AD-70302.1	A-141214.1	AGGAAUUCUCGGAAAGAAA	2005	A-141215.1	UUUCUUUCCGAGAAUUCCUUU	2183

Duple Name	Sense Oligo Name	Senses Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO:
AD-70303.1	A-141214.1	AGGAAUUCUCGGAAAGAAA	2006	A-141216.1	UUUCUUUCCGAGAAUUCCUUU	2184
AD-70304.1	A-141217.1	AGGAAUUCUCGGAAAGAAA	2007	A-141218.1	UUUCUUUCCGAGAAUUCCUUU	2185
AD-70305.1	A-141219.1	AGGAAUUCUCGGAAAGAAA	2008	A-141220.1	UUUCUUUCCGAGAAUUCCUUU	2186
AD-70306.1	A-141221.1	AGGAAUUCUCGGAAAGAAA	2009	A-141220.1	UUUCUUUCCGAGAAUUCCUUU	2187
AD-70307.1	A-141222.1	AAAGGAAUUCUCGGAAAGAAA	2010	A-141223.1	UUUCUUUCCGAGAAUUCCUUU	2188
AD-70308.1	A-141224.1	AAAGGAAUUCUCGGAAAGA	2011	A-141225.1	UCUUUCCGAGAAUUCCUUUUU	2189
AD-70309.1	A-141224.1	AAAGGAAUUCUCGGAAAGA	2012	A-141226.1	UCUUUCCGAGAAUUCCUUUUU	2190
AD-70310.1	A-141227.1	AAAGGAAUUCUCGGAAAGA	2013	A-141228.1	UCUUUCCGAGAAUUCCUUUUU	2191
AD-70311.1	A-141229.1	AAAGGAAUUCUCGGAAAGA	2014	A-141230.1	UCUUUCCGAGAAUUCCUUUUU	2192
AD-70312.1	A-141231.1	AAAGGAAUUCUCGGAAAGA	2015	A-141230.1	UCUUUCCGAGAAUUCCUUUUU	2193
AD-70313.1	A-141232.1	UCAAAGGAAUUCUCGGAAAGA	2016	A-141233.1	UCUUUCCGAGAAUUCCUUUGA	2194

Table 32. Modified Sense and Antisense Strand Sequences of HDV dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5’3’	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5’3’	SEQ ID NO:
AD-67176.1	A-134242.1	uscsccaaGfaAfGfGfccaaaagagaL96	2195	A-134243.1	usCfsucuUfuuggccuUfcUfugggasgsa	2373
AD-67177.1	A-134244.1	asgsuuucUfuGfAfUfcuucuucuuuL96	2196	A-134245.1	asAfsagaAfgaagaucAfaGfaaacususg	2374
AD-67178.1	A-134246.1	uscsuuuuCfuGfAfUfaaagaggagaL96	2197	A-134247.1	usCfsuccUfcuuuaucAfgAfaaagasgsu	2375
AD-67179.1	A-134248.1	asgsugguAfaAfGfAfguauuggaaaL96	2198	A-134249.1	usUfsuccAfauacucuUfuAfccacususu	2376

Duplex Name	Sense Oligo Name	Sense Sequence (5'3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3'	SEQ ID NO:
AD-67180.1	A-134250.1	csuscaagUfuUfCfUfugaucuucuuL96	2199	A-134251.1	asAfsgaaGfaucaagaAfaCfuugagsgsu	2377
AD-67181.1	A-134252.1	asasggccAfaAfAfGfagaauacaaaL96	2200	A-134253.1	usUfsuguAfuucucuuUfuGfgccuuscsu	2378
AD-67182.1	A-134254.1	gsasccucAfaGfUfUfucuugaucuuL96	2201	A-134255.1	asAfsgauCfaagaaacUfuGfaggucsgsa	2379
AD-67183.1	A-134256.1	asgsgccaAfaAfGfAfgaauacaagaL96	2202	A-134257.1	usCfsuugUfauucucuUfuUfggccususc	2380
AD-67184.1	A-134258.1	csgsauagAfgAfAfUfcgagagaaaaL96	2203	A-134259.1	usUfsuucUfcucgauuCfuCfuaucgsgsa	2381
AD-67185.1	A-134260.1	gsasuagaGfaAfUfCfgagagaaaagL96	2204	A-134261.1	csUfsuuuCfucucgauUfcUfcuaucsgsg	2382
AD-67186.1	A-134262.1	ascscccacAfaAfUfCfucucuagauuL96	2205	A-134263.1	asAfsucuAfgagagauUfuGfuggguscsc	2383
AD-67187.1	A-134264.1	gsgsauggGfaAfGfAfguauauccuaL96	2206	A-134265.1	usAfsggaUfauacucuUfcCfcauccsgsa	2384
AD-67188.1	A-134266.1	cscsgauaGfaGfAfAfucgagagaaaL96	2207	A-134267.1	usUfsucuCfucgauucUfcUfaucggsasa	2385
AD-67189.1	A-134268.1	uscscgauAfgAfGfAfaucgagagaaL96	2208	A-134269.1	usUfscucUfcgauucuCfuAfucggasasu	2386
AD-67190.1	A-134270.1	usgsagugGfaAfAfCfccgcuuuauuL96	2209	A-134271.1	asAfsuaaAfgcggguuUfcCfacucascsa	2387
AD-67191.1	A-134272.1	cscsuucuUfuCfCfCfgfagaauuccuuL96	2210	A-134273.1	asAfsggaAfuucucggAfaAfgaaggsasc	2388
AD-67192.1	A-134274.1	csusgugaGfuGfGfAfaacccgcuuuL96	2211	A-134275.1	asAfsagcGfgguuuccAfcUfcacagsgsu	2389
AD-67193.1	A-134276.1	gsascccaCfaAfAfUfcucucuagauL96	2212	A-134277.1	asUfscuaGfagagauuUfgUfgggucscsc	2390
AD-67194.1	A-134278.1	gscsugggAfaAfCfAfucaaaggaauL96	2213	A-134279.1	asUfsuccUfuugauguUfuCfccagcscsa	2391
AD-67195.1	A-134280.1	ascsaguuGfgAfAfGfgcucaaggagL96	2214	A-134281.1	usGfsucaAfccuuccgAfgUfuccucsusu	2392
AD-67196.1	A-134282.1	asasggagGfaGfGfAfacgaguccgaL96	2215	A-134283.1	usUfsccuCfcuccuugCfuCfaggcuscsu	2393
AD-67197.1	A-134284.1	uscsuccuCfuUfUfAfucagaaaagaL96	2216	A-134285.1	usCfsuuuUfcugauaaAfgAfggagascsu	2394
AD-67198.1	A-134286.1	uscscagcAfgUfCfUfccucuuuauaL96	2217	A-134287.1	usAfsuaaAfgaggagaCfuGfcuggascsu	2395
AD-67199.1	A-134288.1	asasagaaGfaAfGfAfucaagaaacuL96	2218	A-134289.1	asGfsuuuCfuugaucuUfcUfucuuusgsu	2396
AD-67200.1	A-134290.1	asasgaagAfaGfAfUfcaagaaacuuL96	2219	A-134291.1	asAfsguuUfcuugaucUfuCfuucuususg	2397

Duplex Name	Sense Oligo Name	Sense Sequence (5'3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3'	SEQ ID NO:
AD-67201.1	A-134292.1	gsasggucGfaAfAfAfucccuggcuaL96	2220	A-134293.1	usAfsgccAfgggauuuUfcGfaccucsasa	2398
AD-67202.1	A-134294.1	cscscuggCfuGfGfGfaaacaucaaaL96	2221	A-134295.1	usUfsugaUfguuucccAfgCfcagggsasu	2399
AD-67203.1	A-134296.1	csusggcuGfgGfAfAfacaucaaagaL96	2222	A-134297.1	usCfsuuuGfauguuucCfcAfgccagsgsg	2400
AD-67204.1	A-134298.1	usgsgcugGfgAfAfAfcaucaaaggaL96	2223	A-134299.1	usCfscuuUfgauguuuCfcCfagccasgsg	2401
AD-67205.1	A-134300.1	gsgscuggGfaAfAfCfaucaaaggaaL96	2224	A-134301.1	usUfsccuUfugauguuUfcCfcagccsasg	2402
AD-67206.1	A-134302.1	uscsaaagGfaAfUfUfcucggaaagaL96	2225	A-134303.1	usCfsuuuCfcgagaauUfcCfuuugasusg	2403
AD-67207.1	A-134304.1	usgsggaaAfcAfUfCfaaaggaauuaL96	2226	A-134305.1	usAfsauuCfcuuugauGfuUfucccasgsc	2404
AD-67208.1	A-134306.1	gsgsaaacAfuCfAfAfaggaauucuaL96	2227	A-134307.1	usAfsgaaUfuccuuugAfuGfuuuccscsa	2405
AD-67209.1	A-134308.1	gsasaacaUfcAfAfAfggaauucucaL96	2228	A-134309.1	usGfsagaAfuuccuuuGfaUfguuucscsc	2406
AD-67210.1	A-134310.1	asasacauCfaAfAfGfgaauucucgaL96	2229	A-134311.1	usCfsgagAfauuccuuUfgAfuguuuscsc	2407
AD-67211.1	A-134312.1	csasucaaAfgGfAfAfuucucggaaaL96	2230	A-134313.1	usUfsuccGfagaauucCfuUfugaugsusu	2408
AD-67212.1	A-134314.1	asasaggaAfuUfCfUfcggaaagaaaL96	2231	A-134315.1	usUfsucuUfuccgagaAfuUfccuuusgsa	2409
AD-67213.1	A-134316.1	asasggaaUfuCfUfCfggaaagaagaL96	2232	A-134317.1	usCfsuucUfuuccgagAfaUfuccuususg	2410
AD-67214.1	A-134320.1	gsgscccuCfgAfGfAfacaagaagaaL96	2233	A-134321.1	usUfscuuCfuuguucuCfgAfgggccsusu	2411
AD-67215.1	A-134322.1	gscsccucGfaGfAfAfcaagaagaaaL96	2234	A-134323.1	usUfsucuUfcuuguucUfcGfagggcscsu	2412
AD-67216.1	A-134324.1	cscscucgAfgAfAfCfaagaagaagaL96	2235	A-134325.1	usCfsuucUfucuuguuCfuCfgagggcsc	2413
AD-67217.1	A-134326.1	gsgsaaagGfaAfAfAfgaagaguagaL96	2236	A-134327.1	usCfsuacUfcuucuuuUfcCfuuuccsusc	2414
AD-67218.1	A-134328.1	asasggaaAfaGfAfAfgaguagccgaL96	2237	A-134329.1	usCfsggcUfacucuucUfuUfuccuususc	2415
AD-67219.1	A-134330.1	gsgsggguGfuGfAfAfcccccucgaaL96	2238	A-134331.1	usUfscgaGfgggguucAfcAfcccccsasa	2416
AD-67220.1	A-134332.1	gsgsguguGfaAfCfCfcccucgaagaL96	2239	A-134333.1	usCfsuucGfaggggguUfcAfcacccscsc	2417
AD-67250.1	A-134346.1	Y44AGUUUCUUGAUCUUCUUCUUu	2240	A-134347.1	AAAGAAGAAGAUCAAGAAACUUg	2418

Duplex Name	Sense Oligo Name	Sense Sequence (5'3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3')	SEQ ID NO:
AD-67251.1	A-134348.1	Y44UCUUUUCUGAUAAAGAGGAGAa	2241	A-134349.1	UCUCCUCUUUAUCAGAAAAGAGu	2419
AD-67252.1	A-134350.1	Y44AGUGGUAAAGAGUAUUGGAAa	2242	A-134351.1	UUUCCAAUACUCUUUACCACUUu	2420
AD-67253.1	A-134352.1	Y44CUCAAGUUUCUUGAUCUUCUu	2243	A-134353.1	AAGAAGAUCAAGAAACUUGAGGu	2421
AD-67254.1	A-134354.1	Y44AAGGCCAAAAGAGAAUACAAa	2244	A-134355.1	UUUGUAUUCUCUUUUGGCCUUCu	2422
AD-67255.1	A-134358.1	Y44AGGCCAAAAGAGAAUACAAGa	2245	A-134359.1	UCUUGUAUUCUCUUUUGGCCUUc	2423
AD-67256.1	A-134360.1	Y44CGAUAGAGAAUCGAGAGAAAa	2246	A-134361.1	UUUUCUCUCGAUUCUCUAUCGGa	2424
AD-67257.1	A-134362.1	Y44GAUAGAGAAUCGAGAGAAAAg	2247	A-134363.1	CUUUUCUCUCGAUUCUCUAUCGg	2425
AD-67258.1	A-134364.1	Y44ACCCACAAAUCUCUCUAGAUu	2248	A-134365.1	AAUCUAGAGAGAUUUGUGGGUCc	2426
AD-67259.1	A-134366.1	Y44GGAUGGGAAGAGUAUAUCCUa	2249	A-134367.1	UAGGAUAUACUCUUCCCAUCCGa	2427
AD-67260.1	A-134368.1	Y44CCGAUAGAGAAUCGAGAGAAa	2250	A-134369.1	UUUCUCUCGAUUCUCUAUCGGAa	2428
AD-67261.1	A-134370.1	Y44UCCGAUAGAGAAUCGAGAGAa	2251	A-134371.1	UUCUCUCGAUUCUCUAUCGGAAu	2429
AD-67262.1	A-134372.1	Y44UGAGUGGGAAACCCGCUUUAUu	2252	A-134373.1	AAUAAAGCGGGUUUCCACUCACa	2430
AD-67263.1	A-134374.1	Y44CCUUCUUUCCGAGAAUUCCUu	2253	A-134375.1	AAGGAAUUCUCGGAAAGAAGGAc	2431
AD-67264.1	A-134376.1	Y44CUGUGAGUGGGAAACCCGCUUu	2254	A-134377.1	AAAGCGGGUUUCCACUCACAGGu	2432
AD-67265.1	A-134378.1	Y44GACCCACAAAUCUCUCUAGAu	2255	A-134379.1	AUCUAGAGAGAUUUGUGGGGUCCc	2433
AD-67266.1	A-134380.1	Y44GCUGGGAAACAUCAAAGGAAu	2256	A-134381.1	AUUCCUUUGAUGUUUCCCAGCCa	2434
AD-67267.1	A-134382.1	Y44ACAGUUGGAAGGCUCAAGGAg	2257	A-134383.1	UGUCAACCUUCCGAGUUCCUCUu	2435
AD-67268.1	A-134384.1	Y44AAGGAGGAGGAACGAGUCCGa	2258	A-134385.1	UUCCUCCUCCUUGCUCAGGCUCu	2436
AD-67269.1	A-134386.1	Y44UCUCCUCUUUAUCAGAAAAGa	2259	A-134387.1	UCUUUUCUGAUAAAGAGGAGACu	2437
AD-67270.1	A-134388.1	Y44UCCAGCAGUCUCCUCUUUAUa	2260	A-134389.1	UAUAAAGAGGAGACUGCUGGACu	2438
AD-67271.1	A-134390.1	Y44AAAGAAGAAGAUCAAGAAACu	2261	A-134391.1	AGUUUCUUGAUCUUCUUCUUUGu	2439

Duplex Name	Sense Oligo Name	Sense Sequence (5'3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3')	SEQ ID NO:
AD-67272.1	A-134392.1	Y44AAGAAGAAGAUCAAGAAACUu	2262	A-134393.1	AAGUUUCUUGAUCUUCUUCUUUg	2440
AD-67273.1	A-134394.1	Y44GAGGUCGAAAAUCCCCUGGCUa	2263	A-134395.1	UAGCCAGGGAUUUUCGACCUCAa	2441
AD-67274.1	A-134396.1	Y44CCCUGGCUGGGAAACAUCAAa	2264	A-134397.1	UUUGAUGUUUCCCAGCCAGGGAu	2442
AD-67275.1	A-134398.1	Y44CUGGCUGGGAAACAUCAAAGa	2265	A-134399.1	UCUUUGAUGUUUCCCAGCCAGGg	2443
AD-67276.1	A-134400.1	Y44UGGCUGGGAAACAUCAAAGGa	2266	A-134401.1	UCCUUUGAUGUUUCCCAGCCAGg	2444
AD-67277.1	A-134402.1	Y44GGCUGGGAAACAUCAAAGGAa	2267	A-134403.1	UUCCUUUGAUGUUUCCCAGCCAg	2445
AD-67278.1	A-134404.1	Y44UCAAAGGAAUUCUCGGAAAGa	2268	A-134405.1	UCUUUCCGAGAAUUCCUUUGAUg	2446
AD-67279.1	A-134406.1	Y44UGGGAAACAUCAAAGGAAUUa	2269	A-134407.1	UAAUUCCUUUGAUGUUUCCCAGc	2447
AD-67280.1	A-134408.1	Y44GGAAACAUCAAAGGAAUUCUa	2270	A-134409.1	UAGAAUUCCUUUGAUGUUUCCCa	2448
AD-67281.1	A-134410.1	Y44GAAACAUCAAAGGAAUUCUCa	2271	A-134411.1	UGAGAAUUCCUUUGAUGUUUCCc	2449
AD-67282.1	A-134414.1	Y44CAUCAAAGGAAUUCUCGGAAa	2272	A-134415.1	UUUCCGAGAAUUCCUUUGAUGUu	2450
AD-67283.1	A-134416.1	Y44AAAGGAAUUCUCGGAAAGAAa	2273	A-134417.1	UUUCUUUCCGAGAAUUCCUUUGa	2451
AD-67284.1	A-134418.1	Y44AAGGAAUUCUCGGAAAGAAGa	2274	A-134419.1	UCUUCUUUCCGAGAAUUCCUUUg	2452
AD-67285.1	A-134420.1	Y44ACGAAGGAAGGCCCUCGAGAa	2275	A-134421.1	UUCUCGAGGGCCUUCCUUCGUCg	2453
AD-67286.1	A-134422.1	Y44GGCCCUCGAGAACAAGAAGAa	2276	A-134423.1	UUCUUCUUGUUCUCGAGGGCCUu	2454
AD-67287.1	A-134424.1	Y44GCCCUCGAGAACAAGAAGAAa	2277	A-134425.1	UUUCUUCUUGUUCUCGAGGGCCu	2455
AD-67288.1	A-134426.1	Y44CCCUCGAGAACAAGAAGAAGa	2278	A-134427.1	UCUUCUUCUUGUUCUCGAGGGCCc	2456
AD-67289.1	A-134428.1	Y44GGAAAGGAAAAAGAAGAGUAGa	2279	A-134429.1	UCUACUCUUCUUUUUCCUUUUCCUc	2457
AD-67290.1	A-134430.1	Y44AAGGAAAAAGAAGAGUAGCCGa	2280	A-134431.1	UCGGCUACUCUUCUUUUUCCUUUUc	2458
AD-67291.1	A-134432.1	Y44GGGGGUGUGAACCCCCUCGAa	2281	A-134433.1	UUCGAGGGGGGUUCACACCCCCCAa	2459
AD-67292.1	A-134434.1	Y44GGGUGUGAACCCCCUCGAAGa	2282	A-134435.1	UCUUCGAGGGGGUUCACACCCCCc	2460

Duplex Name	Sense Oligo Name	Sense Sequence (5'3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3')	SEQ ID NO:
AD-70224.1	A-141079.1	asasacUfuGfAfGfgucgaaaauaL96	2283	A-141080.1	usAfsuuuUfcgaccucAfaGfuuususu	2461
AD-70225.1	A-141081.1	asasacuuGfAfGfgucgaaaauaL96	2284	A-141082.1	usAfsuuuucgaccucAfaGfuuususu	2462
AD-70226.1	A-141083.1	asasacuuGfaGfgucgaaaauaL96	2285	A-141084.1	usAfsuuuucgaccdTcAfaguuususu	2463
AD-70227.1	A-141085.1	asasacuuGfaGfgucga(Agn)aauaL96	2286	A-141084.1	usAfsuuuucgaccdTcAfaguuususu	2464
AD-70228.1	A-141086.1	asGfsaaacuuGfaGfguCfgAfaaausa	2287	A-141087.1	usAfsuuuucgAfcCfucAfaGfuuucsu	2465
AD-70229.1	A-141090.1	gsasucaaGfAfAfacuugagguaL96	2288	A-141091.1	usAfsccucaaguuucUfuGfaucsusu	2466
AD-70230.1	A-141092.1	gsasucaaGfaAfacuugagguaL96	2289	A-141093.1	usAfsccucaaguudTcUfugaucsusu	2467
AD-70231.1	A-141094.1	gsasucaaGfaAfacuug(Agn)gguaL96	2290	A-141093.1	usAfsccucaaguudTcUfugaucsusu	2468
AD-70232.1	A-141095.1	asAfsgaucaaGfaAfacUfuGfaggusa	2291	A-141096.1	usAfsccucaaGfuUfucUfuGfaucusu	2469
AD-70233.1	A-141097.1	uscsaaGfaAfAfCfuugaggucgaL96	2292	A-141098.1	usCfsgacCfuCfAfaguuUfcUfugasusu	2470
AD-70234.1	A-141097.1	uscsaaGfaAfAfCfuugaggucgaL96	2293	A-141099.1	usCfsgacCfucaaguuUfcUfugasusu	2471
AD-70235.1	A-141100.1	uscsaagaAfAfCfuugaggucgaL96	2294	A-141101.1	usCfsgaccucaaguuUfcUfugasusu	2472
AD-70236.1	A-141102.1	uscsaagaAfaCfuugaggucgaL96	2295	A-141103.1	usCfsgaccucaagdTuUfcuugasusu	2473
AD-70237.1	A-141104.1	uscsaagaAfaCfuugagdCucgaL96	2296	A-141103.1	usCfsgaccucaagdTuUfcuugasusu	2474
AD-70238.1	A-141105.1	usCfsaagaAfaCfuugaGfgUfcgsa	2297	A-141106.1	usCfsgaccucAfaGfuuUfcUfugsa	2475
AD-70239.1	A-141107.1	asasgaUfcAfAfGfaaacuugagaL96	2298	A-141108.1	usCfsucaAfgUfUfucuuGfaUfcuususu	2476
AD-70240.1	A-141107.1	asasgaUfcAfAfGfaaacuugagaL96	2299	A-141109.1	usCfsucaAfguuucuuGfaUfcuususu	2477
AD-70241.1	A-141110.1	asasgaucAfAfGfaaacuugagaL96	2300	A-141111.1	usCfsucaaguuucuuGfaUfcuususu	2478
AD-70242.1	A-141112.1	asasgaucAfaGfaaacuugagaL96	2301	A-141113.1	usCfsucaaguuucdTuGfaucuususu	2479
AD-70243.1	A-141114.1	asasgaucAfaGfaaacu(Agn)gagaL96	2302	A-141113.1	usCfsucaaguuucdTuGfaucuususu	2480
AD-70244.1	A-141115.1	asGfsaagaucAfaGfaaAfcUfugagsa	2303	A-141116.1	usCfsucaaguUfuCfuuGfaUfcuucsu	2481

Duplex Name	Sense Oligo Name	Sense Sequence (5'3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3'	SEQ ID NO:
AD-70245.1	A-141117.1	csasucAfaAfGfGfaauucucggaL96	2304	A-141118.1	usCfscgaGfaauuccuUfuGfaugsusu	2482
AD-70246.1	A-141119.1	csasucaaAfGfGfaauucucggaL96	2305	A-141120.1	usCfscgagaauuccuUfuGfaugsusu	2483
AD-70247.1	A-141121.1	csasucaaAfgGfaauucucggaL96	2306	A-141122.1	usCfscgagaauucdCuUfugaugsusu	2484
AD-70248.1	A-141123.1	csasucaaAfgGfaauuc(Tgn)cggaL96	2307	A-141122.1	usCfscgagaauucdCuUfugaugsusu	2485
AD-70249.1	A-141124.1	asAfscaucaaAfgGfaaUfuCfucggsa	2308	A-141125.1	usCfscgagaaUfuCfcuUfuGfaugusu	2486
AD-70250.1	A-141126.1	asuscaAfgAfAfAfcuugaggucaL96	2309	A-141127.1	usGfaccUfcAfAfguuuCfuUfgaususu	2487
AD-70251.1	A-141126.1	asuscaAfgAfAfAfcuugaggucaL96	2310	A-141128.1	usGfaccUfcaaguuuCfuUfgaususu	2488
AD-70252.1	A-141129.1	asuscaagAfAfAfcuugaggucaL96	2311	A-141130.1	usGfaccucaaguuuCfuUfgaususu	2489
AD-70253.1	A-141131.1	asuscaagAfaAfcuugaggucaL96	2312	A-141132.1	usGfaccucaagudTuCfuugaususu	2490
AD-70254.1	A-141133.1	asuscaagAfaAfcuugadCgucaL96	2313	A-141132.1	usGfaccucaagudTuCfuugaususu	2491
AD-70255.1	A-141134.1	asGfsaucaagAfaAfcuUfgAfggucsa	2314	A-141135.1	usGfaccucaAfgUfuuCfuUfgaucsu	2492
AD-70256.1	A-141136.1	gsasagAfuCfAfAfgaaacuugaaL96	2315	A-141137.1	usUfscaaGfuuucuugAfuCfuucsusu	2493
AD-70257.1	A-141138.1	gsasagauCfAfAfgaaacuugaaL96	2316	A-141139.1	usUfscaaguuucuugAfuCfuucsusu	2494
AD-70258.1	A-141140.1	gsasagauCfaAfgaaacuugaaL96	2317	A-141141.1	usUfscaaguuucudTgAfucuucsusu	2495
AD-70259.1	A-141142.1	gsasagauCfaAfgaaac(Tgn)ugaaL96	2318	A-141141.1	usUfscaaguuucudTgAfucuucsusu	2496
AD-70260.1	A-141143.1	asAfsgaagauCfaAfgaAfaCfuugasa	2319	A-141144.1	usUfscaaguuUfcUfugAfuCfuucusu	2497
AD-70261.1	A-141145.1	csasaagGfaAfUfUfcucggaaagL96	2320	A-141146.1	csUfuucCfgAfGfaauuCfcUfuugsasu	2498
AD-70262.1	A-141145.1	csasaagGfaAfUfUfcucggaaagL96	2321	A-141147.1	csUfuucCfgagaauuCfcUfuugsasu	2499
AD-70263.1	A-141148.1	csasaaggaAfUfUfcucggaaagL96	2322	A-141149.1	csUfuuccgagaauuCfcUfuugsasu	2500
AD-70264.1	A-141150.1	csasaaggaAfuUfcucggaaagL96	2323	A-141151.1	csUfuuccgagaadTuCfcuuugsasu	2501
AD-70265.1	A-141152.1	csasaaggaAfuUfcucgdCaaagL96	2324	A-141151.1	csUfuuccgagaadTuCfcuuugsasu	2502

Duplex Name	Sense Oligo Name	Sense Sequence (5'3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3'	SEQ ID NO:
AD-70266.1	A-141153.1	asUfscaaaggaAfuUfcuCfgGfaaasg	2325	A-141154.1	csUfuuccgaGfaAfuuCfcUfuugasu	2503
AD-70267.1	A-141155.1	csasagAfaAfCfUfugaggucgaaL96	2326	A-141156.1	usUfscgaCfcUfCfaaguUfuCfuugsasu	2504
AD-70268.1	A-141155.1	csasagAfaAfCfUfugaggucgaaL96	2327	A-141157.1	usUfscgaCfcucaaguUfuCfuugsasu	2505
AD-70269.1	A-141158.1	csasagaaAfCfUfugaggucgaaL96	2328	A-141159.1	usUfscgaccucaaguUfuCfuugsasu	2506
AD-70270.1	A-141160.1	csasagaaAfcUfugaggucgaaL96	2329	A-141161.1	usUfscgaccucaadGuUfucuugsasu	2507
AD-70271.1	A-141162.1	csasagaaAfcUfugagg(Tgn)cgaaL96	2330	A-141161.1	usUfscgaccucaadGuUfucuugsasu	2508
AD-70272.1	A-141163.1	asUfscaagaaAfcUfugAfgGfucgasa	2331	A-141164.1	usUfscgaccuCfaAfguUfuCfuugasu	2509
AD-70273.1	A-141165.1	gsasagAfaGfAfUfcaagaaacuuL96	2332	A-141166.1	asAfsguuUfcUfUfgaucUfuCfuucsusu	2510
AD-70274.1	A-141165.1	gsasagAfaGfAfUfcaagaaacuuL96	2333	A-141167.1	asAfsguuUfcuugaucUfuCfuucsusu	2511
AD-70275.1	A-141168.1	gsasagaaGfAfUfcaagaaacuuL96	2334	A-141169.1	asAfsguuucuugaucUfuCfuucsusu	2512
AD-70276.1	A-141170.1	gsasagaaGfaUfcaagaaacuuL96	2335	A-141171.1	asAfsguuucuugadTcUfucuucsusu	2513
AD-70277.1	A-141172.1	gsasagaaGfaUfcaaga(Agn)acuuL96	2336	A-141171.1	asAfsguuucuugadTcUfucuucsusu	2514
AD-70278.1	A-141173.1	asAfsgaagaaGfaUfcaAfgAfaacusu	2337	A-141174.1	asAfsguuucuUfgAfucUfuCfuucusu	2515
AD-70279.1	A-141175.1	gsgsaaAfcAfUfCfaaaggaauuaL96	2338	A-141176.1	usAfsauuCfcUfUfugauGfuUfuccsusu	2516
AD-70280.1	A-141175.1	gsgsaaAfcAfUfCfaaaggaauuaL96	2339	A-141177.1	usAfsauuCfcuuugauGfuUfuccsusu	2517
AD-70281.1	A-141178.1	gsgsaaacAfUfCfaaaggaauuaL96	2340	A-141179.1	usAfsauuccuuugauGfuUfuccsusu	2518
AD-70282.1	A-141180.1	gsgsaaacAfuCfaaaggaauuaL96	2341	A-141181.1	usAfsauuccuuugdAuGfuuucsusu	2519
AD-70283.1	A-141182.1	gsgsaaacAfuCfaaagg(Agn)auuaL96	2342	A-141183.1	usAfsauuccuuugdAuGfuuuccsusu	2520
AD-70284.1	A-141184.1	usGfsggaaacAfuCfaaAfgGfaauusa	2343	A-141185.1	usAfsauuccuUfuGfauGfuUfucccsa	2521
AD-70285.1	A-134288.1	asasagaaGfaAfGfAfucaagaaacuL96	2344	A-141186.1	asGfsuuuCfuUfUfGfaucuUfcUfucuuusgsu	2522
AD-70286.1	A-141187.1	asasagaagaAfGfAfucaagaaacuL96	2345	A-141188.1	asGfsuuucuugaucuUfcUfucuuusgsu	2523

Duplex Name	Sense Oligo Name	Sense Sequence (5'3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3'	SEQ ID NO:
AD-70287.1	A-141189.1	asasagaagaAfgAfucaagaaacuL96	2346	A-141190.1	asGfsuuucuugaudCuUfcuucuuusgsu	2524
AD-70288.1	A-141191.1	asasagaagaAfgAfucaag(Agn)aacuL96	2347	A-141190.1	asGfsuuucuugaudCuUfcuucuuusgsu	2525
AD-70289.1	A-141192.1	asAfsagaagaAfgAfucAfaGfaaacsu	2348	A-141193.1	asGfsuuucuuGfaUfcuUfcUfucuusu	2526
AD-70290.1	A-141194.1	asgsaaGfaAfGfAfucaagaaacuL96	2349	A-141195.1	asGfsuuuCfuUfGfaucuUfcUfucususu	2527
AD-70291.1	A-141194.1	asgsaaGfaAfGfAfucaagaaacuL96	2350	A-141196.1	asGfsuuuCfuugaucuUfcUfucususu	2528
AD-70292.1	A-141197.1	asgsaagaAfGfAfucaagaaacuL96	2351	A-141198.1	asGfsuuucuugaucuUfcUfucususu	2529
AD-70293.1	A-141199.1	asgsaagaAfgAfucaagaaacuL96	2352	A-141200.1	asGfsuuucuugaudCuUfcuucususu	2530
AD-70294.1	A-141201.1	asgsaagaAfgAfucaag(Agn)aacuL96	2353	A-141200.1	asGfsuuucuugaudCuUfcuucususu	2531
AD-70295.1	A-141202.1	asGfsaagaAfgAfucaaGfaAfacsu	2354	A-141203.1	asGfsuuucuuGfaUfcuUfcUfucsu	2532
AD-70296.1	A-141204.1	asascaUfcAfAfAfggaauucucaL96	2355	A-141205.1	usGfsagaAfuUfCfcuuuGfaUfguususu	2533
AD-70297.1	A-141204.1	asascaUfcAfAfAfggaauucucaL96	2356	A-141206.1	usGfsagaAfuuccuuuGfaUfguususu	2534
AD-70298.1	A-141207.1	asascaucAfAfAfggaauucucaL96	2357	A-141208.1	usGfsagaauuccuuuGfaUfguususu	2535
AD-70299.1	A-141209.1	asascaucAfaAfggaauucucaL96	2358	A-141210.1	usGfsagaauuccudTuGfauguususu	2536
AD-70300.1	A-141211.1	asascaucAfaAfggaau(Tgn)cucaL96	2359	A-141210.1	usGfsagaauuccudTuGfauguususu	2537
AD-70301.1	A-141212.1	asAfscaucAfaAfggaaUfuCfucsa	2360	A-141213.1	usGfsagaauuCfcUfuuGfaUfgusu	2538
AD-70302.1	A-141214.1	asgsgaAfuUfCfUfcggaaagaaaL96	2361	A-141215.1	usUfsucuUfuCfCfgagaAfuUfccususu	2539
AD-70303.1	A-141214.1	asgsgaAfuUfCfUfcggaaagaaaL96	2362	A-141216.1	usUfsucuUfuccgagaAfuUfccususu	2540
AD-70304.1	A-141217.1	asgsgaauUfCfUfcggaaagaaaL96	2363	A-141218.1	usUfsucuuuccgagaAfuUfccususu	2541
AD-70305.1	A-141219.1	asgsgaauUfcUfcggaaagaaaL96	2364	A-141220.1	usUfsucuuuccgadGaAfuuccususu	2542
AD-70306.1	A-141221.1	asgsgaauUfcUfcggaa(Agn)gaaaL96	2365	A-141220.1	usUfsucuuuccgadGaAfuuccususu	2543
AD-70307.1	A-141222.1	asAfsaggaauUfcUfcgGfaAfagaasa	2366	A-141223.1	usUfsucuuucCfgAfgaAfuUfccuusu	2544

Duplex Name	Sense Oligo Name	Sense Sequence (5'3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'3'	SEQ ID NO:
AD-70308.1	A-141224.1	asasagGfaAfUfUfcucggaaagaL96	2367	A-141225.1	usCfsuuuCfcGfAfgaauUfcCfuuususu	2545
AD-70309.1	A-141224.1	asasagGfaAfUfUfcucggaaagaL96	2368	A-141226.1	usCfsuuuCfcgagaauUfcCfuuususu	2546
AD-70310.1	A-141227.1	asasaggaAfUfUfcucggaaagaL96	2369	A-141228.1	usCfsuuuccgagaauUfcCfuuususu	2547
AD-70311.1	A-141229.1	asasaggaAfuUfcucggaaagaL96	2370	A-141230.1	usCfsuuuccgagadAuUfccuuususu	2548
AD-70312.1	A-141231.1	asasaggaAfuUfcucgg(Agn)aagaL96	2371	A-141230.1	usCfsuuuccgagadAuUfccuuususu	2549
AD-70313.1	A-141232.1	usCfsaaaggaAfuUfcuCfgGfaaagsa	2372	A-141233.1	usCfsuuuccgAfgAfauUfcCfuuugsa	2550

Table 33. HDV Single dose screen in Cos7 Cells against Antigenome and Genome constructs

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-67176.1	79.3		89.6	59.9		77.2	22.9		3.4	9.10		20.6
AD-67177.1	101.5		99.1	39.3		83.6	39.7		14.9	10.76		28.7
AD-67178.1	99.6		97.4	90.5		86.7	12.0		20.1	40.51		41.6
AD-67179.1	84.8		88.6	63.2		81.3	12.1		15.2	19.52		28.2
AD-67180.1	77.7		108.6	41.0		93.2	15.1		26.8	13.25		33.6
AD-67181.1	169.3		92.9	47.9		92.3	6.2		8.9	8.51		25.0
AD-67182.1	62.1		102.6	43.6		86.3	11.4		22.0	12.92		28.7
AD-67183.1	89.3		105.0	65.4		99.2	13.9		14.7	4.65		34.1

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-67184.1	49.9		102.4	96.4		102.7	10.6		16.4	25.05		20.7
AD-67185.1	87.1		87.7	80.9		81.0	23.9		4.5	18.19		19.7
AD-67186.1	102.4		109.4	81.7		82.6	20.2		12.7	12.49		4.9
AD-67187.1	72.0		93.2	87.7		95.4	7.4		11.1	13.69		23.2
AD-67188.1	68.1		110.5	127.7		97.3	12.8		14.9	18.70		26.5
AD-67189.1	84.3		95.9	104.7		98.9	16.9		15.0	23.72		26.2
AD-67190.1	104.5		115.3	110.5		101.5	2.7		7.2	24.53		5.1
AD-67191.1	72.9		99.8	72.8		92.2	8.4		8.1	21.61		14.0
AD-67192.1	124.2		106.4	96.4		113.5	25.6		18.2	22.27		36.9
AD-67193.1	91.3		105.3	105.2		121.4	12.8		21.7	25.63		41.9
AD-67194.1	39.4		84.8	97.5		105.9	9.0		3.1	12.31		32.6
AD-67195.1	90.1		110.7	87.5		98.7	11.7		10.4	20.22		11.7
AD-67196.1	75.5		107.9	102.1		85.5	3.6		5.5	2.71		14.3
AD-67197.1	68.8		98.6	80.2		96.0	5.3		13.5	15.68		23.1
AD-67198.1	49.5		101.2	106.2		111.0	2.7		12.7	20.42		15.9
AD-67199.1	27.1		72.4	162.7		87.3	4.9		11.4	23.09		21.3
AD-67200.1	24.1		76.7	128.5		114.2	4.0		4.2	13.53		24.3
AD-67201.1	58.1		88.6	100.1		86.0	9.9		17.3	26.61		24.4
AD-67202.1	30.3		92.1	107.3		110.5	4.1		13.6	27.71		17.9
AD-67203.1	55.3		99.6	109.7		99.8	4.0		8.0	28.27		26.2
AD-67204.1	71.6		101.4	111.1		107.5	7.7		12.0	49.72		29.2

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-67205.1	54.2		95.4	89.3		90.2	6.4		14.7	17.80		24.2
AD-67206.1	52.4		89.9	106.9		103.8	12.6		7.0	19.47		36.2
AD-67207.1	57.6		99.4	102.6		109.5	11.1		21.9	9.58		9.6
AD-67208.1	31.2		89.5	87.4		102.7	3.5		8.9	15.91		27.3
AD-67209.1	47.1		90.6	97.8		78.5	6.3		10.7	18.03		25.7
AD-67210.1	31.7		95.4	96.2		92.8	1.8		14.4	14.11		28.7
AD-67211.1	25.4		79.8	110.2		93.4	6.5		9.5	23.84		27.4
AD-67212.1	32.2		93.9	90.7		67.7	4.9		5.8	22.15		23.4
AD-67213.1	49.2		97.7	97.4		75.5	5.2		9.6	17.73		25.9
AD-67214.1	56.8		92.7	97.8		99.4	9.6		10.1	20.89		21.4
AD-67215.1	39.0		99.2	92.1		95.4	11.3		5.5	6.57		11.4
AD-67216.1	57.1		94.3	91.2		87.5	12.6		11.9	18.27		25.2
AD-67217.1	63.4		101.4	90.0		96.2	2.1		10.7	15.73		25.7
AD-67218.1	65.8		97.3	91.1		86.6	10.3		7.1	11.42		21.2
AD-67219.1	77.3		100.4	83.9		89.8	1.7		7.8	18.81		40.2
AD-67220.1	78.8		102.2	87.6		77.8	15.2		7.9	7.96		11.5
AD-67250.1	85.3		92.4	29.0		74.3	4.4		11.6	3.72		22.8
AD-67251.1	90.2		104.3	101.9		91.7	25.6		18.0	25.07		12.2
AD-67252.1	99.6		108.6	59.6		64.9	8.2		3.6	18.48		13.7
AD-67253.1	50.0		94.6	32.9		73.5	3.3		15.8	14.44		18.2
AD-67254.1	193.2		106.8	63.7		68.2	47.1		6.1	22.95		12.7

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-67255.1	83.7		94.5	38.2		66.7	25.7		13.7	8.71		9.5
AD-67256.1	88.8		94.3	113.1		106.0	22.8		9.4	37.17		26.3
AD-67257.1	51.8		101.2	105.6		93.7	5.2		4.6	26.52		22.3
AD-67258.1	81.6		102.7	92.2		92.4	24.4		6.2	39.25		26.5
AD-67259.1	113.1		104.8	81.2		71.9	18.8		16.7	30.40		9.6
AD-67260.1	52.1		112.2	67.4		74.6	8.1		8.8	4.21		10.8
AD-67261.1	73.8		96.2	96.1		99.5	15.5		9.1	7.96		17.8
AD-67262.1	110.6		106.5	127.0		115.6	28.0		15.3	20.20		3.0
AD-67263.1	69.4		96.3	49.7		63.1	16.7		8.8	4.00		14.8
AD-67264.1	180.6		104.6	100.1		94.9	43.2		22.3	37.56		10.7
AD-67265.1	122.5		117.0	119.1		99.4	23.3		19.7	13.26		18.6
AD-67266.1	24.3		59.3	115.8		83.6	1.0		3.8	34.70		14.0
AD-67267.1	83.4		113.8	92.6		82.7	13.9		11.8	26.19		10.6
AD-67268.1	99.2		101.2	81.0		94.9	14.9		11.1	21.50		22.4
AD-67269.1	42.9		81.7	179.2		86.0	6.6		6.5	50.94		13.2
AD-67270.1	33.9		85.7	125.2		85.6	5.9		10.8	24.45		12.7
AD-67271.1	29.6		53.4	156.0		115.8	8.9		10.9	27.82		27.2
AD-67272.1	13.4		46.3	136.9		128.4	2.3		4.4	23.52		30.3
AD-67273.1	15.9		57.5	105.1		82.1	4.6		4.4	26.59		13.3
AD-67274.1	25.8		60.4	86.8		93.4	2.0		10.2	14.89		22.4
AD-67275.1	46.5		75.4	131.1		94.3	12.3		9.5	46.37		18.0

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-67276.1	20.8		59.4	87.7		88.0	2.2		4.4	10.78		18.2
AD-67277.1	17.2		49.5	93.4		92.4	2.3		4.7	44.06		19.4
AD-67278.1	25.1		61.3	131.5		94.8	8.4		6.8	30.75		10.0
AD-67279.1	21.9		78.3	111.7		126.9	4.8		10.8	19.15		15.0
AD-67280.1	32.8		78.1	154.7		109.4	4.3		9.1	52.47		29.4
AD-67281.1	32.8		60.0	163.7		110.1	2.4		9.0	20.60		8.6
AD-67282.1	19.3		47.6	129.1		109.7	4.8		9.1	18.16		21.2
AD-67283.1	34.5		71.8	193.2		112.4	14.0		1.1	66.11		10.7
AD-67284.1	51.2		76.9	126.9		94.1	5.2		7.9	40.77		26.8
AD-67285.1	17.3		68.6	66.9		110.7	1.9		10.7	26.05		25.3
AD-67286.1	29.5		85.5	87.3		125.7	5.8		10.7	13.51		30.7
AD-67287.1	33.1		83.5	84.9		98.8	12.2		22.2	33.27		12.9
AD-67288.1	14.4		75.1	73.3		109.2	6.5		5.2	15.93		16.1
AD-67289.1	38.8		66.4	97.9		102.0	15.6		7.9	22.42		19.0
AD-67290.1	20.9		81.2	89.6		112.4	4.8		9.3	11.83		8.4
AD-67291.1	47.8		100.0	107.1		95.2	4.7		19.7	16.82		9.3
AD-67292.1	99.9		99.3	129.3		136.5	22.7		20.8	39.06		58.4
AD-70224.1	46.5	68.6	94.0	99.6	113.6	103.5	10.2	8.7	2.5	13.9	6.9	10.0
AD-70225.1	49.1	76.2	88.9	129.9	107.0	87.1	7.2	0.8	5.3	45.3	6.1	10.0
AD-70226.1	65.3			105.4			8.0			23.9		
AD-70227.1	37.7	58.7	84.3	173.3	111.4	101.7	8.2	3.2	5.1	15.5	6.2	5.5

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-70228.1	102.4	120.8	107.9	46.3	52.2	82.3	10.7	5.7	14.3	11.4	2.5	3.6
AD-70229.1	32.0	65.6	96.9	92.1	107.2	89.6	3.5	0.1	1.9	6.8	25.7	5.5
AD-70230.1	36.7	66.7	99.2	91.8	100.1	91.9	3.9	11.4	13.6	8.2	14.9	12.5
AD-70231.1	26.7	62.6	86.3	97.3	93.9	122.5	4.0	5.2	2.4	25.6	2.7	35.5
AD-70232.1	16.1	35.2	74.5	40.1	33.3	73.9	2.7	2.7	0.9	15.0	6.5	10.9
AD-70233.1	24.8	71.7	96.0	91.8	106.4	96.2	1.6	1.0	1.2	10.4	9.2	0.5
AD-70234.1	29.6	63.0	90.2	89.4	102.5	99.9	5.1	0.1	3.5	8.5	3.4	6.3
AD-70235.1	35.4	84.5	104.2	66.6	101.9	97.8	10.5	4.2	9.7	13.0	1.2	6.2
AD-70236.1	39.4	64.8	95.2	86.3	88.3	94.0	3.7	3.0	0.6	5.7	3.8	6.7
AD-70237.1	22.1	49.4	83.8	96.2	90.3	87.6	2.5	0.1	1.7	19.1	9.4	2.8
AD-70238.1	26.5	61.4	91.8	60.9	72.9	82.7	1.6	0.8	6.7	6.8	5.4	1.9
AD-70239.1	73.7			99.1			8.4			20.7		
AD-70240.1	80.0			82.4			11.0			8.3		
AD-70241.1	82.5			107.4			2.1			21.9		
AD-70242.1	69.5			90.2			4.7			28.9		
AD-70243.1	33.5	57.4	102.5	109.3	106.4	80.7	3.5	1.2	8.2	20.7	17.0	2.8
AD-70244.1	78.5	93.3	99.9	22.6	29.3	85.9	6.2	1.8	4.5	4.2	5.0	12.2
AD-70245.1	44.7	71.2	83.2	109.7	91.5	98.5	3.3	2.4	1.5	15.1	3.9	8.9
AD-70246.1	42.1	79.0	104.6	97.2	99.0	97.0	1.0	4.9	13.5	25.8	4.7	6.9
AD-70247.1	60.3			75.2			6.5			6.8		
AD-70248.1	20.9	45.2	82.2	86.5	121.9	98.2	2.1	6.9	3.8	10.0	3.5	2.4

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-70249.1	72.4	93.6	96.7	39.9	38.5	81.3	4.2	6.7	1.5	13.1	7.4	10.1
AD-70250.1	35.0	72.9	97.5	81.7	91.4	89.8	3.6	3.1	16.8	8.5	2.0	5.2
AD-70251.1	47.2	71.8	92.6	87.6	101.5	105.3	5.5	6.5	0.8	15.2	1.3	11.8
AD-70252.1	55.7			76.2			6.7			6.1		
AD-70253.1	61.5			68.8			3.9			16.5		
AD-70254.1	30.0	65.2	93.1	83.1	92.0	111.6	0.8	1.4	2.6	9.8	15.2	0.8
AD-70255.1	65.0	89.1	90.4	32.6	50.7	92.0	5.2	1.6	2.6	6.9	8.8	2.0
AD-70256.1	17.9	45.9	89.0	107.7	126.3	95.6	2.3	0.6	6.6	17.1	24.6	5.3
AD-70257.1	19.7	41.8	78.1	129.4	104.7	96.7	3.8	0.7	3.3	28.2	16.6	3.9
AD-70258.1	25.9	51.4	83.8	120.5	104.3	77.0	2.6	3.8	8.2	25.8	0.2	7.1
AD-70259.1	15.0	26.0	70.3	131.5	117.4	100.5	1.7	0.2	4.0	16.7	3.8	1.0
AD-70260.1	21.9	43.2	85.4	44.7	39.3	103.6	4.3	1.3	6.4	9.1	0.9	6.7
AD-70261.1	83.1			82.1			4.2			10.7		
AD-70262.1	85.6			81.5			7.2			13.1		
AD-70263.1	92.8			116.9			4.7			50.7		
AD-70264.1	82.3			120.8			5.5			53.7		
AD-70265.1	38.1	72.2	95.2	130.9	98.6	90.5	4.1	1.7	3.1	29.9	0.8	12.6
AD-70266.1	85.8			90.3			11.3			19.5		
AD-70267.1	15.0	27.8	66.1	123.5	122.8	103.0	1.3	1.7	2.8	15.5	4.1	8.8
AD-70268.1	15.8	30.3	78.2	116.7	123.1	103.6	2.0	1.8	1.8	22.7	6.9	5.3
AD-70269.1	16.0	27.9	71.1	106.9	99.8	99.2	1.7	1.7	0.6	19.5	9.4	26.9

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-70270.1	26.1	54.7	89.1	108.8	102.8	93.4	4.5	4.4	10.6	31.1	1.6	14.2
AD-70271.1	15.3	26.8	62.8	115.4	125.0	106.9	3.3	2.3	4.3	13.5	6.9	3.7
AD-70272.1	15.1	33.3	82.3	53.3	31.4	70.3	2.7	2.1	11.2	15.0	5.3	12.4
AD-70273.1	26.8	49.5	85.2	150.6	133.9	111.2	4.9	6.4	2.1	36.0	0.4	0.6
AD-70274.1	23.2	36.5	87.3	177.6	123.0	109.8	2.8	0.5	0.6	27.1	2.2	21.5
AD-70275.1	20.0	43.9	76.1	178.4	151.1	103.7	4.1	4.7	3.0	32.2	10.4	8.1
AD-70276.1	32.1	43.6	84.1	183.4	130.6	103.7	1.8	2.3	2.1	14.9	12.2	5.0
AD-70277.1	29.7	33.8	73.2	213.9	140.7	109.2	6.4	4.9	2.8	20.5	8.8	9.7
AD-70278.1	25.2	41.4	73.6	57.8	56.0	95.3	5.9	1.1	5.7	12.1	2.6	4.7
AD-70279.1	79.8			135.6			7.4			22.9		
AD-70280.1	100.4			162.0			14.5			17.5		
AD-70281.1	82.2			161.7			13.1			10.6		
AD-70282.1	105.1			116.2			5.3			16.9		
AD-70283.1	64.4			134.4			7.7			23.3		
AD-70284.1	95.4			106.1			11.0			5.3		
AD-70285.1	91.6			119.4			11.6			7.9		
AD-70286.1	84.0			110.3			8.8			21.6		
AD-70287.1	89.9			96.7			4.4			7.5		
AD-70288.1	29.1	74.0	89.9	199.1	132.5	110.7	5.8	7.8	2.7	58.5	26.6	2.8
AD-70289.1	32.7	44.4	84.5	77.7	60.0	76.8	5.3	1.4	2.6	11.4	5.7	1.4
AD-70290.1	102.0			102.6			13.6			17.4		

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-70291.1	100.7			110.1			8.5			24.6		
AD-70292.1	76.7			131.9			5.2			20.2		
AD-70293.1	99.7			131.7			5.8			29.7		
AD-70294.1	103.1			124.4			5.4			35.4		
AD-70295.1	101.8			125.9			6.3			10.4		
AD-70296.1	104.3			106.8			11.1			11.9		
AD-70297.1	105.2			105.8			6.0			38.9		
AD-70298.1	103.1			99.9			5.0			19.1		
AD-70299.1	108.1			100.9			12.9			24.6		
AD-70300.1	109.6			80.5			13.3			8.3		
AD-70301.1	99.4			97.2			7.0			11.0		
AD-70302.1	101.9			104.6			17.2			27.3		
AD-70303.1	77.1			133.0			6.4			27.0		
AD-70304.1	119.5			108.7			12.4			10.3		
AD-70305.1	91.1			115.5			8.2			22.6		
AD-70306.1	103.7			140.3			15.5			19.8		
AD-70307.1	75.4			98.0			3.8			14.0		
AD-70308.1	105.5			136.1			7.7			30.9		
AD-70309.1	109.5			92.8			23.5			14.6		
AD-70310.1	111.6			109.7			11.5			27.1		
AD-70311.1	101.8			111.5			10.2			25.1		

Duplex	10nM Avg Anti	1nM Avg Anti	0.1nM Avg Anti	10nM Avg Genome	1nM Avg Genome	0.1nM Avg Genome	10nM SD Anti	1nM SD Anti	0.1nM SD Anti	10nM SD Genome	1nM SD Genome	0.1nM SD Genome
AD-70312.1	113.8			91.0			8.3			26.1		
AD-70313.1	114.8			76.9			10.0			13.1		

Table 34. Dose response screen in Cos7 cells

Duplex	Antigenome, IC50 nM	Genome, IC50 nM	Antigenome hot spot, IC50 nM	Genome hot spot, IC50 nM
AD-67176.1	not achieved	not achieved		
AD-67177.1	not achieved	not achieved		
AD-67180.1	not achieved	3124.5		
AD-67181.1	not achieved	>10000		
AD-67182.1	not achieved	>10000		
AD-67199.1	288.0	not achieved		
AD-67200.1	466.2	not achieved		
AD-67202.1	>10000	not achieved		
AD-67208.1	not achieved	not achieved		
AD-67210.1	>10000	not achieved		
AD-67211.1	1100.8	not achieved		
AD-70228.1			not achieved	23.0
AD-70232.1	405.4	5002.4	33.7	9.0
AD-70238.1			273.9	122.4
AD-70244.1		1589.9	not achieved	14.7
AD-70249.1		2377.2	2376.2	13.1
AD-70255.1			3082.4	26.1
AD-70259.1	173.2			
AD-70260.1	419.2	7185.0	50.3	8.7
AD-70266.1			not achieved	476.4
AD-70267.1	185.7			
AD-70268.1	174.6			
AD-70269.1	267.6			
AD-70271.1	119.6			
AD-70272.1	364.8	3400.4	30.2	22.2
AD-70274.1	258.8			
AD-70277.1	190.6			
AD-70278.1			57.1	30.6
AD-70284.1			not achieved	4670.5
AD-70289.1			not achieved	not achieved
AD-70295.1			61.8	39.4
AD-70301.1			not achieved	not achieved
AD-70307.1			2329.6	379.0
AD-70313.1			not achieved	not achieved

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. A double stranded RNAi agent for inhibiting expression of hepatitis D virus (HDV) in a cell, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region selected from the group consisting of

(a) a 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;

(b) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:31, 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:32;

(c) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:33, 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:34;

(d) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:35, 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:36;

(e) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:37, 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:38;

(f) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:39, 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;

(g) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:41, 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:42;

(h) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:43, 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:44; and

(i) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2551, 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:2552;

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

3. The double stranded RNAi agent of claim 1, wherein one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the sense strand.

4. 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.

5. The double stranded RNAi agent of claim 1, wherein said 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 Table 11, 12, 31, and 32.

6. The double stranded RNAi agent of claim 1, wherein said 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 sense and antisense strands of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, and AD-70275.1.

7. The double stranded RNAi agent of claim 1, wherein said 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 sense and antisense strands of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, and AD-70295.1.

8. The double stranded RNAi agent of any one of claims 1-7, wherein 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.

9. The double stranded RNAi agent of any claim 1, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

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

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

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

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

14. The double stranded RNAi agent of claim 1, wherein the double-stranded region is 23-27 nucleotide pairs in length.

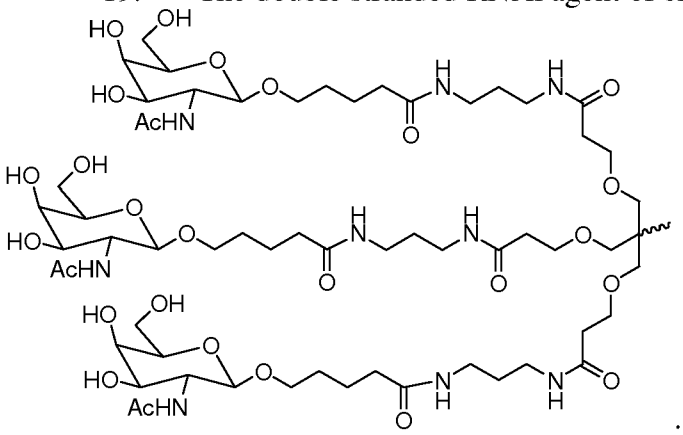
15. The double stranded RNAi agent of claim 1, wherein the double-stranded region is 19-21 nucleotide pairs in length.

16. The double stranded RNAi agent of claim 1, wherein the double-stranded region is 21-23 nucleotide pairs in length.

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

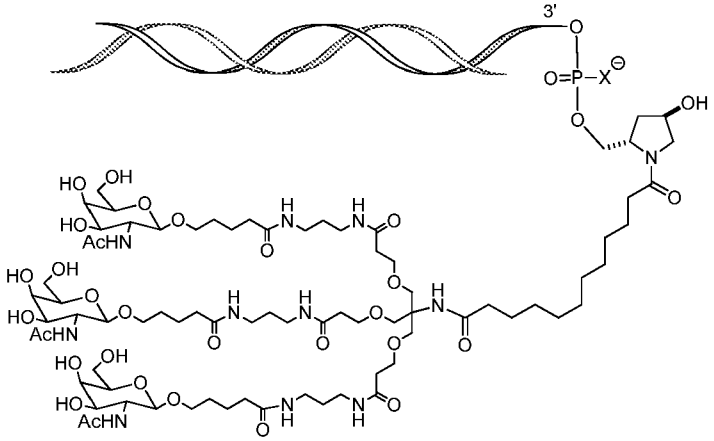
18. The double stranded RNAi agent of claim 1, wherein each strand has 19-30 nucleotides.

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



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20. The double stranded RNAi agent of claim 1, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

21. The double stranded RNAi agent of claim 1, wherein said RNAi agent is selected from the group of RNAi agents listed in any one of Table 11, 12, 31, and 32.

22. The double stranded RNAi agent of claim 1, wherein said RNAi agent is selected from the consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

23. A double stranded RNAi agent for inhibiting expression of hepatitis D virus (HDV) 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 any one of the sense sequences provided in any one of Table 11, 12, 31, and 32, and said antisense strand comprises any one of the antisense sequences provided in any one of Table 11, 12, 31, and 32,

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.

24. The double stranded RNAi agent of claim 23, wherein said RNAi agent is selected from the consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

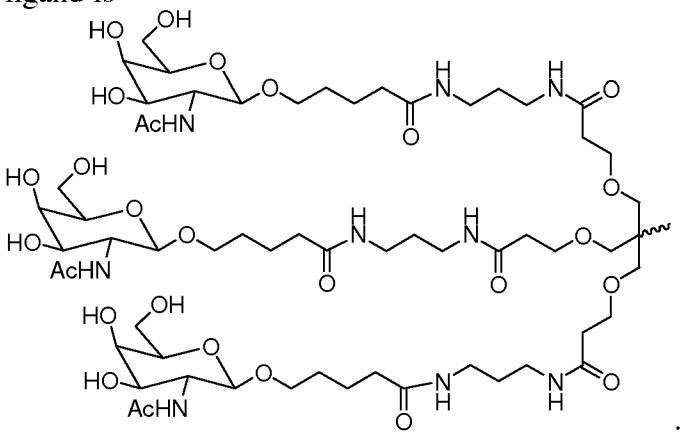
25. The double stranded RNAi agent of claim 23, wherein said RNAi agent is selected from the consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, and AD-70295.1.

26. The double stranded RNAi agent of claim 23, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

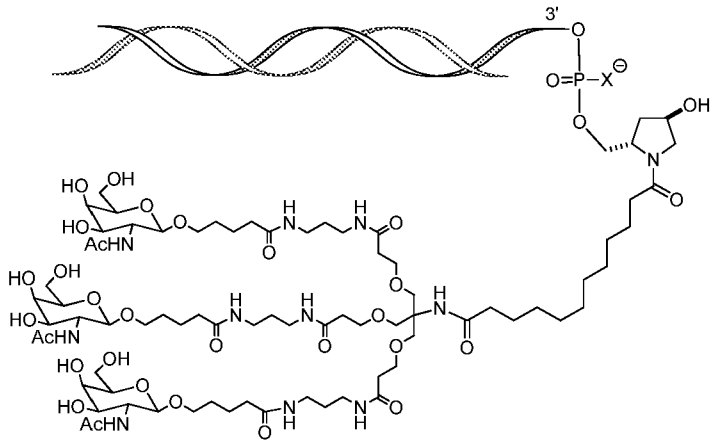
27. The double stranded RNAi agent of claim 23, wherein at least one of said modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, 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.

28. The double stranded RNAi agent of claim 27, wherein the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

29. The double stranded RNAi agent of any one of claims 23-28, wherein the ligand is



30. The double stranded RNAi agent of claim 29, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

31. A composition comprising two or more double stranded RNAi agents for inhibiting expression of hepatitis D virus (HDV) in a cell, wherein each double stranded RNAi agent independently comprises a sense strand and an antisense strand forming a double-stranded region, selected from the group consisting of

(a) a 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;

(b) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:31, 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:32;

(c) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:33, 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:34;

(d) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:35, 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:36;

(e) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:37, 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:38;

(f) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:39, 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;

(g) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:41, 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:42;

(h) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:43, 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:44; and

(i) a sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2551, 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:2552;

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.

32. The composition of claim 31, wherein one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the antisense strand.

33. The composition of claim 31, wherein one or more of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the sense strand.

34. The composition of claim 31, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

35. The composition of claim 31, wherein said 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 provided in any one of Table 11, 12, 31, and 32.

36. The composition of claim 31, wherein said RNAi agent is selected from the consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

37. The composition of claim 31, wherein said RNAi agent is selected from the consisting of any one of AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, and AD-70295.1.

38. The composition of any one of claims 31-37, wherein 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.

39. A composition for inhibiting expression of hepatitis D virus (HDV) in a cell, said 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 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 any one of the sense sequences provided in any one of Table 11, 12, 31, and 32, and

wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences provided in any one of Table 11, 12, 31, and 32.

40. The composition of claim 39, wherein said first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-

70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

41. The composition of claim 39, wherein said first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

42. The composition of claim 39, wherein all of the nucleotides of said first and second sense strand and/or all of the nucleotides of said first and second antisense strand comprise a modification.

43. The composition of claim 39, wherein 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.

44. A double stranded RNAi agent comprising the RNAi agents provided in any one of Table 11, 12, 31, and 32.

45. A vector for expression of a double stranded RNAi agent of claim 1 or claim 23.

46. A cell containing the double stranded RNAi agent of laim 1 or claim 20.

47. A pharmaceutical composition comprising the double stranded RNAi agent of any one of claims 1 and 23, or the composition of any one of claims 31 and 39, or the vector of claim 45.

48. The pharmaceutical composition of claim 47, wherein double stranded RNAi agent is administered in an unbuffered solution.

49. The pharmaceutical composition of claim 48, wherein said unbuffered solution is saline or water.

50. The pharmaceutical composition of claim 47, wherein said double stranded RNAi agent is administered with a buffer solution.

51. The pharmaceutical composition of claim 50, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

52. The pharmaceutical composition of claim 51, wherein said buffer solution is phosphate buffered saline (PBS).

53. A method of inhibiting Hepatitis D virus (HDV) gene expression in a cell, the method comprising:

(a) contacting the cell with the double stranded RNAi agent of any one of claims 1 and 23, or the composition of any one of claims 31 and 39, or the vector of claim 45, or the pharmaceutical composition of any one of claims 47-52; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an HDV gene, thereby inhibiting expression of the HDV gene in the cell.

54. The method of claim 53, wherein the HDV is selected from the group consisting of HDV-1, HDV-2, HDV-3, HDV-4, HDV-5, HDV-6, HDV-7, HDV-8,and a combination thereof.

55. The method of claim 53 or 54, further comprising inhibiting HBV gene expression in a cell.

56. The method of claim 55, wherein the cell is contacted with any one of the double-stranded iRNA agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28.

57. A method of inhibiting replication of a Hepatitis D virus (HDV) in a cell, the method comprising:

(a) contacting the cell with the double stranded RNAi agent of any one of claims 1 and 23, or the composition of any one of claims 31 and 39, or the vector of claim 45, or the pharmaceutical composition of any one of claims 47-52; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an HDV gene, thereby inhibiting replication of the HDV in the cell.

58. The method of 57 49, wherein the HDV is selected from the group consisting of HDV-1, HDV-2, HDV-3, HDV-4, HDV-5, HDV-6, HDV-7, HDV-8,and a combination thereof.

59. The method of claim 57 or 58, further comprising inhibiting HBV replication in a cell.

60. The method of claim 57, wherein the cell is contacted with any one of the double-stranded iRNA agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28.

61. The method of claim 54 or 57, wherein said cell is within a subject.

62. The method of claim 61, wherein the subject is a human.

63. The method of claim 54, wherein the HDV 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%.

64. The method of claim 47, wherein replication of HDV 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%.

65. A method of reducing the level of a Hepatitis D virus (HDV) antigen in a subject infected with HDV, comprising administering to the subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1 and 23, or the composition of any one of claims 31 and 39, or the vector of claim 45, or the pharmaceutical composition of any one of claims 47-52, thereby reducing the level of the HBV antigen in the subject.

66. A method of reducing the viral load of Hepatitis D virus (HDV) in a subject infected with HBV, comprising administering to the subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1 and 23, or the composition of any one of claims 31 and 39, or the vector of claim 45, or the pharmaceutical composition of any one of claims 47-52, thereby reducing the viral load of HDV in the subject.

67. The method of claim 65 or 66, wherein the HDV is selected from the group consisting of HDV-1, HDV-2, HDV-3, HDV-4, HDV-5, HDV-6, HDV-7, HDV-8, and a combination thereof.

68. The method of any of claims 65-67 further comprising inhibiting HBV replication in a cell.

69. The method of claim 68, wherein the cell is contacted with any one of the double-stranded iRNA agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28.

70. A method of treating a subject having a Hepatitis D virus (HDV) infection, comprising administering to the subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1 and 23, or the composition of any one of claims 31 and 39, or the vector of claim 45, or the pharmaceutical composition of any one of claims 47-52, thereby treating said subject.

71. A method 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,

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

wherein said sense strand comprises any one of the sense sequences provided in any one of Table 11, 12, 31, and 32, and said antisense strand comprises any one of the antisense sequences provided in any one of Table 11, 12, 31, and 32, and,

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.

72. The method of claim 71, wherein the HDV is selected from the group consisting of HDV-1, HDV-2, HDV-3, HDV-4, HDV-5, HDV-6, HDV-7, HDV-8,and a combination thereof.

73. The method of claim 71 or 72, further comprising inhibiting HBV replication in a cell.

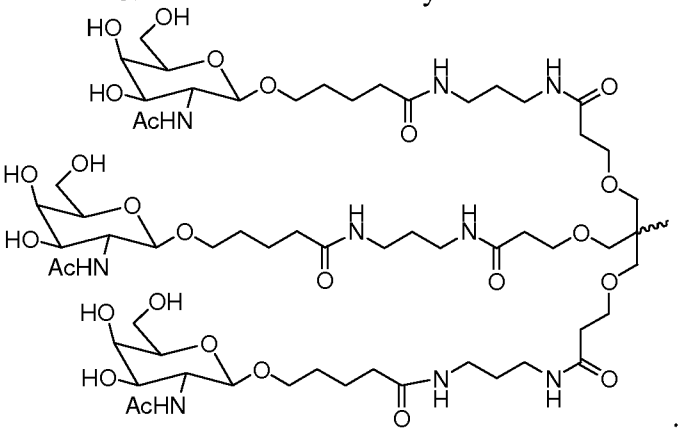
74. The method of claim 73, wherein the cell is contacted with any one of the double-stranded iRNA agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28.

75. The method of any one of claims 71-74, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

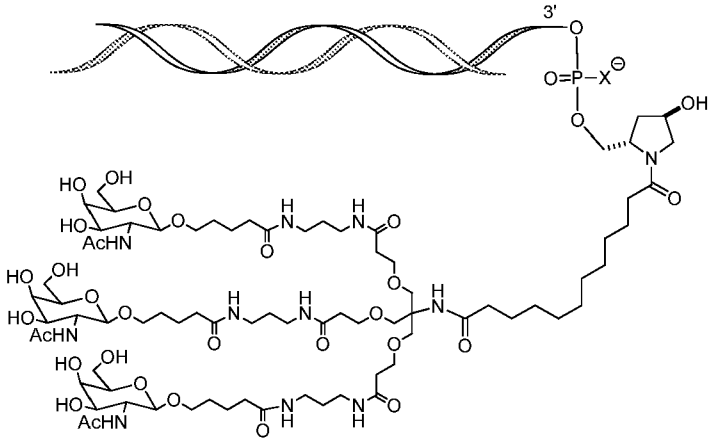
76. The method of any one of claims 71-74, wherein at least one of said modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an 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.

77. Themethod of claim 76, wherein the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

78. The method of any one of claims 71-74, wherein the ligand is



79. The method of claim 78, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

80. A method 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 D virus (HDV) in a cell, said 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 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 any one of the sense sequences provided in any one of Table 11, 12, 31, and 32,

and wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences provided in any one of Table 11, 12, 31, and 32, thereby treating the subject.

81. A method of treating a subject having a Hepatitis D virus (HDV)-associated disorder, comprising administering to the subject a therapeutically effective amount of a composition for inhibiting expression of hepatitis D virus (HDV) in a cell, said 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 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;

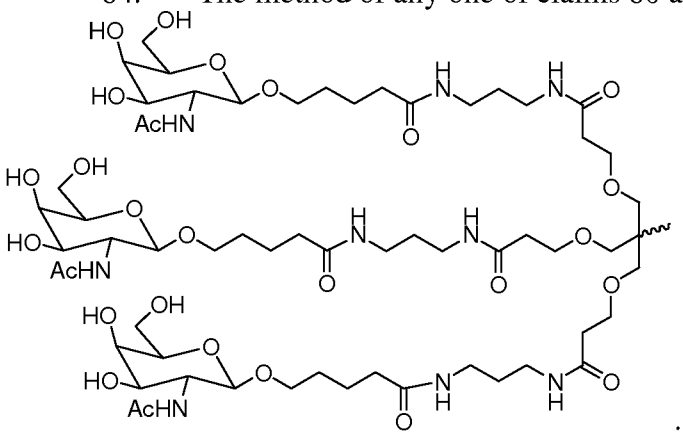
wherein the first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences provided in any one of Table 11, 12, 31, and 32, and

wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences provided in any one of Table 11, 12, 31, and 32, thereby treating the subject.

82. The method of any one of claims 80 and 81, wherein all of the nucleotides of said first and second sense strand and all of the nucleotides of said first and second antisense strand comprise a modification.

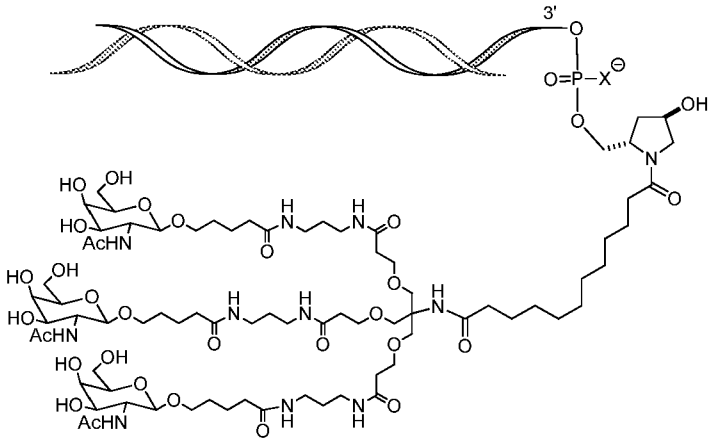
83. The method of any one of claims 80 and 81, wherein at least one of said modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, 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.

84. The method of any one of claims 80 and 81, wherein the ligand is



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85. The method of any one of claims 84, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

86. The method of any one of claims 80 and 81, wherein the subject is a human.

87. The method of claim 80 or 81, wherein the HDV is selected from the group consisting of HDV-1, HDV-2, HDV-3, HDV-4, HDV-5, HDV-6, HDV-7, HDV-8,and a combination thereof.

88. The method of claim 80 or 81, wherein the first and second sense strands each independently comprise a sequence selected from the group consisting of any one of the sense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

89. The method of claim 80 or 81, wherein the first and second antisense strands each independently comprise a sequence selected from the group consisting of any one of the antisense sequences from AD-70260.1, AD-70232.1, AD-70249.1, AD-70244.1, AD-70272.1, AD-70228.1, AD-70255.1, AD-70278.1, AD-70295.1, AD-67200.1, AD-67211.1, AD-67199.1, AD-67202.1, AD-67208.1, AD-67210.1, AD-70259.1, AD-70267.1, AD-

70272.1, AD-70271.1, AD-70268.1, AD-70269.1, AD-70232.1, AD-70256.1, AD-70257.1, or AD-70275.1.

90. The method of claim 80 or 81, further comprising inhibiting HBV replication in a cell.

91. The method of claim 90, wherein the cell is contacted with any one of the double-stranded iRNA agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28.

92. The method of any one of claims 71, 80 or 81, wherein 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.

93. The method of claim 92, wherein the double stranded RNAi agent is administered at a dose of about 10 mg/kg to about 30 mg/kg.

94. The method of claim 92, wherein the double stranded RNAi agent is administered at a dose of about 3 mg/kg.

95. The method of claim 92, wherein the double stranded RNAi agent is administered at a dose of about 10 mg/kg.

96. The method of claim 92, wherein the double stranded RNAi agent is administered at a dose of about 0.5 mg/kg twice per week.

97. The method of any one of claims 71, 80 or 81 wherein the double stranded RNAi agent is administered at a fixed dose of about 50 mg to 200 mg.

98. The method of any one of claims 71, 80 or 81, wherein the double stranded RNAi agent is administered subcutaneously.

99. The method of any one of claims 71, 80 or 81, wherein the double stranded RNAi agent is administered intravenously.

100. The method of any one of claims 71, 80 or 81, wherein said RNAi agent is administered in two or more doses.

101. The method of any one of claims 71, 80 or 81, wherein said 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.

102. The method of any one of claims 71, 80 or 81, wherein said RNAi agent is administered twice per week.

103. The method of any one of claims 71, 80 or 81, wherein said RNAi agent is administered every other week.

104. The method of any one of claims 71, 80 or 81, further comprising administering to the subject an additional therapeutic agent.

105. The method of claim 104, wherein 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 covalently closed circular (ccc) HBV DNA inhibitor, a double-stranded iRNA agent targeting HBV, and a combination of any of the foregoing.

106. The method of any one of claims 71, 80 or 81, further comprising administering to the subject a reverse transcriptase inhibitor.

107. The method of any one of claims 71, 80 or 81, further comprising administering to the subject a reverse transcriptase inhibitor and an immune stimulator.

108. The method of claim 106 or 107, wherein the a 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.

109. The method of claim 107, wherein 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 a Toll-like receptor 7 (TLR7) agonist.

110. The method of claim 105, wherein the double-stranded iRNA agent targeting HBV is any one of the agents provided in any one of Tables 3, 4, 6, 14, 15, 24, 25, 27, and 28.

111. The method of claim 110, wherein the agent is AD-65403, AD-66810, or a combination thereof.