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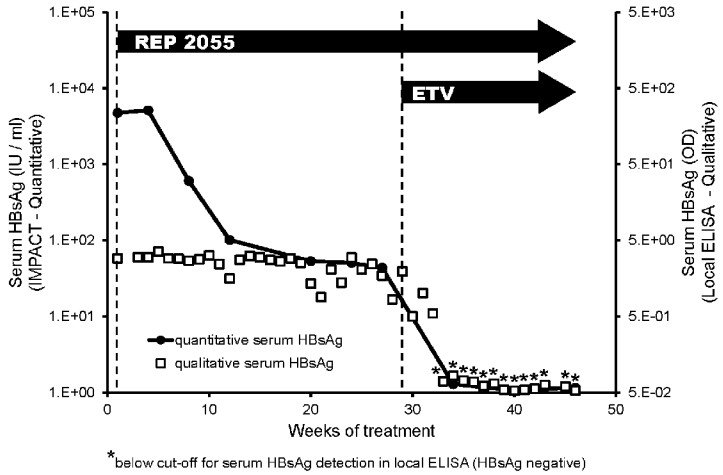
(54) **Title**: METHODS FOR THE TREATMENT OF HEPATITIS B AND HEPATITIS D VIRUS INFECTIONS

Fig. 1



(57) **Abstract**: It is disclosed a method for treating hepatitis B virus infection or hepatitis B virus / hepatitis delta virus co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent that comprises at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent that comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor.

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METHODS FOR THE TREATMENT OF HEPATITIS B AND HEPATITIS D VIRUS INFECTIONS

TECHNICAL FIELD

[0001] The present description relates to methods of treating a subject with hepatitis B virus (HBV) infection or HBV / hepatitis delta virus (HDV) co-infection comprising administering a first pharmaceutically acceptable phosphorothioated nucleic acid polymer formulation and a second pharmaceutically acceptable nucleoside/nucleotide analog formulation that inhibits the HBV polymerase.

BACKGROUND ART

[0002] HBV afflicts 400 million individuals worldwide and causes an estimated 600,000 deaths each year from complications arising from HBV infection. While several antiviral treatments are approved for use, none of these is able to elicit a therapeutically effective immune response capable of providing durable control of infection except in a small fraction of patients undergoing treatment.

[0003] HBV infection results in the production of two different particles: 1) the infectious HBV virus itself (or Dane particle) which includes a viral capsid assembled from the HBV core antigen protein (HBcAg) and is covered by the HBV surface antigen (HBsAg) 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 HBV 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).

[0004] HDV uses HBsAg to form its viral structure (Taylor, 2006, Virology, 344: 71-76) and as such, HDV infection can only occur in subjects with concomitant HBV infection. While the incidence of HDV co-infection in asymptomatic HBV carriers and chronic HBV-related liver disease is low in countries with a low incidence of HBV infection, it is a significant complication in HBV-infected subjects in countries with a high incidence of HBV infection and can increase the rate of progression of liver disease to liver cirrhosis. The unmet medical need in HBV infection is even more

pressing in HBV/HDV co-infected subjects; there is no specific approved agent that directly targets the HDV virus and patient response even to combination therapy with approved agents for HBV treatment is poorer than in patients with HBV monoinfection (Wedemeyer et al., 2014, Oral abstract 4, 49th Annual Meeting of the European Association for the Study of the Liver, April 9-14, London, UK).

[0005] The current approved treatments for HBV include interferon-α or thymosin α1-based immunotherapies and the suppression of viral production by inhibition of the HBV polymerase by nucleoside / nucleotide analogs. HBV polymerase inhibitors are effective in reducing the production of infectious virions but have little to no effect in reducing HBsAg or only very slowly reduce HBsAg with long term treatment in a limited number of patients (Fung et al., 2011, Am. J. Gasteroenterol., 106: 1766-1773; Reijnders et al., 2011, J. Hepatol., 54: 449-454; Charuworn et al., 2014, Poster abstract 401, 48th Annual Meeting of the European Association for the Study of the Liver, April 24-28, Amsterdam, The Netherlands). The primary effect of HBV polymerase inhibitors is to block the transformation of pre-genomic viral mRNA into partially double stranded DNA, which is present in infectious virions. Interferon based immunotherapy can achieve a reduction of infectious virus and removal of HBsAg from the blood but only in a small percentage of treated subjects.

[0006] HBsAg in the blood can sequester anti-HBsAg antibodies and allow infectious viral particles to escape immune detection which is likely one of the reasons why HBV infection remains a chronic condition. In addition HBsAg, HBeAg and HBcAg all have immuno-inhibitory properties as discussed below and the persistence of these viral proteins in the blood of patients following the administration of any of the currently available treatments for HBV as described above likely has a significant impact in preventing patients from achieving immunological control of their HBV infection.

[0007] Although the three primary HBV proteins (HBsAg, HBeAg and HBcAg) all have immuno-inhibitory properties (see below), HBsAg comprises the overwhelming majority of HBV protein in the circulation of HBV infected subjects and is likely the primary mediator of inhibition of the host immune response to HBV infection. While the removal of HBeAg, appearance of anti-HBe or reductions in serum viremia are not correlated with the development of sustained control of HBV infection off

treatment, the removal of serum HBsAg from the blood (and appearance of free anti-HBsAg antibodies) in HBV infection is a well-recognized excellent prognostic indicator of antiviral response on treatment which will lead to control of HBV infection off treatment (although this only occurs in a small fraction of patients receiving immunotherapy or HBV polymerase inhibitors). Thus, while reduction of all three major HBV proteins (HBsAg, HBeAg and HBcAg) may result in the optimal removal of inhibitory effect, the removal of HBsAg is essential and its removal alone is likely sufficient to remove the bulk of the inhibition of immune function in subjects with HBV infection.

[0008] Another critical feature of chronic HBV infection is the establishment of a stable reservoir of HBV genetic information in the nucleus of infected cells called covalently closed circular DNA (cccDNA). cccDNA exists in multiple copies within the nucleus as an extrachromosomal episome which functions as the transcriptional template for the production of mRNA encoding all viral proteins and immature genomes (pre-genomic mRNA) for the production of new virions. After encapsidation in the cytoplasm, the immature pre-genomic mRNA is converted into a mature, partially double stranded DNA genome by the HBV polymerase (which is co-encapsidated with the pregenomic mRNA), thereby rendering the mature HBV genome competent to establish or replenish a cccDNA reservoir in naïve or previously infected cells. The end of the infectious process consists of the delivery of this partially double stranded genomic HBV template into the nucleus and its conversion to cccDNA.

[0009] cccDNA can be replenished in the nucleus of infected cells via nuclear import of HBV capsids containing mature HBV genomes which replenish the cccDNA copy number. This nuclear cccDNA replenishment is accomplished by two mechanisms: direct nuclear import of assembled capsids from the cytoplasm or re-infection of previously infected hepatocytes with subsequent shuttling of the internalized capsids into the nucleus (Rabe et al., 2003, Proc. Natl. Acad. Sci. USA, 100: 9849-9854). The transcriptional inhibition or elimination of this genomic HBV reservoir in the nucleus is critical to the establishment of long term control of HBV infection following treatment.

[0010] Long term treatment with nucleoside/nucleotide HBV polymerase inhibitors can reduce cccDNA copy number within the nucleus, consistent with the ability of HBV polymerase inhibitors to block replenishment of cccDNA by nuclear import of capsids containing mature HBV genomes. However, while the cccDNA copy number per hepatocyte is reduced, it still remains transcriptionally active thus HBsAg levels remain largely unaffected (Werle-Lapostolle et al., 2004, Gastroenterol., 126: 1750-1758; Wong et al., 2013, Clin. Gastroenterol. Hepatol., 11: 1004-1010; Wong et al., 2014, Poster abstract 1074, 49th Annual Meeting of the European Association for the Study of the Liver, April 9-14, London, UK). cccDNA can be transcriptionally inactivated by immune-mediated processes (Belloni et al., 2012, J. Clin. Inv., 122: 529-537) but the ability of the immune response to provoke cytokine responses required for cccDNA inactivation is likely blocked by persistently circulating HBsAg as described in U.S. 2014/0065102 (which is incorporated herein by reference in its entirety) and is consistent with the ineffectiveness of immunotherapies in treating HBV infection.

[0011] As such, there exists a clear unmet medical need for a treatment regimen which can elicit a durable immunological control of HBV infection in a large proportion of patients receiving this treatment.

SUMMARY

[0012] In accordance with the present description there is now provided a composition comprising a first pharmaceutically acceptable agent which comprises at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside/nucleotide analog HBV polymerase inhibitor for treating HBV infection or HBV / HDV co-infection in a subject.

[0013] It is also provided a composition comprising a first pharmaceutically acceptable agent which comprises a chelate complex of at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor for treating HBV infection or HBV / HDV co-infection in a subject.

[0014] It is further provided the use of a first pharmaceutically acceptable agent which comprises at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside/nucleotide analog HBV polymerase inhibitor for treating HBV infection or HBV/HDV co-infection in a subject.

[0015] It is additionally provided the use of a first pharmaceutically acceptable agent which comprises at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor in the manufacture of a medicament for treating HBV infection or HBV / HDV co-infection in a subject.

[0016] It is further provided the use of a first pharmaceutically acceptable agent which comprises a chelate complex of at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor for treating HBV infection or HBV / HDV co-infection in a subject.

[0017] It is additionally provided the use of a first pharmaceutically acceptable agent which comprises a chelate complex of at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor in the manufacture of a medicament for treating HBV infection or HBV / HDV co-infection in a subject.

[0018] In another embodiment, it is provided a composition comprising a first pharmaceutically acceptable agent which comprises a chelate complex of one or more nucleic acid polymers selected from the following:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO 13;
SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;

and a second pharmaceutically acceptable agent which comprises one or more of the following:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;
entricitabine;
clevudine;
besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;
valtorcitabine; and
any nucleoside / nucleotide analog which inhibits the HBV polymerase

for the treatment of HBV infection or HBV / HDV co-infection.

[0019] In an embodiment, it is provided the use of a first pharmaceutically acceptable agent which comprises a chelate complex of one or more nucleic acid polymers selected from the following:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO 13;

SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;

and a second pharmaceutically acceptable agent which comprises one or more of the following:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;
entricitabine;
clevudine;
besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;
valtorcitabine; and
any nucleoside / nucleotide analog which inhibits the HBV polymerase,

for the treatment of HBV infection or HBV / HDV co-infection.

[0020] In another embodiment, it is provided the use of a first pharmaceutically acceptable agent which comprises a chelate complex of one or more nucleic acid polymers selected from the following:

SEQ ID NO: 2;

SEQ ID NO: 10;

SEQ ID NO 13;

SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;

and a second pharmaceutically acceptable agent which comprises one or more of the following:

lamivudine;

adefovir dipivoxil;

entecavir;

telbivudine;

tenofovir disoproxil fumarate;

entricitabine;

clevudine;

besifovir;

tenofovir alafenamide fumarate;

AGX-1009;

elvucitabine;

lagociclovir valactate;

pradefovir mesylate;

valtorcitabine; and

any nucleoside / nucleotide analog which inhibits the HBV polymerase,

in the manufacture of a medicament for the treatment of HBV infection or HBV / HDV co-infection.

[0021] In another embodiment, the nucleic acid polymer comprises a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC.

[0022] In another embodiment, the nucleic acid polymer comprises a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising the repeats of the sequence CA.

[0023] In another embodiment, the nucleic acid polymer comprises a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising the repeats of the sequence TG.

[0024] In another embodiment, the nucleic acid polymer comprises a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising the repeats of the sequence GT.

[0025] In another embodiment, the phosphorothioated nucleic acid polymer further comprises at least one 2' ribose modification.

[0026] In another embodiment, the phosphorothioated nucleic acid polymer further comprises all riboses having a 2' modification.

[0027] In another embodiment, the phosphorothioated nucleic acid polymer further comprises at least one 2' O methyl ribose modification.

[0028] In another embodiment, the phosphorothioated nucleic acid polymer further comprises all riboses having the 2' O methyl modification.

[0029] In another embodiment, the phosphorothioated nucleic acid polymer further comprises at least one 5'methylcytosine.

[0030] In another embodiment, the phosphorothioated nucleic acid polymer further comprises all cytosines present as 5'methylcytosine.

[0031] In another embodiment, the phosphorothioated nucleic acid polymer further comprises at least one 2' ribose modification and at least one 5' methylcytosine.

[0032] In another embodiment, the phosphorothioated nucleic acid polymer further comprises all riboses having the 2' O methyl modification and all cytosines present as 5'methylcytosine.

[0033] In another embodiment, the nucleic acid polymer is selected from the group consisting of SEQ ID NOs: 1-20.

[0034] In another embodiment, the nucleic acid polymer is prepared as an oligonucleotide chelate complex comprising an oligonucleotide selected from the group consisting of SEQ ID NOs: 1-20.

[0035] In another embodiment, the nucleic acid polymer is an oligonucleotide consisting of SEQ ID NO: 2.

[0036] In another embodiment, the nucleic acid polymer is prepared as an oligonucleotide chelate complex comprising SEQ ID NO: 2.

[0037] In another embodiment, the nucleic acid polymer is an oligonucleotide consisting of SEQ ID NO: 10.

[0038] In another embodiment, the nucleic acid polymer is prepared as an oligonucleotide chelate complex comprising SEQ ID NO: 10.

[0039] In another embodiment, the nucleic acid polymer is an oligonucleotide consisting of SEQ ID NO: 13.

[0040] In another embodiment, the nucleic acid polymer is prepared as an oligonucleotide chelate complex comprising SEQ ID NO: 13.

[0041] In an embodiment, the chelate complex is a calcium chelate complex.

[0042] In another embodiment, the chelate complex is a magnesium chelate complex.

[0043] In an additional embodiment, the chelate complex is a calcium / magnesium chelate complex.

[0044] In a further embodiment, the first and second pharmaceutically acceptable agents are formulated within the same pharmaceutical composition.

[0045] In a further embodiment, the first and second agents are formulated within separate pharmaceutical compositions.

[0046] In a further embodiment, the first and second agents are formulated for a simultaneous administration.

[0047] In a further embodiment, the first and second agents are formulated for an administration by a different route.

[0048] In a further embodiment, the first and second agents are formulated for an administration using one or more of the following: oral ingestion, aerosol inhalation, subcutaneous injection, intravenous injection and intravenous infusion.

[0049] In a further embodiment, the nucleic acid polymer is at least one of:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO: 13;
SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT.

[0050] In a further embodiment, the following nucleic acid polymers can be further formulated as an oligonucleotide chelate complex:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO 13;
SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;

a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG; and
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT.

[0051] In another embodiment, the nucleoside/nucleotide analog HBV polymerase inhibitor comprises one or more of the following:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;
entricitabine;
clevudine;
besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;
valtorcitabine; and
any nucleoside / nucleotide analog which inhibits the HBV polymerase.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] Fig. 1 illustrates the synergistic effect of combination therapy with the NAP REP 2055 (SEQ ID NO: 2) and entecavir (ETV) on the reduction of serum levels of HBsAg.

[0053] Fig. 2A illustrates the antiviral activity of NAPs administered to infected Pekin ducks as calcium chelate complexes with DHBV measured by monitoring serum DHBsAg at the end of treatment by ELISA

[0054] Fig. 2B illustrates the antiviral activity of NAPs administered to infected Pekin ducks as calcium chelate complexes with DHBV assessed by monitoring liver DHBV DNA at the end of treatment by quantitative PCR.

[0055] Fig. 3A illustrates the levels of DHBV DNA in the serum of ducks treated 28 days with normal saline at: A) pre-treatment, B) when treatment is half completed, C) end of treatment, D) one month after treatment and E) two months after treatment. The lower limit of quantification (LLOQ) is 3.1×10^4 VGE / ml. Values < LLOQ were set at 3×10^3 VGE / ml. VGE = viral genome equivalents.

[0056] Fig. 3B illustrates the levels of DHBV DNA in the serum of ducks treated for 28 days with tenofovir disoproxil fumarate (TDF) at: A) pre-treatment, B) when treatment is half completed, C) end of treatment, D) one month after treatment and E) two months after treatment. The lower limit of quantification (LLOQ) is 3.1×10^4 VGE / ml. Values < LLOQ were set at 3×10^3 VGE / ml. VGE = viral genome equivalents.

[0057] Fig. 3C illustrates the levels of DHBV DNA in the serum of ducks treated for 28 days with REP 2139-Ca at: A) pre-treatment, B) when treatment is half completed, C) end of treatment, D) one month after treatment and E) two months after treatment. The lower limit of quantification (LLOQ) is 3.1×10^4 VGE / ml. Values < LLOQ were set at 3×10^3 VGE / ml. VGE = viral genome equivalents.

[0058] Fig. 3D illustrates the levels of DHBV DNA in the serum of ducks treated for 28 days with REP 2139-Ca and TDF at: A) pre-treatment, B) when treatment is half completed, C) end of treatment, D) one month after treatment and E) two months after treatment. The lower limit of quantification (LLOQ) is 3.1×10^4 VGE / ml. Values < LLOQ were set at 3×10^3 VGE / ml. VGE = viral genome equivalents.

[0059] Fig. 3E illustrates the levels of DHBV DNA in the serum of ducks treated for 28 days with REP 2139-Ca, TDF and entecavir (ETV) at: A) pre-treatment, B) when treatment is half completed, C) end of treatment, D) one month after treatment

and E) two months after treatment. The lower limit of quantification (LLOQ) is 3.1 x 10^4 VGE / ml. Values < LLOQ were set at 3 x 10^3 VGE / ml. VGE = viral genome equivalents.

DETAILED DESCRIPTION

[0060] It is provided herein a combination therapy against HBV infection which consists of administering a first pharmaceutically acceptable agent capable of removing HBsAg from the blood and a second pharmaceutically acceptable agent which inhibits the HBV polymerase. Such a combination treatment allows recovery of host immune function (by removal of serum HBsAg) which in turn leads to the immune-mediated transcriptional inactivation of cccDNA and or reduction of cccDNA copy number in infected hepatocytes while simultaneously blocking replenishment of cccDNA via nuclear import of capsids containing mature HBV genomes or the production of infectious virus (by inhibiting the HBV polymerase). The combined synergistic effects of these two agents can accelerate the antiviral response to therapy and or the elimination of cccDNA from infected cells, thus shortening the time of therapy required to obtain sustained suppression of infection off treatment. Importantly, these effects can be achieved in the absence of immunotherapy. This combination treatment will be effective in HBV monoinfection and HBV/HDV co-infection.

[0061] HBsAg plays a key role in HBV infection and HBV/HDV co-infection. Aside from its role as an essential structural component for virion formation, HBsAg is also released in large amounts into the blood of infected subjects in the form of subviral particles (SVPs), which lack the viral capsid and genome and which appear to function primarily to deliver HBsAg into the blood. SVPs are secreted from infected cells in 1,000-10,000 fold excess over virus secretion which allows SVPs to effectively sequester HBsAg antibodies (anti-HBs) so that HBV or HDV virus in the blood can escape recognition by adaptive immunity. Several studies have also suggested that HBsAg may also function to directly block activation of adaptive and innate immune responses to HBV infection (Cheng et al., 2005, Journal of hepatology, 43:4 65-471; Op den Brouw et al., 2009, Immunology, 126: 280-289; Vanlandschoot et al., 2002, The Journal of general virology, 83: 1281-1289; Wu et al., 2009, Hepatology, 49: 1132-1140; Xu et al., 2009, Molecular immunology, 46:

2640-2646). The presence of this functionality in human HBV infection and its impact on the activity of immunotherapeutic agents and the additional applicability of these antiviral effects in HBV/HDV co-infection has been previously described in US 2014/0065102 A1, which is incorporated herein by reference in its entirety. Although HBeAg and HBcAg have also been shown to have immuno-inhibitory properties (Kanda et al., 2012, J. Inf. Dis., 206: 415-420; Lang et al., 2011, J. Hepatol., 55: 762-769; Gruffaz et al., 2013, J. Hepatol., 58 (supp1), p s155, Abstract 378), these are likely of minimal impact given the very small proportion of HBeAg and HBcAg in relation to HBsAg in the blood.

[0062] Nucleosid /nucleotide analog inhibitors of HBV polymerase (NRTI's) are a well-known class of antiviral agents whose activity against HBV infection occurs by the same mechanism of action: this class of compounds act as immediate or delayed chain terminators by competing with natural nucleotide substrates during elongation of the DNA chain (Menendez-Arias et al., 2015 Curr. Op. Virol. 8: 1-9). This class of compounds can retain the fundamental core nucleotide / nucleoside core structure consisting of a nitrogenous base and sugar or can be acyclic nucleotides or can lack the sugar or pseudo sugar ring or can have a phosphonate group replacing the α-phosphate and can have many other additional modifications present as described in Michailidis et al., 2012 Int. J. Biochem. Cell. Biol. 44: 1060-1071 and De Clercq et al., 2010 Viruses 2: 1279-1305.

[0063] Duck HBV virus (DHBV)-infected ducks are an accepted model of HBV infection and have been used in the evaluation of several HBV NRTIs currently used to treat human patients (Schultz et al., 2004, Adv Virus Res, 63:1-70; Foster et al., 2005, J Virol, 79:5819-5832; Nicoll et al,. 1998, Antimicrob Agents Chemother., 42:3130-3135). Nucleic acid polymers (NAPs) that are phosphorothioated have been shown to have antiviral activity in DHBV infected ducks (Noordeen et al., 2013 Anti-Microb. Agents Chemother. 57: 5291- 5298 and 5299 – 5306) which is not derived from any direct immunostimulatory mechanisms. Moreover, therapeutic intervention with the NAP REP 2055 (SEQ ID NO:2) in previously established DHBV infection *in vivo*, REP 2055 led to the clearance of serum duck HBsAg (DHBsAg) which was accompanied by transcriptional inactivation of cccDNA and reduction in cccDNA copy number (Noordeen et al., 2009, Abstract 88 HEPDART meeting Dec 6 – 9, HI,

USA). This inactivation and elimination of cccDNA is caused by removal of DHBsAg-mediated repression of the host immune function, which can then inactivate and clear cccDNA from infected cells by recognized, immune-mediated mechanisms (Levrero et al., 2009, J. Hepatol., 51: 581-592; Belloni et al., 2012, J. Clin. Inv., 122: 529-537).

[0064] NAPs effectively remove HBsAg from the blood of human patients are as described in US 2014/0065102. In an accepted preclinical model of HBV infection (duck HBV infected Pekin ducks), NAP treatment resulted in the elimination of serum duck HBsAg (DHBsAg) and the restoration of immune function in the absence of serum DHBsAg was able to both transcriptionally inactivate and eliminate cccDNA from infected hepatocytes (Noordeen et al., 2009, Abstract 88, HEPDART meeting Dec 6-10, HI, USA). Thus, removal of HBsAg from the serum of HBV infected patients is expected to have the same effect on cccDNA inactivation in infected human hepatocytes *in situ*.

[0065] Therefore, it is described herein an effective means for more rapidly establishing control of serum viremia or for establishing durable control of cccDNA activity and or its elimination from HBV infected hepatocytes which consists of a novel combined approach whereby HBsAg is reduced or eliminated from the blood by the use of a pharmaceutically acceptable phosphorothioated NAP formulation and replenishment of cccDNA and production of infectious virus is blocked by a second pharmaceutically acceptable nucleotide/nucleoside analog formulation inhibiting the HBV polymerase. This combined approach has the following novel and important benefits:

1) it combines the ability of an improved host immune function (caused by removal of serum HBsAg) to transcriptionally inactivate and or reduce cccDNA copy number within the cell with the blockade of cccDNA replenishment (by preventing capsids containing mature genomes from entering the nucleus (by inhibition of HBV polymerase activity) or production of infectious virions (my preventing the transformation of pregenomic RNA into partially double stranded DNA within the HBV capsid;

2) it has a synergistic effect on reducing the duration of treatment required to remove, eliminate or establish transcriptional suppression of cccDNA or control of serum viremia from infected hepatocytes in the liver because of the overlapping effects of said two pharmaceutically acceptable agents; and

3) it does not require the use of an immunotherapy (as taught to be specifically required in U.S. 2014/0065102) to achieve sustained control of HBV infection after treatment which would be an important therapeutic improvement, given the poor tolerability of immunotherapy in many patients.

[0066] The improved antiviral effects with methods described above will have the same therapeutic benefit in patients with HBV mono-infection and HBV/HDV co-infection as HDV infection cannot exist in the absence of HBV infection as described above.

[0067] Therefore, in the absence of any current treatment regimen which can either eliminate or establish durable control of cccDNA activity without the use of immunotherapy in a large proportion of patients, it is provided herein for the first time an effective combination treatment against HBV infection and HBV/HDV co-infection which simultaneously reduces or clears HBsAg from the blood and which blocks cccDNA replenishment in the nucleus of HBV infected cells. These effects can be achieved by the use of a pharmaceutically acceptable phosphorothioated NAP formulation used in combination with a pharmaceutically acceptable nucleoside / nucleotide analog HBV polymerase inhibitor.

[0068] This novel combination approach is effective in the absence of immunotherapy, which has the important advantages of improving the tolerability of treatment and reducing the incidence of hematological and other side effects known to occur with immunotherapy.

[0069] The term oligonucleotide (ON) refers to an oligomer or polymer of ribonucleic acid (RNA) and/or deoxyribonucleic acid (DNA). This term includes ONs composed of modified nucleobases (including 5'methylcytosine and 4'thiouracil), sugars and covalent internucleoside (backbone) linkages as well as ONs having non-naturally-occurring portions which function similarly. Such modified or substituted ONs may be preferable over native forms because of desirable

properties such as, for example, reduced immunoreactivity, enhanced cellular uptake, enhanced affinity for the nucleic acid target (in the context of antisense ONs, siRNAs and shRNAs) and / or increased stability to nuclease-mediated degradation. ONs can also be double stranded. ONs also include single stranded molecules such as antisense oligonucleotides, Speigelmers and aptamers and miRNAs, as well as double stranded molecules such as small interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs).

[0070] ONs can include various modifications, e.g., stabilizing modifications, and thus can include at least one modification in the phosphodiester linkage and/or on the sugar, and/or on the base. For example, the ON can include, without restriction, one or more modifications, or be fully modified so as to contain all linkages or sugars or bases with the recited modifications. Modified linkages can include phosphorothioate linkages and phosphorodithioate linkages. While modified linkages are useful, the ONs can include phosphodiester linkages. Additional useful modifications include, without restriction, modifications at the 2'-position of the sugar including 2'-O-alkyl modifications such as 2'-O-methyl modifications, 2' O-methoxyethyl (2' MOE), 2'-amino modifications, 2'-halo modifications such as 2'-fluoro; acyclic nucleotide analogs. Other 2' modifications are also known in the art and can be used such as locked nucleic acids. In particular, the ON has modified linkages throughout or has every linkage modified, e.g., phosphorothioate; has a 3'- and/or 5'-cap; includes a terminal 3'-5' linkage; the ON is or includes a concatemer consisting of two or more ON sequences joined by a linker(s). Base modifications can include 5'methylation of the cytosine base (5' methylcytosine or in the context of a nucleotide, 5' methylcytidine) and/or 4'thioation of the uracil base (4'thiouracil or in the context of a nucleotide, 4'thiouridine). Different chemically compatible modified linkages can be combined where the synthesis conditions are chemically compatible such as having an oligonucleotide with phosphorothioate linkages, a 2' ribose modification (such as 2'O-methylation) and a modified base (such as 5'methylcytosine). The ON can further be completely modified with all of these different modifications (e.g. each linkage phosphorothioated, each ribose 2' modified and each base being modified).

[0071] As encompassed herein, the term “nucleic acid polymer” or NAP is any single stranded ON which contains no sequence specific functionality, either to hybridize with a nucleic acid target or adopt a sequence specific secondary structure which results in binding to a specific protein. The biochemical activity of NAPs are not dependent on Toll-like receptor recognition of ONs, hybridization with a target nucleic acid or aptameric interaction requiring a specific secondary/tertiary ON structure derived from a specific order of nucleotides present. NAPs can include base and or linkage and or sugar modifications as described above. NAPs require phosphorothioation to have antiviral activity. Exemplary antiviral NAP compounds are listed in Table 1:

Table 1

Examples of antiviral NAPs which can be useful in the current disclosure.

Nucleic acid type	Sequence (5' – 3')	Modifications
DNA	$(dAdC)_{20}$ (SEQ ID NO: 2)	All linkages PS
DNA	$(dCdA)_{20}$ (SEQ ID NO: 1)	All linkages PS
DNA	$(dA\text{-}5'MedC)_{20}$ (SEQ ID NO: 3)	All linkages PS
DNA	$(5'MedC\text{-}dA)_{20}$ (SEQ ID NO: 4)	All linkages PS
RNA	$(AC)_{20}$ (SEQ ID NO: 5)	All linkages PS All riboses with 2'OMe modification
RNA	$(CA)_{20}$ (SEQ ID NO: 6)	All linkages PS All riboses with 2'OMe modification
DNA	$(dTdG)_{20}$ (SEQ ID NO: 7)	All linkages PS
DNA	$(dGdT)_{20}$ (SEQ ID NO: 8)	All linkages PS
RNA	$(5'MeC\text{-}A)_{20}$ (SEQ ID NO: 9)	All linkages PS All riboses with 2'OMe modification
RNA	$(A\text{- }5'MeC)_{20}$ (SEQ ID NO: 10)	All linkages PS All riboses with 2'OMe modification
RNA / DNA	$(A\text{-}5'MedC)_{20}$ (SEQ ID NO: 11)	All linkages PS All riboses on riboadenosine are 2'OMe modified
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 12)	All linkages PS All riboses with 2'OMe modification except riboadenosines at positions 13 and 27 (which are 2'H)
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 13)	All linkages PS All riboses with 2'OMe modification except riboadenosines at positions 11, 21 and 31 (which are 2'H)
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 14)	All linkages PS All 5'MeC riboses are 2'OMe modified
RNA / DNA	$(dA\text{-}5'MeC)_{20}$ (SEQ ID NO: 15)	All linkages PS All 5'MeC riboses are 2'OMe modified
RNA / DNA	$(5'MedC\text{-}A)_{20}$ (SEQ ID NO: 16)	All linkages PS All A riboses are 2'OMe modified
RNA	$(5'MeC\text{-}A)_{20}$ (SEQ ID NO: 17)	All linkages PS All riboses with 2'OMe modification except riboadenosines at positions 14 and 28 (which are 2'H)
RNA	$(5'MeC\text{-}A)_{20}$ (SEQ ID NO: 18)	All linkages PS All riboses with 2'OMe modification except riboadenosines at positions 10, 20 and 30 (which are 2'H)
RNA	$(5'MeC\text{-}A)_{20}$ (SEQ ID NO: 19)	All linkages PS All 5'MeC riboses are 2'OMe modified
RNA / DNA	$(5'MeC\text{-}dA)_{20}$ (SEQ ID NO: 20)	All linkages PS All 5'MeC riboses are 2'OMe modified

dA = deoxyadenosine, A = adenosine, dC = deoxycytidine, C = cytidine, dT = deoxythymidine, dG = deoxyguanosine, PS = phosphorothioate, 2'OMe = 2' O methyl, 5'MeC = 5'methylcytosine-modified cytidine, 5'MedC = 5'methylcytosine-modified deoxycytidine

[0072] In the present disclosure, the term "ON chelate complex" refers to two or more ONs linked intermolecularly by a divalent or multivalent metal cation and can

occur with single or double stranded ONs. ON chelate complexes neutralize the inherent chelation properties of ONs which can contribute to administration-related side effects with these compounds. The administration of ON chelate complexes is a method of administering an ON to a subject where administration-related side effects associated with un-chelated ONs (which are ONs administered as sodium salts as is commonly used in the art) are mitigated as described in U.S. 8,513,211 and 8,716,259, which are incorporated herein by reference in their entirety. These side effects may include shivering, fever and chills with intravenous infusion or induration, inflammation and pain at the injection site with subcutaneous administration. The administration of ON chelate complexes does not interfere with the biochemical activity of ONs when used normally as sodium salts. Thus any NAP described herein can be optionally prepared as an ON chelate complex without affecting its biochemical activity.

[0073] ON chelate complexes may contain diverse multivalent metal cations including calcium, magnesium, cobalt, iron, manganese, barium, nickel, copper, zinc, cadmium, mercury and lead. It is further demonstrated that chelation of these multivalent metal cations results in the formation of ON chelate complexes comprised of two or more ONs linked via metal cations and occur with ONs greater than 6 nucleotides in length, and in the presence of ONs with either phosphodiester or phosphorothioate linkages. ONs can optionally have each linkage phosphorothioated. Chelation also occurs with ONs containing 2' modifications (such as 2' O methyl) at the ribose or containing modified bases such as 5'methylcytosine or 4-thiouracil. These 2' modifications can be present on one or more or all riboses and modified bases can be present on one or more bases or be universally present on each base (i.e. all cytosines are present as 5'methylcytosine). Additionally, the ON chelate complexes can comprise ONs which contain multiple modifications such as each linkage phosphorothioated, each ribose 2' modified and each base modified. ON modifications compatible with ON chelate complex formation are further defined above. Moreover, the chelation of the metal cations is not dependent on the sequence of nucleotides present but instead relies on the physiochemical features common to all ONs.

[0074] While the formation of ON chelate complexes can be achieved with any divalent metal cation, ON chelate complexes intended for use as medications should preferably contain only calcium and or magnesium but could also contain iron, manganese, copper or zinc in trace amounts and should not include cobalt, barium, nickel, cadmium, mercury, lead or any other divalent metal not listed herein.

[0075] As described in U.S. 2014/0065192, the removal of HBsAg from the blood of infected patients by phosphorothioated NAPs results in a partial restoration of the immune response which in turn removes HBV e-antigen (HBeAg) from the blood and results in substantial reduction of levels of virus in the blood during treatment but these antiviral effects are not maintained in most patients after treatment is stopped. While this partial restoration of the immune response (in the absence of HBsAg and other viral antigens) can lead to the establishment of durable immunological control of HBV infection after treatment is stopped in a small proportion of patients, it is desirable to establish durable immunological control of infection in an even larger proportion of patients. An improvement in the proportion of patients that achieve durable immunological control after treatment can be achieved by using phosphorothioated NAPs in combination with other antiviral agents to improve the speed and potency of antiviral response to treatment. It would be desirable to avoid the use of immunotherapies as such as interferon-based treatment or other immunotherapies as these are typically associated with side effects which make therapy more difficult to tolerate for patients.

[0076] The term “removal of HBsAg from the blood” as used herein means any statistically significant reduction of the concentration HBsAg in the blood relative to pre-treatment HBsAg blood concentrations as measured by the Abbott Architect™ quantitative HBsAg assay or other clinically accepted quantitative measure of serum HBsAg.

[0077] Exemplary effective dosing regimens for phosphorothioated NAPs follow those typically used for other phosphorothioated ONs (such as antisense oligonucleotides) as described in U.S. 2014/0065102; the routine use of weekly parenteral administration of 100-500 mg of compound is well established in the art to result in the achievement of therapeutically active levels of these compounds in the liver as described for the NAPs in example I below and for a phosphorothioated

antisense ON causing the degradation of a liver specific mRNA (for apolipoprotein B100) as described in Akdim et al. (2010, Journal of the American College of Cardiology, 55: 1611-1618).

[0078] Therefore, according to the disclosures presented herein, it is useful to treat a subject with HBV infection or HBV/HDV co-infection with a pharmaceutically acceptable phosphorothioated NAP formulation combined with a pharmaceutically acceptable nucleoside/nucleotide HBV polymerase inhibitor.

[0079] It is also useful to administer both pharmaceutically acceptable agents in the same pharmaceutical composition or to administer both pharmaceutically acceptable agents in separate pharmaceutical compositions at the same time or at different times.

[0080] It is useful to administer the pharmaceutically acceptable agents by the same or different routes of administration.

[0081] In order to provide the best possible antiviral response in a subject, it may be necessary to use more than one HBV polymerase inhibitors to maximally block the HBV polymerase and thus have maximal greater effect on blocking the replenishment of cccDNA. Thus one or more HBV polymerase inhibitors can be selected from the following nucleoside analogs:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;
entricitabine;
clevudine;
besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;

valtorcitabine; and

any nucleoside / nucleotide analog which inhibits the HBV polymerase

[0082] The compositions described herein may be administered by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; buccally; parenterally, such as by subcutaneous, intravenous, injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); by inhalation; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories or enema; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The present compositions may, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release may be achieved by the use of suitable pharmaceutical compositions, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps. Thus, the above compositions may be adapted for administration by any one of the following routes: oral ingestion, inhalation, subcutaneous injection, , intravenous injection or infusion, or topically.

[0083] The present disclosure will be more readily understood by referring to the following example.

EXAMPLE I

Effect of combination NAP / ETV therapy on serum HBsAg

[0084] A pharmaceutically acceptable formulation of the NAP REP 2055 (SEQ ID NO: 2) was administered to a patient with chronic HBV infection by once weekly IV infusion of 400mg. The serum HBsAg response in this patient was monitored real-time each week using a qualified, on-site qualitative ELISA. This ELISA method is very sensitive to low levels of HBsAg but cannot accurately quantify any significant HBsAg concentration in the blood. Although no detectable reduction in serum HBsAg was observed using this HBsAg assay during REP 2055 monotherapy (Fig. 1, squares), this patient experienced a very mild (~ 1 log) drop in serum virema (serum HBV DNA), indicating that some sort of antiviral response had occurred. Therefore, after 29 weeks of REP 2055 monotherapy, this patient received HBV polymerase

inhibition therapy in addition to the existing REP 2055 therapy which consisted of 0.5mg of entecavir taken orally every day.

[0085] Immediate reductions in serum HBsAg were detected by the qualitative assay within two weeks of starting combination REP 2055 / ETV therapy and serum HBsAg became undetectable in the qualitative ELISA within 4 weeks after starting combination treatment (Fig. 1, squares). This synergistic control of serum HBsAg with combined REP 2055 / ETV treatment was maintained over many weeks of treatment.

[0086] To confirm the synergistic activity of combination REP 2055 / ETV therapy on suppression of HBsAg, serum samples from this patient were re-analyzed using the IMPACT platform to accurately quantitate serum HBsAg levels as described in de Neit et al. (2014, Antiviral Ther., 19: 259-267). This quantitative analysis revealed an initial ~ 2 log reduction of serum HBsAg occurred with REP 2055 monotherapy (Fig. 1 circles), which was not detectable by the qualitative ELISA and which was likely the cause of the observed ~ 1 log drop in viremia on REP 2055 monotherapy described above. Importantly, serum HBsAg reduction in this patient reached a plateau where significant serum HBsAg was stably present starting from 10 weeks of REP 2055 treatment until the start of combination REP 2055/ETV therapy at 29 weeks of treatment. With the onset of combination REP 2055/ETV treatment, the quantitative analysis of serum HBsAg demonstrated an almost identical and rapid reduction in serum HBsAg as observed with the onsite qualitative test, with these additional reductions exceeding 1.5 logs which were also accomplished within 4 weeks after the start of combination REP 2055/ETV treatment.

[0087] The persistence of low levels of serum HBsAg in the presence of REP 2055 monotherapy is an indication that cccDNA was still present in the liver of this patient which was transcriptionally active. The very rapid additional clearance of serum HBsAg with the addition of ETV to existing REP 2055 therapy is an indication that a synergistic effect on cccDNA transcriptional control and or elimination had occurred. Importantly, the development of this additional control of cccDNA occurred much more rapidly than observed with HBV polymerase inhibitors used in monotherapy, requiring only 4 weeks to achieve. Therefore, these observations are a demonstration of the novel, synergistic antiviral effect of serum HBsAg reduction (in

this case achieved using the NAP REP 2055) when combined with an HBV polymerase inhibitor (in this case entecavir).

EXAMPLE II

Antiviral effects of various NAPs in DHBV infected Pekin Ducks

[0088] Various NAPs comprising different nucleic acid modifications were tested in DHBV infected Pekin ducks to establish their antiviral activity. These NAPs are REP 2055 (SEQ ID NO: 2), REP 2139 (SEQ ID NO: 10), REP 2163 (SEQ ID NO: 11) and REP 2165 (SEQ ID NO: 13). Table 2 provides a chemical description of these NAPs.

Table 2

Description of NAPs used in Example II

NAP	Sequence	Oligonucleotide modifications present
REP 2055 (SEQ ID NO: 2)	$(dAdC)_{20}$	Each linkage is phosphorothioated
REP 2139 (SEQ ID NO: 10)	$(A, 5'MeC)_{20}$	Each linkage is phosphorothioated Every ribose is 2'O methylated
REP 2163 (SEQ ID NO: 11)	$(A, 5'MedC)_{20}$	Each linkage is phosphorothioated Only the riboses in adenosine are 2'O methylated
REP 2165 (SEQ ID NO: 13)	$(A, 5'MeC)_{20}$	Each linkage is phosphorothioated Every ribose is 2'O methylated except adenosines at positions 11, 21 and 31 where riboses are 2'OH.

dA = deoxyriboadenosine
dC = deoxyribocytidine
A = riboadenosine
5'MeC = ribo-5' methylcytidine
5'MedC = deoxyribo=5' methylcytidine

[0089] Three-day-old Pekin ducklings were infected with $2x10^{11}$ viral genome equivalents (VGE)/ml of DHBV. NAP treatment was started 11 days later after infection had become established. NAPs were administered via intraperitoneal injection with 10mg/kg of NAPs (formulated as calcium chelate complexes) 3 times/week for three weeks followed by analysis of antiviral effect at the end of treatment. A control group was treated with normal saline via the same route of administration and with the same dosing regimen. Antiviral activity was assessed by monitoring serum DHBsAg by ELISA (Fig. 2A) and liver DHBV DNA by quantitative PCR (Fig. 2B).

[0090] All NAPs resulted in reductions in serum DHBsAg and liver DHBV DNA, demonstrating that different NAPs containing diverse oligonucleotide modifications will have comparable antiviral effect. This in turn indicates that the synergistic antiviral activity observed with the use of a specific NAP and one or more nucleoside analog based HBV polymerase inhibitors (as observed with REP 2055 and entecavir in Example I above) will occur with any other phosphorothioated NAP and also with any said phosphorothioated NAP formulated as a chelate complex (as described in U.S. 8,513,211 and 8,716,259).

EXAMPLE III

Antiviral effects of NAPs in combination with TDF and ETV in DHBV infected Pekin Ducks

[0091] The antiviral effect of combined treatment with the calcium chelate complex of REP 2139 (REP 2139-Ca) and TDF or REP 2139-Ca and TDF and ETV in DHBV infected Pekin ducks was examined by assessing changes in the levels of serum and liver DHBV DNA during and after treatment by quantitative PCR. Infection of ducks was carried out as described in Example II except that treatment was started one month after infection. Treatment regimens were as follows:

1) Normal saline given by IP injection 3 times per week for 4 weeks
2) TDF, given 15 mg / day by oral gavage for 28 days
3) REP 2139-Ca, given 10mg/kg by IP injection, 3 times per week for 4 weeks.
4) REP 2139-Ca and TDF (as dosed above)
5) REP 2139-Ca and TDF (as dosed above) and ETV given 1 mg / day by oral gavage for 28 days.

[0092] Serum DHBV DNA was assessed pre-treatment (time point A), at day 14 of treatment (time point B), at the end of treatment (time point C) and one and two months after treatment was stopped (follow-up, time point D and E).

[0093] In the normal saline treated group, no control of DHBV DNA was observed during treatment, although DHBV DNA became spontaneously controlled in 3 ducks in this group during the follow-up (Fig. 3A). In the TDF-treated group serum DHBV DNA was reduced in all ducks but control was not achieved in all ducks until the end of treatment. DHBV DNA rebounded in all ducks in this group during follow-up (Fig. 3B). In the REP 2139-Ca treated group, no change in DHBV DNA was observed in two ducks throughout the study, DHBV DNA was controlled at the end of treatment

in only two ducks and became spontaneously controlled in two additional ducks during the follow-up (Fig. 3C). When REP 2139-Ca was combined with TDF control of DHBV DNA occurred in all but one duck halfway through treatment and was generally more rapid than the control achieved in the groups treated with either REP 2139-Ca or TDF alone. When REP 2139-Ca was combined with TDF and ETV, DHBV was controlled in all ducks halfway through the treatment (Fig. 3E). The proportion of ducks that maintained control of serum DHBV DNA during follow-up was greater with combined REP 2139-Ca and TDF (or TDF and ETV) than with TDF or REP 2139-Ca alone (Figs. 3D and E).

[0094] These observations teach that the on-treatment antiviral response in HBV infection can be improved synergistically by combining REP 2139-Ca and TDF or TDF and ETV and can lead to improved sustained virologic response off-treatment compared to that achieved with REP 2139-Ca or TDF alone. The synergistic activity seen in the above example can be reliability expected to occur with any NAP active against HBV as described herein and with any nucleotide / nucleoside analog based HBV polymerase inhibitor as described herein. Further, NAPs used in combination with more than one nucleoside / nucleotide HBV polymerase inhibitor can also be used with similarly productive synergistic antiviral effect.

[0095] The synergistic effects of combined NAP / TDF / ETV treatment led to the improved speed of antiviral response, demonstrating the potential for shorter treatment regimens capable of achieving sustained virologic response off-treatment. This potential could also be realized with any combination of NAP and nucleotide / nucleoside analog HBV polymerase inhibitor treatment as described herein.

[0096] Therefore, these observations teach that any pharmaceutically acceptable phosphorothioated NAP formulation which reduces or removes HBsAg from the blood (as described in U.S. 2014/0065102 and U.S. 8,008,269, 8,008,270 and 8,067,385) could be combined with any nucleoside/nucleotide HBV polymerase inhibitor as listed above and be expected to achieve a synergistic effect on the speed with which control of serum viremia can be achieved and or the transcriptional inactivation and or elimination of HBV cccDNA. The synergistic effects observed also teach that lower doses of said pharmaceutically acceptable agents could be combined and still achieve a synergistic activity with a useful antiviral effect.

[0097] Given the synergistic antiviral effects observed above with one phosphorothioated NAP used in combination with one nucleoside/nucleotide HBV polymerase inhibitor, a superior synergistic effect could also be achieved with one or more phosphorothioated NAPs used in combination with one or more nucleoside/nucleotide HBV polymerase inhibitors as described above.

[0098] The above description is meant to be exemplary only, and one skilled in the art will recognize that changes may be made to the embodiments described without departing from the scope of the invention as defined by the appended claims. Still other modifications which fall within the scope of the present invention, as defined in the appended claims, will be apparent to those skilled in the art, in light of a review of this disclosure, without departing from the scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a first pharmaceutically acceptable agent which comprises a chelate complex of at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor for treating HBV infection or HBV / HDV co-infection in a subject.

2. A composition comprising a first pharmaceutically acceptable agent which comprises a chelate complex of one or more nucleic acid polymers selected from the following:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO 13;
SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;

and a second pharmaceutically acceptable agent which comprises one or more of the following:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;
entricitabine;

clevudine;
besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;
valtorcitabine; and
any nucleoside / nucleotide analog which inhibits the HBV polymerase,

for the treatment of HBV infection or HBV / HDV co-infection.

3. The composition of claim 1 or 2, wherein the nucleic acid polymer further comprises at least one 2' ribose modification.

4. The composition of any one of claims 1-3, wherein the nucleic acid polymer further comprises all riboses having a 2' modification.

5. The composition of any one of claims 1-4, wherein the nucleic acid polymer further comprises at least one 2' O methyl ribose modification.

6. The composition of any one of claims 1-5, wherein the nucleic acid polymer further comprises all riboses having the 2' O methyl modification.

7. The composition of any one of claims 1-6, wherein the nucleic acid polymer further comprises at least one 5'methylcytosine.

8. The composition of any one of claims 1-7, wherein the nucleic acid polymer further comprises all cytosines present as 5'methylcytosine.

9. The composition of any one of claims 1-8, wherein the nucleic acid polymer further comprises at least one 2' O methyl ribose modification and at least one 5' methylcytosine.

10. The composition of any one of claims 1-9, wherein the nucleic acid polymer further comprises all riboses having the 2' O methyl modification and all cytosines present as 5'methylcytosine.

11. The composition of any one of claims 1-10, wherein the chelate complex is a calcium chelate complex.

12. The composition of any one of claims 1-10, wherein the chelate complex is a magnesium chelate complex.

13. The composition of any one of claims 1-10, wherein the chelate complex is a calcium / magnesium chelate complex.

14. The composition of any one of claims 1-13, wherein said first and second pharmaceutically acceptable agents are formulated within the same pharmaceutical composition.

15. The composition of any one of claims 1-13, where said first and second pharmaceutically acceptable agents are formulated within separate pharmaceutical compositions.

16. The composition of any one of claims 1-13, wherein said first and second pharmaceutically acceptable agents are administered simultaneously.

17. The composition of any one of claims 1-13, wherein said first and second pharmaceutically acceptable agents are administered by a different route.

18. The composition of any one of claims 1-17, wherein said first and second pharmaceutically acceptable agents are administered using one or more of the following: oral ingestion, aerosol inhalation, subcutaneous injection, intravenous injection and intravenous infusion.

19. Use of a first pharmaceutically acceptable agent which comprises a chelate complex of at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside /

nucleotide analog HBV polymerase inhibitor for treating HBV infection or HBV / HDV co-infection in a subject.

20. Use of a first pharmaceutically acceptable agent which comprises a chelate complex of at least one phosphorothioated nucleic acid polymer and a second pharmaceutically acceptable agent which comprises at least one nucleoside / nucleotide analog HBV polymerase inhibitor in the manufacture of a medicament for treating HBV infection or HBV / HDV co-infection in a subject.

21. Use of a first pharmaceutically acceptable agent which comprises the chelate complex one or more nucleic acid polymers selected from the following:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO 13;
SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;

and a second pharmaceutically acceptable agent which comprises one or more of the following:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;
entricitabine;
clevudine;

besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;
valtorcitabine; and
any nucleoside / nucleotide analog which inhibits the HBV polymerase,

for the treatment of HBV infection or HBV / HDV co-infection.

22. Use of a first pharmaceutically acceptable agent which comprises the chelate complex of one or more nucleic acid polymers selected from the following:

SEQ ID NO: 2;
SEQ ID NO: 10;
SEQ ID NO 13;
SEQ ID NOs: 1, 3-9, 11, 12 and 14-20;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG and
a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;

and a second pharmaceutically acceptable agent which comprises one or more of the following:

lamivudine;
adefovir dipivoxil;
entecavir;
telbivudine;
tenofovir disoproxil fumarate;

entricitabine;
clevudine;
besifovir;
tenofovir alafenamide fumarate;
AGX-1009;
elvucitabine;
lagociclovir valactate;
pradefovir mesylate;
valtorcitabine; and
any nucleoside / nucleotide analog which inhibits the HBV polymerase,

in the manufacture of a medicament for the treatment of HBV infection or HBV/HDV co-infection.

23. The use of any one of claims 19-22, wherein the nucleic acid polymer further comprises at least one 2' ribose modification.

24. The use of any one of claims 19-23, wherein the nucleic acid polymer further comprises all riboses having a 2' modification.

25. The use of any one of claims 19-24, wherein the nucleic acid polymer further comprises at least one 2' O methyl ribose modification.

26. The use of any one of claims 19-25, wherein the nucleic acid polymer further comprises all riboses having a 2' O methyl modification.

27. The use of any one of claims 19-26, wherein the nucleic acid polymer further comprises at least one 5'methylcytosine.

28. The use of any one of claims 19-27, wherein the nucleic acid polymer further comprises all cytosines present as 5'methylcytosine.

29. The use of any one of claims 19-28, wherein the nucleic acid polymer further comprises at least one 2' O methyl ribose modification and at least one 5' methylcytosine.

30. The use of any one of claims 19-29, wherein the nucleic acid polymer further comprises all riboses having the 2' O methyl modification and all cytosines present as 5'methylcytosine.

31. The used of any one of claims 19-30, wherein the chelate complex is a calcium chelate complex.

32. The use of any one of claims 19-30, wherein the chelate complex is a magnesium chelate complex.

33. The use of any one of claims 19-30, wherein the chelate complex is a calcium / magnesium chelate complex.

34. The use of any one of claims 19-33, wherein said first and second pharmaceutically acceptable agents are formulated within the same pharmaceutical composition.

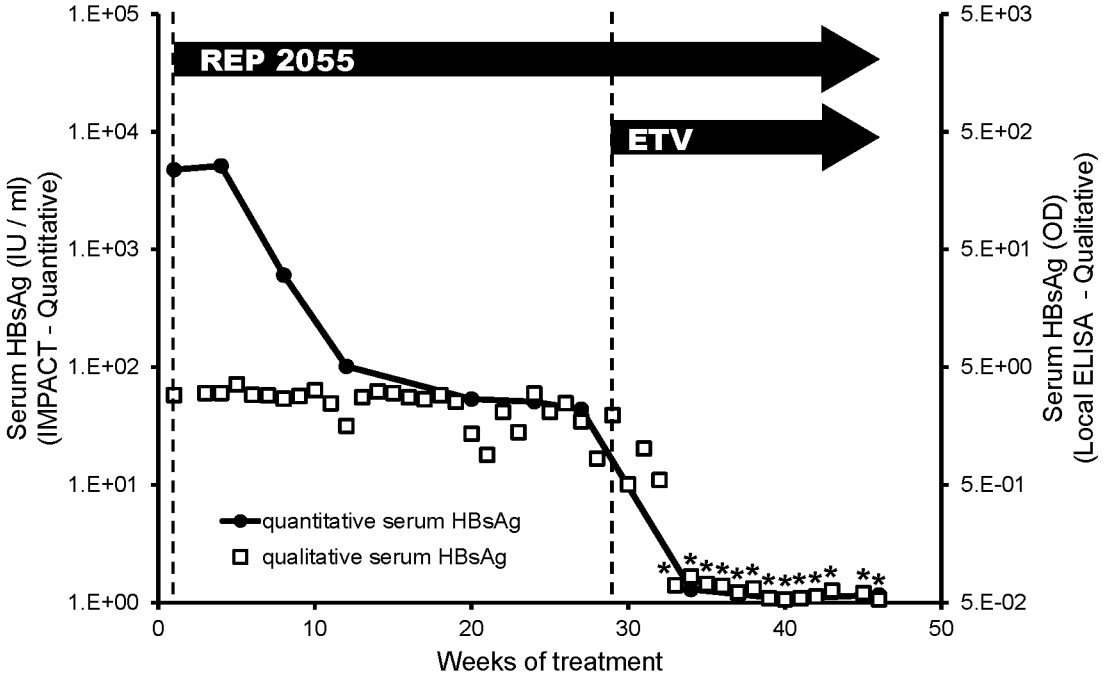
35. The use of any one of claims 19-33, wherein said first and second agents are formulated within separate pharmaceutical compositions.

36. The use of any one of claims 19-33, wherein said first and second agents are formulated for a simultaneous administration.

37. The use of any one of claims 19-33, wherein said first and second agents are formulated for an administration by a different route.

38. The use of any one of claims 19-37, wherein said first and second agents are formulated for an administration using one or more of the following: oral ingestion, aerosol inhalation, subcutaneous injection, intravenous injection and intravenous infusion.

Fig. 1



*below cut-off for serum HBsAg detection in local ELISA (HBsAg negative)

Fig. 2A

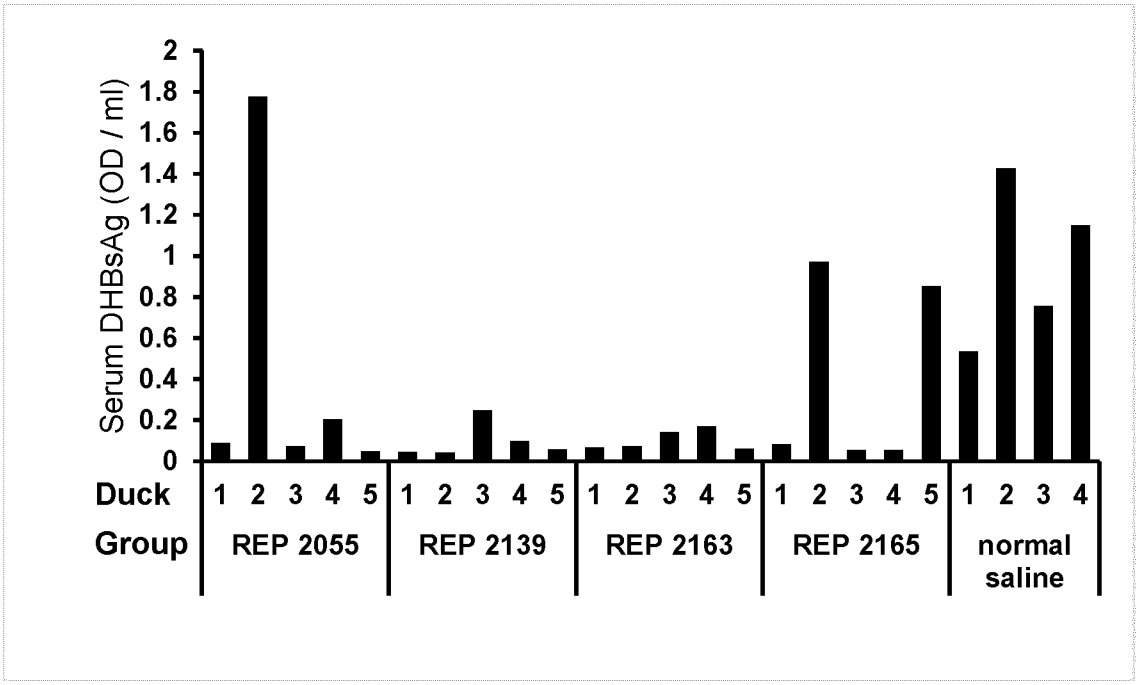

Serum DHBsAg (OD / ml)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Duck
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4
Group
REP 2055
REP 2139
REP 2163
REP 2165
normal saline

Fig. 2B

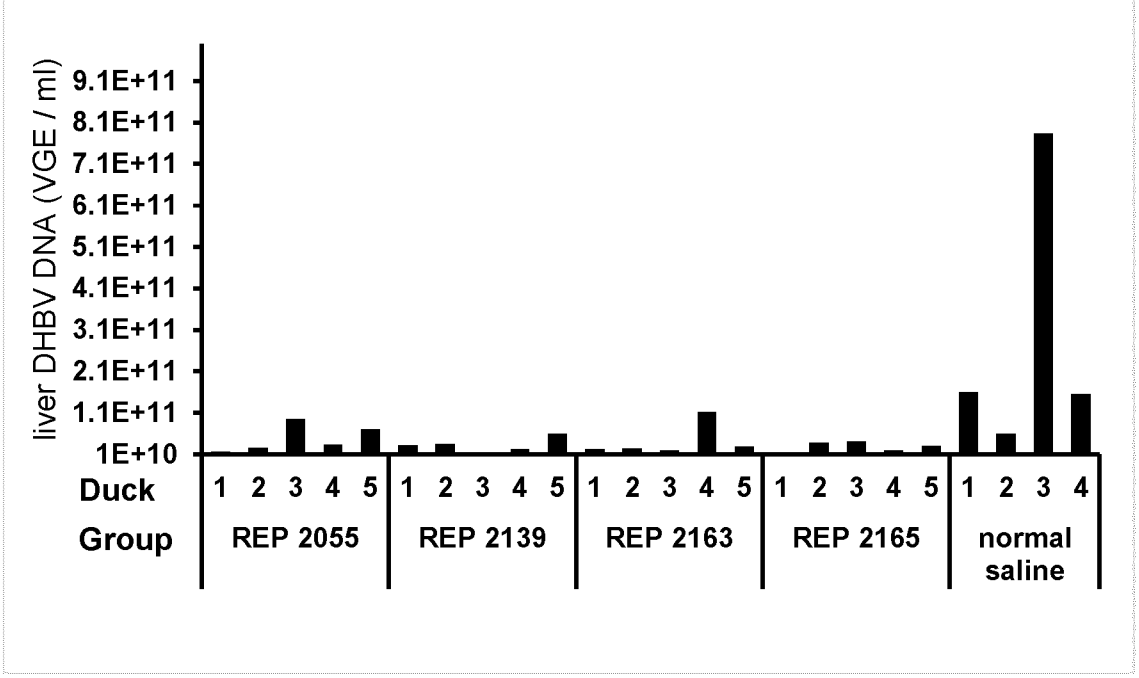

liver DHBV DNA (VGE / ml)
9.1E+11
8.1E+11
7.1E+11
6.1E+11
5.1E+11
4.1E+11
3.1E+11
2.1E+11
1.1E+11
1E+10
Duck
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4
Group
REP 2055
REP 2139
REP 2163
REP 2165
normal saline

Fig. 3A

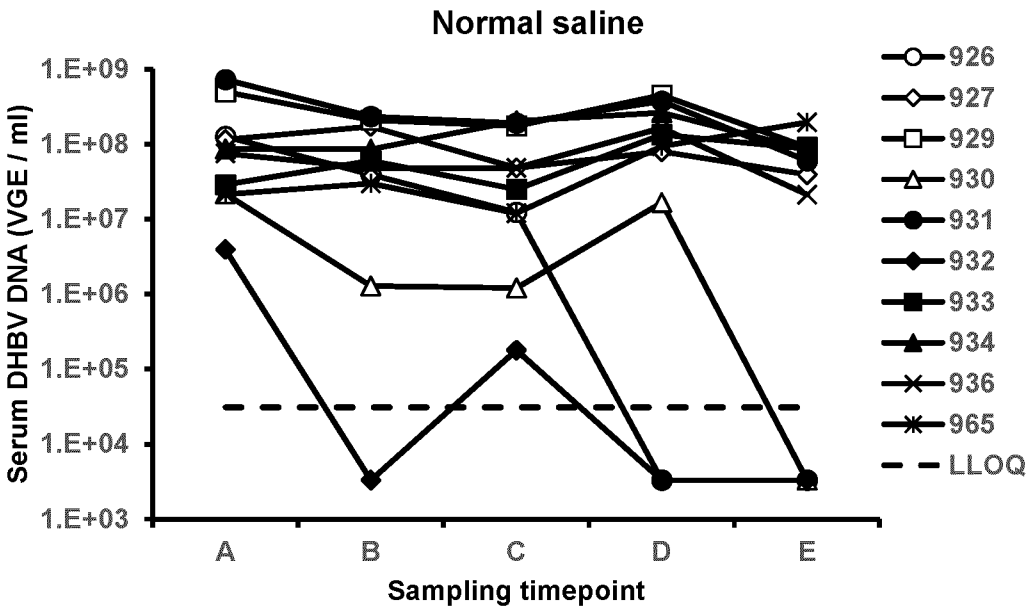

Normal saline
Serum DHBV DNA (VGE / ml)
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
A
B
C
D
E
Sampling timepoint
926
927
929
930
931
932
933
934
936
965
LLOQ

Fig. 3B

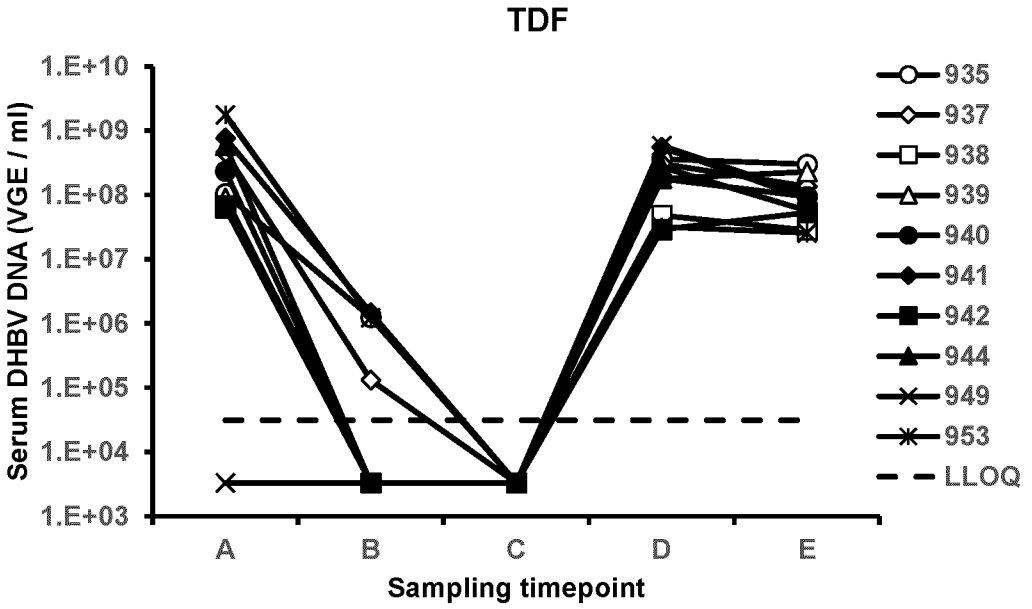

TDF
Serum DHBV DNA (VGE / ml)
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
A
B
C
D
E
Sampling timepoint
935
937
938
939
940
941
942
944
949
953
LLOQ

Fig 3C

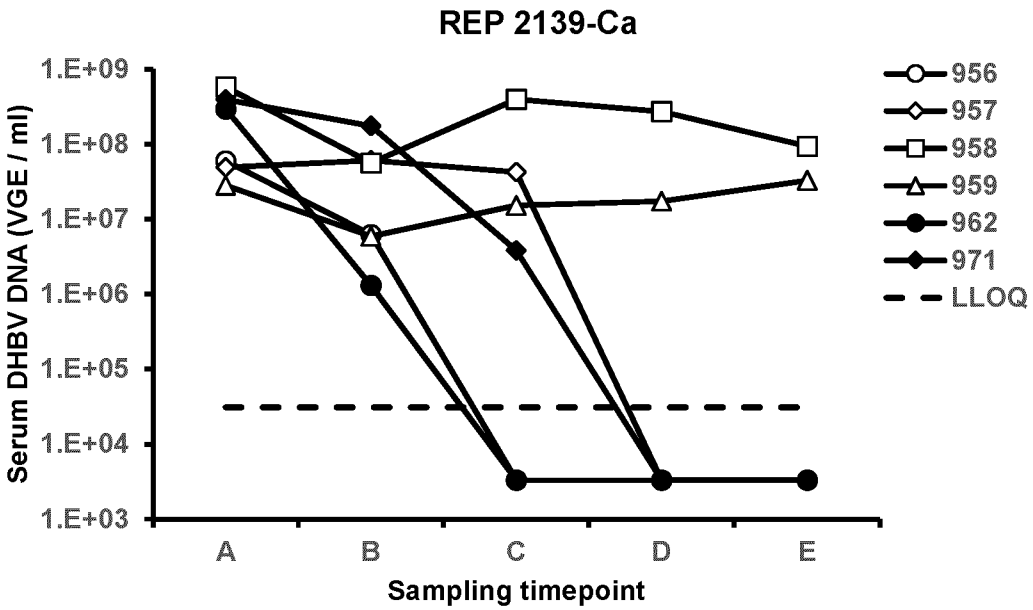

REP 2139-Ca
Serum DHBV DNA (VGE / ml)
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
A
B
C
D
E
Sampling timepoint
956
957
958
959
962
971
LLOQ

Fig. 3D

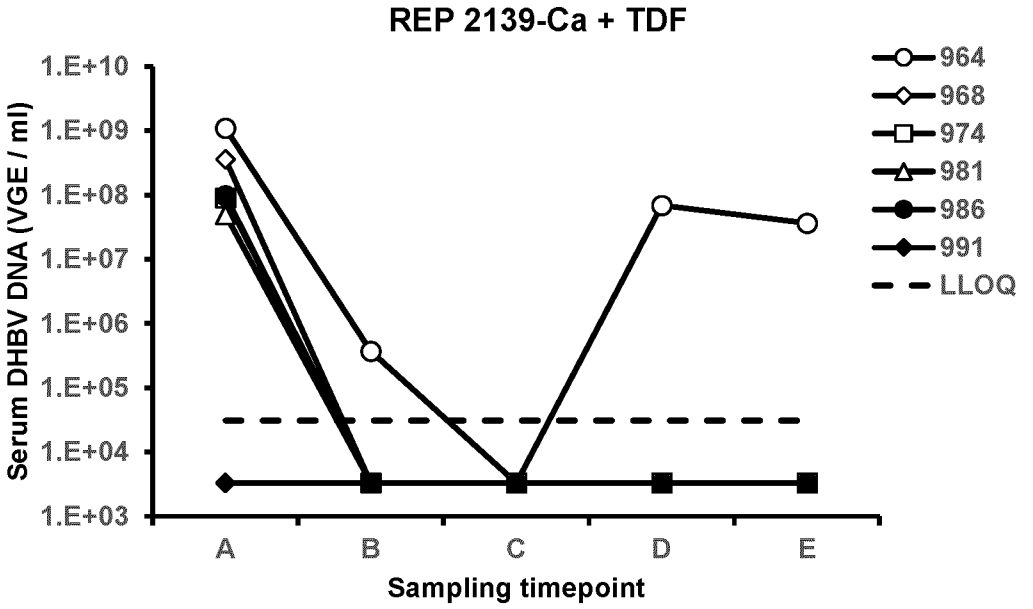
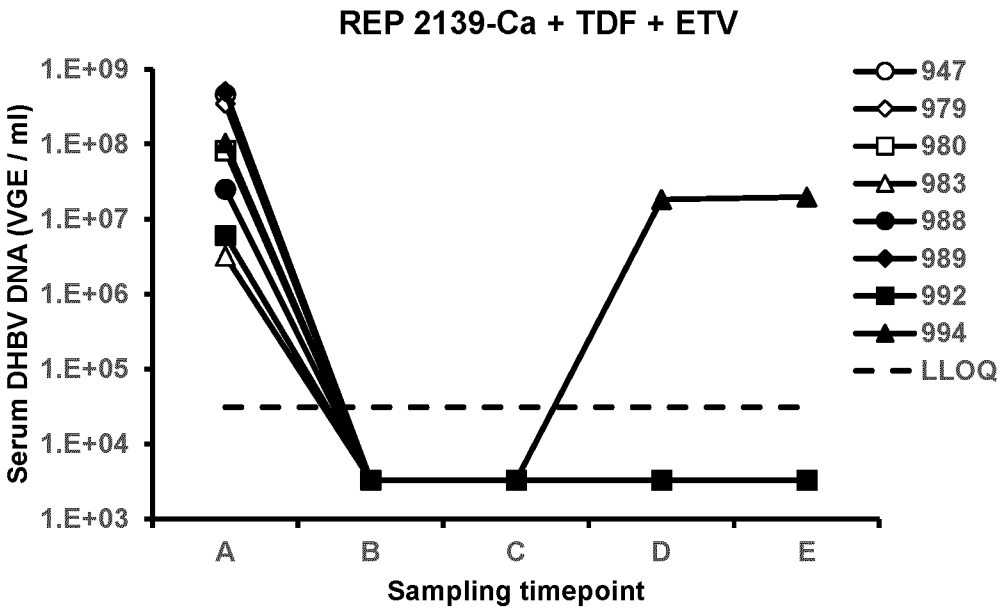

REP 2139-Ca + TDF
Serum DHBV DNA (VGE / ml)
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
A
B
C
D
E
Sampling timepoint
964
968
974
981
986
991
LLOQ

Fig. 3E


REP 2139-Ca + TDF + ETV
Serum DHBV DNA (VGE / ml)
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
A
B
C
D
E
Sampling timepoint
947
979
980
983
988
989
992
994
LLOQ

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2015/050626

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 31/7088*** (2006.01) , ***A61K 31/522*** (2006.01) , ***A61K 31/675*** (2006.01) , ***A61P 31/20*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 31/7088*** (2006.01) , ***A61K 31/522*** (2006.01) , ***A61K 31/675*** (2006.01) , ***A61P 31/20*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Biosis, CAplus, Medline, FAMPAT, Canadian Patent Database; Keywords: phosphorothioate(s); chelate complex(es); hepatitis; HBV; polymerase inhibitor(s); (lami-, telbi-, cle-)vudine; (ade-, teno-, besi-)fovir; (entri-, elvu-, valtor-)citabine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2013/170386 A1 (BAZINET, M. and VAILLANT, A.) 21 November 2013 (21-11-2013) - the entire document	1-38
X	WO2014/032176 A1 (BAZINET, M. and VAILLANT, A.) 6 March 2014 (06-03-2014) - cited in the description; the entire document	1-38
A	WO2012/075114 A2 (HAMMOND, N. et al) 7 June 2012 (07-06-2012)	

☐ Further documents are listed in the continuation of Box C.

☑ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
	"&" document member of the same patent family

Date of the actual completion of the international search 07 October 2015 (07-10-2015)	Date of mailing of the international search report 19 October 2015 (19-10-2015)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Michael W. De Vouge (819) 997-2952

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2015/050626

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2013170386A1	21 November 2013 (21-11-2013)	AU2013262416A1	18 December 2014 (18-12-2014)
		CA2873529A1	21 November 2013 (21-11-2013)
		CL2014003134A1	13 February 2015 (13-02-2015)
		CO7131387A2	01 December 2014 (01-12-2014)
		CR20140527A	15 December 2014 (15-12-2014)
		CU20140133A7	30 March 2015 (30-03-2015)
		DOP2014000264A	31 January 2014 (31-01-2014)
		EA201401278A1	30 April 2015 (30-04-2015)
		EP2849798A1	25 March 2015 (25-03-2015)
		IL235548A	29 January 2015 (29-01-2015)
		JP2015517504A	22 June 2015 (22-06-2015)
		KR20150013309A	04 February 2015 (04-02-2015)
		MX2014014021A	10 February 2015 (10-02-2015)
		PH12014502551A1	21 January 2015 (21-01-2015)
		SG11201407599SA	30 December 2014 (30-12-2014)
		TW201408308A	01 March 2014 (01-03-2014)
		US2013309201A1	21 November 2013 (21-11-2013)
WO2014032176A1	06 March 2014(06-03-2014)	AU2013308045A1	02 April 2015 (02-04-2015)
		CA2883785A1	06 March 2014 (06-03-2014)
		CN104837501A	12 August 2015 (12-08-2015)
		CR20150107A	06 April 2015 (06-04-2015)
		DOP2015000041A	30 April 2015 (30-04-2015)
		EA201500273A1	30 July 2015 (30-07-2015)
		EP2890403A1	08 July 2015 (08-07-2015)
		KR20150046309A	29 April 2015 (29-04-2015)
		PH12015500361A1	20 April 2015 (20-04-2015)
		TW201408309A	01 March 2014 (01-03-2014)
		US2014065102A1	06 March 2014 (06-03-2014)
		US9133458B2	15 September 2015 (15-09-2015)
		US2014369962A1	18 December 2014 (18-12-2014)
		US2014369963A1	18 December 2014 (18-12-2014)
WO2012075114A2	07 June 2012 (07-06-2012)	WO2012075114A3	10 April 2014 (10-04-2014)

(19)中华人民共和国国家知识产权局

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62/091,943 2014.12.15 US

(85)PCT国际申请进入国家阶段日
2017.01.06

(86)PCT国际申请的申请数据
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(87)PCT国际申请的公布数据
WO2016/004525 EN 2016.01.14

(71)申请人 里普利科股份有限公司
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(74)专利代理机构 北京康信知识产权代理有限责任公司 11240
代理人 张英 宫传芝

(51)Int.Cl.
A61K 31/7088(2006.01)
A61K 31/522(2006.01)
A61K 31/675(2006.01)
A61P 31/20(2006.01)

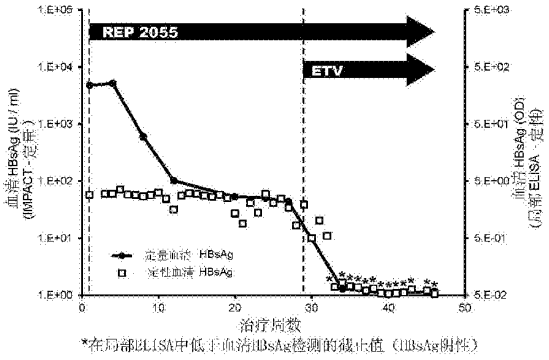
权利要求书4页 说明书18页
序列表6页 附图4页

(54)发明名称

治疗B型肝炎和D型肝炎病毒感染的方法

(57)摘要

公开了治疗B型肝炎病毒感染或B型肝炎病毒/肝炎δ病毒共感染的方法，所述方法包含向需要该治疗的对象施用包含至少一种硫代磷酸酯化核酸聚合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂。



*在局部ELISA中低于血清HBsAg检测的截止值（HBsAg阴性）

CN 106659730 A

1.用于治疗对象中的HBV感染或HBV/HDV共感染的组合物，其包含含有至少一种硫代磷酸酯化核酸聚合物的螯合复合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂。

2.用于治疗HBV感染或HBV/HDV共感染的组合物，其包含第一药学上可接受的药剂，该药剂包含一种或多种选自以下的核酸聚合物的螯合复合物：

SEQ ID NO:2；

SEQ ID NO:10；

SEQ ID NO:13；

SEQ ID NO:1、3-9、11、12和14-20；

包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

和第二药学上可接受的药剂，该药剂包含以下的一种或多种：

拉米夫定；

阿德福韦酯；

恩替卡韦；

替比夫定；

富马酸替诺福韦二吡呋酯；

恩曲他滨；

克来夫定；

贝斯福韦；

富马酸替诺福韦艾拉酚胺；

AGX-1009；

艾夫他滨；

拉昔洛韦瓦拉他特；

甲磺酸帕拉德福韦；

伐托他滨；和

抑制HBV聚合酶的任意核苷/核苷酸类似物。

3.权利要求1或2所述的组合物，其中所述核酸聚合物还包含至少一种2'核糖修饰。

4.权利要求1-3任一项所述的组合物，其中所述核酸聚合物还包含具有2'修饰的所有核糖。

5.权利要求1-4任一项所述的组合物，其中所述核酸聚合物还包含至少一种2'O甲基核糖修饰。

6.权利要求1-5任一项所述的组合物，其中所述核酸聚合物还包含具有2'O甲基修饰的所有核糖。

7.权利要求1-6任一项所述的组合物，其中所述核酸聚合物还包含至少一种5'甲基胞嘧啶。

8.权利要求1-7任一项所述的组合物，其中所述核酸聚合物还包含作为5'甲基胞嘧啶

存在的所有胞嘧啶。

9.权利要求1-8任一项所述的组合物，其中所述核酸聚合物还包含至少一种2'O甲基核糖修饰和至少一种5'甲基胞嘧啶。

10.权利要求1-9任一项所述的组合物，其中所述核酸聚合物还包含具有2'O甲基修饰的所有核糖和作为5'甲基胞嘧啶存在的所有胞嘧啶。

11.权利要求1-10任一项所述的组合物，其中所述螯合复合物是钙螯合复合物。

12.权利要求1-10任一项所述的组合物，其中所述螯合复合物是镁螯合复合物。

13.权利要求1-10任一项所述的组合物，其中所述螯合复合物是钙/镁螯合复合物。

14.权利要求1-13任一项所述的组合物，其中所述第一药学上可接受的药剂和所述第二药学上可接受的药剂被配制在相同药物组合物中。

15.权利要求1-13任一项所述的组合物，其中所述第一药学上可接受的药剂和所述第二药学上可接受的药剂被配制在单独的药物组合物中。

16.权利要求1-13任一项所述的组合物，其中所述第一药学上可接受的药剂和所述第二药学上可接受的药剂被同时施用。

17.权利要求1-13任一项所述的组合物，其中所述第一药学上可接受的药剂和第二药学上可接受的药剂通过不同的途径施用。

18.权利要求1-17任一项所述的组合物，其中所述第一药学上可接受的药剂和所述第二药学上可接受的药剂使用以下的一种或多种进行施用：经口摄取、气溶胶吸入、皮下注射、静脉注射和静脉输注。

19.包含至少一种硫代磷酸酯化核酸聚合物的螯合复合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂用于治疗对象中的HBV感染或HBV/HDV共感染的用途。

20.包含至少一种硫代磷酸酯化核酸聚合物的螯合复合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂在制备用于治疗对象中的HBV感染或HBV/HDV共感染的药物中的用途。

21.第一药学上可接受的药剂和第二药学上可接受的药剂用于治疗HBV感染或HBV/HDV共感染的用途，所述第一药学上可接受的药剂包含一种或多种选自以下的核酸聚合物的螯合复合物：

SEQ ID NO:2；

SEQ ID NO:10；

SEQ ID NO:13；

SEQ ID NO:1、3-9、11、12和14-20；

包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；以及

所述第二药学上可接受的药剂包含以下的一种或多种：

拉米夫定；

阿德福韦酯；

恩替卡韦；

替比夫定；

富马酸替诺福韦二吡呋酯；

恩曲他滨；

克来夫定；

贝斯福韦；

富马酸替诺福韦艾拉酚胺；

AGX-1009；

艾夫他滨；

拉昔洛韦瓦拉他特；

甲磺酸帕拉德福韦；

伐托他滨；和

抑制HBV聚合酶的任意核苷/核苷酸类似物。

22.第一药学上可接受的药剂和第二药学上可接受的药剂在制备用于治疗HBV感染或HBV/HDV共感染的药物中的用途，所述第一药学上可接受的药剂包含一种或多种选自以下的核酸聚合物的螯合复合物：

SEQ ID NO:2；

SEQ ID NO:10；

SEQ ID NO:13；

SEQ ID NO:1、3-9、11、12和14-20；

包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；以及

所述第二药学上可接受的药剂包含以下的一种或多种：

拉米夫定；

阿德福韦酯；

恩替卡韦；

替比夫定；

富马酸替诺福韦二吡呋酯；

恩曲他滨；

克来夫定；

贝斯福韦；

富马酸替诺福韦艾拉酚胺；

AGX-1009；

艾夫他滨；

拉昔洛韦瓦拉他特；

甲磺酸帕拉德福韦；

伐托他滨；和

抑制HBV聚合酶的任意核苷/核苷酸类似物。

23.权利要求19-22任一项所述的用途，其中所述核酸聚合物还包含至少一种2'核糖修饰。

24.权利要求19-23任一项所述的用途，其中所述核酸聚合物还包含具有2'修饰的所有核糖。

25.权利要求19-24任一项所述的用途，其中所述核酸聚合物还包含至少一种2'0甲基核糖修饰。

26.权利要求19-25任一项所述的用途，其中所述核酸聚合物还包含具有2'0甲基修饰的所有核糖。

27.权利要求19-26任一项所述的用途，其中所述核酸聚合物还包含至少一种5'甲基胞嘧啶。

28.权利要求19-27任一项所述的用途，其中所述核酸聚合物还包含作为5'甲基胞嘧啶存在的所有胞嘧啶。

29.权利要求19-28任一项所述的用途，其中所述核酸聚合物还包含至少一种2'0甲基核糖修饰和至少一种5'甲基胞嘧啶。

30.权利要求19-29任一项所述的用途，其中所述核酸聚合物还包含具有2'0甲基修饰的所有核糖和作为5'甲基胞嘧啶存在的所有胞嘧啶。

31.权利要求19-30任一项所述的用途，其中所述螯合复合物是钙螯合复合物。

32.权利要求19-30任一项所述的用途，其中所述螯合复合物是镁螯合复合物。

33.权利要求19-30任一项所述的用途，其中所述螯合复合物是钙/镁螯合复合物。

34.权利要求19-33任一项所述的用途，其中所述第一药学上可接受的药剂和所述第二药学上可接受的药剂被配制在相同药物组合物中。

35.权利要求19-33任一项所述的用途，其中所述第一药剂和所述第二药剂被配制在单独的药物组合物中。

36.权利要求19-33任一项所述的用途，其中所述第一药剂和所述第二药剂被配制用于同时施用。

37.权利要求19-33任一项所述的用途，其中所述第一药剂和所述第二药剂被配制用于通过不同的途径施用。

38.权利要求19-37任一项所述的用途，其中所述第一药剂和所述第二药剂被配制用于使用以下的一种或多种进行施用：经口摄取、气溶胶吸入、皮下注射、静脉注射和静脉输注。

治疗B型肝炎和D型肝炎病毒感染的方法

技术领域

[0001] 本描述涉及治疗具有B型肝炎病毒(HBV)感染或HBV/肝炎δ病毒(HDV)共感染的对象的方法，其包含给予第一药学上可接受的硫代磷酸酯化核酸聚合物制剂和抑制HBV聚合酶的第二药学上可接受的核苷/核苷酸类似物制剂。

背景技术

[0002] HBV折磨全世界4亿个体，并导致每年估计有600,000例因HBV感染引起的并发症的死亡。虽然已经批准一些抗病毒治疗供使用，但这些治疗除在一小部分经历治疗的患者以外都无法引起能够提供感染的持久控制的治疗有效的免疫反应。

[0003] HBV感染导致产生两种不同的颗粒：1)感染性HBV病毒本身(或戴恩粒子(Dane particle))，其包括从HBV核心抗原蛋白(HBcAg)组装且由HBV表面抗原(HBsAg)覆盖的病毒衣壳，和2)亚病毒颗粒(或SVP)，其是包括脂质、胆固醇、胆固醇酯和具有非感染性的中小型形式的HBV表面抗原(HBsAg)的高密度脂蛋白样颗粒。对于所产生的每一种病毒颗粒，1,000至10,000个SVP被释放到血液中。因此，SVP(和其携带的HBsAg蛋白)表示血液中的绝大多数病毒蛋白。HBV感染的细胞也分泌称为HBV e-抗原(HBeAg)的前核心蛋白的可溶性蛋白水解产物。

[0004] HDV使用HBsAg形成其病毒结构(Taylor,2006,Virology,344:71-76)，且因此，HDV感染可仅在具有伴随HBV感染的对象中发生。虽然无症状HBV携带者和慢性HBV相关的肝病中的HDV共感染的发病率在具有低HBV感染发病率的国家中较低，但其在具有高HBV感染发病率的国家中在HBV感染的对象中是显著并发症且可增加肝病至肝硬化的进展速率。在HBV/HDV共感染的对象中，HBV感染的未满足的医疗需求甚至更为紧迫；不存在直接靶向HDV病毒的具体经过批准的药剂，且患者即使对于用于HBV治疗的经批准药剂的组合疗法的反应也比在具有HBV单纯感染的患者中的反应差(Wedemeyer等,2014,Oral abstract 4,49th Annual Meeting of the European Association for the Study of the Liver,April9-14,London,UK)。

[0005] 目前批准的针对HBV的治疗包括基于干扰素-α或胸腺素α1的免疫疗法，和通过核苷/核苷酸类似物抑制HBV聚合酶来抑制病毒产生。HBV聚合酶抑制剂可有效减少感染性病毒体的产生，但对减少HBsAg极少甚至没有效果，或仅在有限数量的患者进行长期治疗下极缓慢减少HBsAg(Fung等,2011,Am.J.Gasteroenterol.,106:1766-1773;Reijnders等,2011,J.Hepatol.,54:449-454;Charuworn等,2014,Poster abstract 401,48th Annual Meeting of the European Association for the Study of the Liver,April 24-28,Amsterdam,The Netherlands)。HBV聚合酶抑制剂的主要作用是阻断前基因组病毒mRNA转化成部分双链的DNA，该DNA存在于感染性病毒体中。基于干扰素的免疫治疗可以实现感染性病毒的减少和从血液去除HBsAg，但仅在小百分比的受治疗的对象中实现。

[0006] 血液中的HBsAg可隔绝抗HBsAg抗体且允许感染性病毒颗粒逃避免疫检测，这可能是HBV感染保持慢性病症的原因之一。另外，HBsAg、HBeAg和HBcAg都具有如以下所述的免疫

抑制性质，且在施用以上所述的针对HBV的目前可用的治疗中的任一个之后，这些病毒蛋白在患者血液中的存留可能对防止患者实现其HBV感染的免疫控制具有显著影响。

[0007] 尽管三种主要HBV蛋白(HBsAg、HBeAg和HBcAg)都具有免疫抑制性质(参见下文)，但HBsAg在HBV感染的对象的循环中占HBV蛋白的绝大多数，并有可能是抑制宿主对HBV感染的免疫反应的主要介导物。尽管HBeAg的去除、抗HBe的出现或血清病毒血症的减少与在停止治疗后HBV感染的持续控制的发展不相关，但在HBV感染中血清HBsAg从血液的去除(和游离抗HBsAg抗体的出现)是治疗时抗病毒反应的公认的优良预后指标，其在停止治疗后，将导致对HBV感染的控制(虽然这仅在一小部分接受免疫治疗或HBV聚合酶抑制剂的患者中发生)。因此，虽然所有三种主要HBV蛋白(HBsAg、HBeAg和HBcAg)的减少可导致抑制效果的最佳去除，但HBsAg的去除是必需的，且其单独去除可能足以去除具有HBV感染的对象中对免疫功能的大部分抑制。

[0008] 慢性HBV感染的另一关键特征是在感染的细胞的细胞核中建立HBV遗传信息的稳定储存库(reservoir)，其被称为共价闭合环状DNA(cccDNA)。cccDNA作为染色体外游离基因以多个拷贝存在于细胞核内，其用作用于产生编码所有病毒蛋白的mRNA的转录模板和用于产生新病毒体的未成熟的基因组(前基因组mRNA)。在细胞质中衣壳化后，将未成熟的前基因组mRNA通过HBV聚合酶(其和前基因组mRNA共同衣壳化)转化为成熟的部分双链DNA基因组，从而在原始细胞或先前感染的细胞中使成熟HBV基因组有能力建立或补充cccDNA储存库。感染过程的结束由这部分双链基因组HBV模板输送至细胞核中以及其转化为cccDNA组成。

[0009] 可在被感染细胞的细胞核中经由细胞核输入含有补充cccDNA拷贝数的成熟HBV基因组的HBV衣壳来补充cccDNA。该细胞核cccDNA补充可通过以下两种机制来实现：从细胞质直接细胞核输入经组装衣壳，或再感染先前感染的肝细胞，且随后使内化衣壳穿梭至细胞核中(Rabe等，2003，Proc.Natl.Acad.Sci.USA，100:9849-9854)。细胞核中该基因组HBV储存库的转录抑制或消除对治疗后建立HBV感染的长期控制是至关重要的。

[0010] 利用核苷/核苷酸HBV聚合酶抑制剂长期治疗可减少细胞核内的cccDNA拷贝数，这和HBV聚合酶抑制剂能够阻断cccDNA通过细胞核输入含有成熟HBV基因组的衣壳的补充一致。然而，虽然cccDNA拷贝数/肝细胞减少，但其仍保持转录活性，因此HBsAg水平在很大程度上不受影响(Werle-Lapostolle等，2004，Gastroenterol.，126:1750-1758；Wong等，2013，Clin.Gastroenterol.Hepatol.，11:1004-1010；Wong等，2014，Poster abstract 1074，49th Annual Meeting of the European Association for the Study of the Liver，April 9-14，London，UK)。可以通过免疫介导的过程将cccDNA转录失活(Belloni等，2012，J.Clin.Inv.，122:529-537)，但免疫反应激发为cccDNA失活所需的细胞因子应答的能力可如U.S.2014/0065102(其通过引用全部并入本文)中所述通过持续循环HBsAg来阻断，并且与免疫疗法在治疗HBV感染的无效性一致。

[0011] 因此，医学上明确需要可以在大比例的接受这种治疗的患者中引发HBV感染的持久免疫控制的治疗方案。

发明内容

[0012] 按照本描述，现在提供用于治疗对象中的HBV感染或HBV/HDV共感染的组合物，其

包含第一药学上可接受的药剂，该药剂包含至少一种硫代磷酸酯化核酸聚合物；和第二药学上可接受的药剂，该药剂包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂。

[0013] 也提供用于治疗对象中的HBV感染或HBV/HDV共感染的组合物，其包含第一药学上可接受的药剂，该药剂包含至少一种硫代磷酸酯化核酸聚合物的螯合复合物；和第二药学上可接受的药剂，该药剂包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂。

[0014] 进一步提供包含至少一种硫代磷酸酯化核酸聚合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂用于治疗对象中的HBV感染或HBV/HDV共感染的用途。

[0015] 另外提供包含至少一种硫代磷酸酯化核酸聚合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂在制备用于治疗对象中的HBV感染或HBV/HDV共感染的药物中的用途。

[0016] 进一步提供包含至少一种硫代磷酸酯化核酸聚合物的螯合复合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂用于治疗对象中的HBV感染或HBV/HDV共感染的用途。

[0017] 另外提供包含至少一种硫代磷酸酯化核酸聚合物的螯合复合物的第一药学上可接受的药剂和包含至少一种核苷/核苷酸类似物HBV聚合酶抑制剂的第二药学上可接受的药剂在制备用于治疗对象中的HBV感染或HBV/HDV共感染的药物中的用途。

[0018] 在另一个实施方式中，提供用于治疗HBV感染或HBV/HDV共感染的组合物，其包含第一药学上可接受的药剂，该药剂包含一种或多种选自以下的核酸聚合物的螯合复合物：

[0019] SEQ ID NO:2；

[0020] SEQ ID NO:10；

[0021] SEQ ID NO:13；

[0022] SEQ ID NO:1、3-9、11、12和14-20；

[0023] 包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0024] 包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0025] 包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

[0026] 包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0027] 和第二药学上可接受的药剂，该药剂包含以下的一种或多种：

[0028] 拉米夫定(lamivudine)；

[0029] 阿德福韦酯(adefovir dipivoxil)；

[0030] 恩替卡韦(entecavir)；

[0031] 替比夫定(telbivudine)；

[0032] 富马酸替诺福韦二吡呋酯(tenofovir disoproxil fumarate)；

[0033] 恩曲他滨(entricitabine)；

[0034] 克来夫定(clevudine)；

[0035] 贝斯福韦(besifovir)；

[0036] 富马酸替诺福韦艾拉酚胺(tenofovir alafenamide fumarate)；

[0037] AGX-1009；

[0038] 艾夫他滨(elvucitabine)；

[0039] 拉昔洛韦瓦拉他特(lagociclovir valactate)；

[0040] 甲磺酸帕拉德福韦(pradefovir mesylate)；

[0041] 伐托他滨(valtorcitabine)；和

[0042] 抑制HBV聚合酶的任意核苷/核苷酸类似物。

[0043] 在实施方式中，提供第一药学上可接受的药剂和第二药学上可接受的药剂用于治疗HBV感染或HBV/HDV共感染的用途，该第一药学上可接受的药剂包含选自以下的一种或多种核酸聚合物的螯合复合物：

[0044] SEQ ID NO:2；

[0045] SEQ ID NO:10；

[0046] SEQ ID NO:13；

[0047] SEQ ID NO:1、3-9、11、12和14-20；

[0048] 包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0049] 包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0050] 包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

[0051] 包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0052] 和该第二药学上可接受的药剂包含以下的一种或多种：

[0053] 拉米夫定；

[0054] 阿德福韦酯；

[0055] 恩替卡韦；

[0056] 替比夫定；

[0057] 富马酸替诺福韦二吡呋酯；

[0058] 恩曲他滨；

[0059] 克来夫定；

[0060] 贝斯福韦；

[0061] 富马酸替诺福韦艾拉酚胺；

[0062] AGX-1009；

[0063] 艾夫他滨；

[0064] 拉昔洛韦瓦拉他特；

[0065] 甲磺酸帕拉德福韦；

[0066] 伐托他滨；和

[0067] 抑制HBV聚合酶的任意核苷/核苷酸类似物。

[0068] 在另一个实施方式中，提供第一药学上可接受的药剂和第二药学上可接受的药剂在制备用于治疗HBV感染或HBV/HDV共感染的药物中的用途，该第一药学上可接受的药剂包含选自以下的一种或多种核酸聚合物的螯合复合物：

[0069] SEQ ID NO:2；

[0070] SEQ ID NO:10；

[0071] SEQ ID NO:13；

[0072] SEQ ID NO:1、3-9、11、12和14-20；

[0073] 包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0074] 包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0075] 包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

[0076] 包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0077] 和该第二药学上可接受的药剂包含以下的一种或多种：

[0078] 拉米夫定；

[0079] 阿德福韦酯；

[0080] 恩替卡韦；

[0081] 替比夫定；

[0082] 富马酸替诺福韦二吡呋酯；

[0083] 恩曲他滨；

[0084] 克来夫定；

[0085] 贝斯福韦；

[0086] 富马酸替诺福韦艾拉酚胺；

[0087] AGX-1009；

[0088] 艾夫他滨；

[0089] 拉昔洛韦瓦拉他特；

[0090] 甲磺酸帕拉德福韦；

[0091] 伐托他滨；和

[0092] 抑制HBV聚合酶的任意核苷/核苷酸类似物。

[0093] 在另一个实施方式中，核酸聚合物包括包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸。

[0094] 在另一个实施方式中，核酸聚合物包括包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸。

[0095] 在另一个实施方式中，核酸聚合物包括包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸。

[0096] 在另一个实施方式中，核酸聚合物包括包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸。

[0097] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含至少一种2'核糖修饰。

[0098] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含具有2'修饰的所有核糖。

[0099] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含至少一种2'O甲基核糖修饰。

[0100] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含具有2'O甲基修饰的所有核糖。

[0101] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含至少一种5'甲基胞嘧啶。

[0102] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含作为5'甲基胞嘧啶存在的所有胞嘧啶。

[0103] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含至少一种2'核糖修饰和至少一种5'甲基胞嘧啶。

[0104] 在另一个实施方式中，硫代磷酸酯化核酸聚合物还包含具有2'O甲基修饰的所有

核糖和作为5'甲基胞嘧啶存在的所有胞嘧啶。

[0105] 在另一个实施方式中，核酸聚合物选自SEQ ID NO:1-20。

[0106] 在另一个实施方式中，核酸聚合物被制备成包含选自SEQ ID NO:1-20的寡核苷酸的寡核苷酸螯合复合物。

[0107] 在另一个实施方式中，核酸聚合物由SEQ ID NO:2组成的寡核苷酸。

[0108] 在另一个实施方式中，核酸聚合物被制备成包含SEQ ID NO:2的寡核苷酸螯合复合物。

[0109] 在另一个实施方式中，核酸聚合物是由SEQ ID NO:10组成的寡核苷酸。

[0110] 在另一个实施方式中，核酸聚合物被制备成包含SEQ ID NO:10的寡核苷酸螯合复合物。

[0111] 在另一个实施方式中，核酸聚合物是由SEQ ID NO:13组成的寡核苷酸。

[0112] 在另一个实施方式中，核酸聚合物被制备成包含SEQ ID NO:13的寡核苷酸螯合复合物。

[0113] 在一个实施方式中，螯合复合物是钙螯合复合物。

[0114] 在另一个实施方式中，螯合复合物是镁螯合复合物。

[0115] 在另外的实施方式中，螯合复合物是钙/镁螯合复合物。

[0116] 在进一步的实施方式中，第一和第二药学上可接受的药剂被配制在相同药物组合物中。

[0117] 在进一步的实施方式中，第一药剂和第二药剂被配制在单独的药物组合物中。

[0118] 在进一步的实施方式中，第一药剂和第二药剂被配制用于同时施用。

[0119] 在进一步的实施方式中，第一药剂和第二药剂被配制用于通过不同的途径施用。

[0120] 在进一步的实施方式中，第一药剂和第二药剂被配制用于使用以下的一个或多个进行施用:经口摄取、气溶胶吸入、皮下注射、静脉注射和静脉输注。

[0121] 在进一步的实施方式中，核酸聚合物是以下的至少一种：

[0122] SEQ ID NO:2；

[0123] SEQ ID NO:10；

[0124] SEQ ID NO:13；

[0125] SEQ ID NO:1、3-9、11、12和14-20；

[0126] 包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0127] 包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0128] 包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸;和

[0129] 包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸。

[0130] 在进一步的实施方式中，下面的核酸聚合物可以进一步被配制成寡核苷酸螯合复合物：

[0131] SEQ ID NO:2；

[0132] SEQ ID NO:10；

[0133] SEQ ID NO:13；

[0134] SEQ ID NO:1、3-9、11、12和14-20；

[0135] 包含序列AC的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0136] 包含序列CA的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；

[0137] 包含序列TG的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸；和

[0138] 包含序列GT的重复的长度为20-120个核苷酸的硫代磷酸酯化寡核苷酸。

[0139] 在另一个实施方式中，核苷/核苷酸类似物HBV聚合酶抑制剂包含下列的一种或多种：

[0140] 拉米夫定；

[0141] 阿德福韦酯；

[0142] 恩替卡韦；

[0143] 替比夫定；

[0144] 富马酸替诺福韦二吡呋酯；

[0145] 恩曲他滨；

[0146] 克来夫定；

[0147] 贝斯福韦；

[0148] 富马酸替诺福韦艾拉酚胺；

[0149] AGX-1009；

[0150] 艾夫他滨；

[0151] 拉昔洛韦瓦拉他特；

[0152] 甲磺酸帕拉德福韦；

[0153] 伐托他滨；和

[0154] 抑制HBV聚合酶的任意核苷/核苷酸类似物。

附图说明

[0155] 图1显示利用NAP REP 2055(SEQ ID NO:2)和恩替卡韦(ETV)的组合治疗对HBsAg的血清水平降低的协同效应。

[0156] 图2A显示作为钙螯合复合物给予被DHBV感染的北京鸭(Pekin duck)的NAP的抗病毒活性，其通过在治疗结束时通过ELISA监测血清DHBsAg来测量。

[0157] 图2B显示作为钙螯合复合物给予被DHBV感染的北京鸭的NAP的抗病毒活性，其通过在治疗结束时通过定量PCR监测肝DHBV DNA来评价。

[0158] 图3A显示在以下时间利用生理盐水治疗28天的鸭血清中DHBV DNA水平：A)治疗前、B)当治疗完成一半时、C)治疗结束时、D)治疗后一个月和E)治疗后两个月。量化的下限(LLOQ)为3.1×10^{4}VGE/ml。小于LLOQ的值设定在3×10^{3}VGE/ml。VGE＝病毒基因组等效物。

[0159] 图3B显示在以下时间利用富马酸替诺福韦二吡呋酯(TDF)治疗28天的鸭血清中DHBV DNA水平：A)治疗前、B)当治疗完成一半时、C)治疗结束时、D)治疗后一个月和E)治疗后两个月。量化的下限(LLOQ)为3.1×10^{4}VGE/ml。小于LLOQ的值设定在3×10^{3}VGE/ml。VGE＝病毒基因组等效物。

[0160] 图3C显示在以下时间利用REP 2139-Ca治疗28天的鸭血清中DHBV DNA水平：A)治疗前、B)当治疗完成一半时、C)治疗结束时、D)治疗后一个月和E)治疗后两个月。量化的下限(LLOQ)为3.1×10^{4}VGE/ml。小于LLOQ的值设定在3×10^{3}VGE/ml。VGE＝病毒基因组等效物。

[0161] 图3D显示在以下时间利用REP 2139-Ca和TDF治疗28天的鸭血清中DHBV DNA水平：

A)治疗前、B)当治疗完成一半时、C)治疗结束时、D)治疗后一个月和E)治疗后两个月。量化的下限(LLOQ)为3.1×10^4VGE/ml。小于LLOQ的值设定在3×10^3VGE/ml。VGE＝病毒基因组等效物。

[0162] 图3E显示在以下时间利用REP 2139-Ca、TDF和恩替卡韦(ETV)治疗28天的鸭血清中DHBV DNA水平:A)治疗前、B)当治疗完成一半时、C)治疗结束时、D)治疗后一个月和E)治疗后两个月。量化的下限(LLOQ)为3.1×10^4VGE/ml。小于LLOQ的值设定在3×10^3VGE/ml。VGE＝病毒基因组等效物。

具体实施方式

[0163] 本文提供抵抗HBV感染的组合治疗,其由施用能够从血液去除HBsAg的第一药学上可接受的药剂和抑制HBV聚合酶的第二药学上可接受的药剂组成。这样的组合治疗允许宿主免疫功能的恢复(通过去除血清HBsAg),其继而引起免疫介导的cccDNA的转录失活和或感染的肝细胞中cccDNA拷贝数的减少,同时经由细胞核输入含有成熟HBV基因组的衣壳或产生感染性病毒(通过抑制HBV聚合酶)阻断cccDNA的补充。这两种药物的组合的协同效应可加速对治疗的抗病毒反应和或消除感染细胞的cccDNA,从而缩短在停止治疗后获得感染的持续抑制所需的治疗时间。重要的是,这些效果可以在不存在免疫治疗时实现。这种组合治疗将对HBV单纯感染和HBV/HDV共感染有效。

[0164] HBsAg在HBV感染和HBV/HDV共感染中起着关键作用。除了HBsAg作为病毒体形成的必要结构组分的作用以外,其也以亚病毒颗粒(SVP)的形式大量释放至被感染的对象的血液中,这些颗粒缺乏病毒衣壳和基因组并且其似乎主要用于将HBsAg输送至血液中。感染细胞以超过病毒分泌过量1,000-10,000倍分泌SVP,从而允许SVP有效隔绝HBsAg抗体(抗HB),使得血液中的HBV或HDV病毒可因适应性免疫而逃避识别。一些研究也已表明,HBsAg还可用来直接阻断HBV感染的适应性和先天免疫反应的活化(Cheng等,2005,Journal of hepatology,43:4 65-471;Op den Brouw等,2009,Immunology,126:280-289;Vanlandschoot等,2002,The Journal of general virology,83:1281-1289;Wu等,2009,Hepatology,49:1132-1140;Xu等,2009,Molecular immunology,46:2640-2646)。该功能性在人类HBV感染中的存在和其对免疫治疗剂的活性的影响和这些抗病毒效果在HBV/HDV共感染中的额外适用性先前已经描述在US 2014/0065102 A1中,其通过引用全部并入本文。虽然HBeAg和HBcAg也已显示出具有免疫抑制性质(Kanda等,2012,J.Inf.Dis.,206:415-420;Lang等,2011,J.Hepatol.,55:762-769;Gruffaz等,2013,J.Hepatol.,58(suppl),ps155,Abstract 378),但是鉴于血液中与HBsAg相关的HBeAg和HBcAg占非常小的部分,这些可能具有最小影响。

[0165] HBV聚合酶(NRTI)的核苷/核苷酸类似物抑制剂是公知类型的抗病毒剂,其抵抗HBV感染的活性通过相同作用机制发生:这类化合物通过在DNA链伸长期间与天然核苷酸底物竞争而充当即时或延迟链终止剂(Menendez-Arias等,2015Curr.Op.Virol.8:1-9)。这类化合物可以保留由含氮碱基和糖组成的基本核心核苷酸/核苷核心结构,或可以是无环核苷酸,或者可以缺少糖或假糖环,或可以具有取代α-磷酸酯的膦酸酯基团且可存在许多其他额外的修饰,如在Michailidis等,2012Int.J.Biochem.Cell.Biol.44:1060-1071和De Clercq等,2010Viruses 2:1279-1305所述。

[0166] 鸭HBV病毒(DHBV)感染的鸭是公认的HBV感染模型，并已用于评估目前用于治疗人类患者的若干HBV NRTI(Schultz等，2004，Adv Virus Res，63:1-70；Foster等，2005，J Virol，79:5819-5832；Nicoll等.1998，Antimicrob Agents Chemother.，42:3130-3135)。已显示出硫代磷酸酯化的核酸聚合物(NAP)在DHBV感染的鸭中具有抗病毒活性(Noordeen等，2013Anti-Microb.Agents Chemother.57:5291-5298and 5299-5306)，这并非源于任何直接免疫刺激机制。此外，在以前建立的体内DHBV感染中利用NAP REP 2055(SEQ ID NO:2)进行治疗介入，REP 2055可清除血清鸭HBsAg(DHBsAg)，这伴有cccDNA的转录失活和cccDNA拷贝数的减少(Noordeen等，2009，Abstract 88HEPDART meeting Dec 6-9，HI，USA)。cccDNA的这种失火和消除通过DHBsAg介导的宿主免疫功能抑制的去除引起，然后可以通过公认的免疫介导的机制失活并清除感染细胞的cccDNA(Levrero等，2009，J.Hepatol.，51:581-592；Belloni等，2012，J.Clin.Inv.，122:529-537)。

[0167] 从人类患者的血液有效去除HBsAg的NAP，如US 2014/0065102中所述。在公认的临床前HBV感染模型(鸭HBV感染的北京鸭)中，NAP治疗可消除血清鸭HBsAg(DHBsAg)，并且在血清DHBsAg的不存在下免疫功能的恢复能够转录失活并消除感染的肝细胞的cccDNA(Noordeen等，2009，Abstract 88，HEPDART meeting Dec 6-10，HI，USA)。因此，预计从HBV感染的患者的血清去除HBsAg对原位感染的人肝细胞中的cccDNA失活具有相同的效果。

[0168] 因此，本文描述用于较快速建立血清病毒血症的控制或用于建立cccDNA活性的持久控制和或消除HBV感染的肝细胞的cccDNA的有效手段，该手段由如下新颖组合方法组成：其中通过使用药学上可接受的硫代磷酸酯化NAP制剂从血液减少或去除HBsAg和通过抑制HBV聚合酶的第二药学上可接受的核苷酸/核苷类似物制剂阻断cccDNA的补充和感染性病毒的产生。这种组合方法具有以下新颖和重要的优点：

[0169] 1)其结合以下能力：改善宿主免疫功能(由血清HBsAg的去除引起)以转录失活细胞内的cccDNA和或减少细胞内的cccDNA拷贝数，和阻断cccDNA的补充(通过防止含有成熟基因组的衣壳进入细胞核(通过抑制HBV聚合酶活性)或感染性病毒体的产生(通过防止前基因组RNA在HBV衣壳内转化为部分双链DNA)；

[0170] 2)其由于所述两种药学上可接受的药剂的重叠效应而对减少自肝中感染肝细胞的去除、消除或建立cccDNA的转录抑制或血清病毒血症的控制所需的治疗持续时间具有协同效应；以及

[0171] 3)其无需使用免疫治疗(如经教导在U.S.2014/0065102中具体所需)，以在治疗后达到HBV感染的持续控制，鉴于免疫治疗在许多患者中耐受性较差，这将是重要的治疗改进。

[0172] 利用上述方法改进的抗病毒效果在具有HBV单纯感染和HBV/HDV共感染的患者中将具有相同的治疗益处，因为在上述HDV感染不存在下，HDV感染不可存在。

[0173] 因此，在可以在大比例的患者中消除或建立cccDNA活性的持久控制而无需使用免疫治疗的任何当前治疗方案不存在下，本文第一次提供抵抗HBV感染和HBV/HDV共感染的有效组合治疗，所述组合治疗同时减少或清除血液的HBsAg且其在HBV感染的细胞的细胞核中阻断cccDNA补充。这些效果可以通过使用与药学上可接受的核苷/核苷酸类似物HBV聚合酶抑制剂组合使用的药学上可接受的硫代磷酸酯化NAP制剂来实现。

[0174] 该新颖组合方法在免疫治疗不存在下是有效的，其具有改善的治疗耐受性和减少

利用免疫治疗发生的已知的血液学和其它副作用的发生率的重要的优点。

[0175] 术语寡核苷酸(ON)是指核糖核酸(RNA)和/或脱氧核糖核酸(DNA)的低聚物或聚合物。该术语包括由修饰的核碱基(包括5'甲基胞嘧啶和4'硫尿嘧啶)、糖和共价核苷间(主链)键构成的ON,以及具有非天然存在的部分的功能类似的ON。这种修饰或取代的ON可以由于诸如以下的期望的性质而优于自然形式:例如,免疫反应性降低、细胞摄取增强、对核酸靶标的亲和力增强(在反义ON、siRNA和shRNA的情况下)和/或对核酸酶介导的降解的稳定性提高。ON也可以是双链的。ON也包括单链分子,诸如反义寡核苷酸、Speigelmers和适配体和miRNA,以及双链分子,诸如小干扰RNA(siRNA)或小发夹RNA(shRNA)。

[0176] ON可以包括各种修饰,例如稳定修饰,并且因此可以在磷酸二酯键中和/或在糖和/或碱基上包括至少一种修饰。例如,ON可包括但不限于,一种或多种修饰,或可完全经修饰以便含有具有所述的修饰的所有键或糖或碱基。经过修饰的键可以包括硫代磷酸酯键和二硫代磷酸酯键。当经过修饰的键有用时,ON可以包括磷酸二酯键。额外有用的修饰包括但不限于,在糖的2'位置的修饰,包括2'-O-烷基修饰,诸如2'-O-甲基修饰、2'O-甲氧基乙基(2'MOE)、2'-氨基修饰、2'-卤素修饰,诸如2'-氟;无环核苷酸类似物。其他2'修饰也是本领域中熟知的并且可加以使用,诸如锁核酸。具体地,ON具有遍及各处的经修饰的键或具有经修饰的每个键,例如,硫代磷酸酯;具有3'-帽和/或5'-帽;包括末端3'-5'键;ON是或包括由两个或多个由连接体结合在一起的ON序列组成的多联体。碱基修饰可包括胞嘧啶碱基的5'甲基化(5'甲基胞嘧啶或在核苷酸的情况下5'甲基胞苷)和/或尿嘧啶碱基的4'硫基化(4'硫尿嘧啶或在核苷酸的情况下4'硫尿苷)。当合成条件是化学相容的,则可组合不同的化学相容的修饰的键,例如具有带有硫代磷酸酯键、2'核糖修饰(例如2'O-甲基化)和经修饰的碱基(例如5'甲基胞嘧啶)的寡核苷酸。可利用所有这些不同的修饰(例如每个硫代磷酸酯化键、每个2'修饰的核糖和每个经修饰的碱基)进一步完全修饰ON。

[0177] 如本文所涵盖的,术语"核酸聚合物"或NAP是不含有与核酸靶标杂交或采用引起与特定蛋白结合的序列特异性二级结构的序列特异性功能性的任一单链ON。NAP的生物化学活性并非取决于ON的Toll样受体识别、与靶标寡核苷酸的杂交或要求源于所存在的核苷酸的特定顺序的特定二级/三级ON结构的适配体相互作用。NAP可包括如上所述的碱基和或键或糖修饰。NAP需要硫代磷酸酯化以具有抗病毒活性。示例性抗病毒NAP化合物列于表1中:

[0178] 表格1

[0179] 可用于本公开的抗病毒NAP的实例

[0180]

核酸类型	序列 (5' – 3')	修饰
DNA	$(dAdC)_{20}$ (SEQ ID NO: 2)	所有键 PS
DNA	$(dCdA)_{20}$ (SEQ ID NO: 1)	所有键 PS
DNA	$(dA\text{-}5'MedC)_{20}$ (SEQ ID NO: 3)	所有键 PS
DNA	$(5'MedC\text{-}dA)_{20}$ (SEQ ID NO: 4)	所有键 PS
RNA	$(AC)_{20}$ (SEQ ID NO: 5)	所有键 PS 所有核糖具有 2'OMe 修饰
RNA	$(CA)_{20}$ (SEQ ID NO: 6)	所有键 PS

[0181]

核酸类型	序列 (5' – 3')	修饰
		所有核糖具有 2'OMe 修饰
DNA	$(dTdG)_{20}$ (SEQ ID NO: 7)	所有键 PS
DNA	$(dGdT)_{20}$ (SEQ ID NO: 8)	所有键 PS
RNA	$(5'MeC\text{-}A)_{20}$ (SEQ ID NO: 9)	所有键 PS 所有核糖具有 2'OMe 修饰
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 10)	所有键 PS 所有核糖具有 2'OMe 修饰
RNA / DNA	$(A\text{-}5'MedC)_{20}$ (SEQ ID NO: 11)	所有键 PS 核糖腺苷上的所有核糖经过 2'OMe 修饰
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 12)	所有键 PS 所有核糖具有 2'OMe 修饰，除了位置 13 和 27 (其为 2'H)的核糖腺苷之外
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 13)	所有键 PS 所有核糖都具有 2'OMe 修饰，除了位置 11、21 和 31 (其为 2'H) 的核糖腺苷之外
RNA	$(A\text{-}5'MeC)_{20}$ (SEQ ID NO: 14)	所有键 PS 所有 5'MeC 核糖经过 2'OMe 修饰
RNA / DNA	$(dA\text{-}5'MeC)_{20}$ (SEQ ID NO: 15)	所有键 PS 所有 5'MeC 核糖经过 2'OMe 修饰
RNA / DNA	$(5'MedC\text{-}A)_{20}$ (SEQ ID NO: 16)	所有键 PS 所有 A 核糖经过 2'OMe 修饰
RNA	$(5'MeC\text{-}A)_{20}$ (SEQ ID NO: 17)	所有键 PS

[0182]

核酸类型	序列 (5’－3’)	修饰
		所有核糖具有 2’OMe 修饰，除了位置 14 和 28 (其为 2’H) 的核糖腺苷之外
RNA	$(5’MeC\text{-}A)_{20}$ (SEQ ID NO: 18)	所有键 PS 所有核糖具有 2’OMe 修饰，除了位置 10、20 和 30 (其为 2’H) 的核糖腺苷之外
RNA	$(5’MeC\text{-}A)_{20}$ (SEQ ID NO: 19)	所有键 PS 所有 5’MeC 核糖经过 2’OMe 修饰
RNA/DNA	$(5’MeC\text{-}dA)_{20}$ (SEQ ID NO: 20)	所有键 PS 所有 5’MeC 核糖经过 2’OMe 修饰

[0183] dA＝脱氧腺苷，A＝腺苷，dC＝脱氧胞苷，C＝胞苷，dT＝脱氧胸苷，dG＝脱氧鸟苷，PS＝硫代磷酸酯，2’OMe＝2’O甲基，5’MeC＝5’甲基胞嘧啶修饰的胞苷，5’MedC＝5’甲基胞嘧啶修饰的脱氧胞苷

[0184] 在本公开中，术语“ON螯合复合物”是指两个或更多个在分子间通过二价或多价金属阳离子连接且可在单链或双链ON下发生的ON。ON螯合复合物中和ON的固有螯合性质，这可有助于利用这些化合物的与施用相关的副作用。ON螯合复合物的施用是向对象施用ON的方法，其中与未螯合ON(其是作为本领域中常用的钠盐来施用的ON)相关联的施用相关的副作用有所减轻，如U.S.8,513,211和8,716,259中所述，其通过引用全部并入本文。这些副作用可包括在静脉内输注或硬结下的寒战、发热和寒冷、在皮下施用下的注射部位的炎症和疼痛。ON螯合复合物的施用在通常作为钠盐使用时并不干扰ON的生物化学活性。因此本文所述的任何NAP可以任选地被制备成ON螯合复合物而不影响其生物化学活性。

[0185] ON螯合复合物可以含有多种不同的多价金属阳离子，包括钙、镁、钴、铁、锰、钡、镍、铜、锌、镉、汞和铅。进一步表明，这些多价金属阳离子的螯合使得形成包含两个或更多个经由金属阳离子连接的ON的ON螯合复合物，且在长度超过6个核苷酸的ON下和在具有磷酸二酯或硫代磷酸酯键的ON存在下发生。ON可以任选地具有每个硫代磷酸酯化的键。螯合也在核糖处含有2’修饰(如2’O甲基)或含有修饰的碱基，如5’甲基胞嘧啶或4-硫尿嘧啶的ON下发生。这些2’修饰可以存在于一个或多个或所有核糖上，且修饰的碱基可以存在于一个或多个碱基上或普遍存在于每个碱基上(即，所有的胞嘧啶都作为5’甲基胞嘧啶存在)。此外，ON螯合复合物可包含含有多个修饰，如每个硫代磷酸酯化的键、每个2’修饰的核糖和每个修饰的碱基的ON。与ON螯合复合物形成相容的ON修饰在上文被进一步定义。此外，金属阳离子的螯合并非取决于所存在的核苷酸的序列而是依赖于所有ON共同的理化特征。

[0186] 虽然ON螯合复合物的形成可用任何二价金属阳离子实现，但是意欲用作医药的ON螯合复合物应优选只包含钙和或镁，但是也可以包含微量的铁、锰、铜或锌且不应包括钴、钡、镍、镉、汞、铅或本文未列出的任何其它二价金属。

[0187] 如U.S.2014/0065192中所述，通过硫代磷酸酯化NAP从感染患者的血液去除HBsAg

使得部分恢复免疫应答，从而从血液去除HBVe-抗原(HBeAg)，并且使得在治疗期间血液中的病毒水平实质上降低，但是这些抗病毒效果在治疗停止后在大多数患者中未得到维持。尽管免疫应答的这部分恢复(在HBsAg及其它病毒抗原不存在下)可使得在停止治疗后在一小部分患者中建立HBV感染的持久免疫学控制，但期望在甚至更大部分患者中建立感染的持久免疫学控制。在治疗后持久免疫学控制的患者的比例的改进可以通过以下来实现：与其它抗病毒药剂组合使用硫代磷酸酯化NAP来改进对治疗的抗病毒反应的速度和效力。可期望避免使用诸如基于干扰素的治疗等免疫治疗或其他免疫治疗，因为这些治疗通常与使患者更加难以耐受治疗的副作用相关。

[0188] 本文所用的术语“从血液去除HBsAg”是指相对于治疗前血液中HBsAg的浓度HBsAg血液中HBsAg浓度的任何统计学显著降低，如通过Abbott Architect™定量HBsAg分析或血清HBsAg的其他临床认可的定量测量测得。

[0189] 针对硫代磷酸酯化NAP的示范性有效的给药方案遵循如U.S.2014/0065102中所述的通常用于其他硫代磷酸酯化ON(如反义寡核苷酸)的那些；本领域中充分确立每周肠胃外施用100-500mg化合物的常规使用，以在肝中实现这些化合物的治疗活性水平，如针对以下实施例I中的NAP和针对引起肝特异性mRNA的降解的硫代磷酸酯化反义ON(对于载脂蛋白B100)所述，如Akdim等(2010,Journal of the American College of Cardiology,55:1611-1618)所述。

[0190] 因此，根据本文中所呈现的公开内容，利用与药学上可接受的核苷/核苷酸HBV聚合酶抑制剂组合的药学上可接受的硫代磷酸酯化NAP制剂治疗具有HBV感染或HBV/HDV共感染的对象是有用的。

[0191] 在同一药物组合物中施用两种药学上可接受的药剂或在单独的药物组合物中同时或在不同时间施用两种药学上可接受的药剂也是有用的。

[0192] 通过相同或不同的施用途径施用药学上可接受的药剂是有用的。

[0193] 为了在对象中提供最可能的抗病毒反应，可能有必要使用一种以上的HBV聚合酶抑制剂以最大限度地阻断HBV聚合酶，且因此对阻断cccDNA的补充具有最大效果。因此，一种或多种HBV聚合酶抑制剂可选自以下核苷类似物：

[0194] 拉米夫定；

[0195] 阿德福韦酯；

[0196] 恩替卡韦；

[0197] 替比夫定；

[0198] 富马酸替诺福韦二吡呋酯；

[0199] 恩曲他滨；

[0200] 克来夫定；

[0201] 贝斯福韦；

[0202] 富马酸替诺福韦艾拉酚胺；

[0203] AGX-1009；

[0204] 艾夫他滨；

[0205] 拉昔洛韦瓦拉他特；

[0206] 甲磺酸帕拉德福韦；

[0207] 伐托他滨;和

[0208] 抑制HBV聚合酶的任意核苷/核苷酸类似物。

[0209] 本文所述的组合物可通过任何合适的手段来施用,例如,经口,如以片剂、胶囊、颗粒或粉末形式;经舌下;经颊;肠胃外,如通过皮下、静脉内、注射或输注技术(例如,以无菌可注射的水或非水溶液或悬浮液);通过吸入;局部,例如以乳膏或软膏的形式;或经直肠,例如以栓剂或灌肠剂的形式;在含有无毒性的药学上可接受的载体或稀释剂的剂量单位制剂中。例如,可以适于立即释放或延长的释放的形式施用本组合物。可通过使用合适的药物组合物或者特别是在延长的释放的情况下通过使用诸如皮下植入物或渗透泵等装置实现立即释放或延长的释放。因此,上述组合物可适于通过以下途径中的任一个来施用:经口摄取、吸入、皮下注射、静脉内注射或输注、或局部。

[0210] 通过参考下面的例子将更容易地理解本公开内容。

[0211] 实施例I

[0212] 组合NAP/ETV治疗对血清HBsAg的效果

[0213] 通过每周一次IV输注400mg将NAP REP 2055(SEQ ID NO:2)的药学上可接受的制剂施用给具有慢性HBV感染的患者。使用合格的现场定性ELISA每周实时监测该患者中的血清HBsAg反应。该ELISA方法对HBsAg的低水平非常敏感,但不能准确地定量血液中的任何显著的HBsAg浓度。虽然在REP 2055单一治疗(图1,正方形)期间中使用此HBsAg分析未观察到血清HBsAg的可检测的降低,但是该患者经历了血清病毒血症(血清HBV DNA)的非常温和的(约1log)下降,这表明已经出现某些抗病毒反应。因此,在29周的REP 2055单一治疗后,该患者除了由每天口服0.5mg恩替卡韦组成的现有REP 2055治疗以外接受HBV聚合酶抑制治疗。

[0214] 在两周的起始组合REP 2055/ETV治疗内通过定性分析检测到血清HBsAg立即减少,且血清HBsAg在开始组合治疗后4周内在定性ELISA中变得检测不到(图1,正方形)。经过许多周的治疗,利用组合的REP2055/ETV治疗对这种血清HBsAg的协同控制得以维持。

[0215] 为证实组合REP 2055/ETV治疗对抑制HBsAg的协同活性,使用IMPACT平台重新分析该患者的血清样品以将血清HBsAg水平准确定量,如de Neit等(2014,Antiviral Ther.,19:259-267)所述。这种定量分析显示利用REP 2055单一治疗发生的血清HBsAg的初始约2log减少(图1圆形),这通过定性ELISA是无法检测的并且这可能是在上述REP 2055单一治疗后所观察到的病毒血症下降约1log的原因。重要的是,该患者中的血清HBsAg减少达到平台期,其中从10周的REP 2055治疗开始直到在29周治疗时开始组合REP 2055/ETV治疗,显著血清HBsAg是稳定存在的。在开始组合REP 2055/ETV治疗下,血清HBsAg的定量分析表明血清HBsAg几乎相同且快速的减少,如利用现场定性测试观察到的,且这些额外的减少超过1.5log,这也可在开始组合REP 2055/ETV治疗后的4周内实现。

[0216] 在REP 2055单一治疗存在下的血清HBsAg低水平的持续性显示具有转录活性的cccDNA仍然存在于该患者的肝中。通过向现有REP 2055治疗中添加ETV对血清HBsAg的非常快速额外清除指示对cccDNA转录控制的协同效应和或已发生消除。重要的是,此额外控制的cccDNA的发生的出现远远快于在单一治疗中所使用的HBV聚合酶抑制剂下所观察到的,其只需要4周来实现。因此,这些观察结果证明当与HBV聚合酶抑制剂(在这种情况下恩替卡韦)组合时,对血清HBsAg减少具有新颖协同抗病毒效果(在这种情况下使用NAP REP 2055

实现)。

[0217] 实施例II

[0218] 各种NAP在DHBV感染的北京鸭中的抗病毒效果

[0219] 在DHBV感染的北京鸭中测试包含不同核酸修饰的各种NAP来建立其抗病毒活性。这些NAP是REP 2055(SEQ ID NO:2)、REP 2139(SEQ ID NO:10)、REP 2163(SEQ ID NO:11)和REP 2165(SEQ ID NO:13)。表2提供这些NAP的化学描述。

[0220] 表2

[0221] 用于实施例II中的NAP的描述

[0222]

NAP	序列	所存在的寡核苷酸修饰
REP 2055 (SEQ ID NO: 2)	$(dAdC)_{20}$	每个键经过硫代磷酸酯化
REP 2139 (SEQ ID NO: 10)	$(A, 5'MeC)_{20}$	每个键经过硫代磷酸酯化 每个核糖经过 2'O 甲基化
REP 2163 (SEQ ID NO: 11)	$(A, 5'MedC)_{20}$	每个键经过硫代磷酸酯化 仅腺苷中的核糖经过 2'O 甲基化
REP 2165 (SEQ ID NO: 13)	$(A, 5'MeC)_{20}$	每个键经过硫代磷酸酯化 每个核糖经过 2'O 甲基化，除了其中核糖是 2'OH 的 11、21 和 31 位置处的腺苷之外

[0223] dA=脱氧核糖腺苷

[0224] dC=脱氧核糖胞苷

[0225] A=核糖腺苷

[0226] 5'MeC=核糖-5'甲基胞苷

[0227] 5'MedC=脱氧核糖=5'甲基胞苷

[0228] 用2×10^{11}个病毒基因组等效物(VGE)/ml的DHBV感染三日龄的北京雏鸭。已经确立在感染后11天后开始NAP治疗。经由每周3次腹膜内注射10mg/kg NAP(配制成钙螯合复合物)达三周施用NAP,随后在治疗结束时分析抗病毒效果。利用生理盐水经由相同的施用途径且利用相同的给药方案处理对照组。通过ELISA监测血清DHBsAg(图2A)和通过定量PCR监测肝DHBV DNA(图2B)来评价抗病毒活性。

[0229] 所有NAP皆导致血清DHBsAg和肝DHBV DNA的减少,这表明含多种不同的寡核苷酸修饰的不同NAP将具有相当的抗病毒效应。这又表明使用特定的NAP和基于一种或多种核苷类似物的HBV聚合酶抑制剂观察到的协同抗病毒活性(如利用上述实施例I中的REP 2055和恩替卡韦观察到的)将在任何其它硫代磷酸酯化NAP下出现并且也在配制成螯合复合物的任何所述硫代磷酸酯化的NAP下出现(如U.S.8,513,211和8,716,259中所述)。

[0230] 实施例III

[0231] NAP与TDF和ETV的组合在DHBV感染的北京鸭中的抗病毒效果

[0232] 通过在治疗期间和在治疗后通过定量PCR评估血清和肝脏DHBV DNA的水平的变化，检查REP 2139的钙螯合复合物(REP 2139-Ca)和TDF或REP 2139-Ca和TDF与ETV的组合治疗在DHBV感染的北京鸭中的抗病毒效果。如实施例II中所述实施鸭的感染，只是治疗在感染后一个月开始。治疗方案如下：

[0233] 1)生理盐水，通过IP注射给予，每周3次，保持4周

[0234] 2)TDF，通过经口胃管灌食法给予15mg/天，保持28天

[0235] 3)REP 2139-Ca，通过IP注射给予10mg/kg，每周3次，保持4周。

[0236] 4)REP 2139-Ca和TDF(如上给药)

[0237] 5)REP 2139-Ca和TDF(如上给药)，和ETV，通过经口胃管灌食法给予1mg/天，保持28天。

[0238] 在治疗前(时间点A)、在治疗的第14天(时间点B)、在治疗结束时(时间点C)和在治疗停止后的一个月和两个月(随访，时间点D和E)评价血清DHBV DNA。

[0239] 在生理盐水治疗组中，在治疗期间没有观察到DHBV DNA的控制，但在随访期间在这组中的3只鸭中DHBV DNA变得自发地受控制(图3A)。在TDF治疗组中，血清DHBV DNA在所有鸭中皆有所减少，但直到治疗结束才在所有鸭中实现控制。在随访期间该组中的所有鸭中的DHBV DNA皆反弹(图3B)。在REP 2139-Ca治疗组中，在整个研究期间在两只鸭中没有观察到DHBV DNA变化，DHBV DNA在治疗结束时仅在两个鸭中得到控制并且在随访期间在两只额外的鸭中变得自发地受控制(图3C)。当将REP 2139-Ca与TDF组合时，DHBV DNA的控制在治疗进行到一半时在除一只鸭以外的所有鸭中发生且通常快于在利用单独REP 2139-Ca或TDF治疗的组中达到的控制。当将REP 2139-Ca与TDF和ETV组合时，可在治疗进行一半时在所有的鸭中控制DHBV(3E)。利用组合的REP 2139-Ca及TDF(或TDF及ETV)比利用单独TDF或REP 2139-Ca在随访期间维持血清DHBV DNA的控制的鸭的比例大。

[0240] 这些观察教导，与利用单独REP 2139-Ca或TDF实现的相比，HBV感染中的治疗过程中的抗病毒反应可通过结合REP 2139-Ca和TDF或TDF和ETV来协同地改进并且可导致在停止治疗后改进持续的病毒学反应。在上述实施例中观察到的协同活性可以可靠地预计利用如本文所述的任何NAP抗HBV活性和利用如本文所述的基于任何核苷酸/核苷类似物HBV聚合酶抑制剂而发生。此外，也可使用与一种以上的核苷/核苷酸HBV聚合酶抑制剂组合使用的NAP，其中具有类似地产生性协同抗病毒效果。

[0241] 组合的NAP/TDF/ETV治疗的协同效应可改进抗病毒反应的速度，这表明了缩短能够在停止治疗后实现持续病毒学反应的治疗方案的潜力。该潜力也可利用如本文所述的NAP和核苷酸/核苷类似物HBV聚合酶抑制剂治疗的任何组合来实现。

[0242] 因此，这些观察结果教导，从血液中减少或移除HBsAg的任何药学上可接受的硫代磷酸酯化NAP制剂(如U.S.2014/0065102和U.S.8,008,269、8,008,270和8,067,385中所述的)可以与如上述所列出的任何核苷/核苷酸HBV聚合酶抑制剂组合并且预计可对可控制血清病毒血症的速度和或HBV cccDNA的转录失活和或消除实现协同效应。所观察到的协同效应也教导，可以组合较低剂量的所述药学上可接受的药剂，并且仍然实现协同活性以及有用的抗病毒效果。

[0243] 鉴于利用与一种核苷/核苷酸HBV聚合酶抑制剂组合使用的一种硫代磷酸酯化NAP在上文观察到的协同抗病毒效果，利用与如上文所述的一种或多种核苷/核苷酸HBV聚合酶

抑制剂组合使用的一种或多种硫代磷酸酯化NAP也可实现优异的协同效应。

[0244] 上面的描述仅仅意欲为示例性的，并且本领域的技术人员将认识到可对所述实施例作出改变而不脱离由所附权利要求定义的本发明的范围。本领域技术人员根据本发明的综述将了解落入如所附权利要求定义的本发明的范围之内的其它修改，而不脱离由所附的权利要求定义的本发明的范围。

序列表

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<120> 治疗B型肝炎和D型肝炎感染的方法

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<151> 2014-07-10

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甲基胞苷

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5' 甲基化和 2'O 甲基核糖修饰

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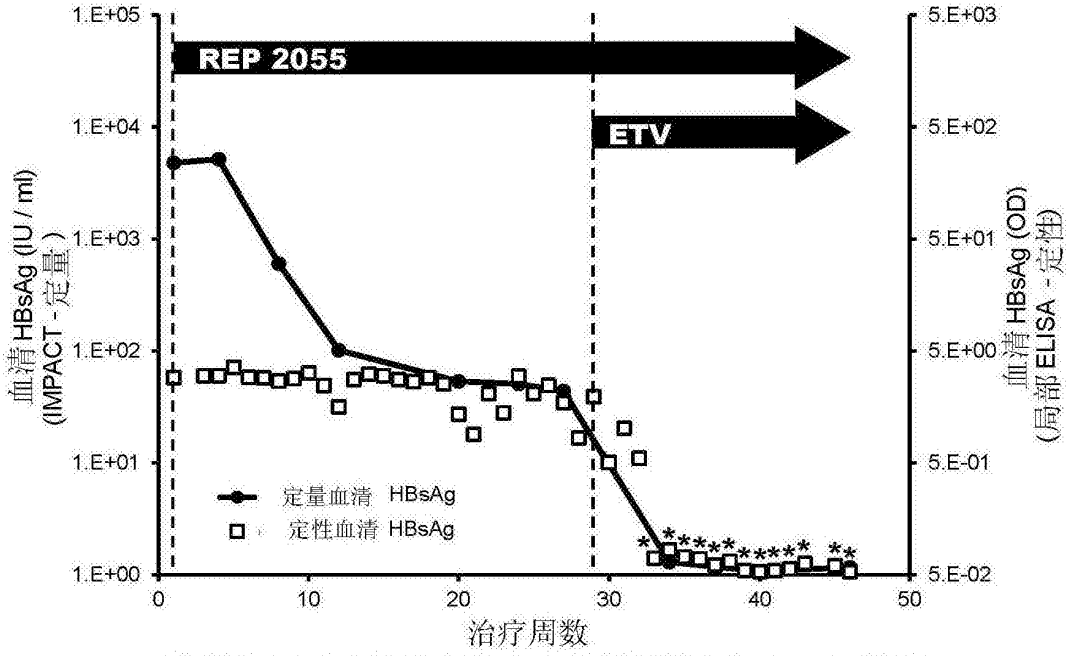
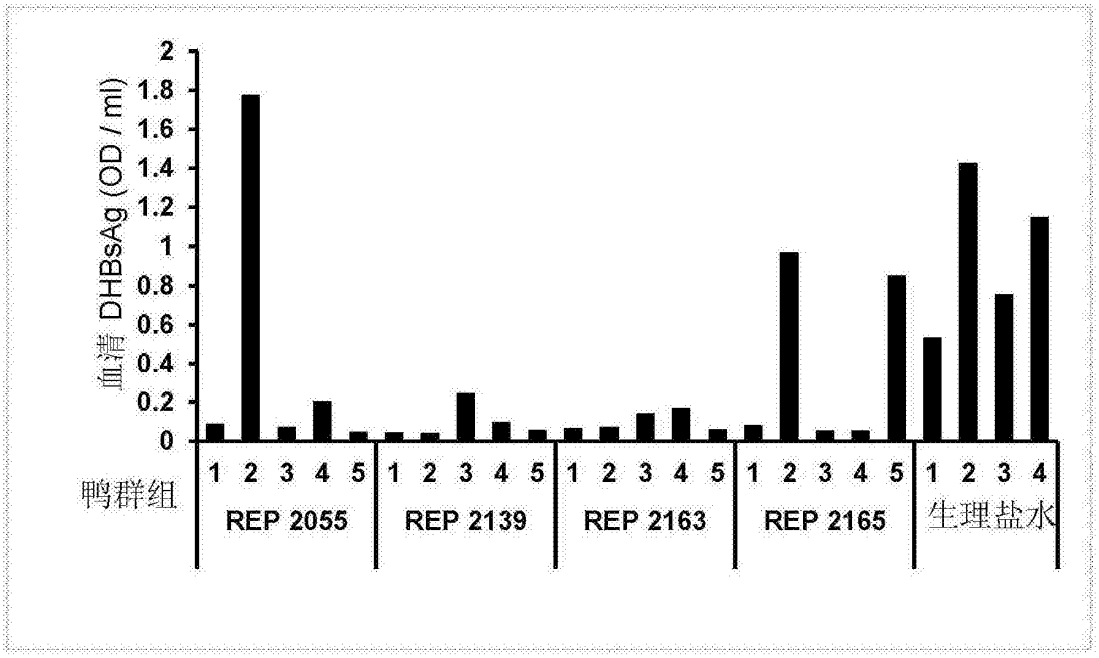


*在局部ELISA中低于血清HBsAg检测的截止值（HBsAg阴性）

图1



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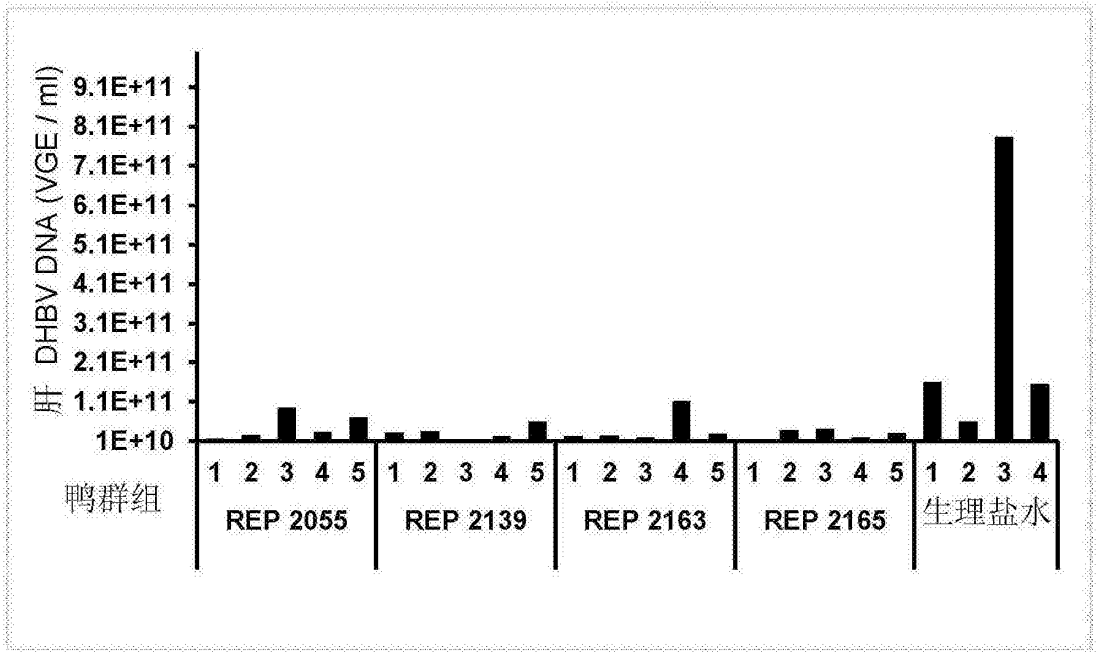
肝 DHBV DNA (VGE / ml)
9.1E+11
8.1E+11
7.1E+11
6.1E+11
5.1E+11
4.1E+11
3.1E+11
2.1E+11
1.1E+11
1E+10
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4
鸭群组
REP 2055
REP 2139
REP 2163
REP 2165
生理盐水

图2B

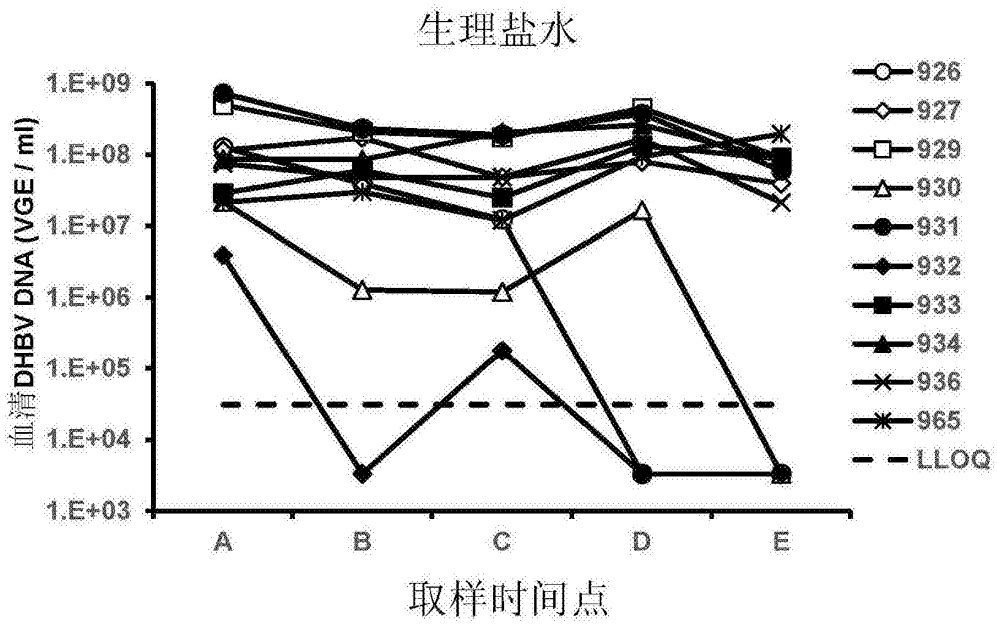
生理盐水
血清DHBV DNA (VGE / ml)
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
A
B
C
D
E
取样时间点
926
927
929
930
931
932
933
934
936
965
LLOQ

图3A

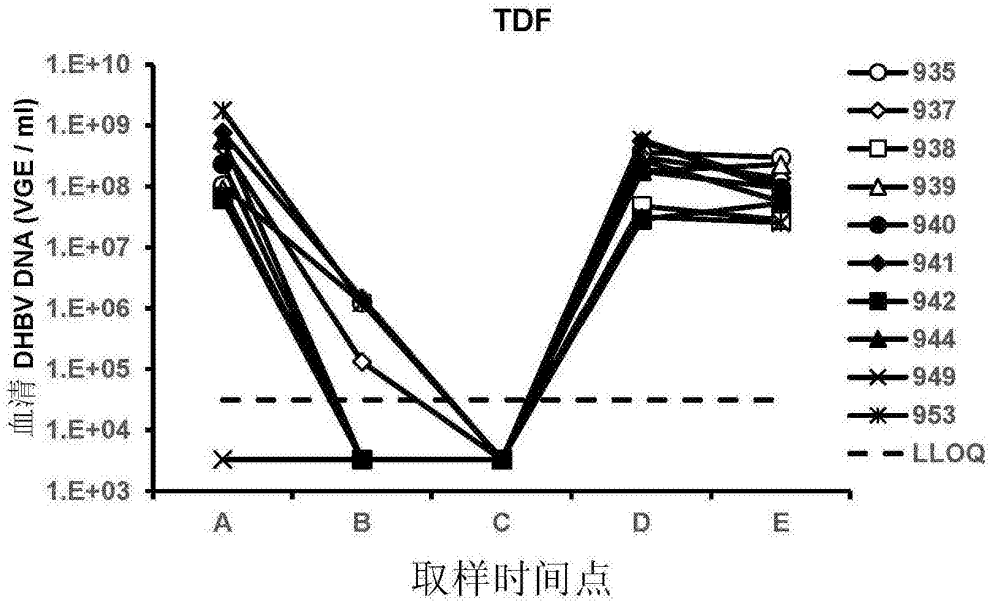
TDF
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
血清 DHBV DNA (VGE / ml)
A
B
C
D
E
取样时间点
935
937
938
939
940
941
942
944
949
953
LLOQ

图3B

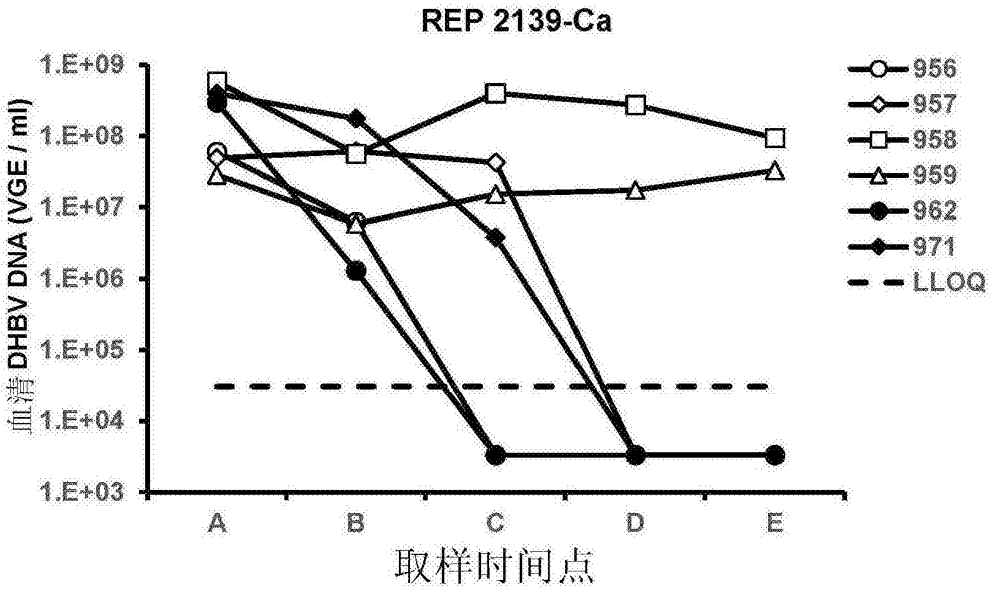
REP 2139-Ca
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
血清 DHBV DNA (VGE / ml)
A
B
C
D
E
取样时间点
956
957
958
959
962
971
LLOQ

图3C

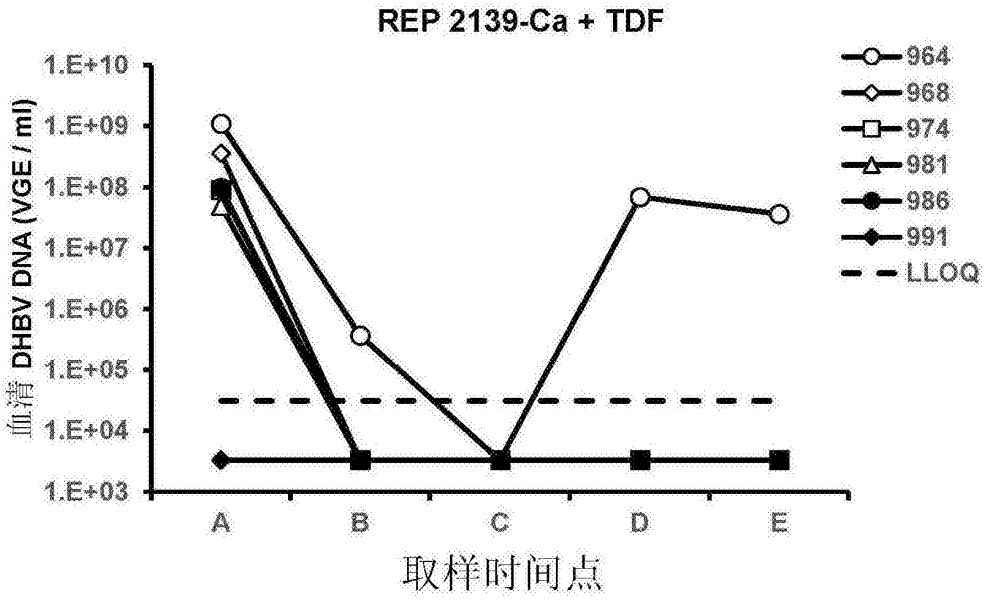
REP 2139-Ca + TDF
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
血清 DHBV DNA (VGE / ml)
A
B
C
D
E
取样时间点
964
968
974
981
986
991
LLOQ

图3D

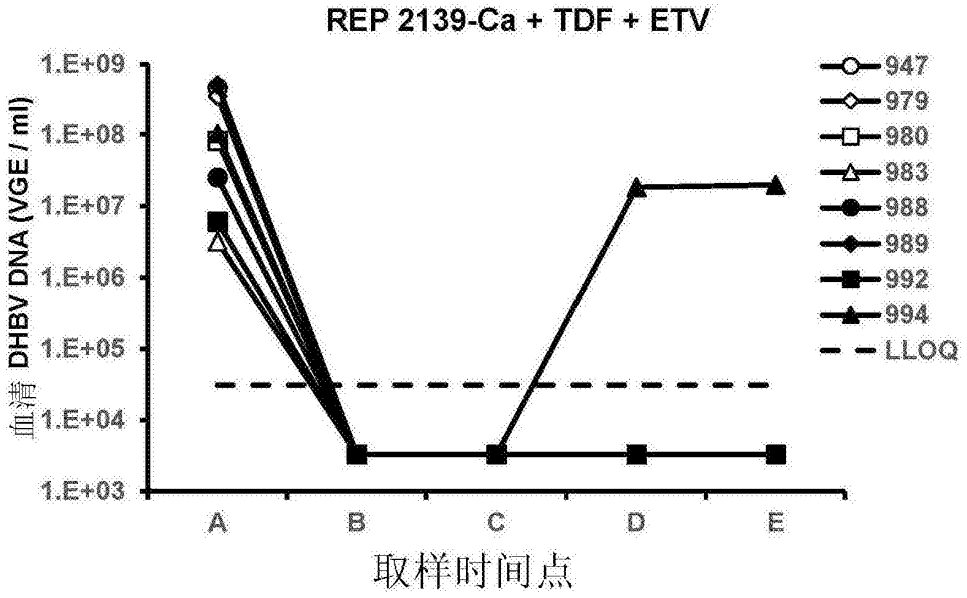
REP 2139-Ca + TDF + ETV
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
血清 DHBV DNA (VGE / ml)
A
B
C
D
E
取样时间点
947
979
980
983
988
989
992
994
LLOQ

图3E