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(54) Title:  COMPOUNDS AND METHODS FOR TREATING VIRAL INFECTIONS

(55) Abstract:  Oligonucleotide conjugates including sequence independent oligonucleotide conjugates having at least one subunit of formula (I) as well as chelates of oligonucleotide conjugates are disclosed. Methods of using such oligonucleotide conjugates and oligonucleotide conjugate chelates for the treatment of viral infections, including HBV infections or HBV/HDV co-infections are also disclosed, the method comprising administering to a subject in need of treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate or chelate thereof and an optional second pharmaceutically acceptable agent which stimulates immune function and an 10 optional third pharmaceutically acceptable agent comprising an antiviral compound.

![Figure 1]

**FIGURE 1**
Declarations under Rule 4.17:
— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))

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COMPOUNDS AND METHODS FOR TREATING VIRAL INFECTIONS

Sequence Listing

This application contains sequences, listed in an electronic Sequence Listing created on August 26, 2015 in a file titled PT65476WO_SEQ_LIST, 3 KB in size and filed herewith, the contents and sequences of which are hereby incorporated by reference herein.

FIELD OF THE INVENTION

[0001] The present description relates to compounds, compositions, and methods for treating a subject having a viral infection with a treatment comprising an oligonucleotide-carbohydrate conjugate suitable for therapeutic delivery. Such oligonucleotide-carbohydrate conjugates can optionally be combined simultaneously or separately with conventional viral treatment regimens.

BACKGROUND

[0002] Hepatitis B virus (HBV) infection is a major worldwide public health problem. Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world. The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma. In addition, HBV carriers can transmit the disease for many years.

[0003] HBV is transmitted through percutaneous or parenteral contact with infected blood, body fluids, and by sexual intercourse. HBV is able to remain on any surface it comes into contact with for about a week, e.g. table-tops, razor blades, blood stains, without losing infectivity. However, HBV cannot cross the skin or the mucous membrane barrier. Some break in this barrier, which can be minimal and insignificant, is required for transmission.

[0004] HBV is a small enveloped DNA virus belonging to the hepadnavirus family. The virus replicates through an RNA intermediate form by reverse transcription, which in practice relates them to retroviruses, like HIV. Although replication takes place in the liver, the virus spreads to the blood where viral proteins and antibodies against them
are found in infected people. HBV is many times more infectious than HIV due to the greater concentrations of HBV virus found in the bloodstream at any given time.

[0005] HBV infection results in the production of two different particles: 1) the HBV virus itself (or Dane particle) which includes a viral capsid assembled from the HBV core antigen protein (HBcAg) and is covered by the hepatitis B surface antigen (HBsAg) and is capable of reinfesting cells and 2) subviral particles (or SVPs) which are high density lipoprotein-like particles comprised of lipids, cholesterol, cholesterol esters and the small and medium forms of the hepatitis B surface antigen (HBsAg) which are non-infectious. For each viral particle produced, 1,000-10,000 SVPs are released into the blood. As such SVPs (and the HBsAg protein they carry) represent the overwhelming majority of viral protein in the blood. HBV infected cells also secrete a soluble proteolytic product of the pre-core protein called the HBV e-antigen (HBeAg).

[0006] The hepatitis D virus (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 fulminant hepatitis. As such, the clear unmet medical need in HBV infection is even more pressing in HBV/HDV co-infected subjects.

[0007] The current conventional methods of treatment for HBV include interferon or thymosin α1-based immunotherapies and the suppression of viral production by inhibition of the HBV polymerase (e.g., "nucsl"). HBV polymerase inhibitors are effective in reducing viral production, but have little to no effect in rapidly reducing HBsAg blood levels or can slowly reduce HBsAg with long term treatment in a limited number of patients (as is the case with tenofovir disoproxil fumarate). Interferon based immunotherapy can achieve a reduction of both viral production and early removal of HBsAg from the blood but only in a small percentage of treated subjects. The generally accepted role of HBsAg in the blood is to 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 is likely having a significant impact in preventing patients from achieving immunological control of their HBV infection.

[0008] Although the three primary HBV proteins (HBsAg, HBeAg and HBCAg) all have immunoinhibitory properties (see below), HBsAg comprises the overwhelming majority of HBV protein in the circulation of HBV infected subjects. Additionally, while the removal (via seroconversion) of HBeAg 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 seroconversion) 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). 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 alone is likely sufficient in and of itself to remove the bulk of the viral inhibition of immune function in subjects with HBV infection.

[0009] Therefore, in the absence of any current treatment regimen which can restore immunological control of HBV in a large proportion of patients, there is a need to be provided with an effective treatment against HBV infection and HBV/HDV co-infection which can restore immunological control in the majority of patients.

[0010] Recently, there has been a growing interest in the potential application of oligonucleotide technology to the treatment of viral disease. Antisense methodology is the complementary hybridization of relatively short oligonucleotides to mRNA or DNA such that the normal, essential functions, such as protein synthesis, of these intracellular nucleic acids are disrupted. Hybridization is the sequence-specific hydrogen bonding via Watson-Crick base pairs of oligonucleotides to RNA or single-stranded DNA. Such base pairs are said to be complementary to one another.

[0011] The naturally-occurring events that alter the expression level of the target sequence, discussed by Cohen (Oligonucleotides: Antisense Inhibitors of Gene Expression, CRC Press, Inc., 1989, Boca Raton, Fla.) are thought to be of two types. The first, hybridization arrest, describes the terminating event in which the oligonucleotide inhibitor binds to the target nucleic acid and thus prevents, by simple steric hindrance, the binding of essential proteins, most often ribosomes, to the nucleic acid. Methyl phosphonate oligonucleotides (Miller et al. (1987) Anti-Cancer Drug Design, 2, 117-128), and .alpha.-anomer oligonucleotides are the two most extensively studied antisense agents which are thought to disrupt nucleic acid function by hybridization arrest.
Another means by which antisense oligonucleotides alter the expression level of target sequences is by hybridization to a target mRNA, followed by enzymatic cleavage of the targeted RNA by intracellular RNase H. A 2'-deoxyribofuranosyl oligonucleotide or oligonucleotide analog hybridizes with the targeted RNA and this duplex activates the RNase H enzyme to cleave the RNA strand, thus destroying the normal function of the RNA. Phosphorothioate oligonucleotides are the most prominent example of an antisense agent that operates by this type of antisense terminating event.

Lebedeva & Stein (Lebedeva et al (2001) Annul Rev Pharmacol 41:403-419) report a variety of non-specific protein binding activity of PS-ODNs, including viral proteins. US Patent No. 8,067,385 ("the '385 patent"), incorporated by reference herein, reports the discovery that oligonucleotides (ONs), e.g., oligodeoxynucleotides (ODNs), can have a broadly applicable, non-sequence complementary (i.e., sequence independent) antiviral activity and that it is not necessary for the oligonucleotide to be complementary to any viral sequence or to have a particular distribution of nucleotides in order to have antiviral activity. Such an oligonucleotide can even be prepared as a randomer, such that there will be at most a few copies of any particular sequence in a preparation, e.g., in a 15 micromol randomer preparation 32 or more nucleotides in length.

The '385 patent reports that different length oligonucleotides have varying antiviral effect, and that the length of antiviral oligonucleotide that produces maximal antiviral effect is in the range of 40-120 nucleotides.

By way of another example, nucleic acid polymers ("NAP") are broad-spectrum antiviral compounds that use the sequence-independent properties of phosphorothioate oligonucleotides as amphipathic polymers to block amphipathic interactions involved in viral entry. See, Noordeen, et al., Antimicrob Agents Chemother. 2013; 57(1 1): 5299-5306.

Such sequence independent oligonucleotide compounds that have antiviral activity have been described and can be made as disclosed in U.S. patents US8,716,259, US8,513,211, US8,318,701, US8,067,385, US8,008,270, US8,008,269, US7,985,581, US7,871,991 and US7,358,068; and in e.g. US Patent Publications US20140065102, US20130296410, US20130171 103 and US20130090299., Inc., the contents of which are all hereby incorporated by reference herein in their entirety. As described in US7,871,991, such phosphorothioate oligonucleotides can have a broadly applicable, non-sequence complementary antiviral activity. Further, different length oligonucleotides have varying
antiviral effects, and the length of the antiviral oligonucleotide that produces maximal antiviral effect is in the range of 40-120 nucleotides.

[0017] The opportunity to use these and other nucleic acid based therapies holds significant promise, particularly if a suitable and effective delivery system is utilized, more particularly if a suitable and effective delivery system is used that is capable of increasing efficacy, aiding in target delivery, increasing oligonucleotide half-life, and potentially improving the therapeutic window of the oligonucleotide therapeutic agent.

[0018] Despite the advances in application of oligonucleotides and oligonucleotide analogs as therapeutics, the need exists for oligonucleotides having improved pharmacological properties, e.g. serum stability, delivery to the right organ or cell and transmembrane delivery. Efforts aimed at improving the transmembrane delivery of nucleic acids and oligonucleotides have utilized protein carriers, antibody carriers, liposomal delivery systems, electroporation, direct injection, cell fusion, viral vectors, and calcium phosphate-mediated transformation. However, many of these techniques are limited by the types of cells in which transmembrane transport is enabled and by the conditions needed for achieving such transport.

[0019] Efficient delivery to cells in vivo requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. One method of achieving specific targeting is to conjugate a targeting moiety to the oligonucleotide agent. The targeting moiety helps in targeting the oligonucleotide agent to the required target site. One way a targeting moiety can improve delivery is by receptor mediated endocytic activity. This mechanism of uptake involves the movement of oligonucleotide agent bound to membrane receptors into the interior of an area that is enveloped by the membrane via invagination of the membrane structure or by fusion of the delivery system with the cell membrane. This process is initiated via activation of a cell-surface or membrane receptor following binding of a specific ligand to the receptor. Many receptor-mediated endocytotic systems are known and have been studied, including those that recognize sugars such as galactose, mannose, mannose-6-phosphate, peptides and proteins such as transferrin, asialoglycoprotein, vitamin B12, insulin and epidermal growth factor (EGF). The Asialoglycoprotein receptor (ASGP-R) is a high capacity receptor, which is highly abundant on liver hepatocytes. The ASGP-R shows a 50-fold higher affinity for N-Acetyl-D-Galactosylamine ("GalNAc") than D-Gal. As used herein, both N-acetyl-D-Galactosylamine and N-acetyl-D-Galactosamine are intended to refer to the same moiety and referenced as
"GalNAc". For purposes of the present specification, N-acetyl-D-Galactosylamine is simply the term for N-acetyl-D-Galactosamine once it is derivatized or conjugated.

[0020] By way of example, certain carbohydrate and lipid conjugates of oligonucleotide compositions and therapeutic agents have been described and can be made as disclosed in U.S. Patents, e.g. US8,598,139, US8,507,455, US8,450,467, US8,344,125 and US8,106,022 and US Patent Publications e.g. US20130338210 and US20140099666; the contents of which are hereby incorporated by reference herein in their entirety.

[0021] Thus, there is a clear need for carbohydrate conjugated oligonucleotide agents for the treatment of hepatitis B and hepatitis B/hepatitis D virus co-infections and other viral infections, that address the shortcomings of the in vivo delivery of previous viral oligonucleotide therapeutics as described above.

SUMMARY OF THE INVENTION

[0022] As described here there is provided an improvement over non-sequence specific oligonucleotides for the treatment of HBV and/or HDV in a subject, wherein the improvement comprises a non-sequence specific oligonucleotide that is conjugated on its 5' or 3' ends with at least one carbohydrate moiety. Therefore, in accordance with the present description there is provided a non-sequence specific oligonucleotide conjugate for the treatment of viral infections in a subject. In some embodiments, the non-sequence specific oligonucleotide conjugate is also effective for the treatment of certain conditions associated with having some viral infections. Such associated conditions could include a viral infection-related reduction in a patient's humoral immune response to the underlying viral infection. By improving the underlying associated condition, the present invention could then demonstrate an indirect antiviral efficacy. In particular, the present invention is directed to treatment the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection in a human subject. The present invention is also directed to non-specific oligonucleotide conjugates having at least one carbohydrate moiety attached to the oligonucleotide, either directly or with a linker ("Linker").

[0023] Therefore, in certain embodiments, the present invention provides a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues.

[0024] In a particular embodiment, there is provided a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising
administering to a subject in need of such treatment a sequence independent oligonucleotide corresponding to any one of SEQ ID NOs: 1-10 conjugated with one or more GalNAc carbohydrate residues.

[0025] In another embodiment, there is provided a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a Gal-Nac-conjugated sequence independent oligonucleotide corresponding to the compound depicted in Scheme 1.

[0026] In another embodiment, the present invention provides a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent comprising a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues in combination with a second pharmaceutically acceptable agent that stimulates immune function.

[0027] In yet another embodiment, the present invention provides a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent comprising a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues in combination with a second pharmaceutically acceptable agent that stimulates immune function and a third pharmaceutically acceptable agent comprising an antiviral compound.

[0028] In still other embodiments, the administration of the sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues inhibits the release of hepatitis B surface antigen (HBsAg) from infected hepatocytes. In a particular embodiment, the administration of the sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues comprises the administration of the compound depicted in Scheme 1.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

[0029] Throughout this application, references are made to various embodiments relating to compounds, compositions, and methods. The various embodiments described are meant to provide a variety of illustrative examples and should not be construed as descriptions of alternative species. Rather it should be noted that the descriptions of various embodiments provided herein may be of overlapping scope. The embodiments
discussed herein are merely illustrative and are not meant to limit the scope of the present invention.

[0030] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention. In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings.

[0031] DEFINITIONS

[0032] As used herein, the term "halo" refers to any radical of fluorine, chlorine, bromine or iodine. The term "alkyl" refers to saturated and unsaturated non-aromatic hydrocarbon chains that may be a straight chain or branched chain, containing the indicated number of carbon atoms (these include without limitation propyl, allyl, or propargyl), which may be optionally inserted with N, O, or S. For example, C\textsubscript{1}-C\textsubscript{10} indicates that the group may have from 1 to 10 (inclusive) carbon atoms in it. The term "alkoxy" refers to an -O-alkyl radical. The term "alkylene" refers to a divalent alkyl (i.e., -R-). The term "alkylenedioxo" refers to a divalent species of the structure -O-R-O-, in which R represents an alkylene. The term "aminoalkyl" refers to an alkyl substituted with an amino. The term "mercapto" refers to an --SH radical. The term "thioalkoxy" refers to an -S-alkyl radical.

[0033] The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like. The term "arylalkyl" or the term "aralkyl" refers to alkyl substituted with an aryl. The term "arylalkoxy" refers to an alkoxy substituted with aryl.

[0034] The term "cycloalkyi" as employed herein includes saturated and partially unsaturated cyclic hydrocarbon groups having 3 to 12 carbons, for example, 3 to 8 carbons, and, for example, 3 to 6 carbons, wherein the cycloalkyi group additionally may be optionally substituted. Cycloalkyi groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

[0035] The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or
furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like. The term "heteroaryalkyl" or the term "heteroarylalkyl" refers to an alkyl substituted with a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy substituted with heteroaryl.

[0036] The term "heterocycl" refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heterocyclic groups include trizolyl, tetrazolyl, piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

[0037] The term "oxo" refers to an oxygen atom, which forms a carbonyl when attached to carbon, an N-oxide when attached to nitrogen, and a sulfoxide or sulfone when attached to sulfur.

[0038] The term "acyl" refers to an alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, or heteroarylcarbonyl substituent, any of which may be further substituted by substituents.

[0039] The term "substituted" refers to the replacement of one or more hydrogen radicals in a given structure with the radical of a specified substituent including, but not limited to: halo, alkyl, alkenyl, alkynyl, aryl, heterocyclyl, thiol, alkylthio, arylthio, alkylthioalkyi, arylthioalkyi, alkylsulfonyl, alkylsulfonylalkyi, arylsulfonylalkyi, alkoxy, aryloxy, aralkoxy, aminocarbonyl, alkylaminocarbonyl, arylaminocarbonyl, alkoxycarbonyl, aryloxy carbonyl, haloalkyl, amino, trifluoromethyl, cyano, nitro, alkylamino, arylamino, alkylaminoalkyi, arylaminoalkyi, aminoalkylamino, hydroxy, alkoxyalkyl, carboxyalkyi, alkoxy carbonylalkyi, aminocarbonylalkyi, acyl, aralkoxy carbonyl, carboxylic acid, sulfonic acid, sulfonyl, phosphonic acid, aryl, heteroaryl, heterocyclic, and aliphatic. It is understood that the substituent can be further substituted.

[0040] Scheme 1 shows the structure of a GalNac-conjugated AC20 (SEQ ID NO: 10) oligonucleotide.

DETAILED DESCRIPTION OF THE DRAWINGS

[0041] Figure 1 shows results of HBV transgenic mice treated with non-Gal-NAc - (AC)20 oligonucleotide and GalNAc -conjugated (AC)20 oligonucleotide.
[0042] Figure 2A shows the changes in HBsAg levels for individual HBV transgenic mice treated with the saline vehicle alone.

[0043] Figure 2B shows the changes in HBsAg levels for individual HBV transgenic mice treated with the non-GalNAc -(AC)20 oligonucleotide (SEQ ID NO: 10) in the saline vehicle.

[0044] Figure 2C shows the changes in HBsAg levels for individual HBV transgenic mice treated with the GalNAc -conjugated (AC)20 oligonucleotide (as depicted in Scheme 1) in the saline vehicle.

Hepatitis B Surface Antigens

[0045] 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. Although several studies have also suggested that HBsAg may also function to directly block activation of adaptive and innate immune responses to HBV infection the presence of this functionality in human HBV infection and HBV/HDV co-infection and its impact on the activity of immunotherapeutic agents has not been investigated or established. HBcAg and HBeAg have also been shown to have immunoinhibitory properties.

[0046] In addition to the recognized activity of excess HBsAg in the blood (as SVPs) to sequester anti-HBs, the ability of HBsAg to block cytokine signaling in some in vitro and in vivo systems suggests that these immuno-inhibitory properties of HBsAg may also be present in HBV infection and HBV/HDV co-infection in human subjects. Due to the large excess of HBsAg in the blood of infected patients, there is likely an effective impairment of many signaling mechanisms critical for optimal immune function (both adaptive and innate). As disclosed in US20140065102, many of these signaling mechanisms are also likely essential for the effects of immunotherapeutic agents to be fully realized and indicated the sizable effect of circulating HBsAg in inhibiting the action of immunotherapeutic agents.

[0047] It is provided herein a treatment against HBV infection and HBV/HDV co-infection which consists of a first pharmaceutically acceptable oligonucleotide conjugate
agent capable of removing HBsAg from the blood and an immunotherapeutic agent which stimulates immune function. Such a combination treatment allows circulating anti-HBsAg antibodies to directly attack the circulating virus and virus producing cells and, in the absence of the immuno-inhibitory properties of HBsAg, is expected to lead to an improvement in the effect of immunotherapy which in turn will result in a greater proportion of patients achieving immunological control of their HBV infection compared with immunotherapy used alone.

[0048] US20140065102 also disclosed that, in addition to the previously described ability to block cytokine signaling in vitro, circulating HBsAg also directly inhibits the function of approved immunotherapies for the treatment of HBV in addition to suppressing the host immune response against HBV infection. Thus, it is expected that a therapy which combines a first agent that is an oligonucleotide conjugate capable of removing HBsAg from the blood and a second agent which stimulates immune function will lead to an improved effect and potentially enable the recovery of immunological control of HBV infection.

[0049] US20140065102 also disclosed that HBsAg in the blood of patients with HBV infection suppresses the biochemical activity of immunotherapeutic agents like thymosin α1 or pegylated interferon α-2a. However, by removing the HBsAg in the blood of patients with HBV infection prior to treatment with thymosin α1 or pegylated interferon α-2a (by pre-treatment with the non-sequence specific oligonucleotide REP2139 - see US20140065102), it was discovered that in an HBsAg negative environment, treatment with either of these two immunotherapeutic agents resulted in an unexpected activation of immunological response (as measured by production of anti-HBsAg antibodies in the blood). This immunological activation was substantially stronger and occurred much more rapidly than normally observed when these immunotherapeutic agents are used in monotherapy. These dramatic responses to immunotherapy are unusual as any kind of positive immunological response is infrequent in patients treated with immunotherapeutic agents used in monotherapy.

[0050] US20140065102 also appears to suggest that removal of HBsAg from the blood of patients with HBV infection or HBV/HDV infection will have a synergistic impact on the ability of any immunotherapeutic agent to elicit a stronger immunological response in many and possibly most patients receiving immunotherapy with a shorter treatment regimen than typically employed. Thus, embodiments of oligonucleotide conjugates as described herein are expected to elicit a similar beneficial and potentially synergistic effect on improving the biochemical activity of any approved or experimental pharmaceutically acceptable immunotherapeutic agent. Such an improvement in the activity of the approved
or experimental immunotherapeutic agent is expected to be realized when the reduction or removal of HBsAg was achieved before immunotherapy, concomitantly with immunotherapy, or when reduction or removal of HBsAg was achieved after immunotherapy had been previously started and is continued.

Oligonucleotides

[0051] Non-sequence specific oligonucleotides or sequence independent oligonucleotides (used interchangeably herein) that have antiviral activity, whether directly or indirectly by improving an associated condition, are described and can be made as disclosed in U.S. patents US8,716,259, US8,513,211, US8,318,701, US8,067,385, US8,008,270, US8,008,269, US7,985,581, US7,871,991 and US7,358,068; and in e.g. US Patent Publications US20140065102, US20130296410, US20130171103 and US20130090299, the contents of which are all hereby incorporated by reference herein in their entirety.

[0052] 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, as well as double stranded molecules such as small interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs).

[0053] 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, phosphorodithioate linkages, and/or methylphosphonate 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’-0-alkyl modifications such as 2’-0-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).

[0054] In the present application, the term "nucleic acid polymer" or NAP is intended to identify 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. Exemplary NAP compounds include:

- 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;
- a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT;
- a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence UG;
- a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GU;
the compound depicted in Scheme 1.

[0055] ON chelate complexes are two or more ONs linked intermolecularly by a
divalent or multivalent metal cation. 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.
These side effects may include shivering, fever and chills with intravenous infusion or
induration, inflammation and pain at the injection site with subcutaneous administration.
Moreover, by preparing ONs as chelated complexes, their pharmacokinetic behavior may be
improved, providing for increased therapeutic performance with similar dosing compared to
un-chelated ONs as described in International application publication no. WO 2012/021985
and U.S. application publication no. 2012/0046348, which are incorporated herein by
reference in their entirety. The administration of ON chelate complexes does not interfere
with the biochemical activity of ONs when used normally as sodium salts. Thus any
antisense ON, siRNA, or NAP as described herein can be optionally prepared as an ON
chelate complex without affecting its biochemical activity.

[0056] 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.
[0057] 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 here.

[0058] ONs can exert their therapeutic effects by numerous mechanisms which are either sequence dependent or sequence independent. Sequence dependent mechanisms are those which require a specific nucleic acid sequence for their activity and where the activity is reduced by one or more alterations in the nucleotide sequence present. This specific sequence may encompass the entire length of the ON or only a portion of it (a sequence motif). Examples of sequence dependent ONs include:

[0059] 1. Antisense ONs either single stranded or double stranded (e.g. synthetic interfering RNA (siRNA) or small hairpin RNA (shRNA)) are designed to target a specific region of a messanger RNA (mRNA) or a micro RNA (miRNA) of interest by a specific hybridization between the antisense ON and sequence in the targeted portion of the mRNA of interest. When antisense ONs are introduced into a cell, they result in the formation of a duplex region on the mRNA or with the miRNA which directs the degradation of this specific mRNA or miRNA by RNase H. When siRNAs is introduced into the cell (or shRNA is expressed in the cell), the antisense strand (or guide strand) is incorporated into the RISC RNA-induced silencing complex) which uses guide-strand targeted hybridization with the complementary region on a target mRNA to effect its cleavage by the catylytic component of the RISC called Argonaute.

[0060] 2. Stearic blocking ONs are single stranded antisense ONs which are complementary to a specific portion of a mRNA or an immature mRNA but which are engineered to not activate RNase H, either by containing 2' modification of every ribose, a modification known to prevent the action of RNase H or by using modified ON chemistry (such as morpholino ONs) which is not recognized by RNase H. The hybridization of these ONs to their target mRNAs results in a double stranded portion which provides stearic hindrance to proteins normally acting on the RNA (such as splicing proteins or ribosomes. Such ONs can be employed to block translation of a particular mRNA or to modify the post-transcriptional splicing and maturation of a particular mRNA.

[0061] 3. Aptamers are ONs which adopt a specific three dimensional conformation capable of specific protein interaction and which do not readily interact with host DNA or
RNA. Aptamers can also include Spiegelmers, which use L-nucleotides instead of D-nucleotides to confer high enzymatic stability to the ON.

[0062] Immunostimulatory ONs contain specific modifications which result in the binding to and activation of toll-like receptors 7, 8 and 9 via a non-CpG motif mediated mechanism and are capable of stimulating immune function (Kandimalla et al. 2011. Cell. Immunol. 270: 126-134; Struthers et al. 2010. Cell. Immunol. 263: 105-113).

[0063] In the design of antisense ONs, siRNAs or shRNAs, the sequence of these molecules is designed to be 100% complementary to the intended target sequence of a specific RNA within the following guidelines:

[0064] Antisense ONs are 15-25 nucleotides in length and contain sequence which is 100% complementary to the intended target sequence.

[0065] The guide strand of siRNA contains one oligoribonucleotide 19-21 nucleotides in length which is 100% complementary to the targeted portion of a mRNA of interest and the passenger strand (the other strand in the duplex) contains the same length of ribonucleotide sequence which is 100% complementary to the guide strand. Both the guide and passenger strand also have two additional deoxythymidine nucleotides on the 3' end of each strand.

[0066] shRNA molecules are produced from an expression vector such as a plasmid or viral based (e.g. lentivirus or adenovirus) expression construct which produces a long RNA which comprises the sequence of the guide and passenger strands (as described above for siRNA but which can be 19-29 nucleotides in length) in one contiguous oligonucleotide but separated by a short non-complementary oligonucleotide sequence designed to form a hairpin. Transcription of RNA from this expression construct results in the formation of a short hairpin RNA which is processed by the dicer enzyme and loaded onto the RISC as described above for siRNA.

[0067] In the present description, the term "antiviral ON" or "antiviral ONC" refers to any antisense ON, siRNA, shRNA or NAP or any ON conjugate, siRNA conjugate, shRNA conjugate or NAP conjugate, which by virtue of its specific biochemical activity (whether sequence dependent or sequence independent) has the ability to directly or indirectly inhibit some aspect of viral replication or to directly or indirectly enhance the host's ability to clear the viral infection by immunological or other mechanisms.

[0068] In the present disclosure, the term "ON chelate complex" or "ONC chelate complex" refers to a complex of two or more ONs or two or more ONCs in solution linked intermolecularily by a divalent metal cation as described in International application
publication no. WO 2012/021985 and U.S. application publication no. 2012/0046348, which are incorporated herein by reference in their entirety. ON or ONC chelate complexes can be formed with antisense ONs or ON conjugates, siRNAs or siRNA conjugates, or NAPs or NAP conjugates.

[0069] Phosphorothioated NAPs are a class of ON-based broad spectrum antiviral agents (see U.S. Pat. Nos. 8,008,269, 8,008,270 and 8,067,385) which also block the formation and release of SVPs from HBV infected hepatocytes (see Example I of US201 400651 02). As SVPs constitute >99.9% of the HBsAg in the blood of patients with HBV, blockage of SVP formation and/or release from infected hepatocytes by NAPs is a highly effective method of removing HBsAg from the blood of patients infected with HBV.

[0070] As described in Example II of US201 400651 02, removal of HBsAg from the blood of infected patients by NAPs results in a partial restoration of the immune response which in turn removes HBV e-antigen (HBeAg) from the blood and substantial reduction of levels of virus in the blood during treatment. However, as disclosed in US201 400651 02, these 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 not sufficient to achieve this control off treatment in the majority of patients treated.

[0071] Aside from NAPs, no other agent has been publicly disclosed which has the ability to rapidly remove HBsAg from the blood in HBV infected human patients. However, there are several other methodologies which could be employed other than the use of NAPs to predictably achieve removal of HBsAg from the blood which are well known in the art. Such methodologies include (but are not limited to) the following:

[0072] A. Using a small molecule approach to target portions of the HBsAg protein or other viral or host factors involved in the formation of SVPs to block the formation of SVPs, block the transport of SVPs through the secretory machinery of the infected cell, block the release of SVPs from infected hepatocytes into the blood or generally block the release of HBsAg from infected cells. Small molecules used in this approach can include triazolopyrimidine derivatives as described in Yu et al. (201 1, J. Med. Chem. 54: 5660-5670) and can include the specific triazolopyrimidine derivatives as described in FIG. 1 which have been shown to block the release of HBsAg from HBV-producing cell-lines. Other small molecules can also be used which target the Apo H protein which may be important for the production of SVPs (as described in Canadian application no. 2,746,981).
B. Using an antisense based ON approach, which includes antisense oligonucleotides, siRNA or shRNA molecules to target specific mRNAs and thereby catalyze their degradation to inhibit the synthesis of HBsAg (i.e. catalyze the degradation of the mRNA which is used to produce the HBsAg protein) or other viral or host factors involved in the formation of SVPs (including Apo H as described in Canadian application no. 2,746,981), the transport of SVPs through the secretory machinery of the infected cell or the release of SVPs from infected hepatocytes into the blood. Such an antisense based approach could also be employed to hybridize with viral or host mRNAs required for the synthesis of proteins important for the formation, intracellular transit or release of SVPs from infected hepatocytes and cause the degradation of these mRNAs by the mechanisms described above. In particular, some antisense-based approaches in HBV may be particularly advantageous as single antisense molecules such as siRNAs can be designed to interfere with all HBV mRNAs produced from the viral genome by hybridizing to a single region on the HBV genome causing the degradation of all mRNAs produced from the HBV genome which simultaneously affects HBsAg, HBeAg and HBCAg synthesis as described in Fu et al. (2008, Acta Pharmacol. Sin. 29: 1522-1528). Two or more antisense molecules could also be used simultaneously either as separate molecules or as molecules produced from a single expression vector introduced into the infected host as described by Snyder et al. (2008, Antiviral Res., 80: 36-44). Examples known in the art for antisense-based inhibition of the synthesis of HBV proteins include:

a. Altritol-modified siRNA lipoplexes (Hean et al., 2010, Artificial DNA: PNA & XNA, 1:17-26).


[0079] C. Using an ON-based aptamer approach (including classical aptamers or Spiegelmers) to target portions of the HBsAg, HBeAg or HBcAg proteins or other viral or host factors (including Apo H) present in the circulation to accelerate their removal from the blood. Classical aptamers and Spiegelmers can be further pegylated as described in Waters et al. 2011 Blood 117: 5514-5522 and Wlotzka et al. 2002 Proc. Nat. Acad. Sci. U.S.A. 99: 8898-8902 to improve their stability and circulating half-life.

[0080] D. Using an antibody based approach to directly target HBsAg and accelerate its removal from the blood.

[0081] 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™ quantative HBsAg assay. This serum HBsAg assay is an accepted standard for the measurement of levels of HBsAg in the blood and is approved for diagnostic use in human patients.

[0082] Examples of ONs which can be useful for preparation of oligonucleotide conjugates in the current disclosure include oligonucleotides comprising SEQ ID NOs 1-10.

[0083] Examples of ONs which can be useful in the current disclosure include oligonucleotides comprising any of SEQ ID NOs. 1-10 wherein the oligonucleotides may further comprise (5'-3') modifications with all linkages being phosphorothioate linkages (PS). Useful oligonucleotides may further comprise base modifications and sugar modifications such as 5'-methylcytosine (5'MeC), 2'-0-methyl (2'OMe) modifications on ribose sugars including, 2'OMeC and/or 2'OMeA. Suitable oligonucleotides may contain an RNA base (e.g. U) and or a ribose sugar, a viral or host mRNA (e.g. Apo H) or a portion of RNA with 2' ribose to a HBV mRNA modification. Other useful oligonucleotides may contain locked nucleic acids (LNA).

[0084] Exemplary effective dosing regimens for the various pharmaceutically acceptable agents described above which can be used to achieve HBsAg removal from the blood are:

[0085] For all NAPs and phosphorothioated antisense oligonucleotides that cause the degradation of HBV or apoH mRNA, 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 NAPs in the
examples below and for a phosphorothioated antisense ON causing the degradation of a liver specific mRNA (for apolipoproteins B100) by Akdim et al. 2010 Journal of the American College of Cardiology 55: 161-1618).

[0086] To achieve therapeutic levels of activity in the liver, siRNAs are typically encapsulated and dosed for the specific application of degrading the mRNA for PCSK9 in the liver of human patients. Encapsulated siRNAs can achieve therapeutic effect in the liver of human patients with parenteral doses ranging from 0.015-0.20 mg/kg and are expected to be able to provide persistent effect with dosing once every two weeks or once monthly.

[0087] shRNA has not yet been used in the human setting but work in vivo in rodents has shown that expression vectors designed to express shRNA, when dosed in mice at comparable concentrations as siRNA, can be equivalently effective as siRNA (McAnuff et al. 2007. Journal of Pharmaceutical Science 96: 2922-2930. It is therefore expected that shRNA dosing, either using viral-based or encapsulation based delivery systems as described above, achieve comparable effect in the liver with dosing regimens comparable to that used for siRNA.

[0088] Aptamers are typically pegylated to achieve improved stability and can be therapeutically effective with weekly parenteral doses of 100-600 µg/kg as described by Waters et al. 201 Blood 117: 5514-5522.

[0089] Speiglemers are also typically pegylated to achieve an improved circulating half-life and can be therapeutically effective with parenteral doses of > 1.2 mg/kg given every other day as described by Riecke et al. 2012 Abstract 2432 presented at the 54th Annual ASH Meeting and Exposition, Atlanta, Ga., U.S.A. In the context of binding to and accelerating the removal of HBsAg or other HBV proteins from the blood of HBV-infected patients, higher doses of both aptamers or Speiglemers may be required due to the high concentrations of HBsAg typically present in patients with HBV infection.

[0090] As described above, immunotherapeutic approaches to the treatment of HBV infection have limited efficacy. One of the limitations of interferon-based monotherapy is the achievement of HBsAg removal from the blood in a very small fraction of patients (Moucari et al., 2009, Antiviral Ther., 14: 1183-1 188; Reijnders et al., 201 J. Hepatol., 54: 449-454). This HBsAg removal may underlie the achievement of durable control of HBV DNA on and off treatment in this small fraction of treated patients (Moucari et al., 2009, Hepatology, 49: 1151-1 157). Another important limitation of interferon-based therapy is that it elicits only a moderate level (<50 mIU/ml) of anti-HBs production in a very small proportion of patients on treatment (Reijnders et al., 201 J. Hepatol., 54: 449-454; Harayiannis et al., 1990, J.
Hepatol., 10: 350-352) after 48 weeks of exposure. These important limitations are likely
critical factors underlying the achievement of a sustained virologic response only in a limited
number of patients after immunotherapy.

[0091] As described above, HBsAg can block signaling pathways important for
cytokine mediated stimulation of immune function. It is well known in the art that many
different classes of immunotherapeutic agents utilize several common signal transduction
pathways to effect immune activation. The disclosures presented herein further show that
many (or most) of these signal transduction pathways used by immunotherapeutic agents
may be also blocked by the action of HBsAg. The disclosures herein indicate that the action
of different immunotherapies are specifically inhibited by the presence of HBsAg and the
therapeutic effects of these different immunotherapies, when provided in a treatment
regimen, are synergistically improved in the absence of HBsAg. Therefore, removal of
HBsAg from the blood would in turn result in a weaker inhibition of signaling pathways
required for optimal activity of numerous different immunotherapeutic agents. Thus,
application of immunotherapy in patients who have previously removed HBsAg in their blood
or who are actively removing HBsAg in the blood while on immunotherapy would likely
experience a similar synergistic impact on the immunostimulatory effect of any
immunotherapy.

[0092] In the present disclosure, the term immunotherapeutic agent refers to a small
molecule or polypeptide or cytokine or hormone which by virtue of its specific biochemical
activity has the ability to directly or indirectly enhance the immune function of the host. The
polypeptide can be naturally derived or recombinant. The polypeptide can be recombinantly
derived from a portion of the naturally occurring polypeptide. The polypeptide can be
pegylated or not.

[0093] The methods for pegylation of polypeptides and the compatibility of
pegylation with the biochemical activity of these polypeptides is well known in the art and
consists of the linking of strands of polyethylene glycol (PEG) to the polypeptide in question
at specific amino acid residues. The primary function of pegylation is to increase the
circulating lifetime of a polypeptide and also to reduce its immunogenicity. These features
improve the tolerability of the polypeptide in question and reduce the frequency of dosing
required for optimal therapeutic effect. It is further known in the art that the attachment of
PEG residues to a polypeptide can be achieved without affecting the specific biochemical
activity of the polypeptide in question. Pegylation is also known to increase the water
solubility of the polypeptide in question, improving its ease of formulation. Numerous
examples of pegylated polypeptides are known in the art and include: Mircera.TM., a pegylated form of erythropoietin; Neulasta™, a pegylated form of human granulocyte colony-stimulating factor; Pegasys™ a pegylated form of human interferon a-2a; Peg-Intron™, a pegylated form of human interferon a-2b; and pegylated interferon λ1 (which is currently in clinical development).

[0094] Additionally, immunotherapeutic agents which have not been previously shown to have useful immunotherapeutic activity in the presence of HBV proteins (e.g. in infected patients, chimpanzees or cellular models) may now be shown to have useful immunotherapeutic activity with the removal of HBsAg from the blood and may be further useful in the treatment of HBV in combination with any agent which removes HBsAg from the blood.

[0095] The demonstration of antiviral activity of any immunotherapeutic agent is generally accepted as an indirect measure of its ability to stimulate immune function such that this stimulated immune function has antiviral affect. Therefore, any immunotherapeutic agent with antiviral activity has an ability to stimulate immune function.


Additionally, the hormone dehydroepiandrosterone (5-androstene-3p-17-one, DHEA) and many of its metabolites (including androstenediol (5-androstene-3p-17p-diol, vAED), androstenetriol (5-androstene-3p-7p-17p triol β AET) have clear, well established immunostimulatory functionality with the capability to improve the development of a

[0097] The measure of stimulation of immune function as described in the current disclosures and in the context of HBV infection is most easily measured by (but not restricted to) changes in the levels of free anti-HBsAg antibodies produced in a patient receiving immunotherapy. The use of the Abbott Architect™ quantitative anti-HBsAg antibody test is a method accepted worldwide for the evaluation levels of free anti-HBsAg antibodies in the serum of patients with chronic HBV infection and the appearance of or increased production of anti-HBsAg antibodies in patients with HBV infection is an accepted surrogate measure of immune response in these patients who receive immunotherapy or HBV polymerase inhibition therapy.

[0098] There are other accepted measures of immune function which may be employed to monitor improvement of immune function in the presence of the combination treatments as described above. These measures may include increases in the transcriptional activity of interferon-response genes or increases in the levels of HBV-specific CD4+ or CD8+ T-cells in the blood or the increased levels of various cytokines in the blood such as IL2 (Liang et al. 2011, Virology Journal 8: 69).

[0099] The use of vaccination against HBV (typically using HBsAg as the antigen) is a well-recognized method for effectively preventing HBV infection and is a method adopted world-wide for the prevention of the spread of HBV infection. However, vaccination against HBV antigens has only a moderate to negligible effect in a therapeutic setting, even when the vaccine combines two different HBV antigens such as HBsAg and HBCAg (Mahtab et al. 2013. J. Hepatol. 58 (supp 1) abstract 760). According to the disclosures provided herein, this poor effect may be due to the circulating levels of HBsAg present in the blood of these patients and therefore the ability of a vaccine to stimulate the production of new antibodies to HBsAg (or to other HBV proteins) may be greatly improved with removal of HBsAg from the blood.
Thus, there are many immunotherapeutic agents known to be able to stimulate immune function which may be of utility when administered before, during or after removal of HBsAg from the blood, these immunotherapeutic agents include (without restriction):

- thymosin α1;
- any α-interferon or pegylated derivatives thereof;
- any β-interferon or pegylated derivatives thereof;
- any γ-interferon or pegylated derivatives thereof;
- any λ-interferon or pegylated derivatives thereof;
- interferon α2a or α-2b or α-N3;
- interferon β-1a or β-1b;
- interferon γ-1b; Interferon λ1 or A2 or A3;
- pegylated interferon α-2a or α-2b or α1 or A2;
- thymic protein A;
- any antiviral cytokine or pegylated derivatives thereof;
- any polypeptide shown to have antiviral activity or immunostimulatory activity;
- an immunostimulatory oligonucleotide such as IMO-2125 and IMO-2055;
- a vaccine targeting any HBV antigen;
- a small molecule Toll-like receptor agonist such as GS-9620, and ANA-773; and
- any hormone shown to have antiviral activity or immunostimulatory activity such as DHEA or its metabolites.

Exemplary effective dosing regimen of immunotherapeutic agents used to achieve stimulation of immune function can include:

- weekly doses of 90-180 μg in the case of Pegasys™ (according to the package insert);
- weekly doses of 1.6 mg in the case of Zadaxin™ (according to the package insert);
- weekly doses of 1. times $10^7$ U in the case of Intron-A™ (according to the package insert);
- weekly doses of 1.5-3.0 μg/kg in the case of pegylated interferon A1 as described in Muir et al., 2010, Hepatology, 52: 822-832;
- similar weekly doses as described above for any cytokine or immunotherapeutic peptide whether pegylated or unpegylated;
weekly doses of 0.16-0.48 mg/kg/week for the non CpG immunostimulatory oligonucleotide IMO-2125 as described in Table 3 of US20140065102, incorporated by reference herein; and

normally proscribed vaccine doses according to the conventional practices in the art and specifically for the HBV vaccines Energix-B™, Recombivax-HB™.

[00102] Therefore, with the disclosures presented herein any of the above recited methods or agents capable of achieving removal of HBsAg in the blood of patients when combined with the stimulation of the host immune function by any of the immunotherapeutic agents recited above would be expected to produce a synergistic effect on the reconstitution of the immune function in patients with HBV infection or HBV/HDV co-infection. In addition to achieving the restoration of an immune function better able to sustain control of infection off treatment, such synergy could also be expected to reduce the dose of one or both agents and even the duration of treatment with either agent required to establish a therapeutically effective immune response in a majority of patients. Example III of US20140065102 illustrates the reported synergistic effect on immunological recovery when removal of HBsAg by the NAP REP 2139 is followed by add-on therapy with either thymosin a 1 or pegylated interferon a-2a.

[00103] In the context of combining an agent which can remove HBsAg from the blood with an immunotherapeutic agent, any amount of HBsAg removal may provide a synergistic improvement in the activity of an immunotherapy and a fractional dose of a particular immunotherapeutic agent may result in comparable or even superior immunostimulatory activity of an immunotherapeutic agent, even if HBsAg is not completely removed. Thus, combining any agent resulting in HBsAg removal from the blood with any immunotherapeutic agent will have a synergistic effect on the action of both agents which has the potential to improve the durability of the antiviral response (host immunological control) off treatment and may also require reduced doses of both agents to achieve a similar or even superior effect than when either is used in monotherapy.

[00104] Therefore, according to the disclosures presented herein, it may be useful to treat a subject with HBV infection or HBV/HDV co-infection with a pharmaceutically acceptable agent which results in the reduction of or clearance of HBsAg from the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent.

[00105] It may also be 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.

[00106] It may also be useful to administer the pharmaceutically acceptable agents by the same or different routes of administration.

[00107] As disclosed herein, the oligonucleotide conjugate compounds and oligonucleotide conjugate chelate compounds that make up embodiments of the invention are based on the discovery that conjugation of a carbohydrate moiety to an oligonucleotide can optimize one or more properties of the oligonucleotide agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the oligonucleotide agent. E.g., the ribose sugar of one or more ribonucleotide subunits of an oligonucleotide can be replaced with another moiety, e.g., a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, i.e., all ring atoms are carbon atoms, or a heterocyclic ring system, i.e., one or more ring atoms may be a heteroatom, e.g., nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, e.g. fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

[00108] The carriers further include (i) at least one "backbone attachment point", preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, e.g. a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, e.g., the phosphate, or modified phosphate, e.g., sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, e.g., a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, e.g., a carbohydrate, e.g. monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, e.g., an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, e.g., a ligand to the constituent ring.

[00109] In one aspect, the invention features, a compound having the structure shown in formula (I)
wherein:
A and B are each independently for each occurrence O, N(R^N), S or absent;
R^N is independently for each occurrence H or C_1-C_6 alkyl;
X and Y are each independently for each occurrence H, OH, a hydroxyl protecting group,
a phosphate group, a phosphodiester group, an activated phosphate group,
an activated phosphite group, a phosphoramidite, a solid support, -P(Z')(Z'')0-nucleoside,
-P(Z')(Z'')0-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside,
-P(Z')(Z'')0-Linker-OP(Z'')(Z'''')0-oligonucleotide, an oligonucleotide, -P(Z')(Z'')-formula (I),
-P(Z')(Z'')-, -Linker-R or is absent;
R is L^G or has the structure shown below:

L^G is independently for each occurrence a ligand including a carbohydrate, a monosaccharide,
a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and Z', Z'', Z''' and Z'''' are each independently for each occurrence O or S.

[00110] The term "Linker" means an organic moiety that connects two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR^8, C(O), C(O)NH, SO, S\_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, aryalkyi, aryalkenyl, aryalkynyl, heteroarylalkyi, heteroarylalkenyl,
heteroarylalkynyl, heterocyclylalkyi, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylaryl, alkylaryl, alkylaryl, alkylaryl, alkylaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), S(O)₂, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the Linker is between 1-24 atoms, preferably 4-24 atoms, preferably 6-18 atoms, more preferably 8-18 atoms, and most preferably 8-16 atoms.

In one embodiment, the Linker is -(P-Q"-R) q-X-(P"-Q""-R") q'-T-, wherein:

P, R, T, P', R' and T are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH, CH₂O ; NHCH(R₈)C(O), -C(0)═-CH(R₈)═NH-, CH=NH-0,

or heterocycle:

Q" and Q"" are each independently for each occurrence absent, -(CH₂)n- , -C(R₁')(R₂')(CH₂)n- , -(CH₂)nC(R₁')(R₂')- , -(CH₂CH₂O)mCH₂CH₂-, or -(CH₂CH₂O)mCH₂CH₂NH-;
X is absent or a cleavable linking group;
R^n is H or an amino acid side chain;
R^1 and R^2 are each independently for each occurrence H, CH₃, OH, SH or N(R^N)_2;
R^m is independently for each occurrence H, methyl, ethyl, propyl, isopropyl, butyl or benzyl;
q, q' and q'' are each independently for each occurrence 0-20 and wherein the repeating unit can be the same or different; and
n is independently for each occurrence 1-20; and m is independently for each occurrence 0-50.

[00112] In one embodiment, the Linker comprises at least one cleavable linking group.

[00113] In certain embodiments, the Linker is a branched Linker. The branchpoint of the branched Linker may be at least trivalent, but may be a tetravalent, pentavalent or hexavalent atom, or a group presenting such multiple valencies. In certain embodiments, the branchpoint is, -N, -N(Q)-C, --0--C, --S--C, --SS--C, -C(0)N(Q)-C, -OC(0)N(Q)-C, -N(Q)C(0)-C, or -N(Q)C(0)O-C; wherein Q is independently for each occurrence H or optionally substituted alkyl. In other embodiment, the branchpoint is glycerol or glycerol derivative.

[00114] Cleavable Linking Groups

[00115] A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least 10 times or more, preferably at least 100 times faster in the target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum).

[00116] Cleavable linking groups are susceptible to cleavage agents, e.g., pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes
that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

[00117] A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing the cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

[00118] A Linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a Linker can depend on the cell to be targeted. For example, liver targeting ligands can be linked to the cationic lipids through a Linker that includes an ester group. Liver cells are rich in esterases, and therefore the Linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

[00119] Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

[00120] In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It may be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least 2, 4, 10 or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

[00121] Redox Cleavable Linking Groups
[00122] One class of cleavable linking groups are redox cleavable linking
groups that are cleaved upon reduction or oxidation. An example of reductively cleavable
linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable
linking group is a suitable "reductively cleavable linking group," or for example is suitable for
use with a particular oligonucleotide moiety and particular targeting agent one can look to
methods described herein. For example, a candidate can be evaluated by incubation with
dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the
rate of cleavage which would be observed in a cell, e.g., a target cell. The candidates can
also be evaluated under conditions which are selected to mimic blood or serum conditions.
In a preferred embodiment, candidate compounds are cleaved by at most 10% in the blood.
In preferred embodiments, useful candidate compounds are degraded at least 2, 4, 10 or
100 times faster in the cell (or under in vitro conditions selected to mimic intracellular
conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular
conditions). The rate of cleavage of candidate compounds can be determined using
standard enzyme kinetics assays under conditions chosen to mimic intracellular media and
compared to conditions chosen to mimic extracellular media.

[00123] Phosphate-Based Cleavable Linking Groups

[00124] Phosphate-based cleavable linking groups are cleaved by agents that
degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate
groups in enzymes such as phosphatases in cells. Examples of phosphate-based
linking groups are --0--P(0)(ORk)-0--, --0--P(S)(ORk)-0--, --0--P(S)(SRk)-0--, --S--
P(0)(ORk)-0--, --S--P(0)(ORk)-S--, --S--P(0)(ORk)-S--, --0--P(S)(ORk)-S--, --S--P(S)(ORk)-S--,
--S--P(0)(ORk)-S--, --0--P(S)(ORk)-S--, --S--P(S)(ORk)-S--. Preferred embodiments are --0--P(0)(OH) --0--,
--0--P(S)(OH) --0--, --S--P(0)(OH) --0--, --S--P(S)(OH) --0--, --0--P(0)(OH) --0--, --S--P(0)(OH)
--0--, --S--P(S)(OH) --0--, --S--P(0)(OH) --0--, --S--P(S)(OH) --0--, --0--P(S)(OH) --0--, --S--
P(0)(OH) --0--, --S--P(S)(OH) --0--, --S--P(0)(OH) --0--, --S--P(S)(OH) --0--, --0--P(S)(OH) --0--. A preferred
embodiment is --0--P(0)(OH) --0--. These candidates can be evaluated using methods
analogous to those described above.

[00125] Acid Cleavable Linking Groups

[00126] Acid cleavable linking groups are linking groups that are cleaved
under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved
in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.5, 5.0, or lower),
or by agents such as enzymes that can act as a general acid. In a cell, specific low pH
organelles, such as endosomes and lysosomes can provide a cleaving environment for acid
cleavable linking groups. Examples of acid cleavable linking groups include but are not
limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have
the general formula -C=NN~, C(0)0, or -OC(O). A preferred embodiment is when the
carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted
alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can
be evaluated using methods analogous to those described above.

[00127] Ester-Based Linking Groups
[00128] Ester-based cleavable linking groups are cleaved by enzymes such
as esterases and amidases in cells. Examples of ester-based cleavable linking groups
include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester
cleavable linking groups have the general formula ~C(0)0~, or -OC(O)~. These
candidates can be evaluated using methods analogous to those described above.

[00129] Peptide-Based Cleaving Groups
[00130] Peptide-based cleavable linking groups are cleaved by enzymes such
as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide
bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.)
and polypeptides. Peptide-based cleavable groups do not include the amide group (--
C(O)NH-). The amide group can be formed between any alkylene, alkenylene or
alkynelene. A peptide bond is a special type of amide bond formed between amino acids to
yield peptides and proteins. The peptide based cleavage group is generally limited to the
peptide bond (i.e., the amide bond) formed between amino acids yielding peptides and
proteins and does not include the entire amide functional group. Peptide-based cleavable
linking groups have the general formula—NHCHR^A(C(0)NHCHR R^B(C(0)—, where R^A and R^B
are the R groups of the two adjacent amino acids. These candidates can be evaluated using
methods analogous to those described above.

[00131] In one embodiment, the oligonucleotide conjugate compound has at
least one subunit of formula (1):
wherein:

A and B are each independently for each occurrence O, N(R^N), S or absent;

R^N is independently for each occurrence H or C_1-C_6 alkyl;

X and Y are each independently for each occurrence H, OH, a hydroxyl protecting group, a phosphate group, a phosphodiester group, an activated phosphate group, an activated phosphite group, a phosphoramidite, a solid support, -P(Z')(Z'')(Z''')-nucleoside, -P(Z')(Z'')-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside, -P(Z')(Z'')-Linker-OP(Z'')(Z''')-oligonucleotide, an oligonucleotide, -P(Z')(Z'')-formula (I), -P(Z')(Z''), -Linker-R or is absent;

R is L^G or has the structure shown below:

\[ \text{Linker-} L^G \]
\[ \text{Linker-} L^G \]
\[ \text{Linker-} L^G \]
\[ \text{Linker-} L^G \]

L^G is independently for each occurrence a ligand including a carbohydrate, a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and

Z', Z'', Z''' and Z'''' are each independently for each occurrence O or S.

[00132] In some embodiments, the formula (I) has the structure
In some embodiments, the formula (I) has the structure

\[ \text{structure diagram} \]

\[ n = 1, 6, 7, 11, 17 \]

\[ m = 1, 6, 7, 11, 17 \]

\[ n = 1, 6, 7, 11, 17 \]
In some embodiments, the formula (I) has the structure
In some embodiments, the formula (I) has the structure

\[ p = 1, 2; \]
\[ q = 1, 5 \text{ and } n = 2, 3, 4, 5 \]
\[ R', R'' = H; R' = H, R'' = Me \]
\[ R', R'' = Me; R' = Me, R'' = H \]
In some embodiments, the formula (I) has the structure

\[ p = 1, 2; \]
\[ q = 1, 5 \text{ and } n = 2, 3, 4, 5 \]
\[ m = 0 \text{ or } 1 \]
\[ R', R'' = H; R' = H, R'' = Me \]
\[ R', R'' = Me; R' = Me, R'' = H \]

[00136] In some embodiments, \( R \) is

\[ i = 2, 3, 5, 9; j = 1, 2; k = 1, 5 \]
\[ s = 0 \text{ or } 1 \text{ and } t = 1, 2, 3 \text{ or } 4 \]
\[ R', R'' = H; R' = H, R'' = Me \]
\[ R', R'' = Me; R' = Me, R'' = H \]

[00137] In some embodiments, \( R \) is
[00138] In some embodiments, R is

[00139] In some embodiments, R is
In some embodiments, R is
In some embodiments, R is

In some embodiments, R is
In some embodiments, R is
[00146] In some preferred embodiments, R is

[00147] In some preferred embodiments, R is

[00148] In some preferred embodiments, formula (I) has the structure
In some embodiments R is

In some embodiments monomer of formula (I) has the structure

In some embodiments monomer of formula (I) has the structure

\[ x = 1-30 \]
\[ y = 1-15 \]
In some embodiments monomer of formula (I) has the structure:
In some embodiments monomer of formula (I) has the structure
[00156] In some embodiments, R is

[00157] In some embodiments, R is
In some embodiments, R is

In some embodiments, R is

In some embodiments, R is

In some embodiments, R is
In some embodiments, R is
In some embodiments, formula (I) has the structure

In some embodiments, formula (I) has the structure

In some embodiments, formula (I) has the structure
[00168] In some embodiments, formula (I) has the structure

[00169] In some embodiments, formula (I) has the structure

[00170] In some embodiments, formula (I) has the structure
In some embodiments, the monomer of formula (I) is linked to the oligonucleotide X or Y through a Linker of formula (C), wherein R is O or S.

[00172] In one aspect, the invention features, an oligonucleotide conjugate agent comprising at least one monomer of formula (I).

[00173] In some embodiments, the oligonucleotide conjugate agent will comprise 1, 2, 3, 4 or 5 monomers of formula (I), more preferably 1, 2 or 3 monomers of formula (I), more preferably 1 or 2 monomers of formula (I), even more preferably only one monomer of formula (I).

[00174] In some embodiments, all the monomers of formula (I) are on the same strand of the oligonucleotide conjugate agent.

[00175] In some embodiments, all monomers of formula (I) in an oligonucleotide conjugate agent are the same.

[00176] In some embodiments, the monomers of formula (I) in an oligonucleotide conjugate agent are all different.

[00177] In some embodiments, only some monomers of formula (I) in an oligonucleotide conjugate agent are the same.

[00178] In some embodiments, the monomers of formula (I) will be next to each other in the oligonucleotide conjugate agent.

[00179] In some embodiments, the monomer of formula (I) will be on the 5'-end, 3'-end, at an internal position, both the 3'- and the 5'-end, both 5'-end and an internal
position, both 3'-end and internal position, and at all three positions (5'-end, 3'-end and an internal position) of the oligonucleotide conjugate agent.

[00180] Oligonucleotide Conjugate Agents

[00181] The oligonucleotide conjugate agent described herein does not contain homology to the target gene, but rather comprises a random or repeating AC or CA sequence and may be referred to as a randomer. The oligonucleotide conjugate or randomer conjugate may be of sufficient length in terms of nucleotides, such that the oligonucleotide conjugate agent, or a fragment thereof, can modulate expression of the target gene. (For ease of exposition the term nucleotide or ribonucleotide is sometimes used herein in reference to one or more monomeric subunits of an oligonucleotide conjugate agent. It will be understood herein that the usage of the term "ribonucleotide" or "nucleotide", herein can, in the case of a modified oligonucleotide or nucleotide surrogate, also refer to a modified nucleotide, or surrogate replacement moiety at one or more positions.) Thus, while the oligonucleotide conjugate agent will not be complementary to a target RNA, it is understood that the oligonucleotide of the oligonucleotide conjugate must be sufficiently long to enable the oligonucleotide conjugate agent, or a cleavage product thereof, to modulate HBs antigen of the virus, for example the HBs and/or HBe antigens of the Hepatitis B virus.

[00182] As discussed elsewhere herein, and in the material incorporated by reference in its entirety, an oligonucleotide conjugate agent will often be modified or include nucleoside surrogates. Regions of the oligonucleotide conjugate agent will often be modified or include nucleoside surrogates. Modification to stabilize one or more 3'- or 5'-termini of an oligonucleotide conjugate agent, e.g., against exonucleases, or to favor the oligonucleotide conjugate agent half-life are also envisioned. Modifications can include C3 (or C6, C7, C12) amino linkers, thiol linkers, carboxyl linkers, non-nucleotide spacers (C3, C6, C9, C12, abasic, triethylene glycol, hexaethylene glycol), special biotin or fluorescein reagents that come as phosphoramidites and that have another DMT-protected hydroxyl group, allowing multiple couplings during RNA synthesis.

[00183] In some embodiments, formula (I) has the structure:
In some embodiments, formula (I) has the structure:
In some embodiments, formula (I) has the structure:

In some embodiments, formula (I) has the formula:
wherein $Y$ is $O$ or $S$ and $n$ is 3-6.

[00187] In some embodiments, formula (I) has the formula:

wherein $Y$ is $O$ or $S$ and $n$ is 3-6.

[00188] In some embodiments, formula (I) has the formula:
In some embodiments, formula (I) has the structure:

wherein X is O or S.

In some embodiments, formula (I) has the structure:
wherein \( R \) is OH or NHCOOH.

[00191] In some embodiments, formula (I) has the structure:

wherein \( R \) is OH or NHCOOH.

[00192] In some embodiments, monomer of formula (I) is linked to the oligonucleotide through a Linker of formula (A):

wherein \( R \) is O or S.
[00193] In some embodiments, formula (I) has the structure

wherein R is OH or NHCOOH.

[00194] In some embodiments, formula (I) has the structure

[00195] In some embodiments, formula (I) has the structure

wherein R is OH or NHCOOH.
In some embodiments, formula (I) has the structure

wherein R is OH or NHCOOH.

In some embodiments, formula (I) has the structure

wherein R is OH or NHCOOH.

In some embodiments, formula (I) has the structure

wherein R is OH or NHCOOH.

In some embodiments, the oligonucleotide X or Y is connected to the at least one subunit of formula (I) at the 3'-end of the oligonucleotide.

In some embodiments, the oligonucleotide X or Y is connected to the at least one subunit of formula (I) at the 5'-end of the oligonucleotide.

In some embodiments, the oligonucleotide X or Y is connected to the at least one subunit of formula (I) at an internal phosphate of the oligonucleotide.
In one particular embodiment there is provided an oligonucleotide conjugate as described herein wherein the oligonucleotide X or Y is connected to formula (I) via a molecule of formula (C):

\[
\begin{align*}
\text{formula (C)}
\end{align*}
\]

wherein \( R \) is O or S.

It may be desirable to modify the oligonucleotide of the oligonucleotide conjugate agent. In some cases a modification which prevents 5'-phosphorylation may be desired, e.g., by modification with a 5'-0-methyl ribonucleotide. Other modifications which prevent phosphorylation can also be used, e.g., simply substituting the 5'-OH by H rather than O-Me.

As used herein, the term "oligonucleotide" refers to a nucleic acid molecule (RNA or DNA) for example of length less than 100, 200, 300, or 400 nucleotides.

Oligonucleotide conjugate agents discussed herein include unmodified oligonucleotides as well as oligonucleotides which have been modified, e.g., to improve efficacy, and polymers of nucleoside surrogates. Unmodified oligonucleotide refers to a molecule in which the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are the same or essentially the same as that which occur in nature, for example as occur naturally in the human body. Modified oligonucleotide refers to a molecule in which one or more of the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are different from that which occur in nature, for example, different from that which occurs in the human body. While they are referred to as modified "oligonucleotides," they will of course, because of the modification, include molecules which are not oligonucleotides. Nucleoside surrogates are molecules in which the deoxyribophosphate or ribophosphate backbone is replaced with a non-deoxyribophosphate...
or nonribophosphate construct e.g., non-charged mimics of the ribophosphate or deoxyribophosphate backbone. Examples of all of the above are discussed herein.

[00202] As nucleic acids are polymers of subunits, many of the modifications described below occur at a position which is repeated within a nucleic acid, e.g., a modification of a base, or a phosphate moiety, or the a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or 5' terminal position, may only occur in a terminal regions, e.g., at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur, e.g., a phosphorothioate modification, at a non-linking O position and may only occur at one or both termini, may only occur in a terminal regions, e.g., at a position on a terminal nucleotide or may occur in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in all positions of the oligonucleotide. The 5' end or ends can be phosphorylated.

[00203] In some embodiments it is possible, e.g., to enhance stability, to include modified nucleotides or nucleotide surrogates. In some embodiments all or some of the cytidine will be modified, e.g., with a modification described herein. Modifications can include, e.g., 5-methyl cytidine, and may include the use of modifications at the 2' OH group of the ribose sugar, e.g., the use of 2'-O-methyl ribose sugars. Other modifications may include deoxythymidine, modifications in the phosphate group, e.g., phosphorothioate modifications.

[00204] Modifications and nucleotide surrogates are discussed below. In some embodiments the nucleotide scaffole of the nucleic acid polymer has the structure of Formula (B):
The scaffold presented above in Formula B represents a portion of an oligonucleic acid. The basic components are the ribose or deoxyribose sugar, the base, the terminal phosphates, and phosphate internucleotide linkers. Where the bases are naturally occurring bases, e.g., adenine, uracil, guanine or cytosine, the sugars are the unmodified 2'-hydroxyl ribose sugar or 2'-H, and W, X, Y, and Z are all O, Formula B represents a naturally occurring unmodified oligonucleotide.

Unmodified oligonucleotides may be less than optimal in some applications, e.g., unmodified oligonucleotides can be prone to degradation by e.g., cellular nucleases. Nucleases can hydrolyze nucleic acid phosphodiester bonds. However, chemical modifications to one or more of the above nucleic acid components can confer improved properties, and, e.g., can render oligonucleotides more stable to nucleases.

Modified nucleic acids and nucleotide surrogates can include one or more of: (i) alteration, e.g., replacement, of one or both of the non-linking (X and Y) phosphate oxygens and/or of one or more of the linking (W and Z) phosphate oxygens (When the phosphate is in the terminal position, one of the positions W or Z will not link the
phosphate to an additional element in a naturally occurring nucleic acid. However, for simplicity of terminology, except where otherwise noted, the W position at the 5' end of a nucleic acid and the terminal Z position at the 3' end of a nucleic acid, are within the term "linking phosphate oxygens" as used herein); (ii) alteration, e.g., replacement, of a constituent of the ribose or deoxyribose sugar, e.g., of the 2' hydroxyl on the ribose sugar; (iii) wholesale replacement of the phosphate moiety (bracket I) with "dephospho" linkers; (iv) modification or replacement of a naturally occurring base; (v) replacement or modification of the sugar-phosphate backbone (bracket II); (vi) modification of the 3' end or 5' end of the oligonucleotide, e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, e.g., a fluorescently labeled moiety, to either the 3' or 5' end of oligonucleotide.

[00208] The terms replacement, modification, alteration, and the like, as used in this context, do not imply any process limitation, e.g., modification does not mean that one must start with a reference or naturally occurring ribonucleic acid and modify it to produce a modified oligonucleic acid but rather modified simply indicates a difference from a naturally occurring molecule.

[00209] It is understood that the actual electronic structure of some chemical entities cannot be adequately represented by only one canonical form (i.e., Lewis structure). While not wishing to be bound by theory, the actual structure can instead be some hybrid or weighted average of two or more canonical forms, known collectively as resonance forms or structures. Resonance structures are not discrete chemical entities and exist only on paper. They differ from one another only in the placement or "localization" of the bonding and nonbonding electrons for a particular chemical entity. It can be possible for one resonance structure to contribute to a greater extent to the hybrid than the others. Thus, the written and graphical descriptions of the embodiments of the present invention are made in terms of what the art recognizes as the predominant resonance form for a particular species. For example, any phosphoroamidate (replacement of a nonlinking oxygen with nitrogen) would be represented by X=0 and Y=N in the above Formula (B).

[00210] The Phosphate Group

[00211] The phosphate group is a negatively charged species. The charge is distributed equally over the two non-linking oxygen atoms (i.e., \( X \) or \( Y \) in Formula B above). However, the phosphate group can be modified by replacing one of the oxygens with a different substituent. One result of this modification to the oligonucleotide phosphate
backbones can be increased resistance of the oligonucleotide to nucleolytic breakdown. Thus while not wishing to be bound by theory, it can be desirable in some embodiments to introduce alterations which result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[00212] Examples of modified phosphate groups include phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. Unlike the situation where only one of X or Y is altered, the phosphorus center in the phosphorodithioates is achiral which precludes the formation of oligoribonucleotides diastereomers. Diastereomer formation can result in a preparation in which the individual diastereomers exhibit varying resistance to nucleases. Further, the hybridization affinity of RNA containing chiral phosphate groups can be lower relative to the corresponding unmodified RNA species. Thus, while not wishing to be bound by theory, modifications to both X and Y which eliminate the chiral center, e.g., phosphorodithioate formation, may be desirable in that they cannot produce diastereomer mixtures. Thus, X can be any one of S, Se, B, C, H, N, or O (R is alkyl or aryl). Thus Y can be any one of S, Se, B, C, H, N, or O (R is alkyl or aryl). Replacement of X and/or Y with sulfur is possible.

[00213] The phosphate linker can also be modified by replacement of a linking oxygen (i.e., W or Z in Formula B) with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylene phosphonates). The replacement can occur at a terminal oxygen (position W (3') or position Z (5')). Replacement of W with carbon or Z with nitrogen is possible.

[00214] **The Sugar Group**

[00215] A modified oligonucleotide can include modification of all or some of the sugar groups of the nucleic acid. E.g., the 2' position can be modified or replaced with a number of different "oxy" or "deoxy" substituents. While not being bound by theory, enhanced stability is expected since the hydroxyl can not be deprotonated to form a 2' alkoxide ion. The 2' alkoxide can catalyze degradation by intramolecular nucleophilic attack on the linker phosphorus atom. Again, while not wishing to be bound by theory, it can be desirable to some embodiments to introduce alterations in which alkoxide formation at the 2' position is not possible.
Examples of "oxy"-2' hydroxyl group modifications include alkoxy or aryloxy (OR, e.g., R=H, alkyl, cycloalkyl, aryl, alky, heteroaryl or sugar); polyethyleneglycols (PEG), (CH₂CH₂O)nCH₂Cl₂OR; "locked" nucleic acids (LNA) in which the 2' hydroxyl is connected, e.g., by a methylene bridge, to the 4' carbon of the same ribose sugar; O-AMINE (AMINE=NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino, ethylene diamine, polyamino) and aminoalkoxy, 0(CH₂)nAMINE, (e.g., AMINE=NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino, ethylene diamine, polyamino). It is noteworthy that oligonucleotides containing only the methoxyethyl group (MOE), (OCH₂CH₂OCH₃, a PEG derivative), exhibit nuclease stabilities comparable to those modified with the robust phosphorothioate modification.

"Deoxy" modifications include hydrogen (i.e., deoxyribose sugars, which are of particular relevance to the overhang portions of partially ds RNA); halo (e.g., fluoro); amino (e.g., NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, or amino acid); NH(CH₂CH₂NH₃+CH₂CH₂-AMINE (AMINE=NH₂; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino), -NHCOO(R=alkyl, cycloalkyl, aryl, alky, heteroaryl or sugar), thiol; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with e.g., an amino functionality. Other substitutents of certain embodiments include 2'-methoxymethyl, 2'-OCH₃, 2'-0-allyl, 2'-C-allyl, and 2'-fluoro.

The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified oligonucleotide can include nucleotides containing e.g., arabinose, as the sugar.

Modified oligonucleotides can also include "abasic" sugars, which lack a nucleobase at C-1'. These abasic sugars can also be further contain modifications at one or more of the constituent sugar atoms.

To maximize nuclease resistance, the 2' modifications can be used in combination with one or more phosphate linker modifications (e.g., phosphorothioate). The so-called "chimeric" oligonucleotides are those that contain two or more different modifications.

Replacement of the Phosphate Group
[00222] The phosphate group can be replaced by non-phosphorus containing connectors (cf. Bracket I in Formula B above). While not wishing to be bound by theory, it is believed that since the charged phosphodiester group is the reaction center in nucleolytic degradation, its replacement with neutral structural mimics should impart enhanced nuclease stability. Again, while not wishing to be bound by theory, it can be desirable, in some embodiment, to introduce alterations in which the charged phosphate group is replaced by a neutral moiety.

[00223] Examples of moieties which can replace the phosphate group include siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrozao, methylenedimethylhydrazo and methylenecarbonylamino, methylenecarbonylamino and methylenemethylimino groups.

[00224] Replacement of Ribophosphate Backbone

[00225] Oligonucleotide-mimicking scaffolds can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates (see Bracket II of Formula B above). While not wishing to be bound by theory, it is believed that the absence of a repetitively charged backbone diminishes binding to proteins that recognize polyanions (e.g., nucleases). Again, while not wishing to be bound by theory, it can be desirable in some embodiment, to introduce alterations in which the bases are tethered by a neutral surrogate backbone.

[00226] Examples include the mophilino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates. In certain embodiments, PNA surrogates may be used.

[00227] Terminal Modifications

[00228] The 3’ and 5’ ends of an oligonucleotide can be modified. Such modifications can be at the 3’ end, 5’ end or both ends of the molecule. They can include modification or replacement of an entire terminal phosphate or of one or more of the atoms of the phosphate group. E.g., the 3’ and 5’ ends of an oligonucleotide can be conjugated to other functional molecular entities such as labeling moieties, e.g., fluorophores (e.g., pyrene, TAMRA, fluorescein, Cy3 or Cy5 dyes) or protecting groups (based e.g., on sulfur, silicon, boron or ester). The functional molecular entities can be attached to the sugar
through a phosphate group and/or a spacer. The terminal atom of the spacer can connect to or replace the linking atom of the phosphate group or the C-3′ or C-5′ O, N, S or C group of the sugar. Alternatively, the spacer can connect to or replace the terminal atom of a nucleotide surrogate (e.g., PNAs). These spacers or linkers can include e.g., \((\text{CH}_2)_n\text{N}^+\), \((\text{CH}_2)_n\text{O}^-\), \((\text{CH}_2)_n\text{S}^-\), \(0(\text{CH}_2\text{CH}_2O)_n\text{CH}_2\text{CH}_2\text{OH}\) (e.g., \(n=3\) or \(6\)), abasic sugars, amide, carboxy, amine, oxyamine, oxyimine, disulfide, thiourea, sulfonamide, or morpholino, or biotin and fluorescein reagents. While not wishing to be bound by theory, it is believed that conjugation of certain moieties can improve transport, hybridization, and specificity properties. Again, while not wishing to be bound by theory, it may be desirable to introduce terminal alterations that improve nuclease resistance. Other examples of terminal modifications include dyes, intercalating agents (e.g., acridines), cross-linkers (e.g., psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g., EDTA), lipophilic carriers (e.g., cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, \(1,3\)-Bis-O(hexadecyl)glycerol, geranylxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, 03-(oleoyl)lithocholic acid, 03-(oleoyl)cholonic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (e.g., antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g., biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu³⁺ complexes of tetraazamacrocycles).

Terminal modifications can be added for a number of reasons, including as discussed elsewhere herein to modulate activity or to modulate resistance to degradation. Terminal modifications useful for modulating activity include modification of the 5′ end with phosphate or phosphate analogs. E.g., in certain embodiments oligonucleotide conjugate agents are 5′ phosphorylated or include a phosphoryl analog at the 5′ prime terminus. Suitable modifications include: 5′-monophosphate \(((\text{HO})_2(0\text{P}-0-5'))\); 5′-diphosphate \(((\text{HO})_2(0\text{P}-0-\text{P}(\text{HO})(0)-0-5'))\); 5′-triphosphate \(((\text{HO})_2(0\text{P}-0-(\text{HO})(0)\text{P}-0-\text{P}(\text{HO})(0)-0-5'))\); 5′-guanosine cap (7-methylated or non-methylated) \((7\text{mG}-0-5'-(\text{HO})(0)\text{P}-0-(\text{HO})(0)\text{P}-0-\text{P}(\text{HO})(0)-0-5'))\); 5′-adenosine cap (Appp), and any modified or unmodified nucleotide cap structure \((N-0-5'-(\text{HO})(0)\text{P}-0-(\text{HO})(0)\text{P}-0-\text{P}(\text{HO})(0)-0-5'))\); 5′-monothiophosphate (phosphorothioate; \((\text{HO})_2(S)\text{P}-0-5')\); 5′-monothiophosphate
(phosphorodithioate; (HO)(HS)(S)P~0-5'), 5'-phosphorothiolate ((HO)2(P-S-5'); any additional combination of oxygen/sulfur replaced monophosphate, diphosphate and triphosphates (e.g., 5'-alpha-thiotriphosphate, 5'-gamma-thiotriphosphate, etc.), 5'-phosphoramidates ((HO)2P~0-NH5', (HO)(NH2)(0) P-O-5'), 5'-alklyphosphonates (R=alkyl=methyl, ethyl, isopropyl, propyl, etc., e.g., RP(OH)(0)-0-5', (OH)2(0) P-5'-CH.sub.2), 5'-alkeletherphosphonates (R=alkylether=methoxymethyl (MeOCH2), ethoxymethyl, etc., e.g., RP(OH)(0)-0-5').

[00230] Terminal modifications can also be useful for increasing resistance to degradation.

[00231] Terminal modifications can also be useful for monitoring distribution, and in such cases the groups to be added may include fluorophores, e.g., fluorescein or an Alexa dye, e.g., Alexa 488. Terminal modifications can also be useful for enhancing uptake, useful modifications for this include cholesterol. Terminal modifications can also be useful for cross-linking an RNA agent to another moiety; modifications useful for this include mitomycin C.

[00232] The Bases

[00233] Adenine, guanine, cytosine and uracil are the most common bases found in RNA. These bases can be modified or replaced to provide RNAs having improved properties. E.g., nucleoside resistant oligoribonucleotides can be prepared with these bases or with synthetic and natural nucleobases (e.g., inosine, thymine, xanthine, hypoxanthine, nubularine, isoguanisine, or tubercidin) and any one of the above modifications. Alternatively, substituted or modified analogs of any of the above bases and "universal bases" can be employed. Examples include 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 5-halouracil, 5-(2-aminopropyl)uracil, 5-amino allyl uracil, 8-halo, amino, thiol, thioalkyl, hydroxyl and other 8-substituted adenines and guanines, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine, 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine, dihydrouracil, 3-deaza-5-azacytosine, 2-aminopurine, 5-alkyluracil, 7-alkylguanine, 5-alkyl cytosine, 7-deazaadenine, N6, N6-dimethyladenine, 2,6-diaminopurine, 5-amino-allyl-uracil, N3-methyluracil, substituted 1,2,4-triazoles, 2-pyridinone, 5-nitroindole,

Generally, base changes are not used for promoting stability, but they can be useful for other reasons, e.g., some, e.g., 2,6-diaminopurine and 2 amino purine, are fluorescent. Modified bases can reduce target specificity. This may be taken into consideration in the design of oligonucleotide conjugate agents.

**Carbohydrate Conjugates**

As used herein, "carbohydrate" refers to a compound which is either a carbohydrate per se made up of one or more monosaccharide units having at least 6 carbon atoms (which may be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which may be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4-9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C₅ and above (preferably C₅-C₆) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (preferably C₅-C₆).

The term "monosaccharide" embraces radicals of allose, altrose, arabinose, cladinose, erythrose, erythrulose, fructose, D-fucitol, L-fucitol, fucosamine, fucose, fuculose, galactosamine, D-galactosaminol, N-acetyl-galactosamine, galactose, glucosamine, N-acetyl-glucosamine, glucosaminol, glucose, glucose-6-phosphate, gulose glyceraldehyde, L-glycero-D-mannos-heptose, glycerol, glycerone, gulose, idose, lyxose, mannosamine, mannose, mannose-6-phosphate, psicose, quinovose, quinovosamine, rhamnitol, rhamnosamine, rhamnose, ribose, ribulose, sedoheptulose, sorbose, tagatose, talose, tartaric acid, threose, xylose and xylulose. The monosaccharide can be in D- or L
configuration. The monosaccharide may further be a deoxy sugar (alcoholic hydroxy group replaced by hydrogen), amino sugar (alcoholic hydroxy group replaced by amino group), a thio sugar (alcoholic hydroxy group replaced by thiol, or C=0 replaced by C=S, or a ring oxygen of cyclic form replaced by sulfur), a seleno sugar, a telluro sugar, an aza sugar (ring carbon replaced by nitrogen), an imino sugar (ring oxygen replaced by nitrogen), a phosphono sugar (ring oxygen replaced with phosphorus), a phospha sugar (ring carbon replaced with phosphorus), a C-substituted monosaccharide (hydrogen at a non-terminal carbon atom replaced with carbon), an unsaturated monosaccharide, an alditol (carbonyl group replaced with CHOH group), aldonic acid (aldehydic group replaced by carboxy group), a ketoaldonic acid, a uronic acid, an aldaric acid, and so forth. Amino sugars include amino monosaccharides, preferably galactosamine, glucosamine, mannosamine, fucosamine, quinovosamine, neuraminic acid, muramic acid, lactosamine, acosamine, bacillosamine, daunosamine, desosamine, forosamine, garosamine, kansamine, kansosamine, myaminose, mycosamine, perosamine, pneumosamine, purpurosamine, rhodosamine. It is understood that the monosaccharide and the like can be further substituted.

[00237] The terms "disaccharide", "trisaccharide" and "polysaccharide" embrace radicals of abequose, acarbose, anucetose, amylopectin, amylose, apiose, arcanose, ascarylose, ascorbic acid, bovinose, cellobiose, cellobiose, cellulose, chacotriose, chalcose, chit in, colitose, cyclodextrin, cymarose, dextrin, 2-deoxyribose, 2-deoxyglucose, diginose, digitalose, digitoxose, evalose, evemitrose, fructooligosachharide, galto-oligosaccharide, gentianose, gentiobiose, glucan, glucogen, glycogen, hamamelose, heparin, inulin, isolevoglucosenone, isomaltose, isomaltotriose, isopanose, kojibiose, lactose, lactosamine, lactosidamine, laminarabiose, levoglucosan, levoglucosenone, .beta.-maltose, maltriose, mannan-oligosaccharide, manninotnose, melezitose, melibiose, muramic acid, mycarose, mycinose, neuraminic acid, nigerose, nojirimycin, noviose, oleandrose, panose, paratose, planteose, pnneverose, raffinose, rhodinose, rutinose, sarmentose, sedoheptulose, sedoheptulosan, solatriose, sorphorose, stachyose, streptose, sucrose, am-trehalose, trehalosamine, turanose, tyvelose, xyllobose, umbelliferose and the like. Further, it is understood that the "disaccharide", "trisaccharide" and "polysaccharide" and the like can be further substituted. Disaccharide also includes amino sugars and their derivatives, particularly, a mycaminose, derivatized at the C-4' position or a 4 deoxy-3-amino-glucose derivatized at the C-6' position.
In one aspect, the invention provides a sequence independent pharmaceutically acceptable oligonucleotide that is conjugated with at least one carbohydrate ligand, e.g., monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, polysaccharide. In one embodiment, these carbohydrate-conjugated sequence independent oligonucleotide target, in particular, the parenchymal cells of the liver. In another embodiment, the carbohydrate-conjugated sequence independent oligonucleotide includes more than one carbohydrate ligand, preferably two or three. In one embodiment, the carbohydrate-conjugated sequence independent oligonucleotide comprises one or more galactose moiety. In another embodiment, the carbohydrate-conjugated sequence independent oligonucleotide includes at least one (e.g., two or three or more) N-Acetyl-Galactosamine (GalNAc) or N-Ac-Glucosamine (GluNAc). In another embodiment, the carbohydrate-conjugated sequence independent oligonucleotide includes at least one (e.g., two or three or more) N-Acetyl-Galactosamine (GalNAc). In another embodiment, the carbohydrate-conjugated sequence independent oligonucleotide includes at least three N-Acetyl-Galactosamine (GalNAc).

Carbohydrate and lipid conjugates of oligonucleotide compositions and therapeutic agents are described and can be made as disclosed in U.S. Patents, e.g. US 8,598,139, US 8,507,455, US 8,450,467, US 8,344,125, and US 8,106,022 and US Patent Publications e.g. US 20130338210 and US 20140099666, the contents of which are hereby incorporated by reference herein in their entirety.

The presently claimed invention involves coupling certain conjugates to the aforementioned sequence independent oligonucleotides for delivery, particularly adding carbohydrate conjugates, and more particularly coupling GalNAc conjugates, to the non-specific oligonucleotides disclosed in the above-cited patents and applications. Such carbohydrate conjugates can be coupled via linkers as described in the cited US patents and patent publications above. The resulting non-sequence specific oligonucleotide antiviral agent conjugates are envisioned to achieve greater efficacy, to exhibit longer half-life inside a patient after administration, and to improve the therapeutic index for the non-sequence specific oligonucleotide antiviral agent. The sequence specific oligonucleotide antiviral agent conjugates, and methods of using them for treatment of conditions associated with virus infections in a subject, are embodiments of the present invention.
In one aspect, the invention provides an oligonucleotide conjugate agent that is conjugated with at least one carbohydrate ligand, e.g., a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, an oligosaccharide, or a polysaccharide. These carbohydrate-conjugated oligonucleotide agents target, in particular, the parenchymal cells of the liver. In one embodiment, the oligonucleotide conjugate agent includes more than one carbohydrate ligand, preferably two or three. In one embodiment, the oligonucleotide conjugate agent comprises one or more galactose moieties. In another embodiment, the oligonucleotide conjugate agent includes at least one (e.g., two or three or more) lactose molecules (lactose is a glucose coupled to a galactose). In another embodiment, the oligonucleotide conjugate agent includes at least one (e.g., two or three or more) N-Acetyl-Galactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (e.g., mannose-6-phosphate). In one embodiment, oligonucleotide conjugate agent comprises at least one mannose ligand, and the oligonucleotide conjugate agent targets macrophages.

In one aspect, the invention features an oligonucleotide conjugate agent comprising a carbohydrate ligand, and the presence of the carbohydrate ligand can increase delivery of the oligonucleotide conjugate agent to the liver. Thus an oligonucleotide conjugate agent comprising a carbohydrate ligand can be useful for targeting a gene for which expression is undesired in the liver. For example, an oligonucleotide conjugate agent comprising a carbohydrate ligand can target a nucleic acid expressed by a hepatitis virus (e.g., hepatitis C, hepatitis B, hepatitis A, hepatitis D, hepatitis E, hepatitis F, hepatitis G, or hepatitis H).

In one embodiment, the carbohydrate-conjugated oligonucleotide conjugate agent targets a gene of the hepatitis C virus. In another embodiment, the oligonucleotide conjugate agent that targets a gene of the hepatitis C virus can be administered to a human having or at risk for developing hepatitis, e.g., acute or chronic hepatitis, or inflammation of the liver. A human who is a candidate for treatment with a carbohydrate-conjugated oligonucleotide conjugate agent, e.g., an oligonucleotide conjugate agent that targets a gene of HCV, can present symptoms indicative of HCV infection, such as jaundice, abdominal pain, liver enlargement and fatigue.

In one embodiment, a carbohydrate-conjugated oligonucleotide conjugate agent targets the 5’ core region of HCV. This region lies just downstream of the ribosomal toe-print straddling the initiator methionine. In another embodiment, an oligonucleotide conjugate agent targets any one of the nonstructural proteins of HCV, such
as NS3, NS4A, NS4B, NS5A, or NS5B. In another embodiment, an oligonucleotide conjugate agent targets the E1, E2, or C gene of HCV.

[00245] In another embodiment, the carbohydrate-conjugated oligonucleotide conjugate agent targets a hepatitis B virus (HBV), and the oligonucleotide conjugate agent has a sequence that is substantially similar to a sequence of a gene of HBV, e.g., the protein X (HBx) gene of HBV.

[00246] Carbohydrate-conjugated oligonucleotide conjugate agents can also be used to treat other liver disorders, including disorders characterized by unwanted cell proliferation, hematological disorders, metabolic disorders, and disorders characterized by inflammation. A proliferation disorder of the liver can be, for example, a benign or malignant disorder, e.g., a cancer, e.g., a hepatocellular carcinoma (HCC), hepatic metastasis, or hepatoblastoma. A hepatic hematologic or inflammation disorder can be a disorder involving clotting factors, a complement-mediated inflammation or a fibrosis, for example. Metabolic diseases of the liver include dyslipidemias and irregularities in glucose regulation. In one embodiment, a liver disorder is treated by administering one or more oligonucleotide conjugate agents that have a sequence that is substantially identical to a sequence in a gene involved in the liver disorder.

[00247] In one embodiment, a carbohydrate-conjugated oligonucleotide conjugate agent targets a nucleic acid expressed in the liver, such as an ApoB RNA, c-jun RNA, beta-catenin RNA, or glucose-6-phosphatase mRNA.

[00248] An oligonucleotide conjugate that targets glucose-6-phosphatase can be administered to a subject to inhibit hepatic glucose production, e.g., for the treatment of glucose-metabolism-related disorders, such as diabetes, e.g., type-2 diabetes mellitus. The oligonucleotide conjugate agent can be administered to an individual at risk for the disorder to delay onset of the disorder or a symptom of the disorder.

[00249] Therefore, in certain embodiments, the present invention provides a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues.

[00250] In another embodiment, the present invention provides a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent comprising a sequence independent oligonucleotide conjugated with one
or more GalNAc carbohydrate residues in combination with a second pharmaceutically acceptable agent that stimulates immune function.

[00251] In yet another embodiment, the present invention provides a method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent comprising a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues in combination with a second pharmaceutically acceptable agent that stimulates immune function and a third pharmaceutically acceptable agent comprising an antiviral compound.

[00252] In still other embodiments, the administration of the sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues, inhibits the release of hepatitis B surface antigen (HBsAg) from infected hepatocytes.

[00253] In some embodiments of the present invention provides a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of a pharmaceutically acceptable oligonucleotide agent conjugate wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00254] In another embodiment there is provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising administering a first pharmaceutically acceptable therapeutic agent that is an oligonucleotide conjugate which removes HBsAg from the blood of the HBV infected host, and a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00255] There is also provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the release of HBsAg from infected cells, and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00256] There is also provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of an effective dosing regimen of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the release of HBV subviral particles from
infected cells, and an effective dosing regimen of a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00257] There is also provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the formation of HBV subviral particles in infected cells, and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00258] There is also provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the synthesis of or lowers the intracellular concentration of HBsAg in infected cells, and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00259] There is also provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of the first pharmaceutically acceptable agent that is an oligonucleotide conjugate and second pharmaceutically acceptable agent as described above, in combination in a single pharmaceutical composition or in two different pharmaceutical compositions given by the same route of administration, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00260] There is also provided a method for the treatment of HBV infection or HBV/HDV co-infection in a subject requiring such treatment, the method comprising the administration of the first pharmaceutically acceptable agent that is an oligonucleotide conjugate and second pharmaceutical agent as described above, simultaneously in a patient, whether given by the same or different routes of administration.

[00261] In accordance with the present description there is herein provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide which removes hepatitis B surface antigen from the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function for
the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00262] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the release of HBsAg from infected cells, and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00263] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the release of HBV subviral particles from infected cells and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00264] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the formation of HBV subviral particles in infected cells and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00265] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which inhibits the synthesis of or lowers the intracellular concentration of HBsAg in infected cells and a second pharmaceutically acceptable immunotherapeutic agent which stimulates immune function for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00266] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which removes hepatitis B surface antigen from the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function in the manufacture of a medicament for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00267] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which removes hepatitis B surface antigen from
the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function in the manufacture of a medicament for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide-carbohydrate conjugate has an oligonucleotide sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[00268] There is also provided the use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which removes hepatitis B surface antigen from the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function in the manufacture of a medicament for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide-carbohydrate conjugate has the structure depicted in Scheme 1.

[00269] In accordance with the present description there is herein provided a composition for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, said composition comprising an effective dose of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which removes hepatitis B surface antigen from the blood and an effective dosing regimen of a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function, wherein the oligonucleotide conjugate is an oligonucleotide-carbohydrate conjugate.

[00270] In an embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood inhibits the formation of HBV subviral particles.

[00271] In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood inhibits the intracellular transit of HBV subviral particles.

[00272] In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood inhibits the release of HBV subviral particles into the blood.

[00273] In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood inhibits the release of hepatitis B surface antigen from the infected cell.

[00274] In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood inhibits the synthesis of HBsAg and or another viral protein.
In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood inhibits the synthesis or function of apolipoprotein H.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood is a nucleic acid polymer comprising a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood is nucleic acid polymer comprising a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising the repeats of the sequence CA.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood is a nucleic acid polymer comprising a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising the repeats of the sequence TG.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood is a nucleic acid polymer comprising a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising the repeats of the sequence GT.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises at least one 2’ ribose modification.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises all riboses having a 2’ modification.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises at least one 2’ O methyl ribose modification.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises all riboses having the 2’ O methyl modification.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises at least one 5’methylcytosine.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises all cytosines present as 5’methylcytosine.
In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises at least one 2′ ribose modification and at least one 5′ methylcytosine.

In another embodiment, the nucleic acid polymer of the oligonucleotide conjugate agent further comprises all riboses having the 2′ O methyl modification and all cytosines present as 5′methylcytosine.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide selected from the group consisting of SEQ ID NOs: 1-10.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood is an oligonucleotide conjugate chelate complex comprising an oligonucleotide selected from the group consisting of SEQ ID NOs: 1-10.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide consisting of SEQ ID NO: 2.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide conjugate chelate complex comprising SEQ ID NO: 2.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide consisting of SEQ ID NO: 3.

In another embodiment, the agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide consisting of SEQ ID NO: 10.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood is an oligonucleotide conjugate chelate complex comprising an oligonucleotide conjugate comprising a sequence selected from the group consisting of SEQ ID NO: 10.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an antisense oligonucleotide conjugate which targets any portion of any HBV mRNA.
In another embodiment, the oligonucleotide conjugate is further formulated as an oligonucleotide conjugate chelate complex.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide aptamer or Speigelmer targeting the hepatitis B surface antigen.

In another embodiment, the oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood comprises an oligonucleotide aptamer or Speigelmer targeting apolipoprotein H.

In another embodiment, the composition comprising an oligonucleotide conjugate agent removing hepatitis B surface antigen from the blood further comprises an antibody or antibody fragment that recognizes the hepatitis B surface antigen.

In another embodiment, the immunotherapeutic agent stimulating immune function comprises one or more compounds selected from the group consisting of: Thymosin α1; Any α-interferon or pegylated derivatives thereof; Any β-interferon or pegylated derivatives thereof; Any γ-interferon or pegylated derivatives thereof; Any λ-interferon or pegylated derivatives thereof; Interferon a-2a or a-2b or a-N3; Interferon β-la or β-1b; Interferon γ-1b; Interferon λ1 or λ2 or A3; Pegylated interferon a-2a or a-2b or λ1 or λ2; Any antiviral cytokine or pegylated derivatives thereof; Thymic protein A; Any polypeptide shown to have antiviral activity or immunostimulatory activity; An immunostimulatory oligonucleotide including IMO-2055 or IMO-2125; A small molecule Toll-like receptor (TLR) agonist including GS-9620 or ANA-773; and Any antiviral or immunostimulatory hormone including DHEA or its metabolites.

In another embodiment, the immunotherapeutic agent stimulating immune function comprises one or more compounds selected from the group consisting of: A non-CpG immunostimulatory oligonucleotide including IMO-2055 or IMO-2125; A small molecule Toll-like receptor (TLR) agonist including GS-9620 or ANA-773; and any antiviral or immunostimulatory hormone including DHEA or its metabolites.

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

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

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

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, intramuscular injection, intraperitoneal injection, intravenous injection and intravenous infusion.

In a further embodiment, the composition comprising an oligonucleotide conjugate agent removing HBsAg from the blood further comprises one or more molecules selected from the group consisting of:

the following triazolopyrimidine derivatives:

\[
\begin{align*}
5-(4\text{-chlorophenyl})-7-(2,6\text{-difluorophenyl})-4,5,6,7\text{-tetrahydro-}
&[1,2,4]\text{triazolo}[1,5-a]\text{pyrimidine} \\
\end{align*}
\]

or

\[
\begin{align*}
7-(2\text{-chloro-6-fluorophenyl})-5-(4\text{-chlorophenyl})-4,5,6,7\text{-}
&\text{tetrahydro-}[1,2,4]\text{triazolo}[1,5-a]\text{pyrimidine} \\
\end{align*}
\]

an oligonucleotide selected from the following:

a. SEQ ID NO: 2;
b. SEQ ID NO: 3;
c. SEQ ID NO: 10;
d. SEQ ID NOs: 1 and 4-9 wherein the oligonucleotide further comprises one or more GalNAc residues;  
a nucleic acid polymer selected from the following:  
e. a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;  
f. a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;  
g. 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 wherein the nucleic acid polymer further comprises one or more GalNAc residues; and  
h. the compound depicted in Scheme 1;  
a Speigelmer or aptamer targeting the hepatitis B surface antigen;  
a Speigelmer or aptamer targeting human apoliporotein H; and  
a second immunotherapeutic agent comprising one or more molecules from the group consisting of:  
i. thymosin α1;  
j. any α-interferon or pegylated derivatives thereof;  
k. any β-interferon or pegylated derivatives thereof;  
l. any γ-interferon or pegylated derivatives thereof;  
m. any λ-interferon or pegylated derivatives thereof;  
n. interferon a-2a or a-2b or a-N3;  
o. interferon β-1a or β-1b;  
p. interferon ν-1b;  
q. interferon λ1 or λ2 or λ3  
r. pegylated interferon a-2a or a-2b or λ1 or λ2;  
s. any antiviral cytokine or pegylated derivatives thereof;  
t. thymic protein A;  
u. any polypeptide shown to have antiviral activity or immunostimulatory activity;  
v. an immunostimulatory oligonucleotide including IMO-2055 or IMO-2125;  
w. a small molecule Toll-like receptor (TLR) agonist including GS-9620 or ANA-773; and
x. any antiviral or immunostimulatory hormone including DHEA or its metabolites.

[00309] In a further embodiment, the following oligonucleotide can be further formulated as an oligonucleotide conjugate chelate complex:

SEQ ID NO: 2;
SEQ ID NO: 3;
SEQ ID NO: 10;
SEQ ID NOs: 1 and 4-9 wherein the oligonucleotide further comprises one or more GalNAc residues;

a nucleic acid polymer selected from the following:

a. a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence AC;
b. a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence CA;
c. a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence TG;
d. a phosphorothioated oligonucleotide from 20-120 nucleotides in length comprising repeats of the sequence GT wherein the nucleic acid polymer of of a., b., c., and/or d. further comprises one or more GalNAc residues; and
e. the compound depicted in Scheme 1.

[00310] In another embodiment, the uses or method of treatments described above further comprise administering or using concurrently a third pharmaceutically acceptable agent selected from the following:

a. tenofovir disoproxil fumarate;
b. entecavir;
c. telbuvidine;
d. adefovir dipivoxil; and
e. lamivudine.

[00311] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises pegylated interferon a-2a.
There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate complex of SEQ ID No: 2 and a second pharmaceutically acceptable agent which comprises pegylated interferon a-2a.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises pegylated interferon a-2a.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises pegylated interferon a-2a.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises pegylated interferon a-2a.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate complex of SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises pegylated interferon a-2a.
SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises thymosin alpha 1.

[0031] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises thymosin alpha 1.

[0031] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises thymosin alpha 1.

[00320] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises thymosin alpha 1.

[00321] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises thymosin alpha 1a.

[00322] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises thymosin alpha 1.

[00323] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the
method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises pegylated interferon α-2b.

[00324] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises pegylated interferon α-2b.

[00325] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises pegylated interferon α-2b.

[00326] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises pegylated interferon α-2b.

[00327] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises pegylated interferon α-2b.

[00328] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises pegylated interferon α-2b.
There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises pegylated interferon λ1.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises pegylated interferon λ1.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises pegylated interferon λ1.

There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises pegylated interferon λ1.
oligonucleotide chelate conjugate complex comprising SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises pegylated interferon λ1.

[00335] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises GS-9620.

[00336] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 2 and a second pharmaceutically acceptable agent which comprises GS-9620.

[00337] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises GS-9620.

[00338] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an oligonucleotide chelate conjugate complex comprising SEQ ID NO: 3 and a second pharmaceutically acceptable agent which comprises GS-9620.

[00339] There is also provided a method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises GS-9620.

[00340] There is also provided method for the treatment of or the use of the following in the treatment of hepatitis B infection or of hepatitis B/hepatitis D co-infection, the method or use comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which comprises an
oligonucleotide chelate conjugate complex comprising SEQ ID NO: 10 and a second pharmaceutically acceptable agent which comprises GS-9620.

[00341] There is also provided a method for the treatment of Hepatitis B infection or of Hepatitis D co-infection, the method comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate comprising at least one subunit of formula (I):

![Formula (I)](image)

[00342] Formula (I)

[00343] wherein:

[00344] A and B are each independently for each occurrence O, N(R^N), S or absent;

[00345] R^N is independently for each occurrence H or C_1-C_6 alkyl;

[00346] X and Y are each independently for each occurrence H, OH, a hydroxyl protecting group, a phosphate group, a phosphodiester group, an activated phosphate group, an activated phosphite group, a phosphoramidite, a solid support, -P(Z')(Z'')-nucleoside, -P(Z')(Z'')-0-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside, -P(Z')(Z'')-0-Linker-OP(Z''')(Z''')-0-oligonucleotide, an oligonucleotide, -P(Z')(Z'')-formula (I), -P(Z')(Z'')-, -Linker-R, or absent;

[00347] R is L^O or has the structure shown below:

![Structure of R](image)

[00348] L^O is independently for each occurrence a ligand including a carbohydrate, a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and Z', Z'', Z''' and Z'''' are each independently for each occurrence O or S; and a second therapeutically active agent.

[00349] In a particular embodiment there is provided a method for the treatment of Hepatitis B infection or of Hepatitis D co-infection as described, the method
comprising administering to a patient in need of such treatment the oligonucleotide conjugate therapeutic composition as described, further wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y comprises a sequence selected from:

\[
\begin{align*}
5'{-}A{-}[CA]_n{-}C{-}3'; \\
5'{-}C{-}[AC]_n{-}A{-}3'; \\
5'{-}T{-}[GT]_n{-}G{-}3'; \\
5'{-}G{-}[TG]_n{-}T{-}3'; \\
5'{-}A{-}[TA]_n{-}T{-}3'; \\
5'{-}T{-}[AT]_n{-}A{-}3'; \\
5'{-}G{-}[CG]_n{-}C{-}3'; \\
5'{-}C{-}[GC]_n{-}G{-}3'; \\
5'{-}C{-}[TC]_n{-}T{-}3'; \\
5'{-}T{-}[CT]_n{-}C{-}3'; \\
5'{-}G{-}[AG]_n{-}A{-}3'; \\
5'{-}A{-}[GA]_n{-}G{-}3';
\end{align*}
\]

wherein \( n \) is 19-59.

[00351] In a particular embodiment there is provided a method for the treatment of Hepatitis B infection or of Hepatitis D co-infection as described, the method comprising administering to a patient in need of such treatment the oligonucleotide conjugate therapeutic composition as described, further wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y comprises any of SEQ ID NOs. 1-10.

[00352] In a particular embodiment there is provided a method for the treatment of Hepatitis B infection or of Hepatitis D co-infection as described, the method comprising administering to a patient in need of such treatment the oligonucleotide conjugate therapeutic composition as described, further wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the following structure:
wherein n is 19-60; and
wherein R² is H or CH₃.

[00353] In a particular embodiment there is provided a method for the treatment of Hepatitis B infection or of Hepatitis D co-infection as described, the method comprising administering to a patient in need of such treatment an oligonucleotide conjugate therapeutic composition wherein the oligonucleotide conjugate has a structure as depicted in Scheme 1.

[00354] In a particular embodiment there is provided a method for the treatment of Hepatitis B infection or of Hepatitis D co-infection as described, the method comprising administering to a patient in need of such treatment the oligonucleotide conjugate therapeutic composition as described, further wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the structure
wherein \( R \) is \( \text{H} \) or \( \text{CH}_3 \).

[00355] In another particular embodiment, there is provided a method for treatment of Hepatitis B infection or of Hepatitis D co-infection in a patient in need thereof, wherein the oligonucleotide conjugate therapeutic composition further comprises a second therapeutically active agent.

[00356] In a particular embodiment there is provided an oligonucleotide conjugate comprising at least one subunit of formula (I):

\[
\begin{array}{c}
\text{X--A} \\
\text{Linke} \\
\text{R}
\end{array}
\]

\[
\begin{align*}
\text{A} & \quad \text{B} \\
\text{Y}
\end{align*}
\]

Formula (I)

wherein:

- \( A \) and \( B \) are each independently for each occurrence \( \text{O} \), \( \text{N}(\text{R}^\text{N}) \), \( \text{S} \) or absent;
- \( \text{R}^\text{N} \) is independently for each occurrence \( \text{H} \) or \( \text{C}_1-\text{C}_6 \) alkyl;
X and Y are each independently for each occurrence H, OH, a hydroxyl protecting group, a phosphate group, a phosphodiester group, an activated phosphate group, an activated phosphite group, a phosphoramidite, a solid support, -P(Z')(Z'')0-nucleoside, -P(Z')(Z'')0-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside, -P(Z')(Z'')0-Linker-OP(Z'')(Z'''')0-oligonucleotide, an oligonucleotide, -P(Z')(Z'')-formula (I), -P(Z')(Z'')-Linker-R or is absent; R is L\(^G\) or has the structure shown below:

\[
\begin{align*}
\text{Linker-}L^G & & \text{Linker-}L^G \\
\text{Linker-}L^G, & & \text{or} \\
\text{Linker-}L^G, & & \\
\text{Linker-}L^G, & & \\
\text{Linker-}L^G & & \\
\end{align*}
\]

L\(^G\) is independently for each occurrence a ligand including a carbohydrate, a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and

Z', Z'', Z''' and Z'''' are each independently O or S for each occurrence.

[00357] Another embodiment provides an oligonucleotide conjugate as described herein, wherein X or Y is independently an oligonucleotide or absent and wherein only X or only Y is present in the oligonucleotide conjugate but not both, further wherein X or Y wherein the oligonucleotide X or Y comprises a sequence selected from:

\[
\begin{align*}
5'\text{-A-}[\text{CA}]_n\text{-C-3'}; \\
5'\text{-C-}[\text{AC}]_n\text{-A-3'}; \\
5'\text{-T-}[\text{GT}]_n\text{-G-3'}; \\
5'\text{-G-}[\text{TG}]_n\text{-T-3'}; \\
5'\text{-A-}[\text{TA}]_n\text{-T-3'}; \\
5'\text{-T-}[\text{AT}]_n\text{-A-3'}; \\
5'\text{-G-}[\text{CG}]_n\text{-C-3'}; \\
5'\text{-C-}[\text{GC}]_n\text{-G-3'}; \\
5'\text{-C-}[\text{TC}]_n\text{-T-3'}; \\
5'\text{-T-}[\text{CT}]_n\text{-C-3'}; \\
5'\text{-G-}[\text{AG}]_n\text{-A-3'}; \and
\end{align*}
\]
5'-A-[GA]_n-G-3',

wherein n is 19-59.

[00358] In a particular embodiment there is provided an oligonucleotide conjugate as described herein, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y comprises any of SEQ ID NOs. 1-10.

[00359] In a particular embodiment there is provided an oligonucleotide conjugate as described herein, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the structure:

wherein n is 19-60; and
wherein R^2 is H or CH_3.

[00360] In a particular embodiment there is provided an oligonucleotide conjugate as described herein, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the structure:
wherein \( R \) is H or CH\(_3\).

[00361] In a particular embodiment there is provided an oligonucleotide conjugate wherein the oligonucleotide has a structure as depicted in Scheme 1.

[00362] In certain embodiments, the oligonucleotide X or Y is connected to at least one subunit of formula (I) at the 3'-end of the oligonucleotide.

[00363] In certain embodiments, the oligonucleotide X or Y is connected to at least one subunit of formula (I) at the 5'-end of the oligonucleotide.

[00364] In certain embodiments, the oligonucleotide X or Y is connected to at least one subunit of formula (I) at an internal phosphate of the oligonucleotide.

[00365] In a particular embodiment there is provided an oligonucleotide conjugate as described herein wherein the oligonucleotide X or Y is connected to formula (I) via a molecule of formula (C):
formula (C)

wherein \( R \) is O or S.

In certain embodiments, the linker is \(-[(P-Q''-R)q-X-(P'-Q'''-R')q.]\)

wherein:

\( P, R, T, P', R' \) and \( T \) are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH\(_2\), CH\(_2\)NH, CH\(_2\)O; NHCH(R\(^a\)C(0), \( \sim C(0)\sim CH(R \sim a)\sim NH\sim \),

\( CH=NH \),

or hetercycle:

\( Q'' \) and \( Q''' \) are each independently for each occurrence absent, \( \sim (CH\(_2\))n\sim \), \( C(R'1)(R'2)(CH\(_2\))n\sim \), \( -(CH\(_2\))nC(R'1)(R'2)\sim \), \( -(CH\(_2\)CH\(_2\)0)\sim \), \( CH=NH \); or

\( (CH\(_2\)CH\(_2\)0)mCH\(_2\)CH\(_2\)NH\sim \);

\( X \) is absent or a cleavable linking group;

\( R^a \) is H or an amino acid side chain;

\( R^1 \) and \( R^2 \) are each independently for each occurrence H, CH\(_3\), OH, SH or N(R\(^N\))\(_2\);

\( R^N \) is independently for each occurrence H, methyl, ethyl, propyl, isopropyl, butyl or benzyl;
q, q’ and q” are each independently for each occurrence 0-20 and wherein the repeating unit can be the same or different; n is independently for each occurrence 1-20; and m is independently for each occurrence 0-50.

In certain embodiment, the Linker comprises at least one cleavable linking group.

In certain embodiments, the Linker is a branched Linker.

One embodiment provides a therapeutic composition for treatment of Hepatitis B infection or of Hepatitis D co-infection for administration to a patient in need of such treatment, wherein the composition comprises a first pharmaceutically acceptable agent that is an oligonucleotide conjugate comprising at least one subunit of formula (I):

\[
\begin{align*}
X & \quad A \\
\text{Linker} & \\
 B & \quad Y
\end{align*}
\]

wherein:

A and B are each independently for each occurrence O, N(R\text{H}), S or absent; 

R\text{H} is independently for each occurrence H or C\text{1-6} alkyl;

X and Y are each independently for each occurrence H, OH, a hydroxyll protecting group, a phosphate group, a phosphodiester group, an activated phosphate group, an activated phosphite group, a phosphoramidite, a solid support, \(\text{-P}(Z')(Z")0-\)nucleoside, \(\text{-P}(Z')(Z")0-\)oligonucleotide, \(\text{-P}(Z')(Z")-\)Linker-\(\text{OP}(Z')(Z")0-\)oligonucleotide, an oligonucleotide, \(\text{-P}(Z')(Z")-\)formula (I), \(\text{-P}(Z')(Z")-\), \(\text{-Linker-R}\) or is absent;

R is \(\text{L}^{\text{G}}\) or has the structure shown below:
L^G is independently for each occurrence a ligand including a carbohydrate, a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and
Z', Z'', Z''' and Z'''' are each independently O or S for each occurrence.

In another particular embodiment there is provided the therapeutic composition as described herein, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y comprises a sequence selected from:

5'-A-[CA]n-C-3';
5'-C-[AC]n-A-3';
5'-T-[GT]n-G-3';
5'-G-[TG]n-T-3';
5'-A-[TA]n-T-3';
5'-T-[AT]n-A-3';
5'-G-[CG]n-C-3';
5'-C-[GC]n-G-3';
5'-C-[TC]n-T-3';
5'-T-[CT]n-C-3';
5'-G-[AG]n-A-3'; and
5'-A-[GA]n-G-3',

wherein n is 19-59.

In another particular embodiment there is provided the therapeutic composition as described herein, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y comprises any of SEQ ID NOs. 1-10.
In another particular embodiment there is provided a therapeutic composition as described herein, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the following structure:

\[
\text{Structure Image}
\]

wherein \( n \) is 19-60; and
wherein \( R^2 \) is H or CH\(_3\).
wherein R is H or CH₃.

[00374] In still another particular embodiment there is provided the therapeutic composition as described, wherein the oligonucleotide has the structure as depicted in Scheme 1.

[00375] In another particular embodiment the therapeutic composition further comprises a second therapeutically active agent.

[00376] In a particular embodiment there is provided the therapeutic composition as described herein, wherein the second therapeutically active agent is selected from:

a. thymosin α1;
b. any α-interferon or pegylated derivatives thereof;
c. any β-interferon or pegylated derivatives thereof;
d. any γ-interferon or pegylated derivatives thereof;
e. any λ-interferon or pegylated derivatives thereof;
f. interferon α-2a or α-2b or α-N3;
g. interferon β-1a or β-1b;
h. interferon v-1b;
i. interferon λ1 or λ2 or A3
j. pegylated interferon a-2a or a-2b or λ1 or λ2;
k. any antiviral cytokine or pegylated derivatives thereof;
i. thymic protein A;
m. any polypeptide shown to have antiviral activity or immunostimulatory activity;
n. an immunostimulatory oligonucleotide including IMO-2055 or IMO-2125;
o. a small molecule Toll-like receptor (TLR) agonist including GS-9620 or ANA-773; and
p. any antiviral or immunostimulatory hormone including DHEA or its metabolites.

[00377] In another particular embodiment, the second therapeutic agent is selected from: ribavirin; an HBV RNA replication inhibitor; an antisense oligomer; an HBV therapeutic vaccine; an HBV prophylactic vaccine; lamivudine (3TC); entecavir; tenofovir; telbivudine (LdT); adeovir; and an HBV antibody therapy (monoclonal or polyclonal).

Pharmaceutical Compositions

[00378] The oligonucleotide conjugates (ONCs) described herein may be in the form of a therapeutic composition or formulation useful for treating (or prophylaxis of) viral diseases, which can be approved by a regulatory agency for use in humans or in non-human animals, and/or against a particular virus or group of viruses. These ONCs may be used as part of a pharmaceutical composition when combined with a physiologically and/or pharmacologically acceptable carrier. The characteristics of the carrier may depend on the route of administration. The pharmaceutical composition of the invention may also contain other active factors and/or agents which enhance activity.

[00379] Administration of the ONCs of the invention used in the pharmaceutical composition or formulation or to practice the method of treating an animal can be carried out in a variety of conventional ways, such as intraocular, oral ingestion, enterally, inhalation, or cutaneous, subcutaneous, intramuscular, intraperitoneal, intrathecal, intratracheal, or intravenous injection.

[00380] The pharmaceutical composition or oligonucleotide conjugate formulation of the invention may further contain other chemotherapeutic drugs for the treatment of viral diseases, such as, without limitation, Rifampin, Ribavirin, Pleconaryl, Cidofovir, Acyclovir, Pencyclovir, Gancyclovir, Valacyclovir, Famiciclovir, Foscarnet, Vidarabine, Amantadine, Zanamivir, Oseltamivir, Resquimod, antiproteases, HIV fusion
inhibitors, nucleotide HIV RT inhibitors (e.g., AZT, Lamivudine, Abacavir), non-nucleotide
HIV RT inhibitors, Doconosol, Interferons, Butylated Hydroxytoluene (BHT) and Hypericin.
Such additional factors and/or agents may be included in the pharmaceutical composition,
for example, to produce a potentially synergistic effect with the ONCs described herein.

[00381] The pharmaceutical composition or oligonucleotide conjugate
formulation of the invention may further contain a polymer, such as, without restriction,
polyanionic agents, sulfated polysaccharides, heparin, dextran sulfate, pentosan polysulfate,
polyvinylalcohol sulfate, acemannan, polyhydroxycarboxylates, cellulose sulfate, polymers
containing sulfonated benzene or naphthalene rings and naphthalene sulfonate polymer,
acetyl phthaloyl cellulose, poly-L-lysine, sodium caprate, cationic amphiphiles, cholic acid.
Polymers are known to affect the entry of virions in cells by, in some cases, binding or
adsorbing to the virion itself. This characteristic of antiviral polymers can be useful in
competing with ONCs for the binding, or adsorption to the virion, the result being an
increased intracellular activity of the ONCs compared to its extracellular activity.

[00382] In order to provide the best possible antiviral response in a subject, it
may be necessary to add to the combination therapies described above a HBV polymerase
inhibitor such as (but are not restricted to): tenofovir disoproxil fumarate, entecavir,
telbuvidine, adefovir dipivoxil or lamivudine. Such antiviral drugs can prevent the replication
of the double stranded viral genome in HBV and lower the concentration of HBV virus in the
blood.

[00383] 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,
intramuscular or 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, intramuscular injection, intraperitoneal
injection, intravenous injection or infusion, or topically.
Routes of Delivery

[00384] For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to unmodified oligonucleotide agents. It may be understood, however, that these formulations, compositions and methods can be practiced with other oligonucleotide agents, e.g., modified oligonucleotide agents, and such practice is within the invention. A composition that includes a oligonucleotide can be delivered to a subject by a variety of routes. Exemplary routes include: intravenous, topical, rectal, anal, vaginal, nasal, pulmonary, ocular.

[00385] The oligonucleotide molecules of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of oligonucleotide and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00386] The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, or intrathecal or intraventricular administration.

[00387] The route and site of administration may be chosen to enhance targeting. For example, to target muscle cells, intramuscular injection into the muscles of interest would be a logical choice. Lung cells might be targeted by administering the oligonucleotide conjugate in aerosol form. The vascular endothelial cells could be targeted by coating a balloon catheter with the oligonucleotide and mechanically introducing the DNA.

[00388] Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the
like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

[00389] Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents can be added.

[00390] Compositions for intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

[00391] Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

[00392] For ocular administration, ointments or droppable liquids may be delivered by ocular delivery systems known to the art such as applicators or eye droppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose or polyvinyl alcohol), preservatives such as sorbic acid, EDTA or benzylchronium chloride, and the usual quantities of diluents and/or carriers.

[00393] **Topical Delivery**

[00394] For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to unmodified oligonucleotide agents. It may be understood, however, that these formulations, compositions and methods can be practiced with other oligonucleotide agents, e.g., modified oligonucleotide agents, and such practice is within the invention. "Topical administration" refers to the delivery to a subject by contacting the formulation directly to a surface of the subject. The most common form of topical delivery is to the skin, but a composition disclosed herein can also be directly applied
to other surfaces of the body, e.g., to the eye, a mucous membrane, to surfaces of a body cavity or to an internal surface.

[00395] As mentioned above, the most common topical delivery is to the skin. The term encompasses several routes of administration including, but not limited to, topical and transdermal. These modes of administration typically include penetration of the skin's permeability barrier and efficient delivery to the target tissue or stratum. Topical administration can be used as a means to penetrate the epidermis and dermis and ultimately achieve systemic delivery of the composition. Topical administration can also be used as a means to selectively deliver oligonucleotides to the epidermis or dermis of a subject, or to specific strata thereof, or to an underlying tissue.

[00396] The term "skin," as used herein, refers to the epidermis and/or dermis of an animal. Mammalian skin consists of two major, distinct layers. The outer layer of the skin is called the epidermis. The epidermis is comprised of the stratum corneum, the stratum granulosum, the stratum spinosum, and the stratum basale, with the stratum corneum being at the surface of the skin and the stratum basale being the deepest portion of the epidermis. The epidermis is between 50 μm and 0.2 mm thick, depending on its location on the body.

[00397] Beneath the epidermis is the dermis, which is significantly thicker than the epidermis. The dermis is primarily composed of collagen in the form of fibrous bundles. The collagenous bundles provide support for, inter alia, blood vessels, lymph capillaries, glands, nerve endings and immunologically active cells.

[00398] One of the major functions of the skin as an organ is to regulate the entry of substances into the body. The principal permeability barrier of the skin is provided by the stratum corneum, which is formed from many layers of cells in various states of differentiation. The spaces between cells in the stratum corneum is filled with different lipids arranged in lattice-like formations that provide seals to further enhance the skin's permeability barrier.

[00399] The permeability barrier provided by the skin is such that it is largely impermeable to molecules having molecular weight greater than about 750 Da. For larger molecules to cross the skin's permeability barrier, mechanisms other than normal osmosis must be used.

[00400] Several factors determine the permeability of the skin to administered agents. These factors include the characteristics of the treated skin, the characteristics of the delivery agent, interactions between both the drug and delivery agent and the drug and
skin, the dosage of the drug applied, the form of treatment, and the post treatment regimen. To selectively target the epidermis and dermis, it is sometimes possible to formulate a composition that comprises one or more penetration enhancers that will enable penetration of the drug to a preselected stratum.

[00401] Transdermal delivery is a valuable route for the administration of lipid soluble therapeutics. The dermis is more permeable than the epidermis and therefore absorption is much more rapid through abraded, burned or denuded skin. Inflammation and other physiologic conditions that increase blood flow to the skin also enhance transdermal adsorption. Absorption via this route may be enhanced by the use of an oily vehicle (inunction) or through the use of one or more penetration enhancers. Other effective ways to deliver a composition disclosed herein via the transdermal route include hydration of the skin and the use of controlled release topical patches. The transdermal route provides a potentially effective means to deliver a composition disclosed herein for systemic and/or local therapy.

[00402] In addition, iontophoresis (transfer of ionic solutes through biological membranes under the influence of an electric field) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 163), phonophoresis or sonophoresis (use of ultrasound to enhance the absorption of various therapeutic agents across biological membranes, notably the skin and the cornea) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 166), and optimization of vehicle characteristics relative to dose position and retention at the site of administration (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 168) may be useful methods for enhancing the transport of topically applied compositions across skin and mucosal sites.

[00403] The compositions and methods provided may also be used to examine the function of various proteins and genes in vitro in cultured or preserved dermal tissues and in animals. The invention can be thus applied to examine the function of any gene. The methods of the invention can also be used therapeutically or prophylactically. For example, for the treatment of animals that are known or suspected to suffer from diseases such as psoriasis, lichen planus, toxic epidermal necrolysis, erythema multiforme, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, Kaposi's sarcoma, pulmonary fibrosis, Lyme disease and viral, fungal and bacterial infections of the skin.

[00404] Pulmonary Delivery
For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to unmodified oligonucleotide conjugate agents. It may be understood, however, that these formulations, compositions and methods can be practiced with other oligonucleotide agents, e.g., modified oligonucleotide agents, and such practice is within the invention. A composition that includes an oligonucleotide agent, can be administered to a subject by pulmonary delivery. Pulmonary delivery compositions can be delivered by inhalation by the patient of a dispersion so that the composition, for example, oligonucleotide, within the dispersion can reach the lung where it can be readily absorbed through the alveolar region directly into blood circulation. Pulmonary delivery can be effective both for systemic delivery and for localized delivery to treat diseases of the lungs.

Pulmonary delivery can be achieved by different approaches, including the use of nebulized, aerosolized, micellar and dry powder-based formulations. Delivery can be achieved with liquid nebulizers, aerosol-based inhalers, and dry powder dispersion devices. Metered-dose devices are may be used. One of the benefits of using an atomizer or inhaler is that the potential for contamination is minimized because the devices are self contained. Dry powder dispersion devices, for example, deliver drugs that may be readily formulated as dry powders. An oligonucleotide composition may be stably stored as lyophilized or spray-dried powders by itself or in combination with suitable powder carriers. The delivery of a composition for inhalation can be mediated by a dosing timing element which can include a timer, a dose counter, time measuring device, or a time indicator which when incorporated into the device enables dose tracking, compliance monitoring, and/or dose triggering to a patient during administration of the aerosol medicament.

The term “powder” means a composition that consists of finely dispersed solid particles that are free flowing and capable of being readily dispersed in an inhalation device and subsequently inhaled by a subject so that the particles reach the lungs to permit penetration into the alveoli. Thus, the powder is said to be "respirable." For example, the average particle size is less than about 10 .mu.m in diameter with a relatively uniform spheroidal shape distribution. In some embodiments, the diameter is less than about 7.5 .mu.m and in some embodiments less than about 5.0 .mu.m. Usually the particle size distribution is between about 0.1 .mu.m and about 5 .mu.m in diameter, sometimes about 0.3 .mu.m to about 5 .mu.m.
The term "dry" means that the composition has a moisture content below about 10% by weight (% w) water, usually below about 5% w and in some cases less it than about 3% w. A dry composition can be such that the particles are readily dispersible in an inhalation device to form an aerosol.

The term "therapeutically effective amount" is the amount present in the composition that is needed to provide the desired level of drug in the subject to be treated to give the anticipated physiological response.

The term "physiologically effective amount" is that amount delivered to a subject to give the desired palliative or curative effect.

The term "pharmacologically acceptable carrier" means that the carrier can be taken into the lungs with no significant adverse toxicological effects on the lungs.

The types of pharmaceutical excipients that are useful as carrier include stabilizers such as human serum albumin (HSA), bulking agents such as carbohydrates, amino acids and polypeptides; pH adjusters or buffers; salts such as sodium chloride; and the like. These carriers may be in a crystalline or amorphous form or may be a mixture of the two.

Bulking agents that are particularly valuable include compatible carbohydrates, polypeptides, amino acids or combinations thereof. Suitable carbohydrates include monosaccharides such as galactose, D-mannose, sorbose, and the like; disaccharides, such as lactose, trehalose, and the like; cyclodextrins, such as 2-hydroxypropyl-beta-cyclodextrin; and polysaccharides, such as raffinose, maltodextrins, dextrans, and the like; alditols, such as mannitol, xylitol, and the like. A group of carbohydrates may include lactose, trehalose, raffinose maltodextrins, and mannitol. Suitable polypeptides include aspartame. Amino acids include alanine and glycine, with glycine being used in some embodiments.

Additives, which are minor components of the composition of this invention, may be included for conformational stability during spray drying and for improving dispersibility of the powder. These additives include hydrophobic amino acids such as tryptophan, tyrosine, leucine, phenylalanine, and the like.

Suitable pH adjusters or buffers include organic salts prepared from organic acids and bases, such as sodium citrate, sodium ascorbate, and the like; sodium citrate may be used in some embodiments.

Pulmonary administration of a micellar oligonucleotide conjugate formulation may be achieved through metered dose spray devices with propellants such as
tetrafluoroethane, heptafluoroethane, dimethylfluoropropane, tetrafluoropropane, butane, isobutane, dimethyl ether and other non-CFC and CFC propellants.

[00417] Oral or Nasal Delivery

[00418] For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to unmodified oligonucleotide agents. It may be understood, however, that these formulations, compositions and methods can be practiced with other oligonucleotide agents, e.g., modified oligonucleotide agents, and such practice is within the invention. Both the oral and nasal membranes offer advantages over other routes of administration. For example, drugs administered through these membranes have a rapid onset of action, provide therapeutic plasma levels, avoid first pass effect of hepatic metabolism, and avoid exposure of the drug to the hostile gastrointestinal (GI) environment. Additional advantages include easy access to the membrane sites so that the drug can be applied, localized and removed easily.

[00419] In oral delivery, compositions can be targeted to a surface of the oral cavity, e.g., to sublingual mucosa which includes the membrane of ventral surface of the tongue and the floor of the mouth or the buccal mucosa which constitutes the lining of the cheek. The sublingual mucosa is relatively permeable thus giving rapid absorption and acceptable bioavailability of many drugs. Further, the sublingual mucosa is convenient, acceptable and easily accessible.

[00420] The ability of molecules to permeate through the oral mucosa appears to be related to molecular size, lipid solubility and peptide protein ionization. Small molecules, less than 1000 daltons appear to cross mucosa rapidly. As molecular size increases, the permeability decreases rapidly. Lipid soluble compounds are more permeable than non-lipid soluble molecules. Maximum absorption occurs when molecules are un-ionized or neutral in electrical charges. Therefore charged molecules present the biggest challenges to absorption through the oral mucosae.

[00421] A pharmaceutical composition of oligonucleotide may also be administered to the buccal cavity of a human being by spraying into the cavity, without inhalation, from a metered dose spray dispenser, a mixed micellar pharmaceutical formulation as described above and a propellant. In one embodiment, the dispenser is first shaken prior to spraying the pharmaceutical formulation and propellant into the buccal cavity.
Devices

For ease of exposition the devices, formulations, compositions and methods in this section are discussed largely with regard to unmodified oligonucleotide agents. It may be understood, however, that these devices, formulations, compositions and methods can be practiced with other oligonucleotide agents, e.g., modified oligonucleotide agents, and such practice is within the invention. An oligonucleotide agent can be disposed on or in a device, e.g., a device which implanted or otherwise placed in a subject.

Exemplary devices include devices which are introduced into the vasculature, e.g., devices inserted into the lumen of a vascular tissue, or which devices themselves form a part of the vasculature, including stents, catheters, heart valves, and other vascular devices. These devices, e.g., catheters or stents, can be placed in the vasculature of the lung, heart, or leg.

Other devices include non-vascular devices, e.g., devices implanted in the peritoneum, or in organ or glandular tissue, e.g., artificial organs. The device can release a therapeutic substance in addition to a oligonucleotide, e.g., a device can release insulin.

Other devices include artificial joints, e.g., hip joints, and other orthopedic implants.

In one embodiment, unit doses or measured doses of a composition that includes oligonucleotide are dispensed by an implanted device. The device can include a sensor that monitors a parameter within a subject. For example, the device can include pump, e.g., and, optionally, associated electronics.

Tissue, e.g., cells or organs can be treated with an oligonucleotide agent ex vivo and then administered or implanted in a subject.

The tissue can be autologous, allogeneic, or xenogeneic tissue. E.g., tissue can be treated to reduce graft v. host disease. In other embodiments, the tissue is allogeneic and the tissue is treated to treat a disorder characterized by unwanted gene expression in that tissue. E.g., tissue, e.g., hematopoietic cells, e.g., bone marrow hematopoietic cells, can be treated to inhibit unwanted cell proliferation.

Introduction of treated tissue, whether autologous or transplant, can be combined with other therapies.

In some implementations, the oligonucleotide treated cells are insulated from other cells, e.g., by a semi-permeable porous barrier that prevents the cells from leaving the implant, but enables molecules from the body to reach the cells and
molecules produced by the cells to enter the body. In one embodiment, the porous barrier is formed from alginate.

[00431] Dosage
[00432] In one aspect, the invention features a method of administering an oligonucleotide agent. In one embodiment, the unit dose is less than 1.4 mg per kg of bodyweight, or less than 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.00005 or 0.000001 mg per kg of bodyweight, and less than 200 nmole of oligonucleotide agent (e.g., about 4.4 times 10.sup.16 copies) per kg of bodyweight, or less than 1500, 750, 300, 150, 75, 15, 7.5, 1.5, 0.75, 0.15, 0.075, 0.015, 0.0075, 0.0015, 0.00075, 0.00015 nmole of RNA agent per kg of bodyweight.

[00433] The defined amount can be an amount effective to treat or prevent a disease or disorder, e.g., a disease or disorder associated with the target oligonucleotide. The unit dose, for example, can be administered by injection (e.g., intravenous or intramuscular), an inhaled dose, or a topical application. In some embodiments dosages may be less than 2, 1, or 0.1 mg/kg of body weight.

[00434] In some embodiments, the unit dose is administered less frequently than once a day, e.g., less than every 2, 4, 8 or 30 days. In another embodiment, the unit dose is not administered with a frequency (e.g., not a regular frequency). For example, the unit dose may be administered a single time.

[00435] In one embodiment, the effective dose is administered with other traditional therapeutic modalities. In one embodiment, the subject has a viral infection and the modality is an antiviral agent other than an oligonucleotide agent. In another embodiment, the subject has atherosclerosis and the effective dose of an oligonucleotide agent is administered in combination with, e.g., after surgical intervention, e.g., angioplasty.

[00436] In one embodiment, a subject is administered an initial dose and one or more maintenance doses of an oligonucleotide conjugate agent. The maintenance dose or doses are generally lower than the initial dose, e.g., one-half less of the initial dose. A maintenance regimen can include treating the subject with a dose or doses ranging from 0.01 to 1.4 mg/kg of body weight per day, e.g., 10, 1, 0.1, 0.01, 0.001, or 0.00001 mg per kg of bodyweight per day. The maintenance doses are, for example, administered no more than once every 5, 10, or 30 days. Further, the treatment regimen may last for a period of time which will vary depending upon the nature of the particular disease, its severity and the overall condition of the patient. In certain embodiments the dosage may be
delivered no more than once per day, e.g., no more than once per 24, 36, 48, or more hours, e.g., no more than once for every 5 or 8 days. Following treatment, the patient can be monitored for changes in his condition and for alleviation of the symptoms of the disease state. The dosage of the compound may either be increased in the event the patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disease state is observed, if the disease state has been ablated, or if undesired side-effects are observed.

The effective dose can be administered in a single dose or in two or more doses, as desired or considered appropriate under the specific circumstances. If desired to facilitate repeated or frequent infusions, implantation of a delivery device, e.g., a pump, semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir may be advisable.

In some cases, a patient is treated with an oligonucleotide agent in conjunction with other therapeutic modalities. For example, a patient being treated for a viral disease, e.g., an HIV associated disease (e.g., AIDS), may be administered an oligonucleotide agent in conjunction with a known antiviral agent (e.g., a protease inhibitor or reverse transcriptase inhibitor). In another example, a patient being treated for cancer may be administered an oligonucleotide agent in conjunction with a chemotherapy.

Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the compound of the invention is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight (see U.S. Pat. No. 6,107,094).

The concentration of the oligonucleotide agent composition is an amount sufficient to be effective in treating or preventing a disorder or to regulate a physiological condition in humans. The concentration or amount of oligonucleotide agent administered will depend on the parameters determined for the agent and the method of administration, e.g., nasal, buccal, pulmonary. For example, nasal formulations tend to require much lower concentrations of some ingredients in order to avoid irritation or burning of the nasal passages. It is sometimes desirable to dilute an oral formulation up to 10-100 times in order to provide a suitable nasal formulation.

Certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an
oligonucleotide agent can include a single treatment or, for example, can include a series of treatments. It will also be appreciated that the effective dosage of a oligonucleotide agent such as a oligonucleotide agent used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein. For example, the subject can be monitored after administering a oligonucleotide agent composition. Based on information from the monitoring, an additional amount of the oligonucleotide agent composition can be administered.

[00442] Dosing is dependent on severity and responsiveness of the disease condition to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compounds, and can generally be estimated based on EC50s found to be effective in in vitro and in vivo animal models.

[00443] The inventors have discovered that oligonucleotide agents described herein can be administered to mammals, particularly large mammals such as nonhuman primates or humans in a number of ways.

[00444] In one embodiment, the administration of the oligonucleotide agent intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary, intranasal, urethral or ocular. Administration can be provided by the subject or by another person, e.g., a health care provider. The medication can be provided in measured doses or in a dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

[00445] Any of the oligonucleotide agents described herein can be administered orally, e.g., in the form of tablets, capsules, gel capsules, lozenges, troches or liquid syrups. Further, the composition can be applied topically to a surface of the oral cavity.

[00446] Any of the oligonucleotide agents described herein can be administered buccally. For example, the medication can be sprayed into the buccal cavity or applied directly, e.g., in a liquid, solid, or gel form to a surface in the buccal cavity. This administration is particularly desirable for the treatment of inflammations of the buccal
cavity, e.g., the gums or tongue, e.g., in one embodiment, the buccal administration is by spraying into the cavity, e.g., without inhalation, from a dispenser, e.g., a metered dose spray dispenser that dispenses the pharmaceutical composition and a propellant.

[00447] Any of the oligonucleotide agents described herein can be administered to ocular tissue. For example, the medications can be applied to the surface of the eye or nearby tissue, e.g., the inside of the eyelid. They can be applied topically, e.g., by spraying, in drops, as an eyewash, or an ointment. Administration can be provided by the subject or by another person, e.g., a health care provider. The medication can be provided in measured doses or in a dispenser which delivers a metered dose. The medication can also be administered to the interior of the eye, and can be introduced by a needle or other delivery device which can introduce it to a selected area or structure. Ocular treatment is particularly desirable for treating inflammation of the eye or nearby tissue.

[00448] Any of the oligonucleotide agents described herein can be administered directly to the skin. For example, the medication can be applied topically or delivered in a layer of the skin, e.g., by the use of a microneedle or a battery of microneedles which penetrate into the skin, but, for example, not into the underlying muscle tissue. Administration of the oligonucleotide agent composition can be topical. Topical applications can, for example, deliver the composition to the dermis or epidermis of a subject. Topical administration can be in the form of transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids or powders. A composition for topical administration can be formulated as a liposome, micelle, emulsion, or other lipophilic molecular assembly. The transdermal administration can be applied with at least one penetration enhancer, such as iontophoresis, phonophoresis, and sonophoresis.

[00449] Any of the oligonucleotide agents described herein can be administered to the pulmonary system. Pulmonary administration can be achieved by inhalation or by the introduction of a delivery device into the pulmonary system, e.g., by introducing a delivery device which can dispense the medication. Certain embodiments may use a method of pulmonary delivery by inhalation. The medication can be provided in a dispenser which delivers the medication, e.g., wet or dry, in a form sufficiently small such that it can be inhaled. The device can deliver a metered dose of medication. The subject, or another person, can administer the medication.

[00450] Pulmonary delivery is effective not only for disorders which directly affect pulmonary tissue, but also for disorders which affect other tissue.
[00451] Oligonucleotide agents can be formulated as a liquid or nonliquid, e.g., a powder, crystal, or aerosol for pulmonary delivery.

[00452] Any of the oligonucleotide agents described herein can be administered nasally. Nasal administration can be achieved by introduction of a delivery device into the nose, e.g., by introducing a delivery device which can dispense the medication. Methods of nasal delivery include spray, aerosol, liquid, e.g., by drops, or by topical administration to a surface of the nasal cavity. The medication can be provided in a dispenser with delivery of the medication, e.g., wet or dry, in a form sufficiently small such that it can be inhaled. The device can deliver a metered dose of medication. The subject, or another person, can administer the medication.

[00453] Nasal delivery is effective not only for disorders which directly affect nasal tissue, but also for disorders which affect other tissue.

[00454] Oligonucleotide agents can be formulated as a liquid or nonliquid, e.g., a powder, crystal, or for nasal delivery.

[00455] An oligonucleotide agent can be packaged in a viral natural capsid or in a chemically or enzymatically produced artificial capsid or structure derived therefrom.

[00456] The dosage of a pharmaceutical composition including a oligonucleotide agent can be administered in order to alleviate the symptoms of a disease state, e.g., disease viral infection, and in particular a Hepatitis B or HBV/HDV co-infection. A subject can be treated with the pharmaceutical composition by any of the methods mentioned above.

[00457] The present disclosure will be more readily understood by referring to the following examples.

[00458] Example I

[00459] NAP Conjugates Inhibit the Transit of HBsAg Out of Cells

[00460] HBsAg has been shown to block many aspects of the immune response to HBV infection. Therefore, elimination of circulating HBsAg may be a critical factor in allowing the restoration of immunocompetence in patients with chronic hepatitis B infection. An efficient method for eliminating HBsAg in the circulation is to prevent the formation and or release of subviral particles (SVPs) from infected cells (SVPs are the major carrier of HBsAg to the blood). The morphogenesis and intracellular transit of SVPs can be modeled in vitro in BHK-21 cells by expressing the small form of the HBsAg protein (sHBsAg) which is the form specifically enriched in SVPs. This model system is considered
to be a surrogate model for the morphogenesis and transit of HBV SVPs. Owing to the
critical role of serum HBsAg in allowing chronicity of HBV infection, the efficacy of
compounds in this model demonstrates their antiviral activity against HBV.

Various NAP conjugate compounds can be tested in sHBsAg-expressing BHK-21 cells including (a) the fully degenerate phosphorothioated NAP conjugates that can be prepared with oligonucleotides comprising SEQ ID NOs. 1-10, (b) a non-phosphorothioated, fully 2’O methylated degenerate oligonucleotide conjugates, as well as oligonucleotide conjugates that can be prepared with a poly AC sequence: (e.g. SEQ ID NO: 2 and SEQ ID NO: 3). These oligonucleotide conjugates can be introduced into BHK-21 cells at the same time as the template RNA for sHBsAg expression using electroporation. Activity in the BHK model system can be assessed by visualizing the location of HBsAg protein inside the BHK-21 cells by immunofluorescence microscopy. Formation of SVPs in the perinuclear space can be visualized by transmission electron microscopy. Oligonucleotide conjugates will be judged to be active if HBsAg is restricted to the perinuclear space and is prevented from transiting to the periphery of the cell (secretion) or if the formation of SVPs is prevented.

Treatment of sHBsAg expressing BHK-21 cells with oligonucleotide conjugates as described herein is expected to demonstrate the ability of oligonucleotide conjugates to block formation of SVPs and the transit of sHBsAg in a sequence independent fashion. The activity of oligonucleotide conjugates is expected to be dependent on the presence of phosphorothioation. Moreover, this ability is predicted to be retained in the presence of 2’ ribose modification (such as for oligonucleotide conjugates that can be prepared with oligonucleotides comprising e.g. SEQ ID NOs 5, 6, 9 and/or 10) and base modification (5’ methylcytosine, such as ONCs that can be prepared with oligonucleotides comprising e.g. SEQ ID nos. 3, 4, 9 and 10).

Expected results will potentially show that within the context of a degenerate sequence and sequences containing repeats of alternating purine/pyrimidine nucleotides such as AC (and therefore also CA) and also such as TG and GT or UG and GU and optionally comprising 2’ ribose modifications and/or base modifications, NAP conjugates will be expected to be able to block the formation of and intracellular transit and secretion of SVPs from infected cells at oligonucleotide lengths from 20-120 nucleotides according to the sequence independent properties of NAPs as described in U.S. Pat. Nos. 8,008,269B2, 8,008,270B2 and 8,067,385B2.
Example II

Effect of HBsAg Clearance from the Blood of HBV Infected Patients on Removal of Other HBV Proteins and Immunological Recovery

Patients chronically infected with HBV were treated with the NAP REP 2055 (also known as REP 9AC, SEQ ID NO: 2) in US20140065102, incorporated by reference herein, to remove HBsAg from their blood. The effect of REP 2055 administration (typically weekly dosing of 400 mg) on HBsAg levels in the blood was monitored using the Abbott Architect™ quantitative HBsAg test and is presented in Table 3 of US201 400651 02.

As disclosed in US201 400651 02, Table 3, the removal of HBsAg from the blood elicited an immunological recovery as evidenced by the additional reduction of circulating HBeAg (measured in two patients by the Abbott Architect™ quantitative HBeAg test—see Table 4 of US201 400651 02, incorporated by reference herein), the appearance of free anti-HBsAg antibodies (as measured by the Abbott Architect. TM. quantitative test—see Table 5 of US201 400651 02, incorporated by reference herein) and reduction of HBV virus in the blood (HBV DNA as measured by the Roche Cobas.TM. quantitative test—see Table 6 of US201 400651 02, incorporated by reference herein).

It is expected that oligonucleotide conjugates as described herein, prepared with oligonucleotides comprising any of SEQ ID NOs 1-10 will have similar properties and will exhibit similar ability to reduce HBsAg and free HBsAg in vivo, as reported in US201 400651 02. Moreover, when used in combination with other HBV or therapeutic agents, oligonucleotide conjugates as described herein are expected to augment immunogenicity and efficacy of the additional therapeutic agent, including

Combination Therapy with an ODN Chelate Complex and Two Different Immunotherapies in the Treatment of Chronic Hepatitis B in Human Patients

Oligonucleotide-10-Ca [ON-10-CA] is a calcium chelate complex of the an oligonucleotide coprizing SEQ ID NO: 10 and can be prepared in normal saline using a ratio of 30 mg of CaCl₂ for every 100 mg of oligonucleotide present (see US201 400651 02 US20140065102). The preparation of ON-1 as a calcium chelate complex can be used to improve the tolerability of ON administration (see International application publication no. WO2012/021985 and U.S. application publication no. 2012/0046348), and does not interfere with specific antiviral activity. ON-10-Ca (which can be administered with weekly 500 mg doses) can clear HBsAg from the blood (and subsequently HBeAg) and HBV virions (HBV...
DNA) in HBV infected patients similarly to other ONs that do not comprise 2'-ribose modifications or modified bases (see Tables 3, 4 and 6 versus 7, 8 and 9 respectively of US20140065102). The results disclosed in US20140065102 demonstrate that NAPs containing both 2' ribose modifications and modified bases (e.g. the ON referred to as REP 2139 of US20140065102) can act to reduce HBsAg in the blood and that ONs prepared as chelate complexes (e.g. the ON-chelate referred to as REP 2139-Ca in US20140065102) can be used to reduce or clear HBsAg from the blood.

[00472] US20140065102 shows the effect of monotherapy with the ON-chelate referred to as REP 2139-Ca on serum HBsAg in patients with chronic HBV infection, (see Table 7 of US20140065102).

[00473] TABLE 8 of US20140065102 shows the effect of ON-chelate induced HBsAg clearance on serum HBeAg levels (for the ON-chelate referred to as REP 2139-CA).

[00474] TABLE 9 of US20140065102 shows the effect of ON-chelate monotherapy on serum HBV DNA (virions) in patients with chronic HBV infection (again for the ON-chelate referred to as REP 2139-Ca).

[00475] As described in Example 11 of US20140065102, the limitation of NAP therapy (or any therapy which can clear HBsAg) is that while the patient's current levels of anti-HBs production are "freed" to clear the virus during NAP therapy, the level of antibody production (and the removal of immuno-inhibition caused by HBsAg) in most patients receiving this treatment is not sufficient to provide complete control of HBV infection after NAP treatment is stopped. As disclosed in US20140065102, REP 2139-mediated HBsAg clearance from the blood achieved the same general levels of anti-HBsAg antibodies in the blood as another ON when used in monotherapy (see Tables 5 and 10 of US20140065102. The results of NAPs used in monotherapy disclosed in US20140065102 identified a likely underlying defect in the ability of the immune system to regenerate a fully competent immune response to the HBV infection even in the absence of the antigenic HBV proteins, which is likely caused by chronic exposure to HBsAg, HBeAg and HBeAg causing durable immunological damage which persists in HBV-infected subjects even after these antigens are cleared from the blood.

[00476] Interferon-based monotherapy typically results in only a moderate level (<50 mlU/ml) of anti-HBsAg antibody in the blood in a very small proportion of patients (<10%) after 48 weeks of therapy (Reijnders et al., 2011, J. Hepatol., 54: 449-454; Harayannis et al., 1990, J. Hepatol., 10: 350-352) and the antiviral effects of thymosin .alpha.1 are similarly limited (Yang et al., 2008, Antiviral Res. 77: 136-141). However, as
disclosed in US20140065102, when either thymosin a1 or pegylated interferon a-2a was added to REP 2139-Ca treatment after HBsAg removal from the blood had been achieved, a significant increase in anti-HBsAg antibody levels was achieved in all patients treated, which greatly exceeded anti-HBsAg levels observed with NAP-mediated HBsAg clearance alone or those reported for immunotherapy alone (see Table 10 of US20140065102). As disclosed in US20140065102, this significant response occurred in 9/9 (100%) of patients treated.

[00477] The results disclosed in US20140065102 demonstrated that certain pharmaceutically acceptable agents which can reduce or clear HBsAg from the blood, when administered in combination with an immunotherapeutic agent, have a beneficial and effect on the stimulation of immune function (such as but not limited to anti-HBsAg antibody production) in patients with chronic HBV. US20140065102 (see Example III) showed that removal of HBsAg from the blood improves the ability of immunotherapy to elicit a strong host derived antiviral immune response and suggests that persistently circulating HBsAg in the blood of patients receiving only immunotherapy is having an inhibitory effect on the activity of the immunotherapy and may underlie the poor performance of accepted immunotherapies in achieving an immunological control of infection which endures off treatment.

[00478] Example III of US20140065102 also showed that the effects on the restoration of immune function occurred in all the patients tested, such that all who were tested achieved anti-HBsAg levels in the blood much more rapidly, and at much greater levels than observed with immunotherapy alone and in cases exceeding the levels of anti-HBsAg antibodies typically observed in healthy, non-infected HBsAg vaccinated individuals.

[00479] The striking effect that HBsAg removal or clearance from the blood has on the effect of conventional immunotherapies that was disclosed in US20140065102 is expected to occur with oligonucleotide conjugates or oligonucleotide conjugate chelates as described herein that have been prepared with oligonucleotides comprising any of e.g. SEQ ID NOs 1-10.

[00480] Example IV

[00481] Analysis of serum HBsAg levels in HBV transgenic mice treated with GalNAc-conjugated oligo-(AC)20 and non-GalNAc-oligo-(AC)20 (SEQ ID NO: 10)

[00482] HBV transgenic mice (Guidotti LG, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. J Virol 1995 Oct 1; 69(10): 6158-6169) were obtained from the Scripps Research Institute and housed at Utah State
University where the treatment and sample collections were performed. Mice (N=10/group) were treated with a single intra peritoneal dose of saline, 5 mg/kg non-GalNAc oligo-(AC)20 (SEQ ID NO: 10), or 5 mg/kg GalNAc-conjugated oligo-(AC)20 (depicted in Scheme 1), (N=9/group for the two oligonucleotide-treated groups), on Day 1 (D1). Blood was collected prior to dosing and on days 5, 9, and 14. Serum samples were stored at -20°C and sent to GlaxoSmithKline, RTP for analysis. Serum HBsAg levels were determined using HBsAg One ELISA (International Immuno Diagnostics, Foster City, CA) according to the manufacturer's instructions. If values were above the limit of quantitation then the sample was diluted in order to be within the linear range of quantitation (3-267 ng/mL). Animals with HBsAg levels below the lower limit of quantitation at Day 1 (D1) were excluded from analysis (1 each from the non-GalNAc oligo-(AC)20 and the GalNAc-conjugated -(AC)20 oligo groups). HBsAg levels were normalized to the Day 1 (D1) value for each mouse and graphed as averages for the treatment group and for individual mice in each treatment group.

[00483] Figure 1 shows the average-fold change in Mean+SE levels in the HBV transgenic mice for the the saline vehicle along (black bars), the non-GalNAc -(AC)20 oligonucleotide (transparent bars) and the GalNac-conjugated -(AC)20 oligonucleotide (grey bars). As can be seen in Figure 1, the average-fold change for the mean+SE level at D1 was the same for the saline vehicle, the non-GalNAc -(AC)20 oligonucleotide (SEQ ID NO: 10) and the GalNac -conjugated (AC)20 oligonucleotide (depicted in Scheme 1). At Day 5 (D5) there is slight increase in average-fold change from that observed for the non-GalNAc -(AC)20 oligonucleotide (SEQ ID NO: 10, transparent bars) and the GalNac -conjugated (AC)20 oligonucleotide (grey bars). The greatest increase in average-fold mean+SE level observed at D5 was for the saline vehicle (black bar). At Day 9 (D9) the average-fold change observed decreases from highest for the non-GalNAc -(AC)20 oligonucleotide (SEQ ID NO: 10), to intermediate for the GalNac -conjugated (AC)20 oligonucleotide (Scheme 1), and least average-fold change observed for the saline vehicle alone. At Day 14 (D14) the saline vehicle alone shows the greatest average-fold change, with the next highest average-fold change of the mean+SE value observed for the non-GalNAc -(AC)20 oligonucleotide (transparent bars), and the least average-fold change observed for the GalNac -conjugated (AC)20 oligonucleotide (grey bars). The average HBsAg levels at Day 1 (D1) observed varied from 3 ng/mL to 5671 ng/mL. Figures 2A, 2B and 2C show the variability in fold change observed for individual HBV transgenic mice in the study for the saline vehicle alone (2A), the non-GalNac -(AC)20 oligonucleotide (2B) and the GalNac -conjugated (AC)20
oligonucleotide (2C), respectively. The HBsAg level changes for the vehicle group are
typical for this transgenic mouse model. As can be observed in Figures 2B and 2C, the fold-change responses in the non-GalNAc -(AC)20 oligonucleotide mice (2B) and GalNAc -cobjugated (AC)20 oligonucleotide mice (2C) are variable. No pattern for non-responders was discerned, in that mice having both low and high initial HBsAg levels were observed to be non-responders. More information is needed to understand the fold-changes observed for individual mice at Day 9 (D9) for both non-GalNAc -(AC)20 oligonucleotide and GalNAc -cobjugated (AC)20 oligonucleotide mice groups.

**SEQUENCE LISTING**

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SEQ ID NO: 10
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ORGANISM: artificial sequence

FEATURE:
OTHER INFORMATION: full phosphorothioate, full 2′ O methylribose, C=5′methylcytidine

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What is claimed is:

1. A method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues.

2. A method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent comprising a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues in combination with a second pharmaceutically acceptable agent that stimulates immune function.

3. A method for the treatment of a hepatitis B infection or hepatitis B/hepatitis D co-infection, the method comprising administering to a subject in need of such treatment a first pharmaceutically acceptable agent comprising a sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues in combination with a second pharmaceutically acceptable agent that stimulates immune function and a third pharmaceutically acceptable agent comprising an antiviral compound.

4. The method of claims 1-3, wherein the administration of the sequence independent oligonucleotide conjugated with one or more GalNAc carbohydrate residues, inhibits the release of hepatitis B surface antigen (HBsAg) from infected hepatocytes.

5. The method of claims 1-4, wherein the first pharmaceutically acceptable agent comprises one or more molecules selected from the group consisting of:

   an oligonucleotide selected from the following:
   
   SEQ ID NO: 2;
   SEQ ID NO: 3;
   SEQ ID NO 10;
   SEQ ID NOs: 1 and 4-9;

   a nucleic acid polymer selected from the following:

   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.

6. The method of claims 2-5, wherein the second pharmaceutically acceptable agent comprises one or more molecules selected from the group consisting of:
   - yhmosin a1;
   - any α-interferon or pegylated derivatives thereof;
   - any β-interferon or pegylated derivatives thereof;
   - any γ-interferon or pegylated derivatives thereof;
   - any λ-interferon or pegylated derivatives thereof;
   - interferon a-2a or a-2b or a-N3;
   - interferon β-1a or β-1b;
   - interferon v-1b;
   - interferon λ.1 or A2 or A3
   - pegylated interferon a-2a or a-2b or A1 or A2;
   - any antiviral cytokine or pegylated derivatives thereof;
   - thymic protein A;
   - any polypeptide shown to have immunostimulatory activity;
   - an immunostimulatory oligonucleotide, including IMO-2055 or IMO-2125;
   - a small molecule Toll-like receptor (TLR) agonist; and
   - any immunostimulatory hormone.

7. The method of claims 3-5, wherein the third pharmaceutically acceptable agent comprises one or more molecules selected from the group consisting of:
   - tenofovir disoproxil fumarate;
   - entecavir;
   - telbuvidine;
   - adefovir dipivoxil; and
   - lamivudine.
8. The method of claim 5, wherein the nucleic acid polymer further comprises at least one 2' ribose modification.

9. The method of claim 5, wherein the nucleic acid polymer further comprises all riboses having a 2' modification.

10. The method of claim 5, wherein the nucleic acid polymer further comprises at least one 2' O methyl ribose modification.

11. The method of claim 5, wherein the nucleic acid polymer further comprises all riboses having the 2' O methyl modification.

12. The method of claim 5, wherein the nucleic acid polymer further comprises at least one 5'methylcytosine.

13. The method of claim 5, wherein the nucleic acid polymer further comprises all cytosines present as 5'methylcytosine.

14. The method of claim 5, wherein the nucleic acid polymer further comprises at least one 2' ribose modification and at least one 5' methylcytosine.

15. The method of claim 5, wherein the nucleic acid polymer further comprises all riboses having the 2' O methyl modification and all cytosines present as 5'methylcytosine.

16. The method of claim 15, wherein the nucleic acid polymer has the structure depicted in Scheme 1.

17. The method of claims 1-16, wherein the oligonucleotide is further formulated as an oligonucleotide chelate complex.

18. The method of claims 2-17, wherein the small molecule Toll-like receptor agonist is GS-9620 or ANA-773.

19. The method of claim 2, wherein said first and second pharmaceutically acceptable agents are formulated within the same pharmaceutical composition.
20. The method of claim 2, where said first and second agents are formulated within separate pharmaceutical compositions.

21. The method of claim 2, wherein said first and second agents are administered simultaneously.

22. The method of claim 2, wherein said first and second agents are administered by a different route of administration.

23. The method of claim 2, wherein said first and second agents are formulated are administered using one or more of the following: oral ingestion, aerosol inhalation, subcutaneous injection, intramuscular injection, intraperitoneal injection, intravenous injection and intravenous infusion.

24. The method of claim 3, wherein said first and second and third pharmaceutically acceptable agents are formulated within the same pharmaceutical composition.

25. The method of claim 3, where said first and second and third agents are formulated within separate pharmaceutical compositions.

26. The method of claim 3, wherein said first and second and third agents are administered simultaneously.

27. The method of claim 3, wherein said first and second and third agents are administered by a different route of administration.

28. The method of claim 3, wherein said first and second and third agents are formulated are administered using one or more of the following: oral ingestion, aerosol inhalation, subcutaneous injection, intramuscular injection, intraperitoneal injection, intravenous injection and intravenous infusion.

29. The method of claim 6, wherein the antiviral or immunostimulatory hormone is DHEA or its metabolites.
30. The method of claim 2, wherein the first pharmaceutically acceptable agent comprises SEQ ID NO: 2 and the second pharmaceutically acceptable agent comprises pegylated interferon a-2a.

31. The method of claim 2, wherein the first pharmaceutically acceptable agent comprises an oligonucleotide chelate complex of SEQ ID NO: 2 and the second pharmaceutically acceptable agent comprises pegylated interferon a-2a.

32. The method of claim 2, wherein the first pharmaceutically acceptable agent comprises SEQ ID NO: 3 and the second pharmaceutically acceptable agent comprises pegylated interferon a-2a.

33. The method of claim 2, wherein the first pharmaceutically acceptable agent comprises an oligonucleotide chelate complex of SEQ ID NO: 3 and the second pharmaceutically acceptable agent comprises pegylated interferon a-2a.

34. The method of claim 2, wherein the first pharmaceutically acceptable agent comprises SEQ ID NO: 10 and the second pharmaceutically acceptable agent comprises pegylated interferon a-2a.

35. The method of claim 2, wherein the first pharmaceutically acceptable agent comprises an oligonucleotide chelate complex of SEQ ID NO: 10 and the second pharmaceutically acceptable agent comprises pegylated interferon a-2a.

36. An oligonucleotide conjugate comprising at least one subunit of formula (I):

\[ \text{Formula (I)} \]

wherein:
A and B are each independently for each occurrence O, -N(R\(^n\)), S or absent;
R\(^n\) is independently for each occurrence H or C\(_1\)-C\(_8\) alkyl;
X and Y are each independently for each occurrence H, OH, a hydroxyl protecting
group, a phosphate group, a phosphodiester group, an activated phosphate group, an
activated phosphite group, a phosphoramidite, a solid support, -P(Z')(Z")0-nucleoside, -
P(Z')(Z")0-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside, -
P(Z')(Z")0-Linker-OP(Z')(Z")0-oligonucleotide, an oligonucleotide, \(\sim\)P(Z')(Z")-formula (I), -
-P(Z')(Z")-, -Linker-R or is absent;
R is L\(_G\) or has the structure shown below:
\[
\begin{align*}
\text{Linker}-L^G \\
\text{Linker}-L^G \\
\text{Linker}-L^G \\
\text{Linker}-L^G \\
\text{Linker}-L^G \\
\text{Linker}-L^G \\
\end{align*}
\]
L\(_G\) is independently for each occurrence a ligand including a carbohydrate, a
monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and
Z', Z", Z''' and Z''" are each independently for each occurrence O or S.

37. The oligonucleotide conjugate of claim 36, wherein the Linker is \(\sim\)[(P-Q"-R) \(_q\) \(\sim\)X \(\sim\)P-P'-Q''-R')\(_q\)']q"-T-, wherein:

P, R, T, P', R' and T are each independently for each occurrence absent, CO, NH, O,
S, OC(O), NHCO(O), CH\(_2\), CH\(_2\)NH, CH\(_2\)0 ; NHCH(R\(^9\)C(0), ...C(0)...CH(R\(^9\))...NH...,
CH=\(N\)-0,
or heterocycle:

Q" and Q"" are each independently for each occurrence absent, ~{(CH₂)n}, ~C(R¹)(R²)(CH₂)n⁻, ~{(CH₂)n}C(R¹)(R²)⁻, ~{(CH₂CH₂O)mCH₂CH₂⁻}, or ~{(CH₂CH₂O)mCH₂CH₂NH⁻};

X is absent or a cleavable linking group;

Rⁿ is H or an amino acid side chain;

R¹ and R² are each independently for each occurrence H, CH₃, OH, SH or N(R²)²;

Rⁿ is independently for each occurrence H, methyl, ethyl, propyl, isopropyl, butyl or benzyl;

q, q' and q" are each independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

n is independently for each occurrence 1-20; and

m is independently for each occurrence 0-50.

38. An oligonucleotide conjugate according to claim 36, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the following structure:
39. A therapeutic composition for treatment of Hepatitis B infection or of Hepatitis D co-infection for administration to a patient in need of such treatment, wherein the composition comprises a first pharmaceutically acceptable agent that is an oligonucleotide conjugate comprising at least one subunit of formula (I):

\[
\text{Formula (I)}
\]

wherein:

\[A\] and \[B\] are each independently for each occurrence \(O, N(R^N), S\) or absent;

\[R^2\] is \(H\) or \(CH_3\).
R is independently for each occurrence H or C₁₋C₆ alkyl;
X and Y are each independently for each occurrence H, OH, a hydroxyl protecting group, a phosphate group, a phosphodiester group, an activated phosphate group, an activated phosphite group, a phosphoramidite, a solid support, -P(Z')(Z")0-nucleoside, -P(Z')(Z")0-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside, -P(Z')(Z")0-Linker-OP(Z')(Z")0-oligonucleotide, an oligonucleotide, -P(Z')(Z")-formula (I), -P(Z')(Z")-Linker-R or is absent;
R is L^G or has the structure shown below:

\[ \text{Linker-}L^G_1, \text{Linker-}L^G_2, \text{Linker-}L^G_3, \text{of} \]

L^G is independently for each occurrence a ligand including a carbohydrate, a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and
Z', Z", Z''' and Z'''' are each independently for each occurrence O or S.

40. The composition of claim 39, wherein the Linker is -[(P-Q''-R')ₜ-X-(P'-Q'''-R')ₜ]₁ₜ-T⁻, wherein:
P, R, T, P', R' and T are each independently for each occurrence absent, CO, NH, O, S, OC(O), NH(O), CH₂, CH₂NH, CH₂O; NHCH(Rₐ)C(0), _C(0)_₋CH(Rₐ)_₋NH₋, CH=N-0,
or heterocycle:
Q'' and Q''' are each independently for each occurrence absent, ~(CH₂)n~, -
C(R¹)(R²)(CH₂)n~, -(CH₂)nC(R¹)(R²)~, -(CH₂CH₂0)mCH₂CH₂~, or -
(CH₂CH₂0)mCH₂CH₂NH~;
X is absent or a cleavable linking group;
Rⁿ is H or an amino acid side chain;
R¹ and R² are each independently for each occurrence H, CH₃, OH, SH or N(Rʰ)²;
Rʰ is independently for each occurrence H, methyl, ethyl, propyl, isopropyl, butyl or benzyl;
q, q' and q'' are each independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;
n is independently for each occurrence 1-20; and
m is independently for each occurrence 0-50.

41. A therapeutic composition according to claim 39, wherein only X or only Y is present
in the oligonucleotide conjugate but not both, wherein X or Y is independently an
oligonucleotide or absent, and wherein the oligonucleotide X or Y has the the
following structure:
42. A method for treating Hepatitis B infection or of Hepatitis D co-infection in a subject in need thereof, the method comprising administering to a patient in need of such treatment a first pharmaceutically acceptable agent that is an oligonucleotide conjugate comprising at least one subunit of formula (I):

wherein \( R^2 \) is H or \( \text{CH}_3 \); and

a second therapeutically active agent.

Formula (I)

wherein:

A and B are each independently for each occurrence O, N(\( R^N \)), S or absent;

\( R^N \) is independently for each occurrence H or \( \text{C}_1-\text{C}_6 \) alkyl;
X and Y are each independently for each occurrence H, OH, a hydroxyl protecting group, a phosphate group, a phosphodiester group, an activated phosphate group, an activated phosphite group, a phosphoramidite, a solid support, \(-P(Z')(Z")0\)-nucleoside, \(-P(Z')(Z")0\)-oligonucleotide, a lipid, a PEG, a steroid, a polymer, a nucleotide, a nucleoside, \(-P(Z')(Z")0\)-Linker-OP(Z"')(Z""")0-oligonucleotide, an oligonucleotide, \(~P(Z')(Z")\)-formula (I), \(-P(Z')(Z")\)-, -Linker-R, or absent; R is \(L^G\) or has the structure shown below:

\[
\text{Linker-L}^G \quad \text{Linker-L}^G \quad \text{Linker-L}^G
\]

\(L^G\) is independently for each occurrence a ligand including a carbohydrate, a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a polysaccharide; and \(Z', Z'', Z'''\) and \(Z''''\) are each independently for each occurrence O or S; and a second therapeutically active agent.

43. The method of claim 42, wherein the Linker is \(-[(P-Q''-R) \_q\cdot X-(P'-Q''''-R') \_q\cdot T]\_q\cdot T\_q\), wherein:

\(P, R, T, P', R'\) and T are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH, CH₂O ; NHCH(Rₐ)C(0), ~C(0)~CH(Rₐ)~NH~. CH=N-N-0,
or heterocycle:

Q" and Q"" are each independently for each occurrence absent, \(\sim (\text{CH}_2)_n\sim\),

\(\text{C}(\text{R}^1)(\text{R}^2)(\text{CH}_2)_m\sim\), \(\sim (\text{CH}_2\text{CH}_3\sim)_m\text{CH}_2\text{CH}_2\sim\), or

\(\sim (\text{CH}_2\text{CH}_2\sim)_m\text{CH}_2\text{CH}_2\text{NH}\sim\);

X is absent or a cleavable linking group;

R\(^a\) is \(\text{H}\) or an amino acid side chain;

R\(^1\) and R\(^2\) are each independently for each occurrence \(\text{H}, \text{CH}_3, \text{OH}, \text{SH}\) or \(\text{N}(\text{R}^\text{N})_2\);

R\(^\text{N}\) is independently for each occurrence \(\text{H}, \text{methyl}, \text{ethyl}, \text{propyl}, \text{isopropyl}, \text{butyl}\) or

benzyl;

q, q' and q" are each independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

n is independently for each occurrence 1-20; and m is independently for each occurrence 0-50.

44. A method for treating Hepatitis B infection or of Hepatitis D co-infection in a subject in need thereof according to claim 3, wherein only X or only Y is present in the oligonucleotide conjugate but not both, wherein X or Y is independently an oligonucleotide or absent, and wherein the oligonucleotide X or Y has the following structure:
45. A method for treating of Hepatitis B infection or of Hepatitis D co-infection in a subject in need thereof according to claim 44, wherein the oligonucleotide conjugate therapeutic composition further comprises a second therapeutically active agent.

46. Use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which removes hepatitis B surface antigen from the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent that stimulates immune function in the manufacture of a medicament for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide-carbohydrate conjugate has an oligonucleotide sequence of any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

47. Use of a first pharmaceutically acceptable agent that is an oligonucleotide conjugate which removes hepatitis B surface antigen from the blood in combination with a second pharmaceutically acceptable immunotherapeutic agent that stimulates
immune function in the manufacture of a medicament for the treatment of hepatitis B infection or hepatitis B/hepatitis D co-infection, wherein the oligonucleotide-carbohydrate conjugate has the structure depicted in Scheme 1
Average Fold change
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FIGURE 1
Figure 2C
INTernational Search Report

A. Classiﬁcation Of Subject Matter

B. Fields Searched

C. Documents Considered to Be Relevant

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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search: 9 November 2015

Date of mailing of the international search report: 19/11/2015

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer:
Kanbier, Titia
## DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>pages 2-4,6,13; claims 1,2,12,13,15,16; figure 18; examples 18,20,21 pages 19,21-23 pages 27,30-39 pages 61-62,71 page 101, paragraph 2 pages 124-125; table 2 pages 7, 59</td>
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<td>WO 2006/042418 A1 (REPLIC0R INC) 27 April 2006 (2006-04-27) page 12, lines 5-19; claims 1-9,11,14,17,21; examples 8,17; sequences 1-3,36, [0043], [0053] - [0055]; claims page 13, line 3 - page 14, line 5 page 15, lines 11-23 page 19, lines 6-11 page 26, lines 17-33 page 31, lines 19-23 page 35, lines 8-15 page 35, line 22 - page 37, line 19 page 37, lines 23-29 page 47, lines 1-14 page 52, lines 9-28 page 55, line 12 - page 56, line 9 page 56, lines 26-31 pages 66-68 page 87, line 27 - page 88, line 4</td>
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<td>WO 02/43771 A2 (CELL WORKS INC; UNIV JOHNS HOPKINS) 6 June 2002 (2002-06-06) paragraphs [0014], [0038], [0041], [0043], [0053] - [0055]; claims</td>
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<td>WO 2010/039548 A2 (ALNYLAM PHARMACEUTICALS INC) 8 April 2010 (2010-04-08)</td>
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