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**Emerging antivirals for the treatment of** **hepatitis B**

WangXY *et al.* Emerging antivirals for hepatitis B

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**Abstract**

Chronic infection with hepatitis B virus (HBV) constitutes a major global public health threat, causing substantial disease burdens such as liver cirrhosis and hepatocellular carcinoma, thus representing high unmet medical needs. Currently available therapies are safe, well tolerated, and highly effective in decreasing viremia and improving measured clinical outcomes with low rates of antiviral resistance. However, long-term management remains a clinical challenge, mainly due to the slow kinetics of HBV surface antigen clearance. In this article, we review emerging antivirals directed at novel targets derived from mechanisms of viral cellular entry, viral replication, viral assembly, and the host immune response, leading to preclinical and clinical trials for possible future therapeutic intervention. The recent therapeutic advances in the development of all categories of HBV inhibitors may pave the way for regimens of finite duration that result in long-lasting control of chronic hepatitis B infection.

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**Key words:** Hepatitis B; Antiviral; Nucleoside analogue; Nucleotide analogue; Non-nucleoside antivirals

**Core tip:** Despite the presence of an effective vaccine, chronic infection with HBV remains a global public health problem. This review summary the emerging antivirals directed at novel targets derived from mechanisms of viral cellular entry, viral replication, viral assembly, and the host immune response, leading to preclinical and clinical trials for possible future therapeutic intervention.

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**INTRODUCTION**

Despite the availability of an effective prophylactic vaccine, hepatitis B virus (HBV) infection remains a global public health problem. Worldwide, over 2 billion people have serological evidence of HBV, and approximately 360 million individuals are chronic HBV surface antigen (HBsAg) carriers[1]. The spectrum of disease and natural history of persistent HBV infection are diverse and variable, ranging from an inactive carrier state to progressive chronic hepatitis B (CHB), which is often associated with the development of cirrhosis and hepatocellular carcinoma (HCC)[2,3]. According to the Global Burden of Disease Study 2010 (GBD 2010), the total number of deaths in 2010 from HBV-related diseases was estimated to be 786000 worldwide[4].

In the last decade, therapeutic options for Chronic Hepatitis B (CHB) have dramatically improved, which has resulted in more patients living with the disease in an inactive state. However, due to the variety of available drugs and the constantly changing guidelines, the management of HBV can be complex.

This article focuses on the current treatment options and various novel antiviral reagents for hepatitis B infection, including HBV life cycle inhibitors and host cellular target directed antiviral reagents.

**HBV** **LIFE CYCLE AND POTENTIALDRUG TARGETS**

HBV, a member of the Hepadnaviridae family of viruses, is a small, enveloped, hepatotropic virus with an approximately 3.2-kilobase (kb) partially double-stranded, relaxed circular (rc) DNA genome[5]. The HBV life cycle is dependent on the reverse transcription of an RNA intermediate copy of the 3.2-kb DNA genome called pregenomic RNA (pgRNA). A simplified outline of the HBV replication cycle, the potential drug targets and the novel antiviral approaches are shown in Figure 1.

The HBV lifecycle has recently been fully described[6-8]. Early events of the viral life cycle include attachment, penetration and uncoating. HBV infection of target cells begins when the virion attaches to the cell surface through interactions between viral envelope proteins and cellular receptors. Evidence suggests that a peptide derived from the pre-S1 region plays a crucial role in HBV binding and is an example of a possible therapeutic target[9]. The host receptor has long remained elusive although a recent report suggests sodium taurocholate cotransporting polypeptide (NTCP), a multiple transmembrane transporter as a candidate receptor[10]. Following binding, the viral particle is internalized into the cytoplasm, where it undergoes a series of transformations involving the disassembly of the lipid bilayer and dissolution of the nucleocapsid[11,12]. Genomic DNA is transported to the cell nucleus as a result of a nuclear localization signal on the capsid protein. Inside the nucleus, the capsid dissociates and the rcDNA is converted into covalently closed circular DNA (cccDNA)[13]. HBV cccDNA is a unique episomal replicative intermediate that is responsible for the persistent infection of hepatocytes and that acts as the template for the transcription of 4 co-terminal mRNAs. These mRNAs are as follows: 3.5-kb pre-core mRNA (pre-C) and pgRNA; a 2.4-kb large surface mRNA; a 2.1-kb middle and small surface mRNA; and a 0.7-kb X mRNA[14, 15]. The slightly longer pre-core 3.5-kb mRNA pre-C mRNA is translated to produce a precore protein that is further proteolytically processed into the HBV e antigen (HBeAg). In addition to serving as mRNA for viral capsid proteins and DNA polymerase, pgRNA serves as the template for the reverse transcriptional synthesis of viral DNA.

The late events in viral replication include viral assembly and release. Following transcription, viral mRNAs are transported to the cytoplasm where translation of the viral proteins, self-assembly of core protein molecules into nucleocapsids, and progeny genome replication occurs. After being transported to the cytoplasm, the pgRNA encodes the viral capsid protein (also termed core protein) and the HBV polymerase. Replication requires the encapsidation of the pgRNA by the core particle. This process is initiated by the interaction of the HBV polymerase with the pgRNA, which triggers encapsidation by the core protein to form the viral nucleocapsid[16]. Inside the lumen of the capsids, the viral polymerase serves as a protein primer to initiate negative-strand DNA synthesis with the pgRNA serving as the template, followed by synthesis of an incomplete plus-strand DNA. At this point the mature nucleocapsid is functionally equivalent to the capsid that entered the cell during primary infection, and has two potential fates. After budding into the endoplasmic reticulum and Golgi apparatus, the nucleocapsid acquires an HBsAg-containing envelope and is released through the secretory pathway as progeny virions[17,18]. Alternatively, nucleocapsids may also deliver their genomes back to the nucleus, causing amplification of the cccDNA pool (a process referred to as the recycling pathway)[19].

These multiple complex steps in the HBV life cycle of cellular entry, disassembly, replication, assembly, and release are all potential targets for novel therapies. Furthermore, complex host-immune responses to viral infection involving both innate and adaptive immunity offer immunological targets for viral control[20]. Combination therapies targeting multiple mechanisms are particularly attractive. The potential targets of representative new antiviral approaches currently undergoing evaluation in vitro, in vivo, or in clinical trials are shown in Figure 1.

**CURRENT ANTIVIRAL REAGENTS**

The goal of therapy for CHB is to improve quality of life and survival by preventing progression of the disease to cirrhosis, decompensated cirrhosis, end-stage liver disease, HCC and death[22]. This goal can be achieved if HBV replication can be suppressed with an effective treatment. The ideal end point is HBsAg loss, which however is infrequently achievable with the currently available anti-HBV agents. A more realistic endpoint is the induction of sustained or maintained virological remission.

Over the last 20 years, 2 interferon (IFN)-based therapies, both host cellular target-directed antiviral reagents, and 5 oral nucleoside/nucleotide analogues (NAs), all of which are HBV life cycle inhibitors, have been approved by the United States Food and Drug Administration (FDA) for the treatment of CHB. These agents include IFN, pegylated interferon (PEG-IFN), lamivudine (LMV), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), and tenofovir disoproxil fumarate (TDF) (Table 1, Figure 2).

**EMERGING ANTIVIRALS FOR HEPATITIS B**

***Inhibitor of HBV DNA polymerase***

**Nucleoside/Nucleotide analogue drugs:** Clevudine [1-(2-deoxy-2-fluoro-β-L-arabinofuranosyl) thymine, L-FMAU], a thymidine nucleoside analogue, has potent antiviral activities against both HBV and Epstein-Barr virus but not HIV. It acts as an inhibitor of the DNA-dependent DNA activity of HBV polymerase, as well as of reverse transcription and priming. Clevudine was approved for the treatment of CHB in South Korea and in the Philippines. However, the use of clevudine has not been endorsed by AASLD or EASL in their hepatitis B treatment guidelines[21,22].

Clevudine has demonstrated potent antiviral activity during therapy and induced a sustained post treatment antiviral effect for 6 months after a 12-week treatment period[23]. A clinical trial of 1-year of clevudine treatment demonstrated it to be effective in suppressing the serum HBV DNA level and in normalizing the ALT level. Viral breakthrough associated with the rtM204I mutation occurred in 1 patient after 9 mo of clevudine treatment[24]. The M204I mutant in HBV RT plays a major role in clevudine resistance and leads to viral breakthrough during long-term clevudine treatment. In vitro drug susceptibility assays have revealed clevudine-associated mutants to be all resistant to lamivudine and usually sensitive to adefovir, entecavir, and tenofovir[25].

In a double-blind study to evaluate the safety and antiviral activity of clevudine (30 mg daily) over a 48-wk period compared with lamivudine (100 mg daily) in 92 HBeAg-positive CHB patients, clevudine was shown to be superior to lamivudine by yielding a higher rate of HBV DNA suppression[26]. In contrast, in a 2-year observational study comparing the clinical efficacy and safety of clevudine and entecavir, clevudine not only was no more effective than entecavir but also was associated with the development of drug resistance and muscle-related problems[27,28].

Clinical trials of long-term therapy with clevudine in the US were terminated due to safety issues, such as drug-induced myopathy[29-31]. Furthermore, clevudine is more potent than is lamivudine, but does not seem to be more potent than entecavir or tenofovir. It has no advantage over the more potent antiviral regimen in current use, and therefore, is unlikely to be more universally approved in most markets given the safety concerns and the availability of the more effective drugs entecavir and tenofovir.

MIV-210 is a prodrug of the nucleoside analogue 3'-fluoro-2', 3'-dideoxyguanosine (FLG) and has high oral bioavailability in humans and potent activity against HBV[32].

Preclinical in vitro and in vivo data reveal MIV-210 to be a good candidate for further testing as an agent against HIV and HBV. In Huh7 cells transiently transfected with different HBV constructs, FLG exhibits a broad spectrum of anti-HBV activity profiles against wild-type, lamivudine-resistant, adefovir-resistant, and lamivudine-plus-adefovir-resistant HBV mutants[33]. A study to determine the dose-related antiviral efficacy and safety of MIV-210 in chronically infected woodchucks showed that oral administration of MIV-210 at 20 or 60 mg/kg of body weight/day induced a rapid virological response[34]. The clinical development of this agent is ongoing. Following favorable plasma levels of MIV-210 and good oral bioavailability in phase I studies, a phase IIa clinical trial has been initiated in South Korea.

Besifovir (LB80380) is an acyclic nucleotide phosphonate with chemical similarity to adefovir and tenofovir. LB80380 is the prodrug of LB80331, which in turn is metabolized to LB80317, the active metabolite with antiviral effects against HBV after further intracellular phosphorylation to the triphosphate form[35].

In a phase Ib dose-finding study of LB80380 in treatment-naïve HBeAg-positive CHB patients, the safety, antiviral activity and pharmacokinetics were assessed[36]. A 3- to 4.2-log reduction of HBV DNA was observed with doses ranging from 30 to 240 mg daily, and viral suppression reached the optimal level when besifovir was administered at dose above 60 mg per day. In a phase IIa clinical study, LB80380 was found to be effective in reducing the viral load in lamivudine-resistant HBeAg-positive CHB patients for a period of 12 wk[37]. In both studies, LB80380 was safe and well tolerated up to a dose of 240 mg daily. In a phase IIb study that compared the safety and antiviral activity of besifovir with entecavir in Asian CHB patients for 48 wk, the proportions of patients who achieved undetectable HBV DNA, HBeAg loss, and HBeAg seroconversion were similar in all 3 groups of patients (besifovir 90 mg daily, besifovir 150 mg daily or entecavir 0.5 mg daily). Two doses of besifovir (90 and 150 mg) were non-inferior to entecavir 0.5 mg daily in treatment-naive CHB patients[38]. No antiviral drug resistance was observed in this study. A phase III study (ClinicalTrials.gov identifier NCT01937806) to assess the antiviral activity and safety of besifovir 150 mg compared with tenofovir 300 mg in CHB patients for 48 wk is not yet open for participant recruitment.

An alternative treatment strategy associated with acyclic nucleotide phosphonates is currently under clinical development, with the goal of increasing oral availability, improving antiviral activity, and decreasing the potential for nephrotoxicity. Tenofovir alafenamide (TAF; formerly GS-7340) is a prodrug of tenofovir diphosphate fumarate (TDF), achieving higher active metabolite concentrations in peripheral blood mononuclear lymphocytes and lymphatic tissues[39].

Inside cells, TAF is initially hydrolyzed to the intermediate tenofovir-alanine conjugate (TFV-Ala). TFV-Ala is converted to the parent tenofovir, which then undergoes subsequent phosphorylations to yield the active tenofovir diphosphate (TFV-DP) metabolite[40]. TFV-DP inhibits the activity of HIV reverse transcriptase by competing with natural substrates and causing DNA chain termination after incorporation into viral DNA[41]. TAF is being studied for the treatment of HIV infection, and is a potent inhibitor of hepatitis B virus. A phase I study to evaluate the safety and efficacy of TAF compared with that of TDF in treatment-naive and experienced adult subjects with chronic hepatitis B virus infection is currently recruiting participants (ClinicalTrials.gov Identifier: NCT01940341).

CMX157 is another investigational prodrug of tenofovir that is currently undergoing a phase I clinical study. CMX157 is a hexadecyloxypropyl conjugate of tenofovir with antiviral activity against HIV and HBV. In vitro, CMX157 is 267-fold more active than tenofovir against HIV-1 and 4.5-fold more active against HBV. CMX157 is orally bioavailable and has no toxicity in rats treated for 7 days at doses ranging from 10 to 100 mg/kg per day[42].

A phase I study to evaluate the safety, tolerability, and pharmacokinetics of a single dose of CMX157 in healthy volunteers has been completed (ClinicalTrials.gov Identifier: NCT01080820). Both TAF and CMX157 represent second-generation tenofovir analogues which may have an improved clinical profile.

AGX-1009 is a novel patented prodrug of tenofovir but is activated by a different molecular side-chain. Ongoing preclinical studies demonstrate good efficacy in inhibiting viral replication, and phase I trials are planned for 2013 in China.

***Non-nucleoside antivirals***

**Inhibitor of viral entry:** Specific inhibition of virus entry represents a rather new and attractive therapeutic concept for controlling acute and chronic infections. The establishment of the HBV-susceptible cell line HepaRG[43] and systems based on primary human hepatocytes (PHHs) and primary Tupaia belangeri hepatocytes have facilitated investigations of the cellular and viral determinants involved in HBV entry and resulted in the discovery of envelope protein-derived entry inhibitors. Both genetic and functional examination identified one domain in the N-terminusof HBV preS1 (amino acids 1-47) that is essential for HBV and hepatitis delta virus (HDV) infectivity.

Myrcludex-B, a synthetic lipopeptide consisting of the authentically myristoylated N-terminal 47 amino acids of the preS1 domain of the large viral envelope protein (L protein), specifically targets hepatocytes and efficiently blocks de novo HBV infection both in vitro[44-47], and in vivo[48]. Humanized chimeric uPA mice were first injected with HBV to permit an initial infection establishment, followed by administration of Myrcludex-B for either 3 d, 3 wk, or 8 wk post-HBV inoculation. Myrcludex-B not only prevented the spread of HBV from infected human hepatocytes in vivo; but also hindered amplification of the cccDNA pool in initially infected hepatocytes[49]. A phase 0/1 clinical study to evaluate the safety, tolerability, pharmacokinetics (PK), and immunogenicity of single ascending doses of Myrcludex-B in healthy volunteers is ongoing[50]. The data to date have shown Myrcludex-B to be very well tolerated in healthy volunteers for all investigated doses of up to 5 mg intravenous (*iv*) injection and 0.8 mg subcutaneous (*sc*) injection.

The entry inhibitor Myrcludex-B has been shown to prevent de novo establishment of HDV infection in vitro[51]. Furthermore, in a successfully established uPA/SCID mice model of HBV/HDV coinfection and superinfection, preclinical antiviral evaluations of the efficacy of Myrcludex-B in inhibiting the establishment of de novo HDV infection in vivo were performed[52].

Currently, the potential role of viral peptide-derived lipoproteins is primarily preventative and limited to post-exposure prophylaxis, the prevention of vertical transmission, and graft reinfection after liver transplant.

***Inhibitor of nucleocapsid formation***

**Heteroaryldihydropyrimidines:** The HBV capsid plays an indispensable role in the virus life cycle, participating in genome packaging, reverse transcription, intracellular trafficking, and maintenance of a stable infection. HBcAg serves as the structural unit in the assembly of the viral nucleocapsid, leading to nucleocapsid formation through a process of self-assembly. Perturbing HBV assembly, and thereby altering either the timing or the geometry of capsid formation, has great potential as an antiviral strategy. Recently, heteroaryldihydropyrimidines (HAPs), a family of assembly effectors, have been identified as highly potent non-nucleosidic inhibitors of HBV replication in vitro and in vivo[53-56]. HAPs act as allosteric effectors to increase the kinetics of assembly and strengthen dimer-dimer association to prevent the proper formation of viral capsids. Additionally, at high concentrations, HAPs misdirect viral assembly[57].

Bay 41-4109, a member of the HAP family, inhibits HBV replication by inducing inappropriate assembly and an aberrant transition to yield virus particles no longer competent for reverse transcription, budding, and/or nuclear transport[58]. In a humanized uPA/SCID HBV mouse model, a decrease in the HBV viral load of approximately 1 log (10) copies/ml was observed after treatment with Bay41-4109 for 5 days. Viral rebound was observed within 5 days of treatment cessation. A phase I study is ongoing, but no clinical results have been reported to date.

GLS4, a small molecular compound that inhibits HBV replication, is also a member of the HAP family. It is a potent inhibitor of the replication of both wild-type and ADV-resistant HBV mutant strains in vitro[59]. GLS4 is currently undergoing a phase I study in China to evaluate its safety, tolerability, and pharmacokinetics, and it exhibits good pharmacological behavior and tolerance. These inhibitors have a potential role as part of future therapeutic regimens against chronic HBV due to their specific mechanisms of action.

**Phenylpropenamides:** Molecules of the phenylpropenamide family of compounds, AT-61 and AT-130, have been shown to inhibit HBV replication in vitro[60,61]. These compounds are specific to HBV and have no activity against related viruses such as duck HBV (DHB), woodchuck HBV (WHV), and human immunodeficiency virus type 1 (HIV-1). Both AT-61 and AT-130 were found to be active against LMV-resistant HBV mutants. Studies with these compounds have shown that the phenylpropenamides appears to effectively inhibit HBV replication by interfering with the encapsidation process at the level of pregenomic RNA encapsidation, producing apparently normal capsids that lacked genetic material[62]. Subsequent studies revealed an assembly effector mechanism underlying the apparent blocking of RNA packaging[63]. Unlike HAPs, the effects of the phenylpropenamides are almost entirely kinetic, affecting only assembly reaction rate and timing while still producing normal capsids, with very little thermodynamic effects on capsid stability. Although this class of compounds has a favorable toxicity profile, clinical trials are still required.

***Inhibitor of HBsAg release***

**REP 9 AC:** Phosphorothioated oligonucleotides (PS-ONs), a novel class of compounds, constitute a promising microbicide approach. Early studies indicated the antiviral activity of PS-ONs to be independent of antisense activity but specifically dependent on their amphipathic characteristic[64]. Thus, PS-ONs with sequence-independent antiviral activity were describedas amphipathic DNA polymers (APs). APs possess broad spectrum antiviral activity, via multiple mechanisms, against a range of viruses, including human HIV-1[65], herpes simplex virus (HSV)[66-68], arenavirus lymphocytic choriomeningitis virus (LCMV)[69], and hepatitis C virus (HCV)[70].

REP 9 AC is a 40-nucleotide polycytidine (alternating adenosine) amphipathic DNA polymer that inhibits the release of HBsAg from infected hepatocytes in HBV infected patients and allows patients to regain durable immunity by eliminating HBsAg-mediated immunosuppression. This agent is currently in a phase I/II clinical trial. The safety and efficacy of REP 9 AC in 8 HBsAg+ patients with HBV DNA levels of 6-12 log10 copies/ml were evaluated[71]. Interim clinical data demonstrated that REP 9AC led to rapid clearance of HBsAg from the serum and development of anti-HBs in as early as 7 d and in no more than 32 wk. Seven of the 8 treated patients exhibited residual to no levels of serum HBsAg and developed anti-HBs. Three of these 7 patients demonstrated durable immunological control over their infections, as evidenced by substantial reductions in serum HBV DNA within 20-27 wk of treatment. REP 9 AC′, a modification of REP 9 AC to reduce pro-inflammatory activity and improve compound stability, shows similar efficacy with respect to HBsAg clearance and HBV DNA suppression with no pro-inflammatory side effects[72]. These results suggest that APs may become an important new tool in the treatment of CHB.

***Small molecules***

**Nitazoxanide (alinia):** Nitazoxanide, a thiazolide anti-infective agent, is active against anaerobic bacteria, protozoa, and a range of viruses in cell culture models, and it is currently in phase II clinical development for the treatment of chronic hepatitis C. Nitazoxanide was approved by the US Food and Drug Administration in 2002 for treating diarrhea caused by *Cryptosporidium* spp. or *Giardia lamblia* in adults and children as young as 12 months[73].

The antiviral activity of nitazoxanide was discovered by serendipity when patients with AIDS and HBV or HCV co-infection were treated for diarrhea. Nitazoxanide is potent inhibitor of HBV and displays synergistic interactions with LMV or ADV against HBV in vitro. Nitazoxanide is also effective against HBV mutants that are resistant to lamivudine and adefovir[74]. In preliminary open-label studies, nitazoxanide alone has shown evidence of efficacy in the treatment of CHB over 1-year course of therapy[75]. Nitazoxanide 500 mg twice daily resulted in a decrease in serum HBV DNA in all 4 HBeAg-positive patients, with undetectable HBV DNA in 2 of the 4 patients, loss of HBeAg in 3 patients, and loss of HBsAg in 1 patient. Nitazoxanide and related agents represent a class of small molecules that modulate host antiviral pathways via protein kinase activation, thereby acting as interferon immune enhancers[76].

Phase II clinical trials have demonstrated the efficacy and safety of nitazoxanide added to peginterferon with or without ribavirin in treating patients with chronic hepatitis C. Multiple clinical trials of nitazoxanide for treating chronic hepatitis C are underway or have been completed; however, no trials of this drug for treating CHB are currently registered with ClinicalTrials.gov.

***Gene silencing***

**DNA-directed RNA Interference:** RNA interference is an evolutionary conserved mechanism that employs short RNAs in association with an effector complex, referred to as the RNA-induced silencing complex (RISC), to regulate gene expression in a sequence-specific manner[77]. Two classes of small RNAs differing in origin and early processing pathways, mediate this process, namely, microRNAs (miRNAs) and small interfering RNAs (siRNAs)[78].

HBV is a promising target for an RNA interference approach because its compact genome lacks significant redundancy. siRNA has shown promise as a potential therapeutic agent by potently knocking down one or more viral transcripts (and consequently, antigens), for prolonged periods, both in vitro and in vivo[79-81].

ddRNAi technology involves inserting a DNA construct into a cell, triggering the production of double stranded RNA (dsRNA), which is then cleaved into siRNA as part of the RNA interference (RNAi) process, causing the destruction of the target mRNA and knocking-down or silencing the target gene expression. Although challenges remain in drug delivery, the most advanced nucleic acid-based therapeutics may enter the clinical realm in the near future.

***ARC-520***

A siRNA-based therapeutic agent called ARC-520 is designed to reduce the expression and release of new viral particles and the viral protein load by the mechanism of RNAi. The siRNAs in ARC-520 intervene at the point of DNA transcription, upstream of where nucleotide and nucleoside analogues act. In transient and transgenic mouse models of HBV infection, a single intravenous injection of ARC-520 targeting HBV sequences caused long-term, multi-log suppression of HBV RNA proteins, and viral DNA.

In a chimpanzee chronically infected with HBV and a high viral titer, a single intravenous injection of 2 mg/kg ARC-520 was well-tolerated and resulted in decreases in serum levels of HBsAg, HBeAg, and HBV DNA[82].

A phase I study of ARC-520 administered intravenously to healthy adult volunteers is being conducted in Melbourne, Australia. Each dose cohort includes 6 subjects randomized at a ratio of 1:2 (placebo: active) to receive a single intravenous injection of either placebo or ARC-520. This phase I trial is expected to be completed in the fourth quarter of 2013, and a phase IIa trial in chronic HBV patients is expected to begin in 2014.

***Immune-based therapies***

**Zadaxin:** Zadaxin (thymosin alpha 1), a synthetic peptide of 28 amino acids, is a substance found naturally in the circulation and produced in the body’s thymus gland. Zadaxin has been evaluated for its immunomodulatory activities and related therapeutic potential in several conditions and diseases, including cancer (such as HCC, lung cancer, and melanoma) and infectious disease (sepsis, infections after bone marrow transplant, lung infections including chronic obstructive pulmonary disorder (COPD), SARS, hepatitis B or C, and HIV). Investigation of Zadaxin’s mechanism of action at the cellular level has revealed both immune-modulating and direct-acting effects.

Interest in using Zadaxin for treating CHB has been based on the drug’s immunomodulating effects, which can trigger lymphocyte maturation, augment T-cell function, and reconstitute immune defects.

A study revealed that Zadaxin added to lamivudine was not superior to lamivudine alone and did not prevent resistance to lamivudine despite increased HBeAg seroconversion[83]. The data from a meta-analysis showed that, compared with IFNα, the benefit of thymosin alpha-1 was not immediately significant at the end of therapy but that the virological, biochemical, and complete response had a tendency to increase or accumulate gradually after the therapy[84]. No persuasive data have yet emerged to suggest a great potential for Zadaxin.

**NOV-205 (****BAM 205)****:** NOV-205 acts as a hepatoprotective agent with immunomodulating and anti-inflammatory properties and, thus, likely requires treatment periods longer than 14 days to affect a clinical response. Its regulatory approval in the Russian Federation under the trade name Molixan® in 2001 was based on clinical studies in 178 Russian hepatitis B and C patients. When used as mono-therapy for 1 mo in hepatitis B and for 2 months in hepatitis C patients, NOV-205 has been shown to greatly reduce/eliminate viral loads and to significantly improve liver function. NOV-205 was also well-tolerated in those studies.

**GS 9620:** Toll-like receptors (TLRs) are components of the innate immune system and serve as a first line of defense against invading pathogens. TLR activation could represent a powerful and novel therapeutic strategy for the treatment of chronic HBV infection[85]. GS-9620, a potent selective TLR-7 agonist, was designed to have rapid clearance and low systemic levels after oral administration. In chimpanzees chronically infected with HBV, the effects of immune activation have been investigated using GS-9620 administered 3 times each week for 4 wk at 1 mg/kg and a 1-wk rest period, for a second cycle of 4 wk at 2 mg/kg[86]. A detailed evaluation was performed that included an assessment of the pharmacokinetics of GS-9620, viral load, IFN-stimulated gene (ISG) expression, cytokine and chemokine levels, and lymphocyte and NK cell activation, as well as safety and tolerability parameters. Short term administration of GS-9620 provided long-term suppression of serum and liver HBV DNA. Serum levels of HBsAg and HBeAg, as well as numbers of HBV antigen-positive hepatocytes, were reduced as hepatocyte apoptosis increased. In parallel, GS-9620 administration induced the production of IFN-α and other cytokines and chemokines, up-regulated ISGs expression, and activated NK-cell and lymphocyte subsets, confirming the activation of TLR-7 signaling.

The safety, tolerability, pharmacokinetics and pharmacodynamics of GS-9620 in 75 healthy volunteers were evaluated with a single ascending-dose up to 12 mg. GS-9620 demonstrated safety and pharmacodynamic activity at doses up to 12 mg and induced an antiviral response before systemic adverse events were observed[87].

The novel approach inhibiting HBV replication and inducing infected cell clearance represents a step toward the development of a true combination therapy for CHB.

**GI-13020:** HBV-specific T cell responses have been shown to have a positive association with infectious status in patients with chronic HBV, with the weakest T cell responses observed in patients with untreated chronic active infection and the strongest T cell responses observed in patients who have achieved seroconversion or cure. GI-13020 is a recombinant yeast-based biological product engineered to express a chimera of HBV X, S, and C antigens. GI-13020 is immunogenic in murine models and has also been used to stimulate human immune cell samples ex vivo to elicit HBV specific T cell responses, which could predict the immune responses in patients dosed with GI-13020.

A phase Ia trial assessing the safety, tolerability, and ability to elicit HBV-specific T cell responses of GI-13020 at various doses and with various regimens in healthy adults is ongoing but is not recruiting participants (ClinicalTrials.gov Identifier: NCT01779505). In the future, GI-13020 could be used in combination with HBV antivirals in an attempt to improve HBsAg seroconversion rates in patients with chronic HBV infection.

**DV-601:** Therapeutic vaccines may promote the resolution of chronic HBV infection through the stimulation of specific cytotoxic T-lymphocyte and B-cell antibody responses against dominant HBV-antigens. DV-601, which comprises recombinant HBsAg and HBcAg, is an investigational therapeutic vaccine. A phase Ib dose-escalation study has been completed to determine whether DV-601 would be well-tolerated and induce HBV-specific virological and immunological responses in CHB patients undergoing concurrent treatment with a nucleoside analogue (ClinicalTrials.gov, Identifier: NCT01023230).

In this initial dose-escalation study of DV-601, 6 injections of DV-601 were administered over a period of 12 weeks. The primary objective was to assess the safety and tolerability of DV-601 by evaluating local and systemic adverse events on Day 99, including changes in laboratory analyses. Secondary objectives were to evaluate the virological response and immunogenicity of DV-601 based on viral load, and humoral and T cell immunological responses. DV-601 appears to be a safe and well-tolerated therapeutic vaccine for the treatment of CHB, and virological response is evident[88].

**CONCLUSION**

Currently available treatments offer major benefits to patients with chronic HBV infection and may prevent liver disease progression to liver cirrhosis, liver failure, and HCC; furthermore, these therapies may improve survival through robust and rapid viral suppression and the persistence of undetectable levels of serum HBV DNA. Currently, more effective and less resistance-prone antiviral agents are available to treat the HBV infection. The need for new antiviral agents that meet the ideal target product profile remains great. The emerging antivirals will provide additional and improved choices for optimized regimen development and will increase the confidence that many patients in more diverse settings will derive the full benefits of CHB treatment.

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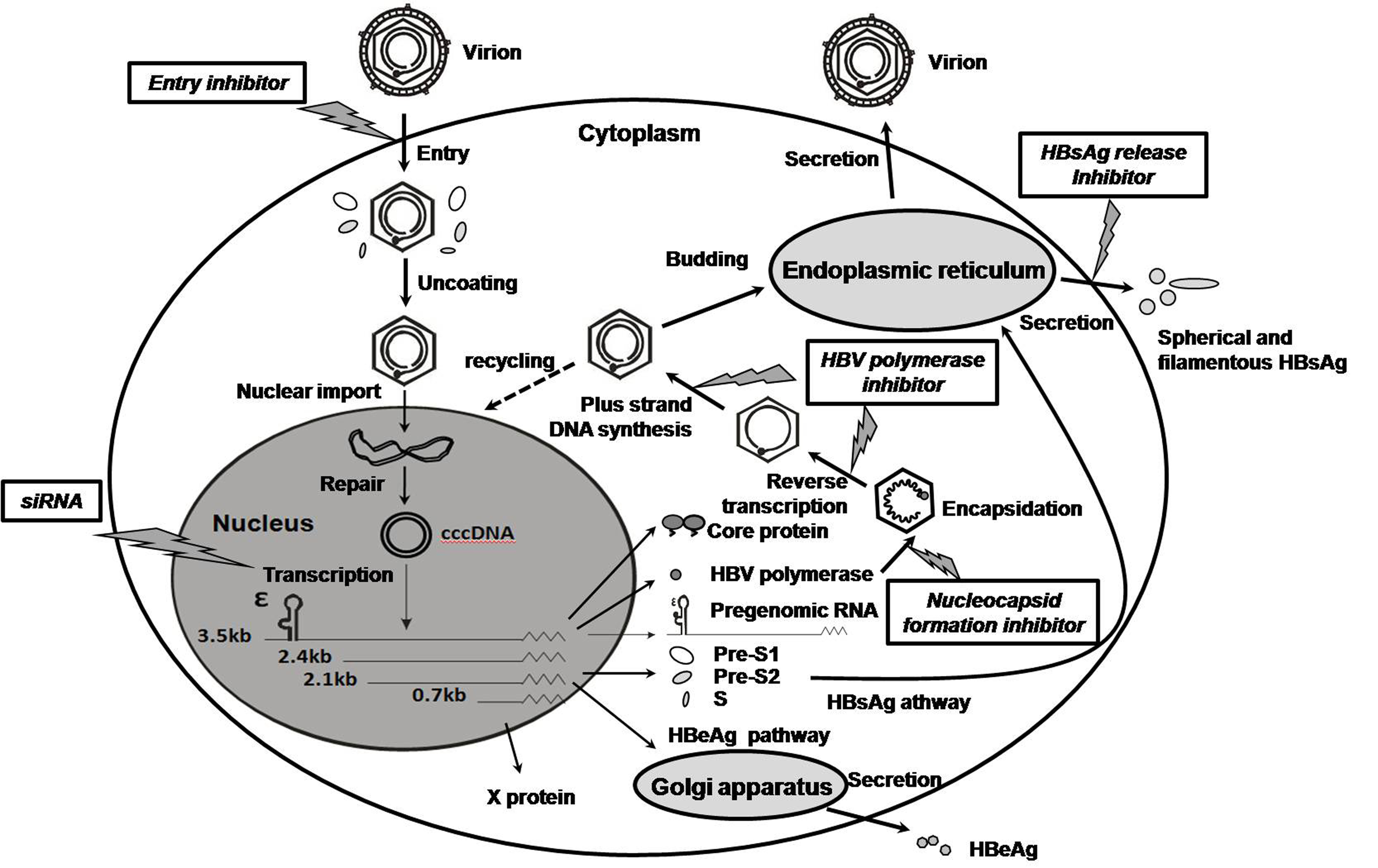
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| **Table 1 Approved antivirals for hepatitis B** | | | |
| **Generic Names** | **Brand Name** | **Manufacturer Name** | **Original Approval or Tentative** **Approval Date** |
| interferon alpha-2b | Intron A | Schering | July, 1992 |
| pegylated interferon | Pegasys | Roche | May 13, 2005 |
| lamivudine | Epivir-HBV | GlaxoSmithKline | December 8, 1998 |
| Adefovir dipivoxil | Hepsera | Gilead Sciences | September 20, 2002 |
| entecavir | Baraclude | Bristol-Myers Squibb | March 29, 2005 |
| telbivudine | Tyzeka | Novartis | October 25, 2006 |
| Tenofovir disoproxil fumarate | Viread | Gilead Sciences | November 8, 2008 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 2 Emerging antivirals for hepatitis B virus treatment** | | | |
| **Family/Drug name** | **Mechanism** | **Status** | **Company** |
| ***Nucleoside/ Nucleotide analogs*** | | | |
| Clevudine | Inhibits viral DNA polymerase | Approved in S. Korea and Philippines | Bukwang/Eisai |
| MIV-210 (Lagociclovir valactate) | Inhibits viral DNA polymerase | Phase II | Medivir/Daewoong |
| Besifovir (LB80380) | Inhibits viral DNA polymerase | Phase IIb | LG Life Sciences |
| Tenofovir Alafenamide (GS-7340) | Inhibits viral DNA polymerase | Phase Ib | Gilead |
| CMX157 | Inhibits viral DNA polymerase | Phase I | Chimerix |
| AGX-1009 | Inhibits viral DNA polymerase | Phase I, China | Agenix |
| ***Non-Nucleoside Antivirals*** | | | |
| Myrcludex-B | entry inhibitor | Phase Ia, Germany | Myr-GmbH |
| Bay 41-4109 | Inhibits viral nucleocapsid | Phase I, Germany | AiCuris |
| GLS 4 | Inhibits viral nucleocapsid | Phase I, China | Sunshine Lake |
| Phenylpropenamides | Inhibits viral encapsidation | Preclinical |  |
| REP 9 AC | HBsAg release inhibitor | Phase Ib | REPLICor Inc. |
| Nitazoxanide (Alinia) | Small Molecule | Preclinical | Romark Labs |
| dd-RNAi compound | Gene silencing | Preclinical | Benitec/Biomics |
| ARC-520 | RNAigene silencer | Phase I | Arrowhead Research |
| ***Immune-based*** | | | |
| Zadaxin  (Thymosin alpha 1) | Immunomodulator | Orphan drug approvalin United States for liver cancer | SciClone |
| NOV-205 (BAM 205) | Immunomodulator | Approved in Russia | Novelos |
| GS-9620 | TLR7-agonist | Phase I | Gilead |
| GI-13020 | HBV antigen | Preclinical | Global Immune |
| DV-601 | Therapeutic HBV vaccine | Phase Ib | Dynavax |

**Figure 1 Hepatitis B Virus replication cycle and potential drug targets.** After HBV virions enter the hepatocyte, they are uncoated and transported to the nucleus, in which cccDNA is generated by repairing the partially double-stranded genome, followed by transcription of the cccDNA into the viral RNA gene products. Encapsidation of the pregenomic RNA occurs in the cytoplasm via a complex interaction among epsilon, core particles, and HBV polymerase. Reverse transcription leading to negative-strand and then positive-strand synthesis occurs within the viral nucleocapsid. After budding into the endoplasmic reticulum and Golgi apparatus, the nucleocapsid acquires an HBsAg-containing envelope and is released through the secretory pathway as progeny virions. Alternatively, nucleocapsids may also recycle their genomes into the nucleus for conversion to cccDNA. Potential inhibitors of the HBV life cycle include inhibitor of viral entry, small interfering double-stranded RNA (siRNA), inhibitor of nucleocapsid formation, inhibitor of HBV DNA polymerase, and inhibitor of HBsAg release.



**Figure 2 Approval dates for chronic hepatitis B therapeutics.**

