Emerging therapeutics and relevant targets for chronic Hepatitis B

Il Han Song
Division of Hepatology, Department of Internal Medicine, Dankook University College of Medicine, Dankook University Hospital, Cheonan, Republic of Korea

ABSTRACT
Chronic hepatitis B virus (HBV) infection is a global health problem for the pursuit of complete virus eradication and immunity acquisition to prevent liver disease progression. Currently, interferon-alpha, with the underlying mechanism of host immunomodulation, and nucleos(t)ide analogs, with the underlying mechanism of inhibition of viral replication, are used for the treatment of patients with chronic hepatitis B. Despite remarkable improvement in the virological, serological, biochemical, and histological response to current therapeutics, we are still far from meeting the therapeutic goals, e.g., clearance of HBV DNA/covalently closed circular DNA (cccDNA) in the serum/liver tissue and seroconversion of hepatitis B surface antigen (HBsAg) to anti-HBs in the present antiviral era. Recently, HBV replication cycle-related, viral RNA interference-based, and host immune-mediated therapeutic targets and relevant anti-HBV agents have been newly introduced and investigated in the preclinical and clinical fields. This review discusses emerging therapeutics and relevant targets in the management of chronic hepatitis B.

Keywords: Chronic hepatitis B, hepatitis B virus, antiviral therapy, therapeutic target

INTRODUCTION
Management guidelines of chronic hepatitis B virus (HBV) infection have been regularly updated according to the recommendations of the American Association for the Study of Liver Diseases (1), European Association for the Study of the Liver (2), and the Asian Pacific Association for the Study of the Liver (3). Current treatment strategies for patients with chronic hepatitis B (CHB) are as follows: interferon alpha-based immunomodulation and nucleos(t)ide analog-based inhibition of viral replication. Interferon alpha is one of the signaling proteins known as cytokines that has several mechanisms of action, including direct antiviral, immunomodulatory, and anti-proliferative effects, while nucleos(t)ide analogs are polymerase inhibitors that target DNA elongation by inhibiting the reverse transcription of HBV. Thymosin alpha-1 and peginterferon lambda have been used as immunomodulatory agents, but their effectiveness was modest and far from satisfactory compared to interferon-alpha (4,5). In the last decade, despite remarkable advances in the therapeutic use of anti-HBV agents such as nucleos(t)ide analogs and interferon-alpha for virological, serological, biochemical, and histological assessment (1-3), complete HBV eradication, e.g., clearance of HBV DNA/covalently closed circular DNA (cccDNA) in the serum/liver tissue and seroconversion of hepatitis B surface antigen (HBsAg) to anti-HBs from the host still remains impossible in the present antiviral era. Therefore, most hepatologists and virologists feel the need for the advent of new anti-HBV agents with different mechanisms. Therefore, this review focuses on the emerging antiviral therapeutics including novel anti-HBV regimens and their related targets involved in the inhibition of virus replication or the modulation of host immune systems in the treatment of chronic HBV infection.

REPLICATION CYCLE OF HBV
HBV is an approximately 3.2 kb-sized partially double-stranded relaxed circular DNA (ds-rcDNA) virus with hepatotropic property, belonging to the family Hepadnaviridae (Figure 1). The replication cycle of HBV has been almost revealed. The life cycle of HBV begins when the HBV virion, Dane particle, attaches to the cell
surface to enter the target hepatocytes. This is mediated by the binding of the pre-S1 region of the viral envelope protein to the cellular receptor sodium taurocholate cotransporting polypeptide (NTCP) (6), a transmembrane transporter. After NTCP-mediated viral entry, the viral nucleocapsid is uncoated and transported into the nucleus, where the viral rcDNA is transformed into cccDNA via repairing process (7), followed by transcription of the cccDNA into the following four viral RNA transcripts: 3.5 kb precore messenger RNA (mRNA) and pregenomic RNA (pgRNA); 2.4 kb large surface mRNA; 2.1 kb middle and small surface mRNA; and 0.7 kb X mRNA. In the cytoplasm, pgRNA serves as the template for reverse transcription, leading to negative-stranded DNA synthesis followed by positive-stranded DNA synthesis within the viral nucleocapsid (8). After budding into the endoplasmic reticulum and Golgi apparatus, the nucleocapsid with dsDNA acquires an HBsAg-containing envelope and is released to infect neighboring hepatocytes through the secretory pathway. Thereafter, the nucleocapsid returns to the nucleus for conversion to cccDNA and amplification, which is responsible for viral persistence in host cells (9).

Figure 2 shows the replication cycle of HBV.

VIRUS REPLICATION CYCLE-RELATED THERAPEUTICS

The complex replication steps of viral entry into the cells, nucleocapsid uncoating, transformation of viral DNA into cccDNA, transcription, encapsidation, reverse transcription, nucleocapsid assembly, and virus particle secretion as progeny virion or recycling to amplify cccDNA during the life cycle of HBV may be all potential targets for the development of novel therapeutics in chronic HBV infection. The new anti-HBV approaches related to virus replication cycle are currently undergoing in vitro/in vivo preclinical studies or early clinical trials (Table 1).

Figure 1. A representative scheme of hepatitis B virus genome. Hepatitis B virus (HBV) genome consists of partially double-stranded DNA with four overlapping open reading frames. ORF: open reading frame; DR: direct repeat domain.

Table 1. Emerging therapeutics and relevant targets for chronic HBV infection

<table>
<thead>
<tr>
<th>Therapeutics (ref.)</th>
<th>Targets/mechanisms</th>
<th>Status (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Replication cycle-based</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myrcludex B (10-12)</td>
<td>Synthetic lipopeptide with domain/ virus entry inhibition</td>
<td>Phase II</td>
</tr>
<tr>
<td>Cyclosporin A (13,14)</td>
<td>Immunosuppressant with NTCP blockade/ virus entry inhibition</td>
<td>Preclinical</td>
</tr>
<tr>
<td>MC2791/MC3119 (15)</td>
<td>Epigenetic modifier of cccDNA/cccDNA inhibition</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Zinc finger proteins (16)</td>
<td>DNA cleavage enzymes/cccDNA inhibition</td>
<td>Preclinical</td>
</tr>
<tr>
<td>CCC-0975/CCC-0346 (17)</td>
<td>Inhibitor of cccDNA formation/cccDNA inhibition</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Besifovir (18-20)</td>
<td>Acyclic nucleotide phosphate/ DNA polymerase inhibition</td>
<td>Phase II</td>
</tr>
<tr>
<td>Tenovir alafenamide (21)</td>
<td>Acyclic nucleotide phosphate/ DNA polymerase inhibition</td>
<td>Phase III</td>
</tr>
<tr>
<td>CMX157 (22)</td>
<td>Hexadecyloxypropyl conjugate of tenovir/DNA polymerase inhibition</td>
<td>Phase II</td>
</tr>
<tr>
<td>Bay-41-4109 (26-28)</td>
<td>Member of HAP family/ nucleocapsid inhibition</td>
<td>Phase I</td>
</tr>
<tr>
<td>GL54 (29)</td>
<td>Member of HAP family/ nucleocapsid inhibition</td>
<td>Phase I</td>
</tr>
<tr>
<td>AT-61/AT-130 (30,31)</td>
<td>Molecule of phenylpropenamide family/nucleocapsid inhibition</td>
<td>Preclinical</td>
</tr>
<tr>
<td>REP 9 AC (32)</td>
<td>Amphiphatic DNA polymer/ inhibition of HBsAg secretion</td>
<td>Phase II</td>
</tr>
<tr>
<td><strong>RNAi-based</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARC-520 (39)</td>
<td>siRNAs targeting HBV transcription/ RNAi-based gene silencing</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>siRNA/ddRNA (40)</td>
<td>siRNA targeting HBV NLS-HBV PRE/RNAi-based gene silencing</td>
<td>Preclinical</td>
</tr>
<tr>
<td><strong>Immune-mediated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS-9620 (44,45)</td>
<td>TLR-7 agonist/TLR-7 signaling activation</td>
<td>Phase II</td>
</tr>
<tr>
<td>Gl-13020 (46)</td>
<td>Recombinant HBV antigens/ HBV-specific T cell activation</td>
<td>Preclinical</td>
</tr>
<tr>
<td>GS-4774/CV-601 (47,48)</td>
<td>Recombinant HBV antigens/ therapeutic vaccines</td>
<td>Phase II</td>
</tr>
<tr>
<td>NAS/VAC (49)</td>
<td>HBsAg/HBcAg-based therapeutic vaccine</td>
<td>Phase III</td>
</tr>
<tr>
<td>Immunogenic vaccine (50)</td>
<td>HBsAg/HBIG complex therapeutic vaccine</td>
<td>Phase III</td>
</tr>
</tbody>
</table>

HBV: hepatitis B virus; NTCP: sodium taurocholate cotransporting polypeptide; cccDNA: covalently closed circular DNA; HAP: heteroaryldihydropyrimidine; HBsAg: hepatitis B surface antigen; RNAi: RNA interference; siRNA: small interfering RNA; HBV NLS: HBV nuclear localization signal; HBV PRE: HBV post-transcriptional regulatory element; TLR: toll-like receptor; HBcAg: hepatitis B core antigen; HBIG: hepatitis B immune globulin

Song IH. Emerging therapeutics for chronic Hepatitis B

Turk J Gastroenterol 2016; 27: 210-5
HBV exposure → Attachment to the cells (hepatocytes) surface
 Binding of HBV virion to cellular receptor (NTCP) → Viral entry into the cells via endocytosis
 Viral nucleocapsid uncoating → Transport into the nucleus
 Transformation of viral DNA into cccDNA → Transcription
 Formation of pgRNA and other viral transcripts → Reverse transcription
 Formation of viral nucleocapsid with HBV DNA → Nucleocapsid assembly
 Viral nucleocapsid binding to ER and Golgi apparatus → Glycosylation/maturity
 Formation of HBsAg envelope-containing viral nucleocapsid → Viral release/exitosis
 Viral particle secretion

**Figure 2.** The replication cycle of hepatitis B virus. NTCP: sodium taurocholate cotransporting polypeptide; cccDNA: covalently closed circular DNA; pgRNA: pregenomic RNA; ER: endoplasmic reticulum

Myrcludex B, a synthetic lipopeptide consisting of a pre-S1 domain structure of the HBV envelope protein, is a prototype of viral entry inhibitor. This peptide efficiently blocks the spread of intrahepatic virus from HBV-infected mice along with the inhibition of cccDNA amplification in hepatocytes (10, 11). A phase IIb clinical trial assessing the safety and tolerability of myrcludex B showed that the virological response rate was higher in the higher dose (10 mg)-treated group than in the lower dose-treated one (12). Cyclosporin A is an immunosuppressive drug clinically used following organ transplantation or in the treatment of autoimmune diseases. Recently, it was shown that cyclosporine A inhibits HBV entry by cyclophilin-independent interference with the NTCP receptor, in which the interaction between the drug and the cellular receptor may be direct and may overlap with a functional binding site of the pre-S1 domain, mediating viral entry (13, 14). Thereafter, cyclosporine A derivatives with minimal influence on the host immune system may provide a new anti-HBV strategy targeting NTCP as a cellular factor.

HBV cccDNA is the most important target in the development of antiviral compounds because cccDNA, a main template of virus replication with a structurally stable frame, is the principal offender of viral persistence in host cells. There are several cccDNA inhibitors, such as hSirt1/2 activator MC2791 and JMJD2 inhibitor MC3119/epigenetic regulators of cccDNA (15); zinc finger proteins/DNA cleavage enzymes (16); and CCC-0975 and CCC-0346/cccDNA formation inhibitors such as sulfonamide compounds (17). However, as the studies for the development of these theoretically attractive compounds targeting cccDNA have been performed at the cell/tissue level, further investigational studies may be necessary at the preclinical and clinical stages.

HBV DNA polymerase is the most popular target of current antiviral agents despite the presence of nucleos(t)ide-related clinical drawbacks such as virus mutation, drug resistance, rebound phenomenon, adverse events, and vague treatment period. Besifovir (LBB0380) (18-20), tenofovir alafenamide (GS-7340) (21), and CMX157 (22) are new nucleos(t)ide analogs that inhibit viral DNA polymerase, which are being investigated under phase II-III clinical trials for their favorable safety profiles and clinical outcomes. Lagociclovir valactate (MIV-210), famciclovir, and pradefovir were suspended from the clinical investigations (23).

Nucleocapsid assembly is initiated by the interaction of the HBV polymerase with pgRNA in the cytoplasm, triggering encapsidation by the core protein to form the viral nucleocapsid. Heteroaryldihydropyrimidines (HAPs), a family of nucleocapsid assembly effectors, have been identified as potent non-nucleoside inhibitors of HBV replication in preclinical studies (24, 25). Bay 4104109 (26-28) and GLS4 (29), members of the HAP family, inhibit HBV replication by inducing inappropriate assembly. The phenylpropanamide derivatives, AT-61 and AT-130, inhibited HBV replication at the stage of viral RNA packaging and also controlled the replication of wild-type and lamivudine-resistant strains of HBV in vitro (30, 31).

HBsAg secretion is known to contribute to the suppression of the host immune system as well as the infection of neighboring hepatocytes. REP 9 AC (32), an amphiphatic DNA polymer, inhibited HBsAg secretion from infected hepatocytes in patients with chronic HBV infection, which resulted in the recovery of innate immunity and cytotoxic T cell response in a phase I clinical study.

**RNA INTERFERENCE-BASED THERAPEUTICS**

RNA interference (RNAi) is a cellular gene-silencing mechanism in which double-stranded RNA (dsRNA) induces the post-transcriptional gene-knockout of corresponding homologous mRNA of host genes (33). Figure 3 depicts the mechanisms of RNAi-mediated gene silencing in mammalian cells. Since Elbashir et al. (34) demonstrated that duplexes of 21-nucleotide RNAs mediate RNAi in cultured mammalian cells, a number of studies have used this gene-silencing mechanism as a tool for gene-knockout techniques for therapeutic application related to inhibiting infectious and genetic diseases as well as a functional study of host genes. There are two classes of small RNAs, microRNAs (miRNAs) and small interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs), which have an effect on the regulation of gene expression in a sequence-specific manner (35).

HBV has been considered as a promising candidate of potentially treatable viruses using the RNAi-based therapeutic approach. After targeting HBV nuclear localization signal (NLS),
Furthermore, this siRNA-induced inhibitory expression vector, especially markedly inhibiting HBV cccDNA expression in HBV-transgenic mice injected with siRNA showed an inhibitory effect on viral replication and antibody production by B cells. The production of cytokines by Th2 facilitates the activation of macrophages, T cell-mediated cytotoxicity, and antibody production by B cells. The production of cytokines by type 2 helper T cells (Th2) facilitates the activation of macrophages, T cell-mediated cytotoxicity, and antibody production by B cells.

siRNA showed an inhibitory effect on viral replication and antigen expression in HBV-transgenic mice injected with siRNA expression vector, especially markedly inhibiting HBV cccDNA amplification (36). Furthermore, this siRNA-induced inhibitory effect was stronger in combination of siRNAs compared with individual use of each siRNA (37). Using a new siRNA delivery system, Wooddell et al. (38) presented that siRNAs targeting different sites of HBV induced multilog repression of viral RNA, viral DNA, and protein and the effect lasted for a long duration. ARC-520, designed to reduce the expression and release of viral particles by RNAi mechanism, suppressed the expression of HBV DNA, HBsAg, and hepatitis B e antigen (HBeAg) in a HBV-infected chimpanzee with a high viral titer (39). ARC-520 was investigated in a clinical trial in which it was intravenously administered to healthy adult volunteers. The results from this phase I trial will be soon published and a phase II trial is expected to be performed thereafter. Recently, three siRNA target sites were selected on HBV post-transcriptional regulatory element (HBV PRE), a conserved RNA region of HBV, through different siRNA designing programs. Functional siRNAs corresponding to these target sites could drastically decrease the expression of HBV transcripts (core, surface, and X) and surface protein without interferon response and cell cytotoxicity in HepG2 cell lines (40). The development of anti-HBV agents as novel RNAi-based therapeutics is anticipated in the future.

**IMMUNE-MEDIATED THERAPEUTICS**

Persistent HBV presence in liver makes the host’s innate immune responses weak and weakly sensitive to HBV during viral infection, resulting in the defect of adaptive immune responses for anti-HBV activity. Figure 4 shows the interaction of HBV-infected hepatocytes and host immune cells and relevant immune-mediated cell control. When the innate immune cells such as dendritic cells (DCs) are exposed to HBV and viral protein from HBV-infected hepatocytes, innate immunity along with toll-like receptor (TLR) triggering is activated, resulting in the induction of an antiviral status on infected cells by production of antiviral cytokines such as type 1 interferon (IFN), NK cell-mediated killing of infected cells, and site recruitment of adaptive immunity. Thereafter, matured DCs migrating to the T cell area of lymphoid organs present viral peptides on HLA class I and class II molecule to CD8 cytotoxic T cells (CTL) and CD4 helper T cells (HTL). CTL and type 1 helper T cells (Th1) secrete antiviral cytokines contributing to cell lysis and killing of intracellular organisms. The production of cytokines by type 2 helper T cells (Th2) facilitates the activation of macrophages, T cell-mediated cytotoxicity, and antibody production by B cells.

HBV inhibits TLR-mediated antiviral signaling in hepatocytes (41). TLRs, known to be components of the innate immune system, are pathogen recognition receptors serving as the first defense mechanism against invading pathogens (42). In chronic HBV infection, the immunostimulative activities, e.g., the production of interferon-alpha and other cytokines/chemokines, upregulation of interferon-stimulated genes (ISGs), and activation of NK cells and CD8 cytotoxic T lymphocytes, are suppressed, while the immunoinhibitory activities, e.g., the expression of programmed death-1 (PD-1)/PD-L1 and cytotoxic T
lymphocyte antigen-4 (CTLA-4)/B7-1 and activation of regulatory T lymphocytes (Treg), are upregulated (43).

GS-9620, a selectively oral agonist of TLR-7, has been shown to activate TLR-7 signaling in immune cells of chimpanzees with chronic HBV infection to induce clearance of HBV-infected hepatocytes, resulting in the induction of immunostimulative activities, long-term suppression of serum and liver HBV DNA, and the decrease of serum levels of virus proteins (HBsAg and HBeAg) and numbers of HBV-positive hepatocytes (44). A phase I study revealed tolerable safety, stable pharmacodynamic activity, and favorable antiviral response of GS-9620 (45). Lan et al. (46) presented that HBV-induced hepatocyte-intrinsic immune tolerance was reversed when a dually functional vector containing both an immunostimulating ssRNA and HBx-silencing shRNA was administered, and the systemic anti-HBV adaptive immune responses, including CD8 T cell cytotoxic activity and anti-HB antibody induction, were efficiently recovered in an interferon-alpha- and TLR-7-dependent manner. GI-13020, a recombinant yeast-based biological product engineered to express a chimera of HBV X, S, and C antigens, is also immunogenic to induce HBV-specific T cell responses. Further progression of this product from the preclinical to clinical stage of study is anticipated in the near future, along with the evaluation of combination effects of HBV antivirals to improve HBsAg seroconversion. GS-4774 (47) and DV-601 (48) are recombinant therapeutic vaccines with HBV antigenicity (X, large S, and Core) that promote the resolution of chronic HBV infection through the stimulation of specific cytotoxic T lymphocyte and B cell antibody response against HBV antigens. In phase I studies, these vaccines seem to be safe and well-tolerated and tolerated as well as to be evident to virological response in the treatment of CHB (47,48). Recently, two phase III clinical trials were conducted to evaluate the safety and efficacy of new formulations of the HBV vaccine, one therapeutic vaccine (NAS-VAC) containing both HBsAg and hepatitis B core antigen (HBcAg) and the other HBsAg-hepatitis B immunoglobulin (HBIG) immunogenic complex, in CHB patients. They were found to be able to improve virological and biochemical responses in these patients (49,50).

**CONCLUSION**

Complete HBV eradication and immunity acquisition, e.g., clearance of HBV DNA/cccDNA in the serum/liver and seroconversion of HBsAg to anti-HBs, are the most important end points of antiviral therapy for chronic HBV infection. Currently, emerging antivirals focused on virus replication cycle-related targets, RNAi-based targets, and immune-mediated targets, although most of the investigation studies are under preclinical and early clinical stages (Table 1). The treatment strategy with these new agents would help steer the current anti-HBV stream for the prevention of liver disease progression to cirrhosis, hepatic decompensation, and hepatocellular carcinoma, subsequently improving the quality of life and survival in patients with chronic HBV infection.

**REFERENCES**

2. European Association For The Study Of The Liver. EASL Clinical practice guidelines: Management of chronic hepatitis B virus infection. EASL. J Hepatol 2012; 57: 167-85. [CrossRef]
amide compounds as specific inhibitors of hepatitis B virus co-
valently closed circular DNA formation. Antimicrob Agents Che-
mother 2012; 56: 4277-88. [CrossRef]
dose-finding study of oral LB80380 versus entecavir in Asian patients 
with chronic hepatitis B. Antivir Ther 2006; 11: 977-83. [CrossRef]
19. Yuen MF, Han KH, Um SH, et al. Antiviral activity and safety of 
LB80380 in hepatitis B e antigen-positive chronic hepatitis B pa-
tients with lamivudine-resistant disease. Hepatology 2010; 51: 767-76. [CrossRef]
trial of besiforvir (LB80380) versus entecavir in Asian patients with 
chronic hepatitis B. Gut 2014; 63: 996-1004. [CrossRef]
[(S)-[[(S)-1-
(isopropoxy carbonyl)ethyl]amino][phenoxyphosphinyl]-ethoxy] 
propyl]adenine (GS-7340) and other tenofovir phosphonami-
de prodrugs by human proteases. Mol Pharmacol 2008; 74: 92-
100. [CrossRef]
22. Painter GR, Almond MR, Trost LC, et al. Evaluation of hexadecyloxy-
propyl-9-R-[2-(Phosphonomethoxyl)propyl]-adenine, CMX157, as a 
potential treatment for human immunodeficiency virus type 1 
and hepatitis B virus infections. Antimicrob Agents Chemother 
2007; 51: 3505-9. [CrossRef]
23. Tang CM, Yau TO, Yu J. Management of chronic hepatitis B in-
fec tion: current treatment guidelines, challenges, and new de-
developments. World J Gastroenterol 2014; 20: 6262-78. [CrossRef]
 virus replication by drug-induced depletion of nucleopasids. 
Science 2003; 299: 893-6. [CrossRef] 
A heteroaryl dihydropyrimidine activates and can misdirect hepati-

citis B virus capsid assembly. Proc Natl Acad Sci U S A 2005; 102: 
8138-43. [CrossRef]
26. Stray SJ, Zlotnick A. Bay 41-4109 has multiple effects on hepatitis 
receptor-mediated innate immune responses in murine paren-
chymal and nonparenchymal liver cells. Hepatology 2009; 49: 
1132-40. [CrossRef]
derivatives AT-61 and AT-130 inhibit replication of wild-type and 
lamivudine-resistant strains of hepatitis B virus in vitro. Antimi-
crob Agents Chemother 2002; 46: 3057-60. [CrossRef]
The phenylpropenamide derivatives AT-130 blocks HBV replica-
tion at the level of viral RNA packaging. Antivir Res 2007; 76: 168-
77. [CrossRef]
30. Mahtab MA, Bazinet M, Vaillant A. REP 9 AC: a potent HBsAg 
release inhibitor that elicits durable immunological control of 
chronic HBV infection. Hepatology 2011; 54(Suppl 1): 478A.
31. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Pa-
tent and specific genetic interference by double-stranded RNA in 

### Song IH. Emerging therapeutics for chronic Hepatitis B

T. Duplexes of 21-nucleotide RNAs mediate RNA interference in 
35. Wilson R, Purcell D, Netter HJ, Revill PA. Does RNA interference 
provide new hope for control of chronic hepatitis B infection? 
Antivir Ther 2009; 14: 879-89. [CrossRef]
[CrossRef].
37. Li G, Fu L, Jiang J, Ping Y, Huang Y, Wang Y. siRNA combinations 
mediated greater suppression of hepatitis B virus replication in 
mice. Cell Biochem Biophys 2014; 69: 641-7. [CrossRef]
RNAi therapeutics for the treatment of chronic hepatitis B virus 
replication. Mol Ther 2013; 21: 973-85. [CrossRef]
39. Sebestyen MG, Wong SC, Trubetskoy V, Lewis DL, Wooddell CJ. Ta-
germed in vivo delivery of siRNA and an endosome-releasing agent 
to hepatocytes. Methods Mol Biol 2015; 1218: 163-86. [CrossRef]
40. Thongthae N, Payungsorn S, Poovorawan Y, T-Thenprasert NP. A 
rational study for identification of highly effective siRNA against 
hepatitis B virus. Exp Mol Pathol 2014; 97: 120-7. [CrossRef]
receptor-mediated innate immune responses in murine paren-
chymal and nonparenchymal liver cells. Hepatology 2009; 49: 
7269-72. [CrossRef]
42. Isogawa M, Robek MD, Furuchi Y, Chisari FV. Toll-like receptor sig-
7269-72. [CrossRef]
43. Su ZJ, Yu XP, Guo RY, et al. Changes in the balance between Treg 
and Th17 cells in patients with chronic hepatitis B. Diagn Micro-
biol Infect Dis 2013; 76: 437-44. [CrossRef]
44. Lanford RE, Guerra B, Chavez D, et al. GS-9620, an oral agonist of 
toll-like receptor-7, induces prolonged suppression of hepatitis 
B virus in chronically infected Chimpanzees. Gastroenterology 
2013; 144: 1508-17. [CrossRef]
and pharmacodynamics of GS-9620, an oral Toll-like receptor 7 
agonist. Antivir Ther 2013; 18: 409-18. [CrossRef]
46. Lan P, Zhang C, Han Q, Zhang J, Tian Z. Therapeutic recovery of 
hepatitis B virus (HBV)-induced hepatocyte-intrinsic immune de-
fect reverses systemic adaptive immune tolerance. Hepatology 
2013; 58: 73-85. [CrossRef]
47. Gagger A, Coeshott C, Apelian D, et al. Safety, tolerability and im-
munogenicity of GS-4774, a hepatitis B virus-specific therapeutic 
vaccine, in healthy subjects: a randomized study. Vaccine 2014; 
32: 4925-31. [CrossRef]
48. Spellman M, Martin JT. Treatment of chronic hepatitis B infection 
with DV-601, a therapeutic vaccine. J Hepatol 2011; 54(Suppl 1): 
s302. [CrossRef]
49. Akbar SM, Al-Mahtab M, Rahman Sm Aguilar JC, Hiasa Y, Mishiro 
S. A phase III clinical trial with a therapeutic vaccine containing 
both HBsAg and HBcAg administered via both mucosal and par-
enteral routes in patients with chronic hepatitis B. Hepatology 
2013; 58(Suppl 1): 647A.
50. Xu DZ, Wang XY, Shen XL, et al. Results of a phase III clinical trial 
with an HBsAg-HBIG immunogenic complex therapeutic vaccine 
for chronic hepatitis B patients: experiences and findings. J Hepa-
tol 2013; 59: 450-6. [CrossRef]