



Review

# Toward a complete cure for chronic hepatitis B: Novel therapeutic targets for hepatitis B virus

Sun Woong Kim<sup>1,\*</sup>, Jun Sik Yoon<sup>2,\*</sup>, Minjong Lee<sup>3</sup>, and Yuri Cho<sup>4</sup>

<sup>1</sup>Department of Internal Medicine, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul; <sup>2</sup>Department of Internal Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan; <sup>3</sup>Department of Internal Medicine, Ewha Womans University College of Medicine, Seoul; <sup>4</sup>Center for Liver and Pancreatobiliary Cancer, National Cancer Center, Goyang, Korea

Hepatitis B virus (HBV) affects approximately 250 million patients worldwide, resulting in the progression to cirrhosis and hepatocellular carcinoma, which are serious public health problems. Although universal vaccination programs exist, they are only prophylactic and not curative. In the HBV life cycle, HBV forms covalently closed circular DNA (cccDNA), which is the viral minichromosome, in the nuclei of human hepatocytes and makes it difficult to achieve a complete cure with the current nucleos(t)ide analogs and interferon therapies. Current antiviral therapies rarely eliminate cccDNA; therefore, lifelong antiviral treatment is necessary. Recent trials for antiviral treatment of chronic hepatitis B have been focused on establishing a functional cure, defined by either the loss of hepatitis B surface antigen, undetectable serum HBV DNA levels, and/or seroconversion to hepatitis B surface antibody. Novel therapeutic targets and molecules are in the pipeline for early clinical trials aiming to cure HBV infection. The ideal strategy for achieving a long-lasting functional or complete cure might be using combination therapies targeting different steps of the HBV life cycle and immunomodulators. This review summarizes the current knowledge about novel treatments and combination treatments for a complete HBV cure. (*Clin Mol Hepatol* 2022;28:17-30)

**Keywords:** Hepatitis B virus; Antiviral therapy; Complete cure

## INTRODUCTION

Hepatitis B virus (HBV) is a small enveloped DNA virus belonging to the Hepadnaviridae family.<sup>1</sup> The structure of the HBV virion is as follows: 3.2 kb of partially double-stranded relaxed circular

DNA (rcDNA) bonded by polymerase, an inner nucleocapsid formed by the core protein (hepatitis B core antigen, HBcAg), and an outer envelope formed by lipid-embedded small, middle, and large surface proteins (hepatitis B surface antigen, HBsAg). HBV is characterized by species specificity, and only humans and chim-

### Abbreviations:

BSV, Besifovir dipivoxil maleate; CAR, chimeric antigen receptor; Cas9, CRISPR-associated protein 9; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CRISPR, clustered regularly interspaced short palindromic repeats; dsDNA, double-stranded DNA; EVT, entecavir; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; mRNA, messenger RNA; NA, nucleos(t)ide analogues; NAP, nucleic acid polymer; NTCP, sodium taurocholate cotransporting polypeptide; PD-1, programmed cell death protein 1; Peg-IFN $\alpha$ , pegylated interferon alpha; pgRNA, pregenomic RNA; rcDNA, relaxed circular DNA; RIG-I, retinoic acid-inducible gene-1; RNAi, RNA interference; siRNA, small interfering RNA; SVP, subviral particle; TALENs, transcription activator-like effector nuclease; TCR, T-cell receptor; TDF, tenofovir disoproxil fumarate; TLR, toll-like receptor; ZFN, zinc finger nuclease

### Corresponding author: Yuri Cho

Center for Liver and Pancreatobiliary Cancer, Research Institute and Hospital, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang 10408, Korea  
Tel: +82-31-920-1605, Fax: +82-31-920-0149  
E-mail: [presh\\_yuri@hanmail.net](mailto:presh_yuri@hanmail.net), [yuricho@ncc.re.kr](mailto:yuricho@ncc.re.kr)  
<https://orcid.org/0000-0002-4488-5352>

\*These authors contributed equally to this work as co-first authors.

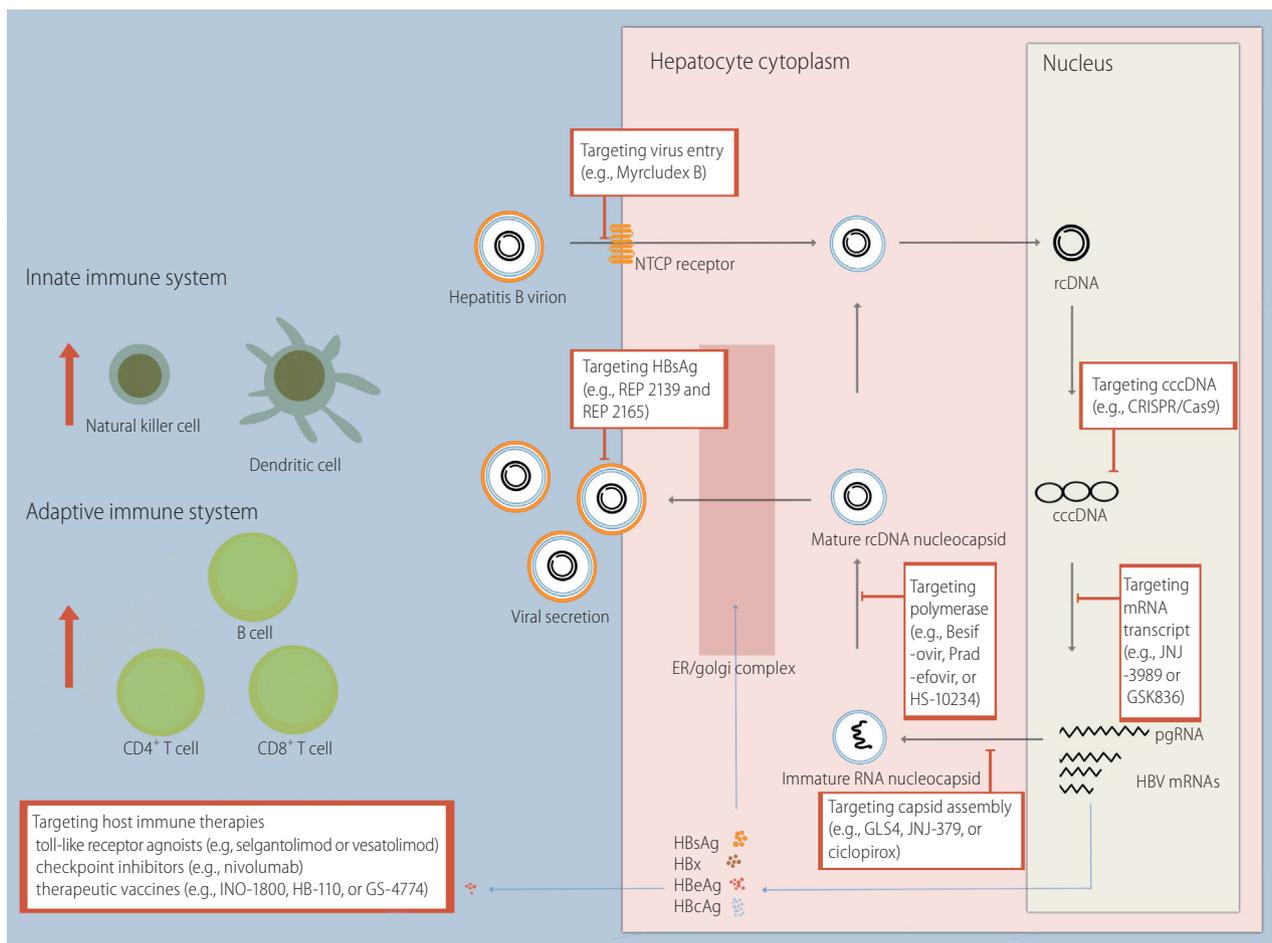
**Editor:** Sang Hoon Ahn, Yonsei University College of Medicine, Korea

**Received:** Mar. 26, 2021 / **Revised:** Jul. 5, 2021 / **Accepted:** Jul. 18, 2021

panzees are susceptible.<sup>2</sup> One of the major factors determining species specificity is the interaction of the preS1 domain of large HBsAg with the sodium taurocholate cotransporting polypeptide (NTCP) of human hepatocytes (Fig. 1).<sup>3</sup>

HBV virions enter the hepatocyte cytoplasm through a specific interaction with NTCP on the basolateral membrane of hepatocytes, followed by a plasma membrane component-mediated pathway (e.g., clathrin adaptor protein AP-2 and caveolin-1).<sup>4,5</sup> Subsequently, translocation of HBV occurs using the endosomal network in the cytoplasm. The gradual decrease in the pH of the endosome (i.e., 6.2 in early endosomes and 5.5 in late endosomes) aids in the fusion of the endosomal membrane with the outer envelope proteins of HBV.<sup>6,7</sup> HBV DNA enters the nucleus after the inner nucleocapsid of HBV is disassembled by the nuclear pore complex.

In the nucleus, HBV DNA is converted from rcDNA to covalently closed circular DNA (cccDNA).<sup>8</sup> cccDNA serves as a template for HBV gene products, including pregenomic RNA (pgRNA), preS1 messenger RNA (mRNA), preS2 mRNA, and X mRNA. The resulting RNAs are released into the cytoplasm. Some of the pgRNA serves as a translation template for the synthesis of the core protein and the polymerase. Another part of the pgRNA, together with the polymerase, forms a capsid using HBcAgs (i.e., encapsidation).<sup>9,10</sup> Inside the capsid, pgRNA is reverse transcribed to rcDNA. PreS1 mRNA, preS2 mRNA, and X mRNA are used to synthesize surface proteins and HBx proteins. Newly synthesized HBV nucleocapsids are either used for intracellular cccDNA amplification or form HBV virions through assembly with surface proteins in the endoplasmic reticulum and are then released to the sinusoidal space. Surface proteins are also released in the form of nonin-



**Figure 1.** HBV life cycle and novel drug targets. NTCP, sodium taurocholate cotransporting polypeptide; rcDNA, relaxed circular DNA; cccDNA, covalently closed circular DNA; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; ER, endoplasmic reticulum; mRNA, messenger RNA; pgRNA, pregenomic RNA; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B envelope antigen; HBcAg, hepatitis B core antigen.

fectious excess subviral particles (SVPs) that do not contain HBV DNA.<sup>11</sup>

The treatment goals for patients with chronic hepatitis B (CHB) are to reduce the mortality rate from chronic liver disease and improve overall survival by preventing the development of cirrhosis and/or hepatocellular carcinoma (HCC). The recommended treatment strategies are as follows: 1) timely initiation of antiviral therapy for patients with CHB; 2) long-term suppression of HBV DNA levels; and 3) HBsAg loss, with or without anti-HBs seroconversion (i.e., a functional cure) as an optimal endpoint.<sup>12-14</sup> Note that a functional cure other than eradication of intrahepatic cccDNA and integrated HBV DNA (i.e., a complete cure) is the endpoint of the latter strategy. This endpoint reflects the therapeutic effects and limitations of the recommended first-line antiviral agents: nucleos(t)ide analogues (NA) and pegylated interferon alpha (Peg-IFN $\alpha$ ).

NA treatment has shown that over 60% of HBV DNA is undetectable after 48 weeks of treatment.<sup>15,16</sup> The effectiveness of NA treatment has been maintained for over 5 years.<sup>17,18</sup> However, the proportion of patients achieving HBsAg loss is only 1.4–3.0% at 48 weeks of treatment. Even NAs have little effect on cccDNA in nature.<sup>19</sup> Peg-IFN $\alpha$  affects the entire replication process of HBV and can also induce degradation of cccDNA.<sup>20</sup> The rate of HBsAg loss exceeds 5% at 48 weeks of treatment.<sup>21,22</sup> However, Peg-IFN $\alpha$  is difficult to use because the frequency of side effects is very high. At 48 weeks of treatment, alopecia, skin manifestations, insomnia, anxiety, and oral symptoms have been reported in 44.0%, 56.0%, 36.0%, 28.9%, and 64.0% of patients, respectively.<sup>23</sup>

Although the risk of CHB-related HCC has been reduced as a result of NA or Peg-IFN $\alpha$  treatment, the risk of HCC remains in patients with CHB.<sup>24-27</sup> CHB is still the leading cause of HCC, causing 44% of HCC cases worldwide and 65–75% of HCC cases in endemic regions like Korea.<sup>13,28</sup> To overcome the limitations of NAs and Peg-IFN $\alpha$  and to achieve a complete cure for CHB, the need for drugs targeting novel therapeutic targets of HBV has emerged.

## NOVLE THERAPEUTICS TARGETING VIRAL LIFE CYCLE

### Targeting virus entry

Strategies to inhibit HBV entry include novel peptides derived

from HBsAg, such as myrcludex B, a myristoylated peptide derived from 47 amino acids within the pre-S1 domain of L-HBsAg.<sup>29</sup> A multicenter, open-label phase 2 clinical trial was performed to evaluate the efficacy and safety of myrcludex B in combination with Peg-IFN2 $\alpha$  for HBV/hepatitis D virus coinfection (Table 1).<sup>30</sup> When myrcludex B was used as a monotherapy for 48 weeks, none of the patients experienced a HBsAg >1 log decline, and HBsAg was undetectable. When myrcludex B was used in combination with Pge-IFN2 $\alpha$  over 48 weeks, HBsAg decline was achieved in 46.7% of the subjects (seven out of 15), indicating a potential role for myrcludex B in future HBV curative regimens.

Small molecules, such as ezetimibe,<sup>31</sup> cyclosporine derivatives,<sup>32</sup> and contravir,<sup>33</sup> have also been evaluated as viral entry inhibitors in many experimental models, and some compounds inhibit entry independently of the NTCP receptor. A phase 1 single ascending dose study for contravir (CRV431) is currently recruiting study subjects to evaluate the safety and pharmacokinetics of CRV431 in combination with TDF.

Monoclonal antibodies against HBsAg epitopes (e.g., HBV-AB<sup>XTL</sup>, HepeX-B<sup>TM</sup>, and 2H5-A14) have been evaluated for their efficacy in blocking HBV entry into hepatocytes and/or accelerating viral clearance from the circulation. These antibodies may have additional antiviral function, as they could confer protection by binding circulating virus.<sup>34-37</sup> However, no clinical trials for monoclonal antibodies against HBsAg are ongoing.

### Targeting capsid assembly

HBV capsid assembly plays a significant role in almost every step of the HBV replication cycle.<sup>38</sup> Notably, the HBV capsid is responsible for trafficking rcDNA to the nucleus, thereby establishing and sustaining cccDNA levels. Moreover, capsid protein is found in hepatocyte nuclei and interacts with host factors that are responsible for transcriptional regulation.<sup>9</sup> Therefore, targeting disruption of the HBV capsid protein could impact cccDNA stability and possibly lead to a complete cure of HBV infection.<sup>39</sup> Because sustained antiviral activity is expected, several capsid assembly modulators have been studied, such as GLS4 (phase 2 clinical trials),<sup>40</sup> NVR 3-778 (phase 2a clinical trials),<sup>41</sup> AB-423 (phase 1 clinical trials),<sup>42</sup> AB-506 (phase 1 clinical trials), JNJ-440 (phase 1 clinical trial),<sup>43</sup> JNJ-56136379 (phase 2 clinical trial),<sup>44</sup> ABI-H0731 (phase 2 clinical trials),<sup>45</sup> ABI-H2158 (phase 2a clinical trials), RO7049389 (phase 2 clinical trials),<sup>46</sup> Bay41-4109 (phase 1 clinical trials),<sup>47</sup> and QL-007 (phase 2 clinical trials). Structurally, these compounds are heteroaryldihydropyrimidines, phenylprope-

**Table 1.** Selected novel agents in development for the treatment of chronic hepatitis B

Drug name	Sponsor	Target	Class	Clinical stage	NCT number
Myrcludex B (bulevirtide)	Hepatera Ltd.	Virus entry	Peptide	II (completed)	NCT02881008 NCT02888106
CRV431	Hepion Pharmaceuticals, Inc.	Virus entry	Analogue of cyclosporine A	I (recruiting)	NCT03596697
GLS4 (morphothiadine)	Sunshine Lake Pharma Co., Ltd.	Capsid assembly	Small molecules	II (recruiting)	NCT04147208
NVR 3-778 (AL-3778)	Novira Therapeutics, Inc.	Capsid assembly	Small molecules	I (completed)	NCT02112799 NCT02401737
AB-506	Arbutus Biopharma Corporation	Capsid assembly	Small molecules	I (terminated)	-
AB-836	Arbutus Biopharma Corporation	Capsid assembly	Small molecules	I (not yet recruiting)	NCT04775797
JNJ-440 (JNJ-64530440)	Alios Biopharma Inc	Capsid assembly	Small molecules	I (completed)	NCT03439488
JNJ-56136379 (JNJ-379)	Janssen Research & Development, LLC	Capsid assembly	Small molecules	II (completed) II (recruiting)	NCT03361956 NCT04667104
ABI-H0731 (vebicovir)	Assembly Bioscience	Capsid assembly	Small molecules	II (completed) II (recruiting)	NCT03577171 NCT04781647
ABI-H2158	Assembly Bioscience	Capsid assembly	Small molecules	II (recruiting)	NCT04398134
EDP-514	Enanta, Pharmaceuticals	Capsid assembly	Small molecules	I (recruiting)	NCT04008004
QL-007	Qilu Pharmaceutical Co., Ltd.	Capsid assembly	Small molecules	II (recruiting)	NCT04157699
ZM-H1505R	Shanghai Zhimeng Biopharma, Inc.	Capsid assembly	Small molecules	I (completed)	NCT04220801
RO7049389 (RG7907)	Hoffmann-La Roche	Capsid assembly	Small molecules	II (recruiting)	NCT04225715
SRI-32007 (CYT997)	-	Capsid assembly	Small molecules	Preclinical	-
Ciclopirox	-	Capsid assembly	Small molecules	Preclinical	-
Besifovir	ILDong Pharmaceutical Co Ltd	Polymerase	Small molecules	III (active, not recruiting) IV (recruiting)	NCT01937806 NCT04202536
Metacavir	Guangzhou Yipinhong Pharmaceutical CO., LTD	Polymerase	Small molecules	II (unknown)	NCT02965859
HS-10234	Jiangsu Hansoh Pharmaceutical Co., Ltd.	Polymerase	Small molecules	III (recruiting)	NCT03903796
Pradefovir	Xi'an Xintong Pharmaceutical Research Co.,Ltd.	Polymerase	Small molecules	III (recruiting)	NCT04543565
ATI-2173	Antios Therapeutics, Inc	Polymerase	Small molecules	I (recruiting)	NCT04248426
GS-5801	Gilead Sciences	cccDNA	Small molecules	I (unknown)	-
REP 2139 and REP 2165	Replicor Inc.	HBsAg	Nucleic acid polymer	II (completed)	NCT02565719
ALG-010133	Aligos Therapeutics	HBsAg	Nucleic acid polymer	I (recruiting)	NCT04485663
ARC-520	Arrowhead Pharmaceuticals	Viral transcript	Small interfering RNA	II (terminated)	NCT02577029
JNJ-3989 (ARO-HBV)	Arrowhead Pharmaceuticals	Viral transcript	Small interfering RNA	II (completed)	NCT03365947

**Table 1.** Continued

Drug name	Sponsor	Target	Class	Clinical stage	NCT number
RG6346	Hoffmann-La Roche	Viral transcript	Small interfering RNA	II (recruiting)	NCT04225715
AB-729	Assembly Biosciences	Viral transcript	Small interfering RNA	II (recruiting)	NCT04820686
VIR-2218 (BRIL-835)	Vir Biotechnology	Viral transcript	Small interfering RNA	II (active, not recruiting)	NCT04507269
GSK3389404(GSK404)	GlaxoSmithKline	Viral transcript	Antisense oligonucleotides	II (completed)	NCT03020745
GSK3228836 (GSK836)	GlaxoSmithKline	Viral transcript	Antisense oligonucleotides	II (active, not recruiting) II (recruiting)	NCT04449029 NCT04676724

NCT, national clinical trial; cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen.

namides, or sulfamoylbenzamides. Recently, a tubulin polymerization inhibitor (SRI-32007) was discovered as an inhibitor of HBV core promoter activity, and preclinical studies have been performed.<sup>48</sup>

Recently, our research group discovered a novel anti-HBV drug, ciclopirox, from U.S. Food and Drug Administration-approved library.<sup>49</sup> Ciclopirox, a synthetic antifungal agent, strongly inhibited HBV replication in cells and mice by blocking assembly of the HBV capsid. The crystal structure of the HBV core protein and the ciclopirox complex revealed a novel binding mode at dimer-dimer interfaces. We also found that ciclopirox synergized with NAs to prevent HBV replication in cells and in a humanized liver mouse model. Orally administered ciclopirox may block HBV capsid assembly effectively and thus provide a novel opportunity to combat chronic HBV infection. Preclinical studies are ongoing for ciclopirox as a novel HBV drug.

### Targeting polymerase

NAs, which are commonly used currently, are antiviral agents that target polymerase. Drugs targeting polymerase and that aim to improve the therapeutic effects or to reduce side effects better than NAs are being developed. Besifovir dipivoxil maleate (BSV) is an acyclic nucleotide phosphonate inhibitor of HBV polymerase.<sup>50</sup> In a phase 3 clinical study, BSV showed comparable efficacy and better safety compared to tenofovir disoproxil fumarate (TDF).<sup>51</sup> Both HS-10234 and pradefovir, which are prodrugs of tenofovir and adefovir, respectively, showed comparable efficacy to TDF in a phase 2 clinical study and are in a phase 3 clinical study.<sup>52</sup> Metacavir, a novel deoxyguanosine analog, is under investigation in a phase 2 clinical trial.<sup>53</sup> ATI-2173 is a new chemical derived from clevudine, and it has the advantage of reducing the risk of clevudine-related myopathy. Currently, a phase 1 clinical trial is in progress.

### Targeting cccDNA

Genome editing tools include zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system.<sup>54,55</sup> The domains that recognize and bind to target double-stranded DNA (dsDNA) sequences in ZFN and TALENs are zinc finger protein and transcription activator-like effector, respectively.<sup>56,57</sup> Both tools require the FokI restriction endonuclease domain to cut the genome. On the contrary, all CRISPR/Cas9 systems use CRISPR RNA (i.e., guide RNA) to find target dsDNA and have a simple working structure that cuts target dsDNA with Cas protein endonuclease.<sup>58</sup> Among these tools, the CRISPR/Cas9 system is the most simplified tool for the development of custom nucleases. In a preclinical study, CRISPR/Cas9 system treatment showed encouraging results, showing that more than 90% of HBV DNA was cleaved by Cas9.<sup>59,60</sup> Subsequently, the removal of the full-length integrated HBV DNA (i.e., sterile eradication) was also successful.<sup>61</sup> Nevertheless, the major obstacle to the clinical application of CRISPR/Cas9 is the possibility of expressing an off-target effect that causes damage to the host DNA rather than the target. As an alternative method, CRISPR/Cas9 nickase only causes a single-strand DNA break, rather than dsDNA breaks.<sup>62</sup> Thus, a pair of properly spaced guide RNA can cause dsDNA breaks and the risk of permanent DNA damage due to off-target effects can be reduced.

In the nucleus, instead of performing genome editing, there are drugs that aim to silence cccDNA transcription. GS-5801 is a pro-drug of a small molecule inhibitor of histone lysine demethylase 5.<sup>63</sup> However, GS-5801 did not show antiviral activity in a humanized mouse model of HBV infection.<sup>64</sup> Other drugs that have blocked cccDNA transcription in the discovery stage are alpha-glucosidase, which suppresses the nuclear transcription factor

Sp1,<sup>65</sup> and dicoumarol, which is a competitive nicotinamide adenine dinucleotide phosphate quinone oxidoreductase that acts as an inhibitor of HBx expression.<sup>66</sup> Peg-IFN $\alpha$  is involved in both the degradation and transcription of cccDNA.<sup>20,67</sup> Peg-IFN $\alpha$  induces degradation of cccDNA through upregulation of APOBEC3A and 3B cytidine deaminases. Peg-IFN $\alpha$  also blocks cccDNA transcription by inducing cccDNA-bound histone hypoacetylation.

## Targeting HBsAg

Nucleic acid polymers (NAPs) have broad spectrum antiviral activity against several enveloped viruses, including HBV.<sup>68</sup> REP 2139 and REP 2165 are NAPs that inhibit the assembly and secretion of HBV SVP. In a phase 2 clinical trial, 48-week combination treatment with TDF, Peg-IFN $\alpha$ , and NAP showed HBsAg loss in 60% of the participants; The functional cure persisted in 35% of the participants during 48 weeks of treatment-free follow up.<sup>69</sup>

## Targeting viral transcript

High levels of viral antigens may be associated with a suppressed host immune response and increased persistence of HBV. The synthesis of viral antigens could potentially be blocked by the use of molecular approaches targeting viral mRNA transcripts, such as RNA interference (RNAi) and antisense oligonucleotides.

Small interfering RNA (siRNA) acts as RNAi, which can target a common 3' terminus shared by all mRNA transcripts and induce the degradation of the transcripts.<sup>70</sup> The degradation of mRNA transcripts can allow the suppression of HBV DNA and viral antigens, including HBsAg, HBeAg, and hepatitis B core-related antigens. Therefore, siRNA can reverse host immune exhaustion caused by high antigenemia, allow restoration of effective HBV-specific immune responses, and finally lead to the seroconversion of HBsAg. ARC-521 was the first siRNA against HBV designed to reduce all mRNA transcripts derived from cccDNA to be investigated in clinical trials.<sup>71</sup> The NA-experienced patients with CHB received four doses of ARC-520 at 1 mg/kg or 2 mg/kg or placebo every 4 weeks. The high-dose group (2 mg/kg) showed a significant reduction in HBsAg levels compared to the placebo group, but absolute HBsAg reductions were moderate in both patients who were hepatitis B envelope antigen (HBeAg)-negative (0.38 log IU/mL) and HBeAg-positive (0.54 log IU/mL). The limited activity was probably due to the high expression level of HBsAg from integrated HBV DNA. Unlike ARC-520, JNJ-3989 (ARO-HBV) can target viral mRNA transcripts from all sources, including cccD-

NA and integrated HBV DNA. In recent studies, 3 subcutaneous doses of JNJ-3989 were administered monthly in CHB patients concomitantly with entecavir or tenofovir.<sup>72</sup> JNJ-3989 with NA therapy was well tolerated and showed a  $\geq 1.0$  log IU/mL reduction in HBsAg in 98% of the patients with CHB.

Antisense oligonucleotides are nucleic acids that are designed to hybridize with target RNAs and block the expression of viral proteins. GSK3389404 is a liver-directed antisense oligonucleotide that inhibits the synthesis of viral proteins, including HBsAg. In a phase 2a clinical trial, subcutaneous injection of GSK3389404 (120 mg/week) for 3 months caused a rapid drop in the levels of HBsAg (0.75 log IU/mL) in patients with CHB.<sup>73</sup>

Major concerns about the agents targeting viral transcripts include the potential toxicity of the delivery platform, the risk of off-target toxicity, and the risk of rebound after treatment due to a lack of cccDNA reduction.

## HOST IMMUNE TARGETING THERAPIES

Adaptive immunity, such as HBV-specific T cell responses, is critical for the clearance of HBV infection. HBV-specific T cells secrete cytokines that induce noncytolytic clearance of HBV and recruitment of other inflammatory immune cells.<sup>74</sup> The innate immune response, such as the production of type I IFNs, is essential for the induction of following HBV-specific immunity. Therefore, the coordination of both innate and adaptive immune responses is important to control HBV infection. Patients with CHB are characterized by a weak innate and adaptive (HBV-specific) immune response.<sup>75</sup> Chronic HBV infection induces impaired cytokine production, T-cell exhaustion characterized by poor cytotoxic activity, and sustained expression of inhibitory receptors.<sup>76</sup> Immunomodulatory therapies focused on the restoration of impaired immune responses have been investigated in several trials as follows.

### Toll-like receptor (TLR) agonists

TLRs are a type of pattern recognition receptor on eukaryotic cells that sense pathogen-associated molecular patterns leading to the production of antiviral mediators.<sup>77</sup> Therefore, TLRs are part of the innate immune system that constitutes the first line of defense against invading microorganisms. TLRs also play a pivotal role in adaptive immune responses by inducing the differentiation of naïve T cells into effector T cells. The activation of TLRs can suppress the replication of HBV and induce the restoration of

HBV-specific adaptive immune responses. Vesatolimod (GS-9620), an oral agonist of TLR-7, activates innate and adaptive immune responses of patients with CHB. In a phase 2 clinical trial, once-weekly oral administration of vesatolimod for 4, 8, or 12 weeks showed a consistent dose-dependent induction of interferon-stimulated gene 15, which is the most sensitive indicator of TLR-7 activation (Table 2).<sup>78</sup> However, vesatolimod therapy did not show a significant decline in HBsAg compared to placebo. Selgantolimod (GS-9688), an oral agonist of TLR-8, induced a sustained antiviral response in woodchuck hepatitis virus (a hepadnavirus that is closely related to HBV)-infected woodchucks.<sup>79</sup> In a phase 2 clinical trial, a combination of selgantolimod (up to 3 mg) once weekly and NA therapy for 24 weeks followed by NA therapy alone for an additional 24 weeks achieved a modest decline in HBsAg levels from baseline and a 5% rate of HBsAg loss.<sup>80</sup>

Similar to TLR agonist, retinoic acid-inducible gene-I (RIG-I) agonist is another compound that can suppress the replication of

HBV by boosting innate immune responses. RIG-I agonist boosts innate immunity directly by activating the intracellular interferon pathway in hepatocytes and also have a direct antiviral effect as a non-nucleotide reverse transcriptase inhibitor.<sup>81</sup> In a phase 2 clinical trial, a combination of inarigivir (SB9200), an oral dinucleotide RIG-I agonist, and tenofovir showed a dose-dependent reduction of HBV DNA.<sup>82</sup> However, further investigation of inarigivir has been stopped due to severe adverse events including the death of one patient in another phase 2 clinical trial of the use of inarigivir 400 mg plus NA in HBeAg-negative patients.<sup>83</sup>

## Checkpoint Inhibitors

In chronic HBV infection, upregulation of checkpoint inhibitors, such as programmed cell death protein 1 (PD-1), is associated with T-cell exhaustion and persistent viral infection. Therefore, checkpoint inhibitors may help to restore T cell function. The in-

**Table 2.** Selected novel immune modulators in development for the treatment of chronic hepatitis B

Drug name	Sponsor	Target	Class	Clinical stage	NCT number
GS-9620 (vesatolimod)	Gilead Sciences	Toll-like receptor 7 agonists	Small molecules	II (completed)	NCT02166047
GS-9699 (selgantolimod)	Gilead Sciences	Toll-like receptor 8 agonists	Small molecules	II (completed)	NCT03615066
RG7854 (RG7020531)	Hoffmann-La Roche	Toll-like receptor 7 agonists	Small molecules	I	NCT04225715
SBT 8320	Silverback Therapeutics	Toll-like receptor 8 agonists	Small molecules	Preclinical	-
Nivolumab	PharmaEssentia	Programmed cell death-1 receptor antagonists	Monoclonal antibody	I (not yet recruiting)	NCT04638439
INO-1800	Inovio Pharmaceuticals	Therapeutic vaccines	DNA vaccines	I (completed)	NCT02431312
HB-110	Genexine, Inc.	Therapeutic vaccines	DNA vaccines	I (completed)	NCT01641536
GS-4774	Gilead Sciences	Therapeutic vaccines	T-cell vaccines	II (completed)	NCT02174276
TG-1050 (T101)	Transgene	Therapeutic vaccines	Adenovirus 5-based vaccines	I (completed)	NCT02428400
HepTcell	Altimune	Therapeutic vaccines	Synthetic peptides	II (recruiting)	NCT04684914
AIC 649	AiCuris	Therapeutic vaccines	A proprietary inactivated parapoxvirus particle preparation	I (completed)	-
VBI-2601 (BR11-179)	Brill Biosciences	Therapeutic vaccines	Recombinant protein vaccine	I/IIb (completed)	-
VTP-300	Vaccitech	Therapeutic vaccines	Heterologous combination of ChAdOx1 and MVA vectors	I/II (recruiting)	NCT04778904
JNJ 64300535	Janssen Sciences Ireland UC	Therapeutic vaccines	DNA vaccines	I (completed)	NCT03463369

NCT, national clinical trial; MVA, modified vaccinia virus Ankara.

duction of uncontrolled hepatitis flares and autoimmunity are the main concerns of this type of therapy. In a phase 1b clinical trial, nivolumab, a PD-1 inhibitor, was administered at either 0.1 or 0.3 mg/kg with or without a HBV therapeutic vaccine (GS-4774) in virally suppressed patients with HBeAg-negative CHB.<sup>84</sup> Patients receiving 0.3 mg/kg of nivolumab with and without GS-4774 at baseline and week 4 showed significant HBsAg declines at week 24 from baseline, and one patient receiving the combination experienced HBsAg loss. There were no serious adverse events. Other checkpoint inhibitors targeting cytotoxic T lymphocyte-associated antigen-5 and CD244/2B4 restored immune function and increased the proliferation of CD8 T cells *in vitro*.<sup>85,86</sup>

### Therapeutic vaccines

Therapeutic vaccination aims to restore HBV-specific immunity by stimulating the host immune response. In previous trials, existing prophylactic vaccines were able to reduce HBV replication in animal models of chronic hepadnaviral infection<sup>87</sup> but failed in patients with CHB.<sup>88,89</sup> These vaccines induced antibodies but failed to induce cytotoxic T cell responses that would be critical for therapeutic efficacy. Alternatively, DNA vaccines coding sequences of HBsAg to induce HBV-specific T cells were investigated but also yielded poor results.<sup>90,91</sup> DNA vaccines against multiple HBV proteins employing heterologous prime-boost regimens are currently under investigation. INO-1800, which is a DNA-based HBV vaccine encoding HBsAg and HBcAg, induced the binding of antibodies to HBs and robust cell-mediated immunity in animals.<sup>92</sup> The use of INO-1800 with or without human interleukin 12 in CHB patients virally suppressed with a NA is in a phase 1 clinical trial (NCT02431312). Combined treatment with HB-110, a DNA vaccine encoding S, L, core, and polymerase proteins, and adefovir was also investigated in a phase 1 clinical trial.<sup>93</sup> HB-110 exhibited a weak capability to induce HBV-specific T cell responses. Other HBV vaccines encoding multiple HBV proteins produced by different vectors, including GS-4774 and TG-1050, have been demonstrated to induce immunogenicity in animal models and healthy individuals.<sup>94,95</sup> However, in a clinical trial, there were no significant differences in the decline in HBsAg levels between the GS-4774 group and the tenofovir group.<sup>94</sup>

### Genetically engineered T cells

To restore adequate HBV-specific T cell immunity, the adoptive transfer of genetically engineered T cells can be a promising strat-

egy.<sup>75</sup> T cells separated from the peripheral blood of patients with CHB were expanded and activated *in vitro*. The expanded T cells were engineered to encode HBV-specific T cell receptors by using viral vectors and then were reinfused to patients with CHB. This approach could be achieved through T-cell receptor (TCR) gene transfer or the use of chimeric antigen receptor (CAR) T cells.<sup>96</sup> TCR-redirection HBV-specific T cells were able to recognize HBV-infected cells in an *in vitro* study.<sup>97</sup> T cells with a CAR specific for HBeAg were also reported to localize to the liver of mice to reduce the replication of HBV with only transient liver damage.<sup>98</sup>

### NEEDS FOR COMBINATION TREATMENT

We reviewed some of the anti-HBV agents targeting virus entry, capsid assembly, polymerase, cccDNA, HBsAg, viral transcripts, or HBV-specific immune responses (immunomodulators). Considering the mechanism of HBV replication and the difficulty in eradicating cccDNA and integrated HBV DNA, combination treatment using two or more drugs with different targets may be more advantageous than monotherapy.

The results of combination treatment with NAs and Peg-IFN $\alpha$  differed depending on the type of NA. Lamivudine and Peg-IFN $\alpha$  combination treatment was inferior to monotherapy.<sup>99-101</sup> However, TDF and Peg-IFN $\alpha$  combination treatment showed a significantly higher proportion of HBsAg loss than monotherapy with TDF or Peg-IFN $\alpha$  after 72 weeks.<sup>102</sup> Recently, a randomized trial found that combination treatment with entecavir (ETV), PEG-IFN $\alpha$ , and sequential vaccination showed a significantly higher proportion of HBsAg loss than monotherapy with ETV after 100 weeks (16.2% vs. 0.0%).<sup>103</sup>

The therapeutic effects of combined treatment with a NA and novel immunomodulators have also been studied. Combination treatment with a NA and GS-4774 did not further reduce HBsAg levels compared to monotherapy with NA in a phase 2 clinical trial.<sup>94</sup> Combination treatment with ETV and thymosin  $\alpha$ -1 did not significantly improve the rate of undetectable HBV DNA or the rate of HBsAg loss after 52 weeks compared to mono ETV treatment.<sup>104</sup> However, in a phase 2 clinical trial, combination treatment with a NA and selgantolimod showed a 5% rate of HBsAg loss after 24 weeks.<sup>80</sup> Similarly, HBsAg loss was observed in 25% of patients at 4 weeks of treatment in a phase 2a clinical trial of NA and GSK3228836 combination treatment.<sup>105</sup> Currently, a phase 2 clinical trial is ongoing to evaluate the efficacy and safety of the combined treatment of NA, GSK3228836, and Peg-IFN $\alpha$ .

Therefore, the combination of direct-acting antivirals and novel immunomodulators might be the ideal strategy to completely cure patients infected by HBV.

## ENDPOINTS OF CLINICAL TRIALS

The main endpoint of recent phase 2/3 clinical trials of novel HBV therapy was the functional cure of HBV (i.e., HBsAg loss) and therefore, quantitative HBsAg is a useful marker for assessing the efficacy of the novel therapy. However, HBsAg is not an ideal marker for very early assessment of the clinical trials because HBsAg may be expressed from cccDNA and integrated HBV DNA. Therefore, circulating viral RNAs or hepatitis B core-related antigen, potential surrogate markers of cccDNA, are also important markers for assessing the novel therapy. In the future clinical trials of novel agents targeting cccDNA elimination, the achievement of complete cure of HBV (i.e., elimination of cccDNA and integrated HBV DNA) will be an important endpoint. Unlike the ease of judgment of the functional cure, the standard evaluation method of the complete cure has not yet been established. Liver biopsy may be used to determine cccDNA elimination.<sup>19</sup> However, it is challenging to accurately determine the absence of cccDNA due to detection limitations.<sup>106</sup>

The appropriate time point for evaluating the treatment response for the phase 3 clinical trial suggested by the expert group was 6 months after the end of treatment.<sup>107</sup> It was agreed that it was an appropriate time to determine if the treatment response persisted. However, in the case of the phase 2 clinical trial, a specific time point was not presented. Considering the mechanism of the novel drug, the evaluation time point can be altered as 6 months after the end of treatment or 6 months after the start of treatment.

## FUTURE DIRECTIONS

The treatment options introduced in this study have different mechanisms of action, and each option has strengths and potential limitations. Therefore, combination therapy is promising for achieving a functional cure for HBV infection. Novel curative agents for HBV can be stratified either as direct regulators of the HBV life cycle (direct-acting antivirals) or indirect regulators of innate and/or HBV-specific immunomodulators. Combination treatment with potent NAs that can effectively suppress viral replication,

one or more direct-acting antiviral, and at least one immunomodulator can be a promising approach for treating chronic HBV infection. In addition, treatments should be individualized by considering different host factors, including treatment history, HBeAg expression, viral load, severity of fibrosis, and genotype. Future trials combining multiple therapeutic agents considering baseline host factors are warranted to find optimal and customized regimens for patients with CHB.

## CONCLUSION

The key obstacle to achieving a complete cure for HBV infection is the presence of cccDNA in the hepatocyte nucleus. The cccDNA acts as a transcription template for all viral RNAs, including pgRNA, and has a very long half-life. The removal of cccDNA is very difficult because cccDNA resides in the nucleus as a stable episomal plasmid-like molecule. Nevertheless, recent advances in technology and expanded knowledge about HBV infection have led to the development of agents targeting cccDNA. In addition, other direct-acting antivirals that can target various steps of the HBV life cycle, including HBV entry, transcription of viral proteins, nucleocapsid assembly, pgRNA packaging, and HBsAg release, are currently under investigation in clinical trials. Another barrier to finding a complete cure for HBV infection is defective host immune responses. Chronic HBV infection induces weak innate and HBV-specific immune responses, such as impaired cytokine production, T-cell exhaustion, and sustained expression of inhibitory receptors. Immunomodulators targeting innate and/or HBV-specific immune responses, including TLR agonists, checkpoint inhibitors, therapeutic vaccines, and engineered T cells, are also currently under development or in clinical trials. The combination of novel agents with or without potent NAs may be a promising strategy for a functional cure of HBV infection. Continued advancements of the novel agents may aid in the elimination of HBV.

## Authors' contribution

SW Kim, JS Yoon, Y Cho wrote the paper, M Lee and Y Cho supervised the whole project, and Y Cho edited the paper.

## Acknowledgements

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Re-

public of Korea (No. HI21C0240), and the National Research Foundation of Korea grant funded by the Korea government (No. 2021R1A2C4001401).

## Conflicts of Interest

The authors have no conflicts to disclose.

## REFERENCES

1. Sekiba K, Otsuka M, Ohno M, Yamagami M, Kishikawa T, Suzuki T, et al. Hepatitis B virus pathogenesis: fresh insights into hepatitis B virus RNA. *World J Gastroenterol* 2018;24:2261-2268.
2. Wieland S, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* 2004;101:6659-6674.
3. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* 2012;1:e00049.
4. Huang HC, Chen CC, Chang WC, Tao MH, Huang C. Entry of hepatitis B virus into immortalized human primary hepatocytes by clathrin-dependent endocytosis. *J virol* 2012;86:9443-9453.
5. Macovei A, Radulescu C, Lazar C, Petrescu S, Durantel D, Dwek RA, et al. Hepatitis B virus requires intact caveolin-1 function for productive infection in HepaRG cells. *J virol* 2010;84:243-253.
6. Elkin SR, Lakoduk AM, Schmid SL. Endocytic pathways and endosomal trafficking: a primer. *Wien Med Wochenschr* 2016;166:196-204.
7. Hayes CN, Zhang Y, Makokha GN, Hasan MZ, Omokoko MD, Chayama K. Early events in hepatitis B virus infection: from the cell surface to the nucleus. *J Gastroenterol Hepatol* 2016;31:302-309.
8. Block TM, Guo H, Guo JT. Molecular virology of hepatitis B virus for clinicians. *Clin Liver Dis* 2007;11:685-706, vii.
9. Diab A, Foca A, Zoulim F, Durantel D, Andrisani O. The diverse functions of the hepatitis B core/capsid protein (HBc) in the viral life cycle: implications for the development of HBc-targeting antivirals. *Antiviral Res* 2018;149:211-220.
10. Klumpp K, Crépin T. Capsid proteins of enveloped viruses as antiviral drug targets. *Curr Opin Virol* 2014;5:63-71.
11. Beck J, Nassal M. Hepatitis B virus replication. *World J Gastroenterol* 2007;13:48-64.
12. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;67:370-398.
13. Korean Association for the Study of the Liver (KASL). KASL clinical practice guidelines for management of chronic hepatitis B. *Clin Mol Hepatol* 2019;25:93-159.
14. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018;67:1560-1599.
15. Chang TT, Gish RG, De Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006;354:1001-1010.
16. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med* 2008;359:2442-2455.
17. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HSM, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010;51:422-430.
18. Buti M, Tsai N, Petersen J, Flisiak R, Gurel S, Krastev Z, et al. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. *Dig Dis Sci* 2015;60:1457-1464.
19. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004;126:1750-1758.
20. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014;343:1221-1228.
21. Luo X, Yu JX, Xie L, Ma WJ, Wang LH. Clinical analysis of polyethylene glycol interferon- $\alpha$  treatment in 155 hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) patients. *Ann Hepatol* 2018;16:888-892.
22. Chan HLY, Wong VWS, Chim AML, Wong GLH, Chan HY, Sung JJY. Treatment of patients with chronic hepatitis B who have failed previous antiviral treatment with pegylated interferon alpha2a (40 kda; PEGASYS). *Antivir Ther* 2008;13:555-562.
23. Yang JF, Kao YH, Dai CY, Huang JF, Hsieh MY, Lin ZY, et al. Comparison of adverse effects related to pegylated interferon-based therapy for patients with chronic hepatitis B and chronic hepatitis C in Taiwan. *Hepatol Int* 2010;4:732-740.
24. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 2013;58:98-107.
25. Lok ASF. Does antiviral therapy for hepatitis B and C prevent hepatocellular carcinoma? *J Gastroenterol Hepatol* 2011;26:221-227.
26. Coffin CS, Rezaeeaval M, Pang JX, Alcantara L, Klein P, Burak KW, et al. The incidence of hepatocellular carcinoma is reduced in patients with chronic hepatitis B on long-term nucleos(t)ide analogue therapy. *Aliment Pharmacol Ther* 2014;40:1262-1269.
27. Kim MA, Kim SU, Sinn DH, Jang JW, Lim YS, Ahn SH, et al. Dis-

- continuation of nucleos(t)ide analogues is not associated with a higher risk of HBsAg seroreversion after antiviral-induced HBsAg seroclearance: a nationwide multicentre study. *Gut* 2020;69:2214-2222.
28. Baecker A, Liu X, La Vecchia C, Zhang ZF. Worldwide incident hepatocellular carcinoma cases attributable to major risk factors. *Eur J Cancer Prev* 2018;27:205.
  29. Schulze A, Schieck A, Ni Y, Mier W, Urban S. Fine mapping of pre-sequence requirements for hepatitis B virus large envelope protein-mediated receptor interaction. *J Virol* 2010;84:1989-2000.
  30. Wedemeyer H, Schoneweis K, Bogomolov P, Voronkova N, Chulanov V, Stepanova T. Final results of a multicenter, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of myrcludex B in combination with PEG-interferon Alpha 2a in patients with chronic HBV/HDV co-infection. *The International Liver Congress; 2019 Apr 10-14; Vienna, Austria. Elsevier, 2019. 81 p.*
  31. Lucifora J, Esser K, Protzer U. Ezetimibe blocks hepatitis B virus infection after virus uptake into hepatocytes. *Antiviral Res* 2013;97:195-197.
  32. Watashi K, Sluder A, Daito T, Matsunaga S, Ryo A, Nagamori S, et al. Cyclosporin A and its analogs inhibit hepatitis B virus entry into cultured hepatocytes through targeting a membrane transporter, sodium taurocholate cotransporting polypeptide (NTCP). *Hepatology* 2014;59:1726-1737.
  33. Armas D, Snyder M, Trepanier D, Ure D, Greytok J, Foster R. FRI-171-A phase 1 single ascending dose study of the safety, tolerability and pharmacokinetics of CRV431. *J Hepatol* 2019;70:e464-e465.
  34. Li D, He W, Liu X, Zheng S, Qi Y, Li H, et al. A potent human neutralizing antibody Fc-dependently reduces established HBV infections. *Elife* 2017;6:e26738.
  35. Zhang TY, Yuan Q, Zhao JH, Zhang YL, Yuan LZ, Lan Y, et al. Prolonged suppression of HBV in mice by a novel antibody that targets a unique epitope on hepatitis B surface antigen. *Gut* 2016;65:658-671.
  36. Neumann AU, Phillips S, Levine I, Ijaz S, Dahari H, Eren R, et al. Novel mechanism of antibodies to hepatitis B virus in blocking viral particle release from cells. *Hepatology* 2010;52:875-885.
  37. Galun E, Eren R, Safadi R, Ashour Y, Terrault N, Keeffe EB, et al. Clinical evaluation (phase I) of a combination of two human monoclonal antibodies to HBV: safety and antiviral properties. *Hepatology* 2002;35:673-679.
  38. Cole AG. Modulators of HBV capsid assembly as an approach to treating hepatitis B virus infection. *Curr Opin Pharmacol* 2016;30:131-137.
  39. Boucle S, Bassit L, Ehteshami M, Schinazi RF. Toward elimination of hepatitis B virus using novel drugs, approaches, and combined modalities. *Clin Liver Dis* 2016;20:737-749.
  40. Zhang H, Zhu X, Chen H, Li X, Hu Y, Wu M, et al. Safety, pharmacokinetics and anti-viral efficacy of novel core protein allosteric modifier GLS4 in patients with chronic hepatitis B: interim results from a 48 weeks phase 2a study. *Hepatology* 2018;68:1454A-1455A.
  41. Yuen MF, Gane EJ, Kim DJ, Weilert F, Yuen Chan HL, Lalezari J, et al. Antiviral activity, safety, and pharmacokinetics of capsid assembly modulator NVR 3-778 in patients with chronic HBV infection. *Gastroenterology* 2019;156:1392-1403.e7.
  42. Eley T, Caamano S, Denning J, Sims K, Larouche R, Symonds W, et al. Single dose safety, tolerability, and pharmacokinetics of AB-423 in healthy volunteers from the ongoing single and multiple ascending dose study AB-423-001. *Hepatology* 2017;66:490A-490A.
  43. Kakuda TN, Yogaratnam JZ, Westland C, Gane EJ, Schwabe C, Patel M, et al. JNJ-64530440, a novel capsid assembly modulator: single- and multiple-ascending dose safety, tolerability and pharmacokinetics in healthy volunteers. *J Hepatol* 2019;70(Suppl 1):e469.
  44. Zoulim F, Yogaratnam J, Vandenbossche JJ, Moscalu I, Streinu-Cercel A, Lenz O, et al. Safety, pharmacokinetics and antiviral activity of a novel hepatitis B virus (HBV) capsid assembly modulator, JNJ-56136379, in patients with chronic hepatitis B (CHB). *Hepatology* 2018;68:47A-48A.
  45. Ma XL, Lalezari J, Nguyuen T, Bae H, Schiff ER, Fung S, et al. Interim safety and efficacy results of the ABI-H0731 phase 2a program exploring the combination of ABI-H0731 with Nuc therapy in treatment-naive and treatment-suppressed chronic hepatitis B patients. *J Hepatol* 2019;70:e130.
  46. Yuen MF, Schwabe C, Tanwandee T, Jin Y, Gao L, Zhou X, et al. RO7049389, a core protein allosteric modulator, demonstrates robust decline in HBV DNA and HBV RNA in chronic HBV infected patients. *J Hepatol* 2019;70:e491.
  47. Brezillon N, Brunelle MN, Massinet H, Giang E, Lamant C, DaSilva L, et al. Antiviral activity of Bay 41-4109 on hepatitis B virus in humanized Alb-uPA/SCID mice. *PLoS One* 2011;6:e25096.
  48. Kalkeri R, Peng J, Huang C, Cai Z, Ptak RG, Suto MJ. HBV core promoter inhibition by tubulin polymerization inhibitor (SRI-32007). *Adv Virol* 2020;2020:8844061.
  49. Kang JA, Kim S, Park M, Park HJ, Kim JH, Park S, et al. Ciclopirox inhibits Hepatitis B Virus secretion by blocking capsid assembly. *Nat Commun* 2019;10:2184.
  50. Lai CL, Ahn SH, Lee KS, Um SH, Cho M, Yoon SK, et al. Phase IIB multicentred randomised trial of besifovir (LB80380) versus entecavir in Asian patients with chronic hepatitis B. *Gut* 2014;63:996-1004.
  51. Ahn SH, Kim W, Jung YK, Yang JM, Jang JY, Kweon YO, et al. Efficacy and safety of besifovir dipivoxil maleate compared with tenofovir disoproxil fumarate in treatment of chronic hepatitis B virus

- infection. *Clin Gastroenterol Hepatol* 2019;17:1850-1859.e4.
52. Zhang H, Hu Y, Wu M, Liu J, Zhu X, Li X, et al. Randomised clinical trial: safety, efficacy and pharmacokinetics of HS-10234 versus tenofovir for the treatment of chronic hepatitis B infection. *Aliment Pharmacol Ther* 2021;53:243-252.
  53. Zhang P, Zhang L, Jiang Z, Wang T, Chen H, Xiong Y, et al. In vitro mitochondrial toxicity of metacavir, a new nucleoside reverse transcriptase inhibitor for treatment of hepatitis B virus. *Antimicrob Agents Chemother* 2010;54:4887-4892.
  54. Bloom K, Maepa MB, Ely A, Arbuthnot P. Gene therapy for chronic HBV-can we eliminate cccDNA? *Genes (Basel)* 2018;9:207.
  55. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* 2013;339:819-823.
  56. Cradick TJ, Keck K, Bradshaw S, Jamieson AC, McCaffrey AP. Zinc-finger nucleases as a novel therapeutic strategy for targeting hepatitis B virus DNAs. *Mol Ther* 2010;18:947-954.
  57. Bloom K, Ely A, Mussolino C, Cathomen T, Arbuthnot P. Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. *Mol Ther* 2013;21:1889-1897.
  58. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 2014;157:1262-1278.
  59. Seeger C, Sohn JA. Complete spectrum of CRISPR/Cas9-induced mutations on HBV cccDNA. *Mol Ther* 2016;24:1258-1266.
  60. Ramanan V, Shlomai A, Cox DB, Schwartz RE, Michailidis E, Bhatta A, et al. CRISPR/Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus. *Sci Rep* 2015;5:10833.
  61. Li H, Sheng C, Wang S, Yang L, Liang Y, Huang Y, et al. Removal of integrated hepatitis B virus DNA using CRISPR-Cas9. *Front Cell Infect Microbiol* 2017;7:91.
  62. Karimova M, Beschoner N, Dammermann W, Chemnitz J, Indenbirken D, Bockmann JH, et al. CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. *Sci Rep* 2015;5:13734.
  63. Gilmore S, Tam D, Dick R, Appleby T, Birkus G, Willkom M, et al. Antiviral activity of GS-5801, a liver-targeted prodrug of a lysine demethylase 5 inhibitor, in a hepatitis B virus primary human hepatocyte infection model. *J Hepatol* 2017;66:S690-S691.
  64. Gilmore S, Ramirez R, Snyder C, Li L. Characterization of a KDM5 small molecule inhibitor with antiviral activity in rodent livers. *Gene Expression Omnibus web site*, <<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166040>>. Accessed 23 May 2021.
  65. Hayakawa M, Umeyama H, Iwadate M, Taguchi YH, Yano Y, Honda T, et al. Development of a novel anti-hepatitis B virus agent via Sp1. *Sci Rep* 2020;10:47.
  66. Cheng ST, Hu JL, Ren JH, Yu HB, Zhong S, Wai Wong VK, et al. Dicoumarol, an NQO1 inhibitor, blocks cccDNA transcription by promoting degradation of HBx. *J Hepatol* 2021;74:522-534.
  67. Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, et al. IFN- $\alpha$  inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. *J Clin Invest* 2012;122:529-537.
  68. Vaillant A. Nucleic acid polymers: broad spectrum antiviral activity, antiviral mechanisms and optimization for the treatment of hepatitis B and hepatitis D infection. *Antiviral Res* 2016;133:32-40.
  69. Bazinet M, Păntea V, Placinta G, Moscalu I, Ceboatarescu V, Cojuhari L, et al. Safety and efficacy of 48 weeks REP 2139 or REP 2165, tenofovir disoproxil, and pegylated interferon alfa-2a in patients with chronic HBV infection naïve to nucleos(t)ide therapy. *Gastroenterology* 2020;158:2180-2194.
  70. Birmingham A, Anderson EM, Reynolds A, Ilsley-Tyree D, Leake D, Fedorov Y, et al. 3' UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nat Methods* 2006;3:199-204.
  71. Yuen MF, Schiefke I, Yoon JH, Ahn SH, Heo J, Kim JH, et al. RNA interference therapy with ARC-520 results in prolonged hepatitis B surface antigen response in patients with chronic hepatitis B infection. *Hepatology* 2020;72:19-31.
  72. Gane E, Locarnini S, Lim TH, Strasser S, Sievert W, Cheng W, et al. Short-term treatment with RNA interference therapy, JNJ-3989, results in sustained hepatitis B surface antigen suppression in patients with chronic hepatitis B receiving nucleos(t)ide analogue treatment. *J Hepatol* 2019;73:S20.
  73. Yuen MF, Heo J, Kumada H, Suzuki F, Suzuki Y, Xie Q, et al. Results after 12 weeks treatment of multiple doses of GSK3389404 in chronic hepatitis B subjects on stable nucleos(t)ide therapy in a phase 2a double-blind, placebo-controlled study. *Hepatology* 2019;70:433A.
  74. Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. *J Hepatol* 2016;64(1 Suppl):S71-S83.
  75. Bertoletti A, Le Bert N. Immunotherapy for chronic hepatitis B virus infection. *Gut Liver* 2018;12:497-507.
  76. Ye B, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis* 2015;6:e1694.
  77. Schnare M, Rollinghoff M, Qureshi S. Toll-like receptors: sentinels of host defence against bacterial infection. *Int Arch Allergy Immunol* 2006;139:75-85.
  78. Janssen HLA, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen AH, et al. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. *J Hepatol* 2018;68:431-440.
  79. Daffis S, Balsitis S, Chamberlain J, Zheng J, Santos R, Rowe W, et al. Toll-like receptor 8 agonist GS-9688 induces sustained efficacy in the woodchuck model of chronic hepatitis B. *Hepatology* 2021;73:53-67.

80. Gane E, Dunbar PR, Brooks A, Zhao Y, Tan S, Lau A, et al. Efficacy and safety of 24 weeks treatment with oral TLR8 agonist, selgantolimod, in virally-suppressed adult patients with chronic hepatitis B: a phase 2 study. *J Hepatol* 2020;73:S52.
81. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, et al. The RNA Sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. *Immunity* 2015;42:123-132.
82. Yuen RMF, Chen CY, Liu CJ, Jeng RWJ, Elkhashab M, Coffin C, et al. Ascending dose cohort study of inarigivir - a novel RIG I agonist in chronic HBV patients: final results of the ACHIEVE trial. *J Hepatol* 2019;70:E47-E48.
83. Agarwal K, Afdhal N, Coffin C, Fung S, Dusheiko G, Foster G, et al. Liver toxicity in the phase 2 catalyst 206 trial of inarigivir 400 mg daily added to a nucleoside in HBV eAg negative patients. *J Hepatol* 2020;73:S125.
84. Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, et al. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: a pilot study. *J Hepatol* 2019;71:900-907.
85. Raziourrouh B, Schraut W, Gerlach T, Nowack D, Grüner NH, Ulsenheimer A, et al. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. *Hepatology* 2010;52:1934-1947.
86. Schurich A, Khanna P, Lopes AR, Han KJ, Peppas D, Micco L, et al. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-prone CD8 T cells in persistent hepatitis B virus infection. *Hepatology* 2011;53:1494-1503.
87. Rollier C, Sunyach C, Barraud L, Madani N, Jamard C, Trepo C, et al. Protective and therapeutic effect of DNA-based immunization against hepatitis B large envelope protein. *Gastroenterology* 1999;116:658-665.
88. Pol S, Nalpas B, Driss F, Michel ML, Tiollais P, Denis J, et al. Efficacy and limitations of a specific immunotherapy in chronic hepatitis B. *J Hepatol* 2001;34:917-921.
89. Vandepapelière P, Lau GK, Leroux-Roels G, Horsmans Y, Gane E, Tawandee T, et al. Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. *Vaccine* 2007;25:8585-8597.
90. Godon O, Fontaine H, Kahi S, Meritet JF, Scott-Algara D, Pol S, et al. Immunological and antiviral responses after therapeutic DNA immunization in chronic hepatitis B patients efficiently treated by analogues. *Mol Ther* 2014;22:675-684.
91. Fontaine H, Kahi S, Chazallon C, Bourguin M, Varaut A, Buffet C, et al. Anti-HBV DNA vaccination does not prevent relapse after discontinuation of analogues in the treatment of chronic hepatitis B: a randomised trial--ANRS HB02 VAC-ADN. *Gut* 2015;64:139-147.
92. Obeng-Adjei N, Hutnick NA, Yan J, Chu JS, Myles DJ, Morrow MP, et al. DNA vaccine cocktail expressing genotype A and C HBV surface and consensus core antigens generates robust cytotoxic and antibody responses in mice and Rhesus macaques. *Cancer Gene Ther* 2013;20:652-662.
93. Yoon SK, Seo YB, Im SJ, Bae SH, Song MJ, You CR, et al. Safety and immunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. *Liver Int* 2015;35:805-815.
94. Gaggar A, Coeshott C, Apelian D, Rodell T, Armstrong BR, Shen G, et al. Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vaccine, in healthy subjects: a randomized study. *Vaccine* 2014;32:4925-4931.
95. Martin P, Dubois C, Jacquier E, Dion S, Mancini-Bourguin M, Godon O, et al. TG1050, an immunotherapeutic to treat chronic hepatitis B, induces robust T cells and exerts an antiviral effect in HBV-persistent mice. *Gut* 2015;64:1961-1971.
96. Spyrou E, Smith CI, Ghany MG. Hepatitis B: current status of therapy and future therapies. *Gastroenterol Clin North Am* 2020;49:215-238.
97. Gehring AJ, Xue SA, Ho ZZ, Teoh D, Ruedl C, Chia A, et al. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *J Hepatol* 2011;55:103-110.
98. Krebs K, Böttinger N, Huang LR, Chmielewski M, Arzberger S, Gasteiger G, et al. T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. *Gastroenterology* 2013;145:456-465.
99. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004;351:1206-1217.
100. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005;352:2682-2695.
101. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005;365:123-129.
102. Marcellin P, Ahn SH, Ma X, Caruntu FA, Tak WY, Elkashab M, et al. Combination of tenofovir disoproxil fumarate and peginterferon  $\alpha$ -2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B. *Gastroenterology* 2016;150:134-144.e10.
103. Lee JH, Lee YB, Cho EJ, Yu SJ, Yoon JH, Kim YJ. Entecavir plus pegylated interferon and sequential hepatitis B virus vaccination increases hepatitis B surface antigen seroclearance: a randomized controlled proof-of-concept study. *Clin Infect Dis* 2021;73:e3308-e3316.

104. Peng D, Xing HY, Li C, Wang XF, Hou M, Li B, et al. The clinical efficacy and adverse effects of entecavir plus thymosin alpha-1 combination therapy versus entecavir monotherapy in HBV-related cirrhosis: a systematic review and meta-analysis. *BMC Gastroenterol* 2020;20:348.
105. Yuen MF, Heo J, Jang JW, Yoon JH, Kweon YO, Park SJ, et al. Hepatitis B virus (HBV) surface antigen (HBsAg) inhibition with is 505358 in chronic hepatitis B (CHB) patients on stable nucleos(t)ide analogue (NA)-naïve CHB patients: phase 2a, randomized, double-blind, placebo-controlled study. *J Hepatol* 2020;73:S49-S50.
106. Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. *Gut* 2015;64:1972-1984.
107. Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: from discovery to regulatory approval. *J Hepatol* 2017;67:847-861.