Advances in new antivirals for chronic hepatitis B

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Abstract
Chronic hepatitis B virus (HBV) infection remains a global health burden. Timely and effective antiviral therapy is beneficial for patients with HBV infection. With existing antiviral drugs, including nucleos(t)ide analogs and interferon-alfa, patients can achieve viral suppression with improved prognosis. However, the rate of hepatitis B surface antigen loss is low. To achieve a functional cure and even complete cure in chronic hepatitis B patients, new antivirals need to be developed. In this review, we summarized the advantages and disadvantages of existing antiviral drugs and focused on new antivirals including direct-acting antiviral drugs and immunotherapeutic approaches.

Keywords: Hepatitis B virus; New antivirals; Functional cure; Complete cure

Introduction
Chronic hepatitis B virus (HBV) infection remains a heavy public health burden worldwide, and it is estimated that individuals infected with HBV account for 5% to 6% of the total population in China.[1] Timely and effective antiviral therapy is beneficial for chronic hepatitis B (CHB) patients. With current antivirals, including nucleos(t)ide analogs (NAs) and interferon alfa (IFN-α), most CHB patients have achieved alanine aminotransferase (ALT) normalization and HBV DNA suppression. Furthermore, these patients are less likely to develop necroinflammation of the liver, leading to a lower incidence of complications such as liver failure and hepatocellular carcinoma (HCC) with an improved prognosis.[2]

Functional or even complete cure is the ideal therapeutic endpoint.[3] However, the annual incidence of spontaneous hepatitis B surface antigen (HBsAg) loss in untreated CHB patients is approximately 1%. [4] Only 1% to 13% of patients with NAs treatment and 3% to 11% with IFN-α therapy can achieve HBsAg loss.[5]

Therefore, the development of new anti-HBV drugs is imperative to achieve the goal. Currently, new antivirals mainly include two categories: direct-acting antiviral drugs [Table 1] and immunotherapeutic approaches [Table 2]. In this review, we summarized the advantages and disadvantages of existing antivirals and focused on new drugs with promising applications.

Current antiviral drugs
Nucleos(t)ide analogues
NAs are a type of reverse transcriptase inhibitor that inhibits the polymerase activity of HBV via incorporating into elongating DNA chain to terminate the DNA reverse transcription. Entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) are recommended as first-line NAs by international guidelines.[6]

Entecavir
ETV is an analog of cyclopentanoylguanosine, which has strong anti-HBV activity and rapidly reduces the viral load of CHB patients. In two phase III clinical trials, for hepatitis e antigen (HBeAg)-negative and HBeAg-positive patients with 48 weeks of ETV treatment, 90% and 67% had undetectable serum HBV DNA, 78% and 68% achieved ALT normalization, while 0% and 2% achieved HBsAg loss, respectively.[6,7] For HBeAg-positive patients with 5-year ETV treatment, 94% achieved HBV DNA <300 copies/mL, 80% achieved ALT normalization, while 5% achieved HBsAg loss.[8]
Tenofovir disoproxil fumarate (TDF) is an acyclic adenine nucleoside analogue (NAs) that inhibits the hepatitis B virus (HBV) polymerase efficiently. In two phase III clinical trials, for HBeAg-negative and HBeAg-positive patients with 8-year of TDF therapy, 99% and 98% had HBV DNA undetectable, 88% and 84% achieved ALT normalization, while 1.1% and 13% achieved HBsAg loss, respectively.[9]

### Table 1: New antivirals targeting different steps of HBV lifecycle.

| Classification by mechanism | Drug name | Mechanism of action | Clinical stage | Sponsor | Notes | Refs |
|-----------------------------|-----------|---------------------|----------------|---------|-------|------|
| Entry inhibitors            | Hepcludex | Specifically block the binding of HBV to NTCP receptor | IIb | Hepatera, Russia with MYR GmbH, Germany | Only nine out of 30 patients receiving both Hepcludex and Peg IFN-α had a >1 log_{10} decrease of HBsAg level. | [20] |
| Targeting viral transcripts | JNJ-3989  | siRNAs              | IIb | Arrowhead Pharma, USA With Janssen | 39 subjects experienced an HBsAg reduction of ≥1.0 log_{10} IU/mL. 22 patients achieved a continuous decrease of HBsAg during the follow-up and the average HBsAg decrease was 1.74 log_{10} IU/mL. | [25] |
|                             | VIR-2218  |                     | II  | Alnylam and VirBiotech, USA | Most trial participants achieved their maximum HBsAg decline at week 16. However, after 24 weeks, a certain rebound of average HBsAg levels was observed in all arms. | [30] |
|                             | IONIS-HBVRx | ASO              | II  | Ionis Pharma, USA with GS | The decrease in HBsAg levels of three patients and the decrease of HBV DNA levels of four patients was >3.0 log_{10} IU/mL. | [33] |
| Capsid assembly inhibitors  | GLS4      | The core protein variant regulator | II  | HEC Pharma, China | GLS4/ritonavir treatment is safe and well-tolerated with good antiviral activity, especially when combined with ETV. | [37] |
|                             | ABI-H0731 | A new capsid assembly inhibitor | II  | Assembly, Biosciences, USA Beigene, China | In CHB patients treated with ABI-H0731 and NAs, the HBV DNA and HBV RNA levels decreased faster and deeper, similar to pgRNA, HBeAg, and HBeAg levels. | [40-42] |
| HBsAg secretion inhibitors  | REP2139   | A nucleic acid polymer | II  | Replicor, Canada | REP 2139-based treatment can achieve high HBsAg clearance and seroconversion rates during treatment, high virological control, and functional cure of HBV infection during long-term follow-up. | [43] |

ALT: Alanine aminotransferase; ASO: Antisense oligonucleotides; CHB: Chronic hepatitis B; ETV: Entecavir; HBeAg: Hepatitis B core-related antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; NAs: Nucleos(t)ide analogs; NTCP: Sodium taurocholate cotransporting polypeptide; PEG IFN-α: Pegylated interferon alfa; pgRNA: Pre-genomic RNA; RTV: Ritonavir; siRNA: Small interfering RNA.
| Classification by mechanism | Drug name | Mechanism of action | Clinical stage | Sponsor | Notes | Refs |
|----------------------------|-----------|---------------------|---------------|---------|-------|------|
| TLR agonists               | GS-9620   | TLR-7 agonist       | II            | Gilead Sciences, USA | In CHB patients, GS-9620 was safe and well-tolerated while no significant decline in HBsAg was observed. | [59] |
|                            | RO7020531 |                     | I             | Roche, Switzerland | RO7020531 was safe and well-tolerated in healthy volunteers with an increase in IFN-α-related cytokine levels and induction of ISGs. | [60] |
|                            | JNJ-4964  |                     | I             | Janssen Pharmaceutica, Belgium | Oral doses of 0.2–1.8 mg appeared to be safe and well-tolerated. | [61] |
|                            | GS-9688   | TLR-8 agonist       | II            | Gilead Sciences, USA | With GS-9688 therapy, three patients at week 24 and four patients at week 48 achieved an HBsAg decline of ≥0.5 log10 IU/mL. | [65] |
| Therapeutic vaccine        | GS-4774   | Expressing HBV-specific antigens including HBsAg, HBcAg, and HBx | III | Gobelimmune with Gilead, USA | GS-4774 did not reduce levels of HBsAg in patients, its strong immune-stimulatory effect on CD8+ T cells might be used in combination with other antiviral agents to boost the antiviral immune response. | [69] |
|                            | ABX-203   | Containing both HBsAg and HBcAg | I | Center for Genetic Engineering and Biotechnology, Cuba | Five patients had a decline to undetectable viral load, and all had liver stiffness measurements <7.8 kPa. | [71] |
|                            | BRII-179  | Induce Th1 type immune response | Ib/Ia | VBI Vaccines, USA with Brii Biosciences | BRII-179 alone or in combination with IFN-α could simultaneously induce B and T cell immune responses. | [74] |
|                            | TG-1050   | Adenovirus serotype 5 delivery | II | Transgene, France | TG-1050 was able to induce HBV-specific cellular immune responses. | [73] |
| IAPs antagonists           | APG-1387  | Mimicking endogenous SMAC molecules to degrade IAPs | II | Gilead Sciences, USA | The phase II clinical trial is still ongoing. | [79] |
| Immune checkpoint inhibitors | ASC22 (KN035) | Anti-PD-1/PD-L1 | IIb | Ascleis Pharma, China | The phase IIb clinical trial is still ongoing. | – |
|                            | Nivolumab |                     | II            | Gilead Sciences, USA | At week 24, 14% (3/22) of the patients had obtained a >0.5 log10 reduction in HBsAg levels. | [84] |
| Monoclonal antibodies      | GC1102    | HBsAg monoclonal antibody | II | Green Cross, South Korea | In a phase I clinical trial, the combination of GC1102 with antiviral drugs could reduce HBsAg titers and increase the chance of functional cure in patients with CHB. | [89] |
|                            | VIR-3434  | RNA gene silencer   | I             | Alnylam and Vir Biotech, USA | The results showed that a single low dose of 6 mg VIR-3434 produced rapid HBsAg reduction and was maintained for 2 weeks after administration. | [91] |
| Other immune approaches    | IMC-I109V | Immune mobilizing monoclonal T cell receptors against the virus | I             | Immunocore, USA | The phase I clinical trial is still ongoing. | – |

B cell: Bursa-dependent lymphocyte; CHB: Chronic hepatitis B; HBcAg: Hepatitis B core antigen; HBsAg: Hepatitis B surface antigen; HBx: Hepatitis B virus X protein; IAPs: Inhibitor of apoptosis proteins; IFN-α: Interferon alfa; ISGs: IFN-stimulated genes; PD-1: Programmed cell death protein 1; PD-L1: Programmed cell death-ligand 1; SMAC: Second mitochondria-derived activator of caspase; T cell: Thymus-dependent lymphocyte; TLR: Toll-like receptor.
Tenofovir alafenamide

TAF, a prodrug of tenofovir, delivers active metabolites to hepatocytes more effectively than TDF. In two phase III clinical trials, for HBeAg-negative and HBeAg-positive patients with 3-year therapy of TAF, 87% and 74% achieved HBV DNA levels lower than 29 IU/mL, 71% and 64% reached ALT normalization, while 0.4% and 1.4% achieved HBsAg loss, respectively.\[10,11\]

In conclusion, NAs exert strong antiviral effects in CHB patients. However, the HBsAg loss rate in patients is low.

Interferon alfa

IFN-α exerts its antiviral effect via exerting direct antiviral activity, enhancing cell-mediated immune responses, or clearing infected hepatocytes.\[12\] In two phase III clinical trials, at the 3-year follow-up after treatment discontinuation, for HBeAg-negative patients with pegylated IFN-α-2a (PEG IFN-α-2a) therapy, 18% had undetectable HBV DNA, 31% had ALT normalization, while 8% had HBsAg loss;\[13\] for patients who received PEG IFN-α-2b therapy, 35% achieved HBeAg seroconversion, 19% had undetectable HBV DNA, while 11% had HBsAg loss.\[14\]

In conclusion, IFN-α has both antiviral and immunomodulatory effects. However, the HBsAg loss rate is still far from satisfactory, especially taking into consideration the adverse events associated with IFN-α therapy.

Future therapy

Interference with viral life cycle and spreading

The complete HBV life cycle includes attachment, entry, decapsulation, transport to the nucleus, covalently closed circular DNA (cccDNA) formation, transcription, translation, coating, assembly, and secretion.\[15\] Direct-acting antiviral drugs have been developed to interfere with HBV replication and spread to achieve a functional cure in CHB patients [Figure 1].

Entry inhibitors

HBV entry into hepatocytes is the first step of HBV infection. Sodium taurocholate cotransporting polypeptide (NTCP), the functional receptor of HBV,\[16\] specifically interacts with the pre-S1 region of the HBV envelope protein to promote efficient HBV entry into hepatocytes.\[17\]
Hepcludex (Bulevirtide, formerly Myrcludex B), the first-in-class entry inhibitor drug for CHB, specifically blocks the binding of HBV to the NTCP receptor. It can also participate in HBV transcription inhibition to prevent the production of new HBV.[18]

In a phase IIb clinical trial, 60 patients with HBV/hepatitis D virus (HDV) co-infection were recruited to assess the safety and efficacy of Hepcludex combined with PEG IFN-α. They were randomized to receive Hepcludex, PEG IFN-α, or a combination of both for 48 weeks, respectively, followed by a 24-week follow-up. At week 48, the normalization of ALT was observed in all Hepcludex treatment arms (21/45). However, only 9 out of 30 patients who received both Hepcludex and PEG IFN-α had a >1 log₁₀ decrease of HBsAg level. No changes in HBsAg were observed in the monotherapy group.[20]

In conclusion, Hepcludex is safe and well-tolerated in CHB patients, it exhibits anti-HBV effects, but its effect on the reduction of HBsAg is limited.[20] At present, Hepcludex is considered an important breakthrough in the treatment of hepatitis D.[21] Although further evidence is required before it can be applied for the treatment of hepatitis B.

**Directly targeting cccDNA**

HBV cccDNA in the nucleus of infected hepatocytes is the template for HBV transcription and subsequent replication, leading to the persistence of HBV infection.[22] Clustered, regularly interspaced, short palindromic repeats (Cas9) — a new gene-editing technique derived from bacteria — can accurately and efficiently target HBV DNA, inhibit viral gene expression, reduce HBV replication, and therefore reduce nuclear cccDNA pools with the potential to completely cure CHB.[23] However, whether gene editing is reversible or persistent, whether it will affect the host genome or mitochondrial DNA are unknown challenges that need to be resolved.

**Targeting viral transcripts**

**Small interfering RNAs (siRNAs)**

The siRNA can be linked with RNA-induced silencing complex (RISC), and after binding with RISC, it can target to cut specific mRNA small fragments of 10 to 11 bases, thereby interrupting the translation process of specific mRNA and silencing the expression of the target gene.[24]

JNJ-3989 (AR0-HBV), composed of two siRNAs, can silence all mRNAs from cccDNA and integrate DNA to reduce all viral products, mostly HBsAg. In a phase II trial, 40 NA-treated or -untreated, HBeAg-positive/negative CHB patients received JNJ-3989 subcutaneous injections (on days 1, 27, and 57) consisting of 100 mg (n = 8), 200 mg (n = 8), 300 mg (n = 16), or 400 mg (n = 8) doses. From day 1, 39 subjects experienced a reduction of HBsAg ≥1.0 log₁₀ IU/mL. Twenty-two patients achieved a continuous decrease of HBsAg during the follow-up and the average HBsAg decrease was 1.74 log₁₀ IU/mL, while HBV RNA, HBeAg, and hepatitis B core-related antigen (HBcrAg) were continuously suppressed in 15, 9, and 10 patients, respectively.[25]

At the EASL 2021 study, it was reported that baseline HBsAg levels and other viral markers, therapeutic status (treated or untreated for NAs), and body mass index did not affect HBsAg reduction. Compared with other markers, the reduction trend of HBsAg was higher than that of any other markers. Furthermore, the decrease in HBeAg, HBcrAg, and HBV RNA was usually associated with a reduction in HBsAg levels.[26]

The APASL 2020 study evaluated JNJ-3989, JNJ-6379, and NAs triglyceride therapy in CHB patients. In the trial, 12 patients received JNJ-3989 (subcutaneous injection of 200 mg on days 1, 29, and 57) and oral JNJ-6379 (250 mg daily) for 12 weeks with continued NAs treatment. On days 85 and 113, HBsAg levels were decreased by 1.4 ± 0.12 log₁₀ IU/mL (12 cases) and 1.9 ± 0.11 log₁₀ IU/mL (7 cases), respectively. The levels of HBV DNA, HBV RNA, HBeAg, and HBcrAg in patients were significantly reduced.[27]

In conclusion, JNJ-3989 is safe and well-tolerated with no serious adverse events observed. CHB patients with JNJ-3989 therapy achieved a strong decline in HBsAg levels and other measurable virological markers. This drug is currently being evaluated in a phase IIb clinical trial.

VIR-2218 (ALN-HBV02), the first siRNA that utilized Enhanced Stabilization Chemistry Plus technology to enhance stability and minimize off-target activity, it targets HBV X gene regions shared by all HBV transcripts, therefore effectively silences all HBV RNA transcripts from cccDNA and integrated DNA.[28] In a phase II trial, 18 HBeAg-negative and 6 HBeAg-positive virally suppressed patients received different doses of VIR-2218 or placebo on the days 1 and 29.[29] At the EASL 2021 study, researchers announced that most trial participants achieved their maximum HBsAg decline at week 16. For HBeAg-negative patients who have received 20, 50, 100, and 200 mg VIR-2218, the maximum mean HBsAg reductions were 1.03, 1.23, 1.50, and 1.65 log₁₀ IU/mL, respectively. For HBeAg-positive participants who have received 50 and 200 mg of VIR-2218, the maximum average reductions in HBsAg were 1.16 and 1.57 log₁₀ IU/mL, respectively. Decreases in quantitative HBeAg and HBcrAg were observed in HBeAg-positive subjects with 200 mg of VIR-2218 therapy. However, after 24 weeks, a certain rebound in average HBsAg levels was observed in all arms of the study.[30]

In another ongoing phase II trial, virally suppressed CHB patients were recruited to assess the antiviral efficacy of VIR-2218 combined with PEG IFN-α-2a treatment. During the 24-week treatment, patients in group 1 received 200 mg VIR-2218 every 4 weeks; patients in group 2 received 200 mg VIR-2218 every 4 weeks with an additional 12-week treatment of 180 mg PEG IFN-α-2a, and other patients in group 3 received VIR-2218 and PEG IFN-α-2a simultaneously. At the EASL 2021 study, the trial report indicated that in group 3, the reduction of HBsAg levels was earlier and greater, and the
average decrease of HBsAg at week 4 was $\geq 1 \log_{10} \text{IU/mL}$.\cite{33}

In conclusion, VIR-2218 is safe and well-tolerated and has direct anti-HBV activity with HBsAg dose-dependently decreased in all patients. However, it remains to be seen whether the VIR-2218 will continue to lower HBsAg levels. This drug is currently being evaluated in a phase II clinical trial.

**Antisense oligonucleotides (ASO)**

ASO are a molecular drug that inhibits gene expression by combining their specific sequence with target gene DNA or mRNA.\cite{32}

IONIS-HBVRx (GSK3228836, ISIS 505358) is an ASO modified by 2'-MOE, it targets all HBV RNAs. In a phase II trial, previously untreated CHB patients with HBV DNA $\geq 2 \times 10^5 \text{IU/mL}$ and HBsAg $> 50 \text{IU/mL}$ were recruited to evaluate the safety and antiviral efficacy of IONIS-HBVRx. On days 1, 4, 8, 11, 15, and 22, IONIS-HBVRx was injected subcutaneously at 150 or 300 mg/dose, and the antiviral effect was evaluated on day 29, following a 6-month TDF treatment. In the 300 mg/time IONIS-HBVRx arm (n = 12), the changes in HBsAg and HBV DNA levels from baseline were $-1.556 \pm 1.379 \log_{10} \text{IU/mL}$. The decrease in HBsAg levels of three patients and the decrease of HBV DNA levels of four patients were both $>3.0 \log_{10} \text{IU/mL}$. Of these, the HBsAg levels of two patients and the HBV DNA level of one patient fell below the lower limit of quantification. However, these three patients with decreased HBsAg levels experienced ALT flares, which were asymptomatic and self-resolved.\cite{33}

In conclusion, IONIS-HBVRx is safe and well-tolerated. After a 4-week treatment, significant inhibition of both HBsAg and HBV DNA was observed in previously untreated CHB patients, indicating that IONIS-HBVRx has significant anti-HBV activity.\cite{33} This drug is currently being evaluated in a phase II clinical trial.

**Capsid assembly inhibitors**

The nucleocapsid composed of core protein is an important structure of HBV. Pre-genomic RNA (pgRNA) and polymerase are first encapsulated before DNA replication can take place. The capsid assembly inhibitors block the nucleocapsid assembly process, thereby affecting viral replication and cccDNA supplementation. Thus, it can be used as a new target of anti-HBV drugs to improve the current clinical resistance of NAs. Capsid assembly inhibitors are divided into two categories, namely, core protein allosteric modulators which lead to misassembled non-capsid core polymers or capsid assembly modulators which form morphologically normal capsids that are devoid of viral nucleic acid.\cite{44}

Morphothiadin (GLS4) is a new hepatitis B drug acting as a core protein variant regulator that interferes with the HBV nucleocapsid assembly. When GLS4 is administered alone, the plasma GLS4 concentration is low because of its short half-life. Combination with ritonavir (RTV) inhibits the activity of liver enzymes and therefore improves GLS4 exposure and its anti-HBV efficacy.\cite{35}

At the AASLD 2019 study, researchers analyzed data from 20 patients in a phase II trial. In this trial, patients received 120 mg GLS4, twice or three times a day for 24 weeks. After a 24-week treatment, the results indicated that in HBV patients, GLS4 exerts multiple antiviral effects and many factors are associated with its antiviral activity, including viral load, baseline ALT, HBeAg-positive, and immune status.\cite{36}

In a phase IIb trial, 250 HBeAg-positive patients were enrolled to assess the safety and efficacy of GLS4/RTV and ETV treatment compared with ETV monotherapy. Patients were divided into two groups: the untreated group (n = 125) and the virally suppressed group (n = 125). In each group, patients were randomized at a ratio of 4:1 and were treated with 120 mg GLS4/100 mg RTV (three times a day) combined with 0.5 mg ETV (daily) or 0.5 mg ETV (daily) monotherapy for 96 weeks (arms A and B in the untreated group, arms C and D in the suppressed group). At the EASL 2020 study, the results of 77 patients with 12-week treatment were reported. In the untreated group, the mean reduction from baseline in HBV DNA, pgRNA, HBsAg, and HBeAg in arm A were 5.02, 2.63, 0.43, and 0.49 log10 IU/mL, respectively; while in arm B, the mean reduction in HBV DNA, pgRNA, HBsAg, and HBeAg were 3.84, 0.27, 0.21, and 0.29 log10 IU/mL, respectively. In the virally suppressed group, the mean reductions from baseline of pgRNA, HBsAg, and HBeAg in arm C were 1.59, 0.11, and 0.17 log10 IU/mL, respectively; while in arm D, mean reductions in pgRNA, HBsAg, and HBeAg were 0.15, 0, and 0.06 log10 IU/mL, respectively. It was obvious that the antiviral efficacy of GLS4/RTV combined with ETV treatment achieved better outcomes than ETV monotherapy.\cite{37}

In conclusion, GLS4/RTV treatment is safe and well-tolerated with good antiviral activity, especially when combined with ETV.\cite{37} This drug is now being evaluated in a phase IIIb clinical trial.

ABI-H0731 (Vebicorvir), a new capsid assembly inhibitor, not only blocks the encapsidation of HBV pgRNA and subsequent DNA replication but also relaxed circular DNA before it is fed into the nucleus to prevent the formation of new cccDNA.\cite{38} In a phase II clinical trial (ABI-H0731-201), 47 HBeAg-positive and 26 HBeAg-negative virally suppressed CHB patients were recruited to receive ABI-H0731 (300 mg, daily) or placebo with NAs for 24 weeks. In another phase II clinical trial (ABI-H0731-202), 25 untreated HBeAg-positive CHB patients were enrolled to receive ABI-H0731 with ETV or ETV monotherapy for 24 weeks.\cite{39}

At the AASLD 2019 study, researchers announced the results of HBeAg-positive CHB patients in the 201 and 202 studies. In the 201 study, in patients receiving ABI-H0731 and ETV treatment, 41% achieved undetectable HBV DNA, HBV RNA $< 35 \text{U/mL}$, and HBeAg $< 1 \text{U/mL}$. In the 202 study, the mean reductions in HBV DNA, HBV RNA,
PEG IFN-α monotherapy for 15 weeks, followed by combined NAs treatment, whereas pgRNA and HBcrAg levels were lower.\[^{[41]}\]

ABI-H0731-211 was an open-label extension study that allowed subjects in the 201 and 202 studies to continue to use ABI-H0731 with NAs treatment for up to 1 year. The subjects included in the study were patients who met the criteria for stopping treatment and have stopped treatment, and their safety and recurrence were evaluated monthly. It was the first study to explore whether ABI-H0731 and NAs treatment in virally suppressed CHB patients could achieve a sustained virological response (SVR) after drug withdrawal.\[^{[42]}\] Unfortunately, this study did not achieve a meaningful SVR rate because 39 of 41 patients have relapsed (defined as quantifiable HBV DNA after stopping treatment).

Recently, a phase II clinical trial was announced in which approximately 60 virally suppressed HBeAg-negative CHB patients would be recruited to assess the safety and antiviral efficacy of ABI-H0731, AB-729 (siRNA), and NAs triple therapy.

In conclusion, ABI-H0731 combined with NAs treatment is safe and presents no severe adverse effects. In CHB patients treated with ABI-H0731 and NAs, the HBV DNA and HBV RNA levels decreased faster and lower, which was similar to pgRNA, HBeAg, and HBcrAg levels.\[^{[40-42]}\] This drug is currently being evaluated in a phase II clinical trial.

HBsAg secretion inhibitors

HBsAg secretion inhibitors inhibit the release of HBsAg from infected hepatocytes, which in turn inhibits the immune response induced by HBsAg and acts as an antiviral agent.\[^{[15]}\]

REP 2139, a nucleic acid polymer, blocks the assembly and secretion of HBV subviral particles to prevent the release of HBsAg. It also clears circulating HBsAg in patients to establish functional control of HBV infection that persists on therapy withdrawal.\[^{[43]}\]

In a phase II clinical trial (REP 301 study), 12 untreated patients with HBV/HDV coinfection received REP 2139-Ca monotherapy for 15 weeks, followed by combined PEG IFN-α-2a therapy for 15 weeks, then followed by PEG IFN-α-2a monotherapy for 33 weeks. REP 301-LTF, a 3.5-year follow-up study, evaluated the long-term stability of the functional control/cure of HBV/HDV coinfection achieved in the REP 301 study.\[^{[43]}\] Eleven patients who completed the REP 301 study were included in the REP 301-LTF study. Of these, 45% of patients had achieved HBsAg levels <0.05 IU/mL and 60% of them maintained the level during the REP 2139-LTF study.\[^{[44]}\]

In another phase II clinical trial (REP 401 study), 40 untreated HBeAg-negative CHB patients were enrolled to assess the efficacy of REP 2139 or REP 2165 combined with TDF and PEG IFN-α-2a therapy. After a 24-week TDF therapy, these patients were randomly divided into an experimental group that a 48-week treatment of REP 2139/REP 2165 combined with TDF and PEG IFN-α-2a was given or a control group that a 24-week control therapy (TDF + PEG IFN-α-2a) followed by a 48-week experimental therapy was given. All patients were followed up for another 48 weeks after treatment. At the end of treatment, 70.6% of patients achieved >1 log\(_{10}\) IU/mL reduction in HBsAg, 67.5% of patients achieved HBsAg levels <1 IU/mL, and 60% of patients achieved HBsAg seroconversion. A total of 34 patients completed the 48-week follow-up, and 17 patients of them achieved HBsAg levels <1 IU/mL, 14 patients achieved HBsAg clearance, and 17 patients achieved HBsAg seroconversion. Overall, 27 patients maintained HBV DNA levels ≤2000 IU/mL, and 20 patients achieved undetectable HBV DNA.\[^{[45]}\]

In conclusion, REP 2139 is safe and well-tolerated. REP 2139-based treatment can achieve high HBsAg clearance and seroconversion rates, high virological control, and functional cure of HBV infection, as well as continuous HBsAg seroconversion during the treatment, and maintain normal liver function during long-term follow-up.\[^{[43]}\] This drug is currently being evaluated in a phase II clinical trial.

**Modulating the host immune response**

During HBV natural infections, due to the inherent ability of the virus to evade recognition, the innate immune system is poorly activated.\[^{[46,47]}\] Moreover, chronic HBV infection leads to T-cell dysfunction (exhaustion),\[^{[48]}\] manifested by poor cytotoxic activity, impaired cytokine production, and expression of inhibitory receptors.\[^{[49,50]}\] Therefore, it is reasonable that after treatment withdrawal, SVR depends not only on sustained viral suppression but also on the induction of an effective antiviral immune response.\[^{[51,52]}\] Targeted immunotherapy strategies, by properly orchestrating the activation of antiviral immunity, may help to achieve long-lasting control, even a functional cure for CHB.\[^{[53]}\] Currently, multiple immunotherapeutic approaches are being developed to achieve the goal of functional cure by restoring immune competence against HBV and HBV-infected hepatocytes\[^{[50,53]}\] [Figure 2].

**Toll-like receptor (TLR) agonists**

TLRs, the first line of defense against invading pathogens, are functionally involved in the recognition of self and non-self-antigens, the maturation of dendritic cells, and the initiation of antigen-specific adaptive immune responses.\[^{[54,55]}\] After binding to TLRs, a cascade of production of IFNs and other cytokines/chemokines is induced, natural killer (NK) cells and cytotoxic T lymphocytes will be activated, thereby innate and adaptive immunity simultaneously activated.\[^{[53]}\] TLR agonists (TLR-7 and TLR-8) are involved in the production of endogenous IFNs, the induction of IFN-stimulated genes, and the activation of other signaling cascades such
as the Janus kinase/signal transducer and activator of transcription signaling pathway, leading to the inhibition of HBV replication.\[56\]

GS-9620, a TLR-7 agonist, increases T-cell and NK-cell responses and reduces the ability of NK to suppress T cells.\[57\] In preclinical studies, GS-9620 achieved a durable suppression of HBV replication through induction of type I IFN.\[58\] In a phase II trial, it was reported that in CHB patients, GS-9620 was safe and well-tolerated while no significant decline in HBsAg was observed.\[59\] The antiviral efficacy of GS-9620 combined with other antiviral drugs has yet to be verified. Other TLR7 agonists which are in clinical trials, such as RO7020531 and TQ-A3334 (INJ-4964), have shown the possibility that might suppress HBV-specific immunity.\[60,61\]

GS-9688 (Selgantolimod), a TLR8 agonist, has the potential to boost responses contributing to viral control and modulation of regulatory mediators.\[62\] In a randomized, blind, placebo-controlled phase I study, GS-9688 was found to be safe and well-tolerated in CHB patients and elicited cytokine responses consistent with target engagement.\[63\] In a phase II clinical study, 24 HBeAg-positive and 24 HBeAg-negative CHB patients were recruited to receive 24 weeks of GS-9688 with NAs therapy, following a 24-week NAs monotherapy. At the AASLD 2020 study, it was reported that HBsAg loss was...
observed in two patients and HBeAg loss in three patients at week 48.\(^\text{[64]}\) In another phase II clinical study, 67 HBeAg-positive/negative CHB patients were randomized (2:2:1) to receive GS-9688 3 mg, 1.5 mg, and placebo once a week with TAF for 24 weeks. At the EASL 2021 study, it was reported that three patients at week 24 and four patients at week 48 achieved an HBeAg decline ≥0.5 log\(_{10}\) IU/mL under the therapy of GS-9688 in the study.\(^\text{[65]}\) Nonetheless, whether the immune system activation induces subsequent autoimmune or hepatitis outbreaks is a concern. This drug is currently being evaluated in a phase II clinical study.

**Therapeutic vaccines**

Therapeutic vaccines, which are aimed to activate the patient’s immune system to combat infectious pathogens, hold promise as an immunotherapeutic approach.\(^\text{[66]}\) Many therapeutic vaccines have been developed,\(^\text{[67]}\) but most have failed to restore HBV-specific immunity in patients.\(^\text{[68]}\) The unfavorable results from the therapeutic vaccines are most likely due to the preferential induction of antibodies, rather than induction cytotoxic T-cell responses.\(^\text{[66]}\) Currently, therapeutic vaccines have been combined with current antiviral drugs in most clinical trials.

GS-4774 is a recombinant, heat-killed, *Saccharomyces cerevisiae* yeast-based vaccine, expressing HBV-specific antigens including HBsAg, hepatitis B core antigen (HBcAg), and hepatitis B virus X protein. In a phase II study, GS-4774 in combination with TDF was evaluated for its ability to boost the anti-HBV immune response. The researchers found that GS-4774 could increase the production of IFN-γ, tumor necrosis factor (TNF), interleukin 2 by CD8\(^+\) T cells exposed to antigenic peptides. Although GS-4774 did not reduce levels of HBsAg in patients, its strong immune-stimulatory effect on CD8\(^+\) T cells might be used in combination with other antiviral agents to boost the antiviral immune response.\(^\text{[69]}\)

HeberNasvac (ABX-203) is a vaccine containing both HBsAg and HBcAg formulated to be administered intranasally. The results from a phase III clinical trial of HeberNasvac were disappointing, no significant superiority in reduction of HBV DNA level and normalization of liver function was observed.\(^\text{[70]}\) Another phase I clinical trial has been conducted in Cuba including six CHB patients who were refractory or incomplete responders to IFN-α. After a 5-year follow-up, HBeAg loss was demonstrated in three HBeAg (+) patients. Five patients had a decline to undetectable viral load, and all had liver stiffness measurements <7.8 kPa.\(^\text{[71]}\) This vaccine is currently being modified and further studies on the efficacy and safety are needed.\(^\text{[72]}\)

In preclinical studies and early clinical studies, BRII-179 was characterized by the induction of a Th1 type immune response.\(^\text{[73]}\) At the EASL 2021 study, it was reported that the therapeutic candidate BRII-179 alone or in combination with IFN-α in a phase Ia2a clinical trial for the treatment of CHB patients could simultaneously induce B and T cell immune responses and was well-tolerated.\(^\text{[74]}\) A clinical phase II study of BRII-179 in combination with the siRNA drug BRII-835 (VIR-2218) is currently underway.

Other vaccines are currently under development, such as TG-1050 [Table 2].\(^\text{[75]}\) Further clinical evaluation of them in combination with other anti-HBV agents is needed. Preliminary data imply that therapeutic vaccines will be effective when administered in a combination approach.

**Inhibitor of apoptosis proteins (IAPs) antagonists**

Members of the IAPs family were first thought to be functionally restricted to inhibition of apoptosis.\(^\text{[76]}\) With further research, it emerged that IAPs are not only gatekeepers of cell death, but may also be involved in the regulation of inflammation, innate, and acquired immunity.\(^\text{[77]}\) Results from animal studies revealed that IAPs play an important role in T cell proliferation and survival in the inflammatory environment of viral infection, suggesting that IAP antagonists may interfere with immune responses.\(^\text{[78]}\)

APG-1387 is the first IAP antagonist-based new drug for hepatitis B treatment in China, and its mechanism of action involves mimicking endogenous second mitochondria-derived activator of caspase molecules to degrade IAPs, thereby inducing and accelerating the process of apoptosis.\(^\text{[79]}\) Preclinical findings found that APG-1387 was able to clear chronic HBV infection in various mouse models with a unique induction of apoptosis and immunoregulation mechanism. Further investigation revealed that this clearance mechanism may be related to the upregulation of the number and function of intrahepatic virus-specific CD8\(^+\) T cells, with a knockout of TNFαs and depletion of either CD4\(^+\) or CD8\(^+\) T cells, the HBV clearance effect of APG-1387 can be completely blocked. To explore the tolerability, safety, and pharmacokinetics/pharmacodynamics of APG-1387 in CHB patients, a phase I study is currently underway which will be completed in October 2021. Another phase II clinical study, evaluating the efficacy in APG-1387 combined with ETV has been initiated, with the first dose completed in June 2020. We hope to see encouraging results.

**Immune checkpoint inhibitors**

The expression of targeting immune checkpoint inhibitor molecules, such as programmed cell death-1 (PD-1) and PD-ligand 1 (PD-L1), is significantly increased in lymphocytes infiltrating the portal area in CHB patients, increases paralleled with the degree of inflammation.\(^\text{[80]}\) Conversely, intrahepatic HBV-specific CD8\(^+\) cells express higher levels of PD-1, and blockade of the PD-1/PD-L1 interaction increased CD8\(^+\) cell proliferation.\(^\text{[81,82]}\) By restoring antiviral T-cell functions, not only in the periphery but also in intrahepatic lymphocytes, anti-PD-1/PD-L1 might be a promising therapeutic candidate for chronic HBV infection.\(^\text{[72]}\)

Among 51 HBV-related HCC patients treated with nivolumab in a phase I trial, all of whom were receiving NAs with HBV DNA level <100 IU/mL, none experienced reactivation of HBV and none experienced anti-HBs
seroconversion. Recently, a phase I pilot study evaluated the safety and immunologic mechanisms of nivolumab treatment with or without GS-4774 in HBeAg-negative CHB patients. At week 24, 14% (3/22) of the patients have obtained a >0.5 log_{10} reduction in HBsAg levels, while one patient receiving the combination achieved HBsAg loss. Thus, these pilot studies support the inclusion of PD-1/PD-L1 blockade in future combination strategies toward a functional cure of chronic HBV infection.

In addition, several immune checkpoint inhibitors (such as ASC22, etc) are currently under evaluation in preclinical or clinical studies. The safety of immune checkpoint inhibitors with prolonged use is an important issue that needs to be addressed. Further testing of these compounds in the clinical setting would indicate whether these may be applied to CHB patients.

Monoclonal antibodies

In recent years, several monoclonal antibodies targeting HBV have been developed and have demonstrated high affinity, specificity, and neutralizing potency. Further studies confirming immunotherapy for HBV infection may require supplementation with broadly neutralizing antibodies. Activated B cells further limit the spread of HBV infection by producing neutralizing antibodies, preventing viral spread, and clearing circulating viruses. Consequently, monoclonal antibodies could be a promising alternative strategy in the prevention and treatment of HBV infection.

GC1102 and VIR-3434 are monoclonal antibodies. The findings indicated that the combination of GC1102 with antiviral drugs could reduce HBsAg titers and increase the chance of functional cure in patients with CHB. GC1102 is undergoing a phase II trial, which will end in November 2021.

At the EASL 2021 study, it was reported that single-dose treatment of VIR-3434 up to 3000 mg was well-tolerated in healthy participants. In addition, preliminary data of the phase Ib trial evaluated the safety, and HBsAg reduction in involved CHB patients were reported. The results showed that a single low dose of 6 mg VIR-3434 produced rapid HBsAg reduction and was maintained for 2 weeks after administration. No safety issues were observed up to the 3000 mg dose in healthy volunteers, and this significant reduction in HBsAg in patients suggested that VIR-3434 has the potential to play an important role in the functional cure of chronic HBV infection. A phase II study of VIR-2218 combined with VIR-3434 in patients with HBV infection was initiated in June 2021.

Other immune approaches

To date, immunotherapeutic approaches for CHB patients have limited efficacy, probably because HBV-specific T cells are rare and have an exhausted phenotype with altered metabolism. Additional cytokines may have some effect and immune restoration by the adoptive transfer of engineered HBV-specific T cells has been proposed. The latest research showed that immune mobilizing monoclonal T cell receptors against virus platform may eliminate infected cells by redirecting endogenous non-HBV-specific T cells or bypassing depleted HBV-specific T cells, which is becoming a promising therapeutic choice for the treatment of CHB patients. In May 2021, Immunocore announced the first CHB patient with IMC-I109V treatment was initiated. Furthermore, a stimulator of IFN genes agonist may also provide additional avenues for immunotherapy.

Conclusions and future perspectives

The limitations of available treatment options for CHB, coupled with the unique virological and immunological mechanisms involved, make the complete cure of HBV infection an unachievable treatment goal. The results of clinical trials show that combined antiviral and immunomodulatory therapy will be an important strategy to improve the effectiveness and the possibility of a functional cure for CHB patients. The key question is whether the combination of antiviral drugs exerts additive or synergistic effects on HBV replication. For example, direct-acting antivirals could be used to decrease viral replication and improve innate immune function. Subsequently, direct-acting antivirals could be used to reduce antigen load, which may contribute to correct immune exhaustion. In addition, immunostimulatory agents could also be added to enhance T-cell-mediated clearance of infected hepatocytes. Finally, combination therapy may allow for the correction of immune impairments while minimizing side effects due to an overwhelming immune response. The safety and antiviral activity of combination therapy are currently being tested in clinical trials, but complete robust data are not available, which are highly anticipated.

In this review, we summarized the new therapeutic strategies for CHB, including direct-acting antiviral drugs that interfere with the viral life cycle and spreading, and immunotherapeutic approaches that modulate the host immune response. The different approaches currently under investigation have varying degrees of therapeutic efficacy. Some drugs achieve superior performance, such as direct-acting antiviral approaches with novel mechanisms of action and may have the ability to reduce cccDNA and HBsAg, leading to the potential for immune recovery. More data from clinical trials are needed for further validation.

In conclusion, we believe that as novel antiviral and immunomodulatory therapies continue to emerge and improve, compared with existing treatments, a functional cure for CHB will be an attainable goal, and dawn is on the horizon.

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Conflicts of interest
None.

References
1. Cornberg M, Lok AS, Terrault NA, Zoulim F, Facalcy F-AHTEC. Guidance for design and endpoints of clinical trials in chronic hepatitis B—report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference (double dagger). J Hepatol 2020;72:539–537. doi:10.1016/j.jhep.2019.11.003.
2. Association CsoDCM, Association CsoHCM. Guidelines for the prevention and treatment of chronic hepatitis B (version 2019) (in Chinese). J Clin Hepatol 2019;35:2648–2669. doi: 10.3969/j.issn.1001-5256.12019.05.027.
3. Asselah T, Louereiro D, Boyer N, Mansouri A. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. Lancet Gastroenterol Hepatology 2019;4:883–892. doi:10.1016/S2513-5317(18)30308-X.
4. Zhou K, Contag C, Whittaker E, Terrault N. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. Lancet Gastroenterol Hepatol 2019;4:227–238. doi:10.1016/S2513-5317(18)30143-0.
5. 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. doi:10.1056/NEJMoa051285.
6. Lai CL, Shouval D, Lok AS, Chang TT, Chienher H, Goodman Z, et al. Entecavir versus lamivudine for HBV-infected chronic hepatitis B patients. N Engl J Med 2006;354:1011–1020. doi:10.1056/NEJMoa051286.
7. Lai CL, Shouval D, Lok AS, Chang TT, Chienher H, Goodman Z, et al. Entecavir versus lamivudine for HBV-infected chronic hepatitis B patients. N Engl J Med 2006;354:1011–1020. doi:10.1056/NEJMoa051288.
8. Marcellin P, Gane EJ, Flisiak R, Trinh HN, Petersen J, Gurul S, et al. Long-term treatment with tenofovir disoproxil fumarate for chronic hepatitis B infection is safe and well tolerated and associated with durable virologic response with no detectable resistance: 8 year results from two phase 3 trials. Hepatology 2014;60 (Suppl 1):313A–314A. doi:10.1002/hep.27486.
9. Marcellin MA, Lok AS. Interferon treatment for hepatitis B. Clin Liver Dis 2016;20:645–665. doi:10.1016/j.cld.2016.06.002.
10. Marcellin P, Bonino F, Lau GK, Farci P, Yurdaiudin C, Piravitvuthi T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology 2009;136:2169–2179 e4. doi:10.1053/j.gastro.2009.03.006.
11. Bister EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, et al. Sustained HBVAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459–467. doi:10.1053/j.gastro.2008.05.031.
12. Zhang XD, Zhao LN. Perspectives in the development of new drugs against hepatitis B virus (in Chinese). J Clin Hepatol 2021;37:993–998. doi:10.1016/j.jclh.2021.04.015.
13. Yan H, Zhong GC, Xu GW, He WH, Jing ZY, Gao ZC, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife 2012;1:e00049. doi:10.7554/ eLife.00049.
14. Yan H, Liu Y, Su J, Li W. NTCP opens the door for hepatitis B virus infection. Antiviral Res 2015;121:24–30. doi:10.1016/antivi- ral.2015.06.002.
15. Schiek A, Muller T, Schulze A, Haberkorn U, Urban S, Mier W. Solid-phase synthesis of the lipopeptide Myr-HBVpreS2-78, a hepatitis B virus entry inhibitor. Molecules 2010;15:4773–4783. doi:10.3390/molecules15074773.
16. Zhao K, Liu S, Chen Y, Yao Y, Zhou M, Yuan Y, et al. Upregulation of HBV transcription by sodium taurocholate cotransporting polypeptide at the postentry step is inhibited by the entry inhibitor Myrcludex B. Emerg Microbes Infect 2018;7:186. doi:10.1038/ s41426-018-0189-8.
17. Wedemeyer H, Schöneweiss K, Bogomolov PO, Voronkova N, Chulanov V, Stepanova T, et al. GS-3–final results of a multicenter, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of myrcludex B in patients with chronic HBV/HDV coinfection. J Hepatol 2019;70:e261. doi:10.1016/j.jhep.2019.05.008.
18. Kang C, Syed Y. Bulevirtide: first approval. Drugs 2020;80:1601–1605. doi:10.1007/100426-020-01400-1.
19. Guo JT, Guo H. Metabolism and function of hepatitis B virus cDNA: implications for antiviral therapy. Antiviral Therapeutical. Antiviral Res 2015;122:91–100. doi:10.1016/ j.antiviral.2015.08.005.
20. Martinez MG, Inchause A, Delberghe E, Chapus F, Zoulmi FJH. Targeting hepatitis B virus with CRISPR: fundamental approaches and clinical applications. Lancet Gastroenterol Hepatol 2019;4:883–892. doi:10.1016/s2468-1253(18)30397-3.
36. Zhang H, Niu JQ, Ding HY, Zhang YJ, Luo L, Li CY, et al. Analysis of factors influencing the efficacy and safety of GSK4 treatment in patients with chronic hepatitis B. Hepatology 2019;70:429A–429A. doi: 10.1002/hep.30941.

37. Zhang M, Zhang J, Tan Y, Xin Y, Gao H, Zheng S, et al. Efficacy and safety of GS4 x/ratonvir combined with entecavir in HBV patients with positive HBV DNA: a randomised, open-label, phase 2b, multicenter study. J Hepatol 2020;73:S878–S880. doi: 10.1016/j.jhep.2018-8278(20)32197-8.

38. Huang Q, Cai D, Yan R, Li L, Zong Y, Guo L, et al. Preclinical profile and characterization of the hepatitisc B virus core protein inhibitor ABH3071. Antimicrob Agents Chemother 2020;64:e01463-20. doi: 10.1128/AAC.01463-20.

39. Ma X, Lalezari J, Nguyen T, Bae H, Schiff ER, Fung S, et al. Interim safety and viremia ce-reduction of the ABH3071 phase 2a program exploring the combination of ABH3071 with Nuc therapy in treatment-naive and treatment-suppressed chronic hepatitis B patients. J Hepatol 2019;70:e130. doi: 10.1002/hep.31033.

40. Yuen MF, Agarwal K, Ma XL, Nguyen T, Schiff ER, Han FH, et al. Antiviral activity and safety of the hepatitis B core inhibitor ABH3071 administered with a nucleos(t)ide reverse transcriptase inhibitor in patients with HBV/HCV coinfection. J Hepatol 2020;73:S51–S52. doi: 10.1016/j.jhep.2018-8278(20)30288-1.

41. Bazinet M, Pantea V, Cebotarescu V, Coughari L, Jimbei P, Albrecht J, et al. Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis delta virus infection in a long-term extension study. J Hepatol 2020;73:S1140. doi: 10.1016/j.jhep.2018-8278(20)30790-X.

42. Fung S, Sulkowski M, Lalezari J, Schiff ER, Dieterich D, Hassanein T, et al. Antiviral activity and safety of the hepatitis B core inhibitor ABH3071 administered with a nucleos(t)ide reverse transcriptase inhibitor in patients with HBeAg-negative chronic hepatitis B infection. J Hepatol 2020;73:S51–S52. doi: 10.1016/j.jhep.2018-8278(20)30288-1.

43. Sulkowski MS, Agarwal K, Fung SK, Yuen RMF, Ma XL, Lalezari JP, et al. Continued Therapy with ABH3071 + NRTI results in sequential reduction/loss of DNA HBV, HBV DNA, HBeAg, HBeAg and HBcAg in HBV positive patients. Hepatology 2019;70:1486A–1487A. doi: 10.1002/hep.31033.

44. Bazinet M, Pantea V, Cebotarescu V, Coughari L, Jimbei P, Anderson M, et al. Persistent control of hepatitis B virus and hepatitis delta virus infection following REP2139-Ca and pegylated interferon therapy in chronic hepatitis B/virus delta virus infection. Hepatol Commun 2021;5:183–202. doi: 10.1002/hep4.1633.

45. Bazinet M, Pantea V, Cebotarescu V, Coughari L, Jimbei P, Anderson M, et al. Persistent control of hepatitis B virus and hepatitis delta virus infection after REP2139-Ca and pegylated interferon therapy in chronic hepatitis B virus/hepatitis delta virus coinfection. Hepatol Commun 2021;5:S189–202. doi: 10.1002/hep4.1633.

46. Bazinet M, Pantea V, Placinta C, Mosca I, Cebotarescu V, Coughari 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 hepatitis B infection naïve to nucleos(t)ide therapy. Gastroenterolo gy 2020;170:S20–2194. doi: 10.1053/j.gastro.2020.02.058.

47. Zoulifi F, Lebosse F, Leverro M. Current treatments for chronic hepatitis B virus infections. Curr Opin Virol 2016;18:109–116. doi: 10.1016/j.coviro.2016.06.004.

48. Maupin MK, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. J Hepatol 2016;64:560–570. doi: 10.1016/j.jhep.2016.01.028.

49. 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. doi: 10.1038/cddis.2015.42.

50. Bertolotti A, Ferrari C. Adaptive immunity in HBV infection. J Hepatol 2016;64:S71–S83. doi: 10.1016/j.jhep.2016.04.015.

51. Mouzannar K, Zoulim F, Bertoletti A. Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat Rev Drug Discov 2019;18:827–844. doi: 10.1038/s41573-019-0037-0.

52. Yang H, Kao JH. Viral hepatitis. HBV cure—can we pin our hopes on immunotherapy? Nat Rev Gastroenterol Hepatol 2015;12:139–151. doi: 10.1038/3158.

53. Yu XQ, Zhang XX. Research progress in host immune response during hepatitis B virus infection. Chin Med J 2020;133:2746–2748. doi: 10.1097/CM9.0000000000001096.
83. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis B virus HBsAg with distinct epitopes suppresses escape mutations. Cell Host Microbe 2020;28:333–349.e6. doi: 10.1016/j.chom.2020.05.010.

84. Hong B, Wen Y, Ying T. Recent progress on neutralizing antibodies against hepatitis B virus and its implications. Infect Disord Drug Targets 2019;19:213–223. doi: 10.2174/187152618666180602123400.

85. Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis B virus HBsAg with distinct epitopes suppresses escape mutations. Cell Host Microbe 2020;28:333–349.e6. doi: 10.1016/j.chom.2020.05.010.

86. Loomba R, Liang TJ. Hepatitis B reactivation associated with immune suppressive and biological modifier therapies: current concepts, management strategies, and future directions. Gastroenterology 2017;152:1297–1309. doi: 10.1016/j.gastro.2017.02.009.

87. Looomba R, Liang TJ. Hepatitis B reactivation associated with immune suppressive and biological modifier therapies: current concepts, management strategies, and future directions. Gastroenterology 2017;152:1297–1309. doi: 10.1016/j.gastro.2017.02.009.

88. Hong B, Wen Y, Ying T. Recent progress on neutralizing antibodies against hepatitis B virus and its implications. Infect Disord Drug Targets 2019;19:213–223. doi: 10.2174/187152618666180602123400.

89. Lee HW, Park JY, Hong T, Park MS, Ahn SH. A prospective, open-label, dose-escalation, single-center, phase 1 study for GC1102, a recombinant human immunoglobulin for chronic hepatitis B patients. Hepatology 2018;68 (Suppl 1):268A–269A. doi: 10.1002/hep.30257.

90. Gupta SV, Arizpe A, Fanget MC, Dholakiya S, Shen L, Pang P, et al. Preliminary pharmacokinetics and safety in healthy volunteers of VIR-3434, a monoclonal antibody for the treatment of chronic hepatitis B infection. J Hepatol 2021;75 (Suppl 2):S733. doi: 10.1016/S0140-6736(21)01843-2.

91. Agarwal K, Yuen MF, Wedemeyer H, Cloutier D, Shen L, Arizpe A, et al. A phase 1 study evaluating the neutralizing, vaccinal monoclonal antibody VIR-3434 in participants with chronic hepatitis B virus infection. J Hepatol 2021;75 (Suppl 2):S288. doi: 10.1016/S0140-6736(21)01842-0.

92. Schurich A, Pallett LJ, Jajbhay D, Wijngaarden J, Otano I, Gill US, et al. Distinct metabolic requirements of exhausted and functional virus-specific CD8 T cells in the same host. Cell Rep 2016;16:1243–1252. doi: 10.1016/j.celrep.2016.06.078.

93. Fiscarco P, Barili V, Montanini B, Acerbi G, Ferracin M, Guerrieri F, et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B patients. Nat Med 2017;23:327–336. doi: 10.1038/nm.4275.

94. Koh S, Kh J, Tham CYL, Yang N, Cecchello E, Chia A, et al. Nonlytic lymphocytes engineered to express virus-specific T-cell receptors limit HBV infection by activating APOBEC3. Gastroenterology 2018;155:180–193.e6. doi: 10.1016/j.gastro.2018.03.027.

95. Fergusson JR, Wallace Z, Connolly MM, Woon AP, Suckling RJ, Hine DW, et al. Immune-mobilizing monoclonal T cell receptors mediate specific and rapid elimination of hepatitis B-infected cells. Hepatology 2020;72:1328–1340. doi: 10.1002/hep.31503.

96. Hu J, Tang L, Cheng J, Zhou T, Li Y, Chang J, et al. Hepatitis B virus nucleocapsid uncoating: biological consequences and regulation by cellular nucleases. Emerg Microbes Infect 2021;10:832–864. doi: 10.1002/emm.21751.2021,1919034.

97. Ning Q, Wu D, Wang GQ, Ren H, Gao ZL, Hu P, et al. Roadmap to functional cure of chronic hepatitis B: an expert consensus. J Viral Hepat 2019;26:1146–1155. doi: 10.1111/jvh.13126.

98. Martinez MG, Villener F, Testoni B, Zoulam F. Can we cure hepatitis B virus with novel direct-acting antivirals? Liver Int 2020;40 Suppl 1:61–69. doi: 10.1111/liv.14364.