Chapter 6
Present and Future Therapies for Chronic Hepatitis B

Yachao Tao, Dongbo Wu, Lingyun Zhou, Enqiang Chen, Changhai Liu, Xiaoqiong Tang, Wei Jiang, Ning Han, Hong Li, and Hong Tang

Abstract Chronic hepatitis B (CHB) remains the leading cause of liver-related morbidity and mortality across the world. If left untreated, approximately one-third of these patients will progress to severe end-stage liver diseases including liver failure, cirrhosis, and hepatocellular carcinoma (HCC). High level of serum HBV DNA is strongly associated with the development of liver failure, cirrhosis, and HCC. Therefore, antiviral therapy is crucial for the clinical management of CHB. Current antiviral drugs including nucleoside/nucleotide analogues (NAs) and interferon-α (IFN-α) can suppress HBV replication and reduce the progression of liver disease, thus improving the long-term outcomes of CHB patients. This chapter will discuss the standard and optimization antiviral therapies in treatment-naïve and treatment-experienced patients, as well as in the special populations. The up-to-date advances in the development of new anti-HBV agents will be also discussed. With the combination of the current antiviral drugs and the newly developed antiviral agents targeting the different steps of the viral life cycle or the newly developed agents modulating the host immune responses, the ultimate eradication of HBV will be achieved in the future.

1 Introduction

Chronic HBV infection remains the leading cause of liver-related morbidity and mortality across the world. CHB patients are at the risk of developing cirrhosis and HCC. The 5-year cumulative incidence of cirrhosis in untreated CHB patients is 8–20%, the 5-year cumulative risk of hepatic decompensation in cirrhotic patients is approximately 20%, and the annual rate of cirrhosis progressing to HCC is 2–5%

Yachao Tao and Dongbo Wu contributed equally to this chapter

Y. Tao · D. Wu · L. Zhou · E. Chen · C. Liu · X. Tang · W. Jiang
N. Han · H. Li (✉) · H. Tang (✉)
Center of Infectious Diseases, Division of Infectious Diseases, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan, China

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[1, 2]. High serum HBV DNA level is associated with the increased risk of cirrhosis and HCC [3, 4]. Cirrhosis patients with low, but detectable, viral load are still at risk of HCC [5]. Thus, antiviral treatment with sustained suppression of HBV DNA is important to relieve underlying liver injury and prevent its progression toward cirrhosis and HCC.

2 Current Antiviral Therapy

2.1 Goals of Antiviral Treatment

The main goals of antiviral therapy are to improve the survival and the quality of life for CHB patients [6, 7]. Through sufficient antiviral treatment, sustained suppression of HBV replication can be achieved, which is associated with ALT normalization and liver histology improvement, thus leading to the reversal of fibrosis or even cirrhosis, and the decrease of hepatic decompensation and HCC [8, 9].

The ultimate goal of antiviral therapy is to cure HBV infection with the elimination of all forms of potentially replicating HBV, which is hardly achievable with current antiviral therapy due to the persistence of cccDNA (the HBV transcriptional template) in the hepatocyte nucleus [2]. However, a "functional cure" defined as the HBsAg loss or seroconversion and sustained HBV DNA suppression [10] is a realistic goal and can be achieved in a proportion of eligible CHB patients with optimization of antiviral strategies such as the different combinations of NAs and Peg-IFN.

2.2 Indications for Antiviral Treatment

The current indications to begin antiviral treatment are generally based on the serum HBV DNA, ALT levels, and the severity of liver disease (assessed by liver biopsy and/or noninvasive tests) (Table 1) [1]. Indications for antiviral treatment should also consider the patients' age, family history of cirrhosis or HCC, and concomitant diseases.

2.2.1 Antiviral Treatment Indications for Non-cirrhotic Patients

Current professional society guidelines recommend the initiation of antiviral treatment in non-cirrhotic CHB patients who are in the immune-active phase, which is defined by an elevation of ALT $\geq$ 2 ULN (upper limits of normal) or significant liver histological disease plus HBV DNA $\geq$ 20,000 IU/mL if HBeAg positive or HBV DNA levels $\geq$ 2000 IU/mL if HBeAg negative [1, 2, 11, 12] (Table 1). The 2018 AASLD guidelines recommended utilizing the ALT levels of 35 U/L for males and

Table 1 Indications for antiviral treatment in CHB patients among different guidelines

	HBeAg positive	HBeAg negative	Compensated cirrhosis	Decompensated cirrhosis
EASL 2017	HBV DNA >2000 IU/mL and ALT>ULN	HBV DNA >2000 IU/mL and ALT>ULN	Any detectable HBV DNA level	Any detectable HBV DNA level
	At least moderate liver necroinflammation or fibrosis	At least moderate liver necroinflammation or fibrosis	Regardless of ALT levels	Regardless of ALT levels
AASLD 2018	HBV DNA >20 000 IU/mL and ALT>2 × ULN	HBV DNA >2000 IU/mL and ALT>2 × ULN	Any detectable HBV DNA level Regardless of ALT levels	HBsAg-positive Regardless of HBV DNA level, HBeAg status, or ALT levels
	HBV DNA >20 000 IU/mL, ULN <ALT≤ 2 × ULN and significant histological disease by liver biopsy or noninvasive testing	HBV DNA >2000 IU/mL, ULN <ALT≤ 2 × ULN and significant histological disease by liver biopsy or noninvasive testing		
	Immune-active CHB with HBV DNA below thresholds, ALT≤2 × ULN and older age (>40 years) or family history of HCC or presence of extrahepatic manifestation	Immune-active CHB with HBV DNA below thresholds, ALT≤2 × ULN and older age (>40 years) or family history of HCC or presence of extrahepatic manifestation		
APASL 2015	HBV DNA>20 000 IU/mL and ALT>2 × ULN	HBV DNA>2000 IU/mL and ALT>2 × ULN	HBV DNA> 2000 IU/mL, regardless of ALT levels or detectable HBV DNA if elevated ALT levels	Any detectable HBV DNA level, regardless of ALT levels
	HBV DNA>20 000 IU/mL, ALT 1–2 × ULN, biopsy if noninvasive tests suggest evidence of significant fibrosis, ALT is persistently elevated, age>35 years or family h/o HCC or cirrhosis. Treat if moderate to severe inflammation or significant fibrosis	HBV DNA>20 000 IU/mL, elevated ALT≤ 2 × ULN, consider biopsy if age > 35 years or family history of HCC or cirrhosis. Treat of moderate to severe inflammation or significant fibrosis		
	HBV DNA< 20 000 IU/mL, consider biopsy if age > 35 years or family history of HCC or cirrhosis. Treat if moderate to severe inflammation or significant fibrosis	HBV DNA< 2000 IU/mL, consider biopsy if age > 35 years or family history of HCC or cirrhosis. Treat if moderate to severe inflammation or significant fibrosis		

(continued)

Table 1 (continued)

	HBeAg positive	HBeAg negative	Compensated cirrhosis	Decompensated cirrhosis
China 2015	HBV DNA>20 000 IU/mL and ALT>2 ×ULN	HBV DNA>2000 IU/mL and ALT>2 ×ULN	Any detectable HBV DNA, regardless of ALT and HBeAg status	Any detectable HBV DNA, regardless of ALT and HBeAg status
	HBV DNA>20 000 IU/mL and ULN <ALT≤ 2×ULN. Consider biopsy and noninvasive fibrosis tests; treat if significant inflammation or fibrosis exists	HBV DNA>2000 IU/mL and ULN <ALT≤ 2×ULN. Consider biopsy and noninvasive fibrosis tests; treat if significant inflammation or fibrosis exists		
	HBV DNA>20 000 IU/mL and normal ALT levels. Consider biopsy and noninvasive fibrosis tests if age > 30 years and family history of HCC or cirrhosis. Treat if moderate to significant inflammation or fibrosis exists	HBV DNA>2000 IU/mL and normal ALT levels. Consider biopsy and noninvasive fibrosis tests if age > 30 years and family history of HCC or cirrhosis. Treat if significant inflammation or fibrosis exists		

25 U/L for females as ULN rather than local laboratory ULN to guide the initiation of antiviral treatment [11]. It needs to be noted that CHB is a dynamic disease and individuals with CHB can transition through different phases with variable levels of HBV DNA, ALT, and HBV antigens, and thus a single ALT, HBV DNA level, and HBV antigens are insufficient to assign phase of infection and/or need for treatment. Serial testing of ALT, HBV DNA, and HBV antigen are required to guide the treatment decisions [11].

For patients unfulfilling the above treatment indications, especially in patients >30 years or with family history of cirrhosis or HCC, liver biopsy or noninvasive test (such as elastography) is recommended to assess the grade of hepatic inflammation or the evidence of fibrosis, thereby helping to determine whether it is necessary to start antiviral treatment. For instance, for patients with HBV DNA >2000 IU/mL and at least moderate fibrosis (assessed by liver biopsy or elastography), antiviral treatment may be initiated even if ALT levels are mildly elevated (ULN <ALT $\leq$ 2 ×ULN) or normal [13]. In addition, antiviral treatment is recommended for CHB patients with extrahepatic manifestations, such as dermatomyositis [14] and vasculitis [15], regardless of ALT level and HBeAg status.

Antiviral treatment is currently not recommended for CHB patients in the immune-tolerant phase, which is defined by persistently normal ALT, high levels of HBV DNA, biopsies showing the absence of significant inflammation or fibrosis, as well as younger age (typically below 30 years old) [16]. However, the likelihood of transitioning from immune-tolerant phase to HBeAg-positive immune-active phase increases with age; the EASL guidelines suggest that patients with normal ALT and high HBV DNA level but older than 30 years may be treated regardless of the severity of liver histological lesions [13]. The recommendation against antiviral treatment for immune-tolerant CHB patients is due to the following reasons: 1) the risk of disease progression in the immune-tolerant phase is very slow; and 2) the current antiviral treatment during this phase is associated with a minimal chance of suppressing HBV replication completely and the potential harms of antiviral drug side effects and development of drug resistance. However, if new and effective anti-HBV drugs for immune-tolerant CHB patients could be developed in the future, these patients may also be treated.

2.2.2 Antiviral Treatment Indications for Cirrhotic patients

For patients with compensated cirrhosis and detectable HBV DNA, indefinite antiviral therapy is recommended to reduce the risk of decompensation, regardless of ALT level and HBeAg status [1, 12, 17]. For HBsAg-positive patients with decompensated cirrhosis, the 2018 AASLD guidelines recommended indefinite antiviral therapy regardless of HBV DNA level, HBeAg status, or ALT level to decrease risk of worsening liver-related complications [11]. Indications for other special populations will be discussed in detail below.

2.3 Current Anti-HBV Drugs

Current anti-HBV drugs can be categorized into two classes: nucleoside/nucleotide analogues (NAs) and interferon-α (IFN-α) [2]. NAs are widely used due to its favorable safety profile, convenient route of administration, and no obvious contraindications as compared with IFN-α. However, the treatment duration for NAs is not definite, and long-term NA therapy may increase the risk of drug resistance, while for IFN-α, there is no drug resistance, and the treatment duration for CHB treatment is relatively definite. IFN-α treatment induces higher rate of HBsAg loss and HBeAg seroconversion compared to NAs. Nevertheless, the administration of IFN-α needs injection and is contraindicated in patients with decompensated cirrhosis or autoimmune disease, pregnant women, and patients with uncontrolled severe depression or psychosis. Thus, when designing an optimal therapy for individual patients, physicians should take account of many factors including patients' characteristics, the estimated duration of treatment, the side effects of chosen drugs, the treatment costs, and the drug resistance.

2.3.1 Nucleoside/Nucleotide Analogues

Antiviral therapy with NAs for CHB has become the primary standard treatment strategy. Currently, there are six NAs approved for CHB antiviral treatment: lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF). The development of NAs is ascribed to the comprehensive understanding of HBV replication process. NAs not only inhibit HBV reverse transcriptase activity but also compete with natural nucleotide substrates for incorporation into the elongating DNA chain, thus interrupting HBV DNA synthesis [18, 19]. Long-term NA therapy can decrease the cccDNA pool of infected hepatocytes by inhibiting the recycling of the nucleocapsids. However, NAs cannot prevent the initial cccDNA formation in newly infected hepatocytes [19].

LAM, ADV, and LdT are the first generation of NAs developed for anti-HBV treatment. These NAs have low barrier to resistance and therefore are liable to develop resistance during long-term treatment. LAM is the first nucleoside analogue approved by the US FDA in 1998 for the treatment of CHB. It competes for cytosine in the synthesis of viral DNA. CHB patients receiving 104 weeks of 100 mg LAM treatment showed 52% rate of virological response [20]. However, long-term LAM therapy led to high rate of drug resistance: 65–70% after 5 years of LAM therapy [21]. Following LAM, ADV was the second NA approved by the US FDA in 2002 for the treatment of CHB. It is a phosphonate acyclic nucleotide analogue of adenosine monophosphate. Following 1-year ADV therapy, the rates of virological response and HBeAg seroconversion were 21% and 12% in HBeAg-positive patients, respectively [22]. And in HBeAg-negative patients, the rates of virological

response and histological improvement were 51% and 64%, respectively [23]. However, long-term ADV treatment also leads to high drug resistance rate (20–29% after 5-year treatment) [24, 25]. LdT is another nucleoside analogue and is the unmodified β-L enantiomer of the naturally occurring nucleoside thymidine. The rates of virological response in HBeAg-positive and HBeAg-negative patients treated with 104 weeks of LdT were 55.6% and 82%, respectively [26]. With a proven safety profile, LdT is a pregnancy category B medication and has been applied to prevent mother-to-child-transmission (MTCT) in mothers with HBV infection [27–29]. However, similar with LAM and ADV, long-term LdT treatment leads to high rate of drug resistance (34% after 3-year LdT therapy) [30]. When drug-resistant mutations occur in CHB patients, the clinical benefit of treatment decreases, and hepatitis flares and even liver failure may occur. Therefore, selecting potent and low-resistant antiviral drugs is highly recommended for treatment-naïve CHB patients.

ETV, TDF, and TAF are potent antiviral NAs with high genetic barrier to HBV resistance, and they are recommended as the first-line oral anti-HBV drugs. ETV is a guanosine nucleoside analogue with selective activity against HBV and has been commercially available since 2005 [31]. The effective concentration (EC_{50}) of ETV is around 4 nM, which is at least 100-fold more potent than LAM or ADV on the suppression of HBV [32]. TDF is an acyclic nucleotide analogue with activity in vitro against retroviruses, including HIV and HBV. It is an orally bioavailable ester prodrug of tenofovir. TDF was approved by the US FDA for the treatment of CHB in 2008 and is categorized as a pregnancy category B drug. TAF is a newly approved drug for the antiviral treatment of CHB in 2017 [1]. It is a new prodrug of tenofovir and exerts more stable concentration in the serum than TDF. Compared with TDF, TAF permits a lower dose in circulating and less systemic exposure, thereby decreasing the renal and bone toxicity.

For the safety profiles of the NAs, TDF has proven to be associated with dose-dependent renal toxicity in animal studies [33]. The first case of TDF-associated nephrotoxicity was reported in 2002 in a patient with HIV [34]. Later, numerous case reports of TDF-induced nephrotoxicity have been published. In 2015, TDF-induced Fanconi syndrome was observed in a CHB patient [35]. This patient developed a progressive chronic kidney disease with serious hypophosphatemia and secondary osteomalacia. After TDF withdrawal and oral supplementation with phosphate and calcitriol, the renal damage gradually resolved. As for TAF, data from phase III registration trials demonstrated that it induced less reduction in the estimated glomerular filtration rate (eGFR) and bone mineral density than TDF [36]. Thus, all patients treated with potent NAs, especially TDF, should periodically monitor clinical indicators, including complete blood count, liver and kidney function tests, serum HBV DNA, and abdominal ultrasound. Liver function tests should be performed every 3–4 months during the first year and every 6 months thereafter. Serum HBV DNA should be determined every 3–4 months during the first year and every 6–12 months thereafter.

2.3.2 Peg-IFN-α

Interferons are central mediators of immune response to viral infections. IFN-α can induce IFN-stimulated genes (ISGs), exerting antiviral functions against a variety of viruses. IFN-α exhibits direct inhibition of HBV DNA replication and clears infected hepatocytes through indirect regulation of the host immunity [37]. As the covalent attachment of polyethylene glycol (Peg) molecules to conventional IFN-α produces a biologically active molecule with a longer half-life, pegylated interferon α (Peg-IFN-α) increasingly replaced conventional IFN-α with improved pharmacokinetic properties [38]. Thus, Peg-IFN-α has been selected as one of the first-line therapies to treat CHB patients. Of note, Peg-IFN-α is prohibited in patients with decompensated cirrhosis.

2.4 Treatment Strategies for Patients Chronically Infected with HBV

The choice of an optimal therapy for individual patient depends on several factors, including age, sex, stage of liver disease, coinfections, treatment duration, side effects, and drug resistance.

2.4.1 NAs for CHB Patients

Potent NAs with high barrier to resistance (ETV, TDF, and TAF) are recommended as the first-line antiviral drugs. Long-term ETV treatment showed good tolerance, a favorable safety profile. The rates of virological responses in HBeAg-positive and HBeAg-negative patients after 1-year ETV therapy were 67% and 90%, respectively [39, 40], and the HBeAg seroconversion was 21% in HBeAg-positive patients [39]. Five years of ETV therapy resulted in 99% cumulative rate of virological response and 53% rate of HBeAg loss in HBeAg-positive patients [41]. The virological response at year 5 reached 100% in HBeAg-negative patients [42]. Similarly, a study evaluating the efficacy of ETV in NA-naïve Egyptian patients reported that the rate of HBV DNA undetectability reached 100% after 5 years of ETV therapy [43].

Among treatment-naïve CHB patients with HBeAg positive and negative, the rates of virological response were 76% and 93% after receiving 48-week TDF, respectively [44], and the virological response increased to 97% in HBeAg-positive patients after 5 years. After 3 years, about 96% of HBeAg-negative patients treated with TDF achieved virological response [45]. Phase III studies comparing TAF to TDF in CHB patients demonstrated that with the anti-HBV efficacy of TAF was non-inferior to that of TDF, but TAF had a better safety profile than TDF in CHB patients [46, 47].

The NAs with low barrier to drug resistance, such as LAM, LdT, and ADV, should be avoided, as this may lead to inappropriate viral suppression and the emergence of multidrug-resistant strains. For treatment-experienced CHB patients with NAs of low barrier to resistance (LAM, ADV, LdT), it is recommended to change to a more potent drug without cross-resistance [1]. The risk of resistance is associated with high baseline HBV DNA levels, a slow decline in HBV DNA, and a previous suboptimal NA treatment [1]. Patients previously treated with LAM, LdT, or ADV often develop high rate of resistance during prolonged treatment. For ADV-experienced patients, high rates of CVR could be achieved after switching to ETV [48]. Although ETV is an excellent inhibitor of HBV reverse transcriptase, it often fails to treat LAM-resistant individuals. Patients carrying LAM-resistant virus strain showed a highly increased ETV-resistance rate (51% *vs.* 1.2% in treatment-naïve patients receiving 5-year ETV therapy) [49, 50], as LAM-resistant mutations contribute to the development of ETV resistance [51]. The LAM mutations, rtM204I/V with or without rtL180M, along with other mutations are frequently detected in patients with ETV resistance [32], and the presence of LAM-resistant mutations leads to several 100-fold increases in ETV resistance.

For NA-experienced patient, TDF also have antiviral efficacy to act as an idea agent for CHB patients with LAM or LdT resistance [52]. Both treatment-naïve and treatment-experienced patients showed a rapid decline in HBV DNA within 3 months of TDF initiation [53]. HBV DNA < 69 IU/mL was achieved in 91% of treatment-naïve patients and 96% of treatment-experienced patients, respectively, demonstrating that TDF showed a rapid and sustained suppression of HBV DNA in CHB patients, irrespective of treatment history. In ADV treatment-experienced CHB patients, TDF had inferior efficacy compared to NA-naïve patients. A total of 92.3% of NA-naïve patients and 84.5% of NA-exposed patients achieved CVR, respectively [54], suggesting that the response of patients with previous ADV switching to TDF monotherapy should be monitored closely.

For patients harboring multiple drug-resistant HBV strains, combination of TDF and ETV seems to be an effective and safe rescue approach [55, 56]. To reduce the emergence of multidrug-resistant strains, combination therapies, especially combination of NAs with low barrier to resistance, such as LAM or LdT with ADV, and sequential monotherapies with agents with a low barrier to resistance are not generally recommended, which may ultimately increase the difficulty and cost of treatment.

2.4.2 Peg-IFN-α for CHB Patients

Peg-IFN-α, an immunomodulatory agent, could enhance host immunity to mount a defense against HBV and modest antiviral action [12]. Peg-IFN-α therapy offers several benefits over NAs for treatment of CHB including a finite duration of therapy and higher rates of anti-HBe and anti-HBs seroconversion with 12 months of therapy [57].

Rate of HBsAg loss was 3–7% following 12 months of Peg-IFN-α treatment, higher than 12-month treatment with current NAs (1% for LAM, 0% for ADV, 2% for ETV, 0.5% for LdT, and 3% for TDF) [58]. With 12-month therapy of Peg-IFN-α for HBeAg-positive CHB patients, the rate of HBV DNA<60–80 IU/ml, anti-HBe seroconversion, ALT normalization, and HBsAg loss were 14%, 32%, 41%, and 3%, respectively [59]. The response rates of 48-week Peg-IFN-α treatment for HBeAg-negative CHB were also evaluated in another multicenter, randomized study; the rate of HBV DNA <60–80 IU/ml, ALT normalization, and HBsAg loss were 19%, 59%, and 4%, respectively [60]. HBsAg loss rarely occurred during Peg-IFN-α therapy in HBeAg-negative CHB patients, but the rate of HBsAg loss progressively increased from 3% at month 6 to 9% at year 3 to 12% at year 5 after Peg-IFN-α discontinuation [58]. IFN-α treatment improved long-term outcomes, including decreased risk of hepatic complication survival and HCC and in CHB patients with sustained response [12, 61]. A 5-year observation cohort study revealed Peg-IFN-α-treated patients showed a lower cumulative incidence of cirrhosis and HCC [62].

The evaluation of predictors for response before and during treatment is very important for CHB patient with Peg-IFN-α therapy. For patients with HBeAg positive, having low HBV DNA (below 2×10^8 IU/mL), genotype A, as well as high serum ALT levels (above 2–5 times ULN) and high activity scores on liver biopsy, Peg-IFN-α could be considered as first-line antiviral agent [1, 58]. For patients with HBeAg negative, genotype D, a combination of no decrease in HBsAg levels and 2 $\log_{10}$ IU/ml reduction of HBV DNA at 12 weeks of Peg-IFN-α therapy predicts no response and should be used as Peg-IFN-α stopping rules [1, 58]. These treatment predictors for the existing antiviral therapies at various time points may be useful to guide initiation and continuation of Peg-IFN-α therapy.

Screening suitable patients prior to treatment is quite important for Peg-IFN-α therapy. Relative or absolute contraindications to IFN-α treatment include Child B/C cirrhosis, cirrhotic hypersplenism, autoimmune hepatitis, hyperthyroidism, coronary artery disease, renal transplant, pregnancy, seizures, severe depression, etc. The side effects of IFN-α are relatively common but are acceptable in most patients. The adverse effects of IFN-α mainly include flu-like symptoms, fatigue, bone marrow suppression, and exacerbation of autoimmune illnesses [19]. Therefore, patients should be closely monitored throughout the therapy. Complete blood counts and serum ALT levels should be monitored monthly, and TSH should be monitored every 3 months. Serum HBV DNA and HBsAg in all CHB patients and HBeAg and anti-HBe in HBeAg-positive patients should be examined at 3, 6, and 12 months of Peg-IFN-α treatment and at 6 and 12 months posttreatment. Patients with the definite therapy course of Peg-IFN-α, despite HBV DNA negative and serological conversion at the end of treatment, require long-term follow-up in case of HBV reactivation. In summary, IFN-α is associated with a broad spectrum of potential adverse effects, and the recommendations to use Peg-IFN-α should balance benefits versus risks, and decisions should be made according to individual patient characteristics and preference.

2.4.3 Combination of NA Plus Peg-IFN-α Therapy for CHB Patients

The current anti-HBV therapy with potent and high genetic barrier NAs can suppress the viral replication to undetectable level in the blood circulation in the majority of CHB patients, preventing the progression of CHB to cirrhosis and markedly decreasing the rates of HBV-related HCC. However, current long-term anti-HBV NAs can rarely achieve the "functional cure" of HBV (HBsAg loss or seroconversion), the best current stopping rule. This goal is hardly achievable by the finite-duration treatment with Peg-IFN, either. Hence, to accomplish the goal of "functional cure" of HBV infection in more CHB patients, the combination of a potent NA with Peg-IFN-α has been investigated. The rationale behind is that the two classes of anti-HBV agents have different mechanism of actions, the advantages of the potent antiviral effect of the NAs and the immunomodulating effect of the Peg-IFN-α, and thus their combination would conceptually result in a synergistic anti-HBV effect.

There are two different ways to combine NA and Peg-IFN: 1) the de novo combination, which means the simultaneous administration of the two agents in treatment-naïve CHB patients, and 2) the sequential combination, which means the "add-on" or "switch-to" strategy to CHB patients who are already on treatment with either drug (Table 2).

de novo Combination Therapy

The initial treatments of LAM plus Peg-IFN and ADV plus Peg-IFN showed less-than-desirable results in treatment-naïve patients [59, 60, 63]. The combination therapy with LdT plus Peg-IFN is prohibited due to a high risk of severe polyneuropathy [58, 64]. The de novo combination of TDF and administration of Peg-IFN have been recently investigated in a global multicenter randomized controlled study (Marcellin) [65]. In this study, 740 treatment-naïve patients with HBe-positive and HBe-negative CHB were randomly assigned to receive TDF plus Peg-IFN-α2a for 48 weeks (group A), TDF plus Peg-IFN-α2a for 16 weeks followed by TDF for 32 weeks (group B), TDF for 120 weeks (group C), or Peg-IFN-α2a for 48 weeks (group D). The rates of HBsAg loss at week 72 (24 weeks posttreatment) in the four groups were 9.1%, 2.8%, 0%, and 2.8%, respectively. In the follow-up study at week 120 (72 weeks posttreatment), the rates of HBsAg loss in the combination group increased from 9.1% to 10.4% [66]. Thus, patients receiving combination of TDF plus Peg-IFN had a higher rate of HBsAg loss than those receiving Peg-IFN or TDF alone. Although the increased rate of HBsAg loss in patients receiving TDF plus Peg-IFN therapy was encouraging, the overall rate of HBsAg at week 120 (10.4%) in the combination group was still relatively low, meaning that approximately 90% of patients did not achieve a sustained immune control. Besides, the benefit of the increased HBsAg loss was mainly associated with HBV genotype A and treatment with TDF plus Peg-IFN in the study [67].

Table 2 Combination of NA plus Peg-IFN therapy for CHB patients

De novo combination therapy

Study design	Country	Year of publication	No. of patients	Type of CHB (HBeAg positive/ negative)	Follow-up	NAs	HBeAg seroconversion (%)	The HBsAg loss (%)	Reference
A secondary analysis of data from study GSUS-174-0149, randomized, open-label, active-controlled, superiority trial (NCT01277601)	Global study of 30 investigators	2016	740	428/312	72 weeks	TDF	NA	6.5%	[65]
Randomized, open-label, active-controlled, multinational, superiority trial (NCT01277601)	Global study of 149 investigators	2016	740	428/312	72 weeks	TDF	25%	9.1%	[67]
Open-label, active-controlled study (NCT01277601)	Global study of 19 investigators	2018	740	428/312	120 weeks	TDF	29.5%	10.4%	[66]
Retrospective study (ISRCTN 77073364)	The Netherlands	2017	92	44/48	5 years	ADV	22%	18%	[63]
Multicenter, randomized, partially double-blind study	67 sites in 16 countries	2005	814	814/0	72 weeks	LAM	32%	3%	[59]
Multicenter, randomized, partially double-blind study	54 sites in 13 countries	2005	552	0/552	72 weeks	LAM	NA	2.8%	[60]
Randomized controlled, open-label trial (NCT00973219)	The Netherlands	2017	151	0/151	72 weeks	TDF or ADV	NA	4%	[68]

Sequential combination therapy									
Study design	Country	Year of publication	No. of patients	Type of CHB (HBeAg positive/ negative)	Follow-up	NAs before switch-to IFN or combination	HBeAg seroconversion (%)	The HBsAg loss (%)	Reference
Open-label, multicenter, randomized controlled trial (NCT00877760)	14 centers in 5 countries in Europe and Asia	2015	185	185/0	96 weeks	ETV	26%	1.18%	[72]
Randomized, controlled, open-label trial (NCT01172392)	France	2017	185	0/185	96 weeks	ETV or TDF or ADV or LAM	NA	7.8%	[69]
Open-label, randomized study (NCT00940485)	China	2014	200	200/0	48 weeks	ETV	14.9%	8.5%	[70]
Open-label, randomized study (NCT01464281)	China	2018	303	0/303	96 weeks	ADV/ETV/ LAM	NA	48 weeks 14.4% 96 weeks 20.7%	[71]

However, a recent randomized controlled, open-label study did not support the use of combination treatment with Peg-IFN and NA in patients with CHB [68]. At week 72, only two patients (4%) in the Peg-IFN plus TDF group and two patients in the Peg-IFN plus ADV group achieved HBsAg loss, compared with none of the patients in the no-treatment group (p=0.377). All four patients with HBsAg loss were included in the group of patients with HBV DNA less than 2000 IU/mL, so the baseline HBV DNA should be taken into account before initial de novo combination therapy.

Sequential Combination Therapy

Sequential combination therapy (including "add-on" and "switch-to" strategy) may be alternative options for CHB patients pursuing a functional cure. Starting with an NA first and then followed by Peg-IFN add-on seems to be a very logic approach to the sequential combination strategy. The concept is that the administration of a potent NA first would quickly halt the viral replication and therefore partially restore the host adaptive immune response, whereas the Peg-IFN add-on later may enhance serological response rates, resulting in more patients achieving a functional cure of CHB [69–71].

The study by Brouwer et al. (ARES study) investigated the "early add-on" strategy by comparing 24 weeks of ETV followed by 24 weeks of Peg-IFN add-on versus 48 weeks of ETV monotherapy for treatment-naïve HBeAg-positive CHB patients [72]. It showed that Peg-IFN add-on therapy led to a higher proportion of HBeAg serological response compared to ETV monotherapy. At week 48, the response defined as HBeAg loss with HBV DNA <200 IU/mL was achieved in 16 of 85 (19%) patients receiving the combination therapy versus 9 of 90 (10%) patients receiving ETV monotherapy. At week 72 (24 weeks posttreatment), the response rate in the combination group increased to 32% (27/85). However, in the ARES long-term follow-up study (the median follow-up duration was 226 weeks), the rates of serological response became comparable between the combination group and the ETV monotherapy group, suggesting that Peg-IFN add-on may lead to accelerated HBeAg loss rather than increased long-term HBeAg loss [73].

The "late add-on" Peg-IFN combination therapy was recently investigated in a multicenter and randomized trial enrolling only HBeAg-negative CHB patients with undetected HBV DNA by at least 1 year of NA treatment (PEGAN study) [69]. In this study, 183 patients were randomized to either continue NA or add on Peg-IFN treatment for 48 weeks. Due to the adverse effects of Peg-IFN, only 65 out of 90 patients in the Peg-IFN-α add-on group completed a full 48-week course of Peg-IFN-α. As the primary endpoint for this study was HBsAg loss at week 96 by intention-to-treat analysis (8% in the Peg-IFN add-on group versus 3% in the NA group, $P = 0.15$), the interpretation of the study results was that Peg-IFN-α add-on was poorly tolerated [69]. However, HBsAg loss rates were significantly higher in the full-dose Peg-IFN-α add-on group than in the NA group, being 11% *vs.* 0%, 11% *vs.* 3%, and 14% *vs.* 4% at week 48, week 96, and week 144, respectively [69].

Secondary post hoc analysis showed that patients who had lower baseline HBsAg titers might benefit more from this add-on strategy to achieve HBsAg loss and anti-HBs seroconversion [69].

The rates of HBsAg loss in the "switch-to" Peg-IFN-α strategy have also been investigated in CHB patients pre-treated with NA. In the "early switch-to" study (OSST trial), 192 HBeAg-positive patients receiving 9 to 36 months of ETV therapy with HBeAg <100 PEIU/ml and HBV DNA≤1000 copies/ml were randomized 1:1 to receive ETV or switch to Peg-IFN-α2a for 48 weeks. At week 48, serological response rates were significantly higher in the Peg-IFN-α group than the ETV group (HBeAg seroconversion 14.9% *vs.* 6.1%; HBsAg loss 8.5% *vs.* 0%) [70]. The study further found that a baseline HBsAg level <1500 IU/ml as the optimal cutoff to predict HBsAg loss and week 12 HBsAg <200 IU/ml were associated with the highest rates of HBsAg loss 77.8% (7/9).

In the "late switch-to" study (New Switch trial), 305 HBeAg-positive patients who achieved HBeAg loss and HBV DNA <200 IU/mL with previous NA treatment (ADV, LAM, or ETV) were randomized 1:1 to receive Peg-IFN for 48 or 96 weeks [71]. The rates of HBsAg loss were achieved in 14.4% (22/153) of patients receiving "switch-to" Peg-IFN for 48 weeks and in 20.7% (31/150) of patients receiving "switch-to" Peg-IFN for 96 weeks. Similar to the OSST study, the New Switch study also found that baseline HBsAg <1500 IU/mL and week 24 HBsAg <200 IU/mL were associated with the highest rates of HBsAg loss 51.4% (18/35) and 58.7% (27/46) at the end of both 48- and 96-week treatment, respectively [71].

In summary, recent studies have demonstrated that the combination of NA with Peg-IFN either simultaneously or sequentially can enhance the rates of HBsAg loss, but the benefits are mainly limited to a relatively small proportion of patients, especially in those with low baseline HBsAg level and on-treatment HBsAg response. Therefore, CHB patients who can benefit from NA and Peg-IFN combination therapy should be carefully evaluated including age, viral load, genotype, baseline ALT, and HBsAg levels. In addition, Peg-IFN stopping rules based on on-treatment HBsAg kinetics should be followed during the treatment to make decisions whether to continue or discontinue Peg-IFN and shift to NA monotherapy. Further investigations are needed to identify the optimal Peg-IFN combination strategy and the subgroup of CHB patients with the highest potential to benefit from the combination treatment.

2.4.4 Treatment Strategies for Special Populations

Patients with HBV-Related Cirrhosis

Patients with evidence of HBV-related cirrhosis and detectable HBV DNA are strong indicators for antiviral treatment, regardless of ALT levels and HBeAg status. The aim of antiviral therapy for patients with compensated cirrhosis is to reduce the risk of disease progression to hepatic decompensation and HCC. It has been demonstrated that the occurrences of death, hepatic decompensation, and HCC were

less frequent in the treated cohort than in the untreated controls, with the 5-year cumulative incidences being 19.4% *vs.* 43.9%, 15.4% *vs.* 45.4%, and 13.8% *vs.* 23.4%, respectively [74]. Taking both efficacy and drug resistance profiles into account, antiviral drugs for HBV-related cirrhosis should be safe and affordable for long-term use to achieve a high rate of sustained HBV suppression with a low risk of drug resistance. Therefore, potent and low drug-resistant NAs (ETV, TDF, and TAF) are preferred for these patients. It has been reported that more than 2 years of ETV treatment for cirrhotic patients led to the improvement of liver function and fibrosis markers [75]. Moreover, up to 5 years of treatment with TDF achieved high rates of hepatic fibrosis regression and even the reversion of cirrhosis [76]. It needs to be noted that although Peg-IFN-α is not contraindicated in patients with compensated cirrhosis, it should be used with caution due to its side effects, and therefore the safer NAs are preferred and recommended [2].

Antiviral therapy in patients with decompensated cirrhosis has been shown to slow disease progression and may delay the burden of liver transplantation. Patients with decompensated cirrhosis and detectable HBV-DNA should be treated urgently, while HBsAg-positive decompensated patients with undetectable HBV-DNA may also receive lifelong antiviral therapy to reduce the risk of aggravation of liver-related complications [11]. ETV and TDF are recommended as the first-line NAs in these patients. Treatment with either ETV or TDF in patients with decompensated cirrhosis improved both hepatic function and Child-Turcotte-Pugh (CTP) and Model for End-Stage Liver Disease (MELD) scores [77, 78]. Although data concerning the use of TAF in these patients are currently insufficient, TAF may also be used in patients with decompensated cirrhosis due to its favorable safety profile. It needs to be noted that long-term antiviral therapy decreases but does not eliminate the risk of HCC in patients with liver cirrhosis [79–81], thus long-term surveillance of HCC still required even with successful antiviral treatment in these patients.

Patients with HBV-Related HCC

High serum HBV DNA is associated with early HCC recurrence after curative resection in patients with HBV-related HCC [82]. In addition, reactivation of HBV may be induced by HCC treatment strategies including curative resection, radiofrequency ablation (RFA), trans-arterial chemoembolization (TACE), and radioembolization [83]. Therefore, antiviral therapy is an important part of the comprehensive treatment for HBV-related HCC. NA treatment following HCC curative resection reduces HCC recurrence and improves the overall survival of patients with advanced HBV-related HCC [84]. Both ETV and TDF are recommended as the first-line antiviral agents for HBV-related HCC patients [17]. As TAF is the latest NA approved for anti-HBV treatment, the experience in its use for HBV-related HCC patients is currently limited. However, with its potent antiviral efficacy and favorable safety profile, it may also be considered for these HBV-related HCC patients.

Patients with Liver Failure Related with HBV Infection

Liver failure is a life-threatening disease with high short-term mortality [85]. It may develop following acute HBV infection or reactivation of chronic HBV infection. HBsAg-positive or HBV DNA-positive patients with liver failure (including acute, subacute, or acute on chronic) should consider NA therapy as soon as possible [86]. ETV, TDF, or TAF are the preferred NAs to improve the survival of CHB patients with liver failure [87, 88]. The beneficial effects were mostly observed in patients with MELD score within 20–30, while the mortality rate in patients with MELD score over 30 is >90% even with prompt antiviral treatment, and thus urgent liver transplantation should be considered in these patients [89].

Patients Undergoing Liver Transplantation Related with HBV Infection

In western countries, only 5–10% of liver transplantation is conducted for patients with HBV-related liver diseases, while in China, approximately 90% of the liver transplantation is due to HBV-related liver diseases [90]. HBV recurrence was a major problem for liver transplantation in the past, and patients with a high HBV viral load preoperatively had a higher risk of HBV reinfection after liver transplantation [91]. Previously, prophylactic therapy with HBIG only showed unfavorable results [92, 93]. With the advent of NA and its combination with HBIG, the risk of the HBV reinfection rate has been reduced to less than 5% [1]. However, the use of HBIG is costly and inconvenient (requires regular parenteral/intramuscular injections). In the current era of potent NAs with high barrier to drug resistance (ETV, TDF, and TAF), the prophylactic therapy with short course and low dose of HBIG or even HBIG-free regimen has been evaluated [93].

In 42 CHB patients with HBV DNA levels <100 IU/mL at the time of liver transplantation, prophylaxis using HBIG (5000 IU daily) intravenously in the anhepatic phase of liver transplantation and then daily for 5 days postoperatively (6 doses total) in combination with long-term NA therapy was highly effective in preventing HBV recurrence, with only 1 patient having detectable HBV DNA at 5 years after liver transplantation [94]. In another Greek study, 28 HBV-related cirrhotic patients with undetectable HBV DNA at the time of liver transplantation, prophylaxis using HBIG (1000 IU IM/day) for 7 days and then monthly for 6 months (13 doses total) plus ETV (n = 11) or TDF (n = 7) was also highly effective with all patients remaining HBsAg/HBV DNA negative during the follow-up period (9–43 months) [95]. A recent study from the University of Hong Kong has shown that HBIG-free prophylaxis using ETV monotherapy for CHB patients after liver transplantation is highly effective at preventing HBV reactivation [96]. In 265 consecutive CHB liver transplant recipients treated with ETV monoprophylaxis without HBIG, 85%, 88%, 87.0%, and 92% remained HBsAg negative at 1, 3, 5, and 8 years of follow-up, respectively, and 100% had undetectable HBV DNA at 8 years after transplantation. Of note, more than 60% of the 265 CHB liver transplant recipients had detectable HBV DNA at the time of liver transplantation. The overall 9-year survival was 85%

without any graft loss or death due to HBV reactivation [96]. Thus, short-course and low-dose HBIG in combination with potent NA or even HBIG-free NA monotherapy can be effective prophylaxis in the prevention of HBV reinfection after liver transplantation.

Of note, both the 2017 EASL guidelines and the 2018 AASLD guidelines stated that CHB patients with HDV and HIV coinfections were at high risk of HBV recurrence, and therefore the lifelong combination of HBIG and NA therapy was recommended as prophylaxis for these patients undergoing liver transplantation [1, 11]. Because of high potency and low rate of resistance with long-term use, ETV, TDF, and TAF are the preferred antiviral drugs for the prophylactic therapy, which should be administered in all CHB patients on the transplant waiting list. After liver transplantation, the duration of the antiviral therapy should be indefinite. After liver transplantation, the duration of the antiviral therapy should be indefinite, regardless of HBsAg, HBeAg, or HBV DNA status [1, 2, 97].

Patients Undergoing Immunosuppressive Therapy or Chemotherapy

After HBV exposure, the virus persists in the liver and other extrahepatic sites for long periods and may reactivate in individuals who receive immunosuppression or chemotherapy [98]. HBV reactivation is characterized by increased serum HBV DNA compared with the baseline level in HBsAg-positive patients or reverse seroconversion from HBsAg negative to HBsAg positive in HBsAg-negative and anti-HBc-positive patients. HBV reactivation causes elevation of ALT and hepatitis flare, which may result in liver failure and even death [99]. Thus, all patients should be screened with HBV markers including HBsAg, anti-HBs, and anti-HBc, prior to immunosuppressive therapy or chemotherapy, particularly in countries or regions with intermediate or high prevalence of HBV. HBsAg-positive patients are at high risk of HBV reactivation and should receive antiviral prophylaxis before immunosuppressive therapy or chemotherapy regardless of the baseline HBV DNA. Prophylactic antiviral therapy should be better initiated 1 week before or at the latest, concurrently at the initiation of immunosuppressive therapy [100]. Potent NAs (ETV, TDF, and TAF) should be preferred for the antiviral prophylaxis for CHB patients undergoing immunosuppressive therapy or chemotherapy [11].

Patients with HBsAg negative and anti-HBc positive are still at risk of HBV reactivation when they receive high-risk treatments, such as immunosuppressive agent rituximab and bone marrow/stem cell transplantation. Prophylactic anti-HBV drugs are recommended for these patients [12, 101]. HBsAg-negative and anti-HBc-positive patients who receive moderate- or low-risk immunosuppressive agents need to be regularly monitored with HBsAg and/or HBV DNA every 1–3 months during and after immunosuppression. Anti-HBV prophylaxis can be initiated at the first sign of HBV reactivation.

Regarding the duration of antiviral prophylaxis, the 2018 AASLD guidelines suggested that antiviral therapy should be at least 6 months (or at least 12 months for patients receiving rituximab) after completion of immunosuppressive therapy [2], whereas EASL recommends the antiviral therapy to be at least 12 months

(18 months for patients receiving rituximab) after cessation of the immunosuppressive treatment [1]. It is suggested that liver function and HBV DNA level should be routinely monitored every 3 to 6 months during prophylaxis and for at least 12 months after NA withdrawal.

Children with HBV Infection

Annually, about 2 million new HBV infections occur in children younger than 5 years old [102]. Exposed infants should be tested for HBsAg at 6–12 months after birth [103]. Most children with chronic HBV infection are in the immune-tolerant phase characterized by high viral load and normal ALT levels and respond poorly to currently available antiviral therapies. Thus, the 2018 AASLD guidelines recommend against the use of antiviral therapy in HBeAg-positive children with persistently normal ALT, regardless of HBV DNA level [2]. Although a rather benign course of CHB during childhood, about 3–5% and 0.01–0.03% of chronic carriers still have a risk of developing cirrhosis or HCC before adulthood, respectively [104]. Therefore, lifelong follow-up is recommended even for inactive carriers, because of the risk of cirrhosis and HCC and reactivation of HBV infection [104]. For children with normal ALT, monitoring should be done every 6 months, and the surveillance of HCC with liver ultrasound is recommended to be performed every 6–12 months, depending on the stage of fibrosis.

In children, the course of the HBV-related liver disease is generally mild, and most of the children do not meet the standard treatment indication. Thus the initiation of the antiviral treatment should be considered with caution [1]. CHB children fulfilling the indication for antiviral treatment should be treated [1, 11]. The antiviral therapy should be immediately initiated for CHB children with advanced liver diseases and cirrhosis [12].

There are several antiviral drugs approved for children with CHB, including conventional IFN-α (≥1 year old), LAM (≥2 years old), ETV (≥2 years old), and TDF (≥12 years old) [11]. The dose and treatment duration for each drug are shown in Table 3. Conventional IFN-α treatment accelerates ALT normalization, HBeAg

Table 3 Antiviral drugs approved for children with chronic HBV infection

Drugs	Ages approved for drug use	Dose	Duration
IFN-α	≥ 1 year	6 MU/m^2 three times per week	6 months
LAM	≥ 2 years	3 mg/kg/day	≥ 1 year
ADV	≥ 12 years	10 mg daily	≥ 1 year
ETV	≥ 2 years	10–30 kg, 0.015 mg/kg/day (maximum 0.5 mg); >30 kg, 0.5 mg daily	≥ 1 year
TDF	≥ 2 years[a]	300 mg daily	≥ 1 year
TAF	≥ 12 years	25 mg daily	≥ 1 year

[a]The European Medicines Agency approves TDF for children ≥2 years, and the US FDA approves for children ≥ 12 year

seroconversion, and viral load reduction in children [105, 106]. Although LAM is permitted for the treatment of CHB children, long-term use of LAM induces drug resistance and subsequent viral breakthrough [107], while ETV or TDF monotherapy has the advantage of high potency and low drug resistance [108, 109]. Thus, ETV and TDF should be preferred [11]. According to the 2017 EASL guidelines, TAF can be used in children ≥12 years old [1]. However, the 2018 AASLD guidelines stated that "TAF has not been studied in children. Thus, there are insufficient data to recommend the use of TAF in children 12 years and older" [11]. For children receiving antiviral treatment, the frequency of monitoring for safety, adherence, and efficacy of drugs should be determined on an individual basis.

Pregnancy with Chronic HBV Infection

When formulating treatment plan for women with CHB at childbearing age, the physician should take account of the effects and safety profile of different antiviral drugs [110]. Among current oral antiviral drugs, LdT and TDF are pregnancy category B medicines and are recommended for use in pregnant women with CHB, while ADV and ETV are pregnancy category C drugs and therefore are limited for use during pregnancy [1]. Although LAM is classified as pregnancy category C medicine, it can also be used in pregnant women with the safety data obtained from its use in pregnant women with HIV. Previous studies proved either LAM [111], LdT [27, 112], or TDF [113] effectively reduced perinatal HBV transmission. However, TDF is preferred with a better resistance profile and more safety data in pregnant women with chronic HBV infection. The 2018 AASLD guidelines stated that "TAF has not been studied in pregnant women. Thus, there are insufficient data to recommend the use of TAF in pregnancy" [11]. Peg-IFN-α is contraindicated for use during pregnancy.

The criteria to initiate antiviral therapy for women at childbearing age are the same as any other individuals with CHB. For women who fulfill the treatment indication and plan a pregnancy in the near future, NAs of category B (especially TDF) are recommended. For CHB patients on NA therapy who become pregnant, category B NAs can be continued (TDF is preferred), while category C NAs should be switched to TDF [2, 17].

Without intervention, 80–90% of infants exposed to HBV during the perinatal period may develop CHB infection [114]. Passive-active immunoprophylaxis, including HBIG and HBV vaccination at birth followed by two additional HBV vaccines within 6 months, is very effective against neonatal HBV exposure, reducing the MTCT rate from 90% to 10% [58]. It should be noted that about 10% immunoprophylaxis failures occur, which are almost exclusively in HBeAg-positive women with high HBV DNA levels.

Although CHB patients in immune-tolerant phase are generally not the indications for current antiviral therapy, accumulating evidences have suggested pregnant women with high HBV DNA level in immune-tolerant phase need antiviral therapy to reduce the risk of MTCT [28, 115–117]. The 2017 EASL and the 2018 AASLD

Table 4 Antiviral therapy to prevent mother-to-child transmission during pregnancy among different guidelines

	EASL 2017	AASLD 2018	China 2015	APASL 2015 update
Screening	Screening for HBsAg in the first trimester of pregnancy is strongly recommended	All pregnant women should be screened for HBsAg, especially those with high risk of HBV infection	-	Antenatal screening HBV in pregnant females is an evidence-based standard of practice
Standard indication	HBV DNA> 200 000 IU/mL or HBsAg levels >4 $\log_{10}$ IU/mL	HBV DNA> 200 000 IU/mL	HBV DNA> 2000 000 IU/mL	HBVDNA > 6–7 $\log_{10}$ IU/mL
Licensed antiviral drugs	TDF and LdT	LAM, LdT, and TDF	LAM, LdT, and TDF	TDF and LdT
	TDF is a preferred choice	TDF is a preferred choice		
The time to start therapy	Starting at weeks 24–28 of gestation	From 28 to 32 weeks of gestation	Starting at weeks 24–28 of gestation	From 28 to 32 weeks of gestation
The time to stop therapy	Continue therapy for up to 12 weeks after delivery	Antiviral therapy is discontinued at birth to 3 months postpartum	Antiviral therapy is discontinued at birth to months postpartum	The NAs could be stopped at birth and when breast-feeding starts. For those with ALT flares detected during the treatment period, continuation of antiviral treatment according to maternal liver disease status may be indicated
Breast-feeding after delivery	Breast-feeding is not contraindicated during maternal NA treatment	Breast-feeding is not prohibited during maternal NA treatment	Breast-feeding is recommended after drug withdrawal	Breast-feeding is discouraged during maternal NA treatment

guidelines recommended that pregnant women with serum HBV DNA > 20,0000 IU/ML should receive antiviral therapy to prevent MTCT [1, 2]. The time to start antiviral treatment to decrease the risk of MTCT varies among different guidelines [1, 11, 17]. Most guidelines recommended to initiate anti-HBV therapy at 24–28 weeks of gestation [1, 17], whereas the 2018 AASLD guidelines recommended at 28–32 weeks [11] (Table 4). Antiviral therapy may stop at delivery or continue for 12 weeks after delivery but should be closely monitored for ALT flares every 3 months for 6 months. As the concentration of TDF in breast milk is minimal with very limited bioavailability, breast-feeding is not contraindicated in HBsAg-positive mothers treated with TDF [1, 2, 12, 17].

Patients Coinfected with HBV and HCV

In CHB patients coinfected with HCV, the risks of the development of cirrhosis and HCC are higher than those with either HBV or HCV mono-infection [118, 119]. The treatment of HBV/HCV coinfection should be individualized based on the HBV and HCV viral loads, ALT levels, and liver fibrosis or cirrhosis assessment.

Anti-HCV therapy is indicated in coinfected patients with positive HCV-RNA. In the IFN-α era, the application of IFN-α plus ribavirin could achieve HCV eradication and HBV suppression in coinfected patients. With the advent of direct-acting antiviral (DAA), IFN-α-free and ribavirin-free DAA treatment has become the mainstream therapy for HCV infection. However, there is a potential risk of HBV reactivation during DAA therapy of patients with HCV/HBV coinfection, and life-threatening consequences have been reported in some individuals [120]. Therefore, during anti-HCV therapy with DAA, coinfected patients should be closely monitored by checking the HBV viral load and ALT levels [121].

In those HBV-/HCV-coinfected patients meeting the standard criteria for HBV treatment, HBV antiviral therapy should be started concurrently with DAA therapy of HCV [1]. The 2018 AASLD guidelines recommended monitoring HBV DNA levels every 4–8 weeks during DAA therapy and for 3 months post DAA treatment in HBsAg-positive patients who do not meet treatment criteria of anti-HBV treatment [11]. The risk of HBV reactivation in patients with HBsAg negative and anti-HBc positive is very low during HCV DAA therapy, but HBsAg and HBV DNA should be tested if ALT levels increase during or after anti-HCV therapy [11, 122]. For those HBV-/HCV-coinfected patients with cirrhosis, HBV antiviral therapy with NAs should be initiated concurrently with DAA therapy [121].

Another important issue that requires special attention is drug-drug interactions (DDI) in the context of combination therapy with NAs and DAAs. Based on the www.hep-druginteractions.org website, ETV can be safely co-administered with currently approved DAAs as no potential clinically significant DDI are indicated, but careful monitoring is still necessary during the therapy. Co-administration of TDF with DAA is either contraindicated or needs dose adjustment and additional monitoring [122].

Patients Coinfected with HBV and HIV

HBV/HIV coinfection induced increased risk of all-cause mortality and liver-related mortality [123]. HIV infection leads to higher HBV DNA levels, lower rates of HBeAg loss, and faster progression to cirrhosis [124].

The current guidelines recommend immediate initiation of antiretroviral therapy (ART) for all people living with HIV, regardless of CD4 cell count [125]. LAM, emtricitabine (FTC), TDF, and TAF are NAs with effective activities against both HIV and HBV. Thus, for patients with HBV/HIV coinfection, the ART regimen should include TDF or TAF plus LAM or FTC as the ART backbone [11]. Of note, these drugs should not be used as a single agent for HBV treatment in HBV-/HIV-

coinfected patients because of the risk of HIV resistance. Patients who are already on effective ART regimen that does not include drugs with antiviral activity against HBV should have the ART regimen altered to include TDF or TAF plus LAM or FTC. It needs to be noted that HBV-/HIV-coinfected patients with liver cirrhosis and low CD4 cell count require careful surveillance of immune reconstitution syndrome and subsequent liver decompensation in the first months after starting ART [1].

2.5 Endpoint of Antiviral Treatment

Deciding the antiviral treatment duration or therapy endpoint for CHB patients is challenging and needs to take account of many factors including the choice of antiviral agents (IFN-α-based or NA-based), sustained suppression of HBV DNA replication, HBeAg status, HBsAg status, and the presence of cirrhosis (compensated or decompensated).

For non-cirrhotic CHB patients, the duration of Peg-IFN-α treatment is generally 48 weeks, whereas the duration of NA-based antiviral therapy is variable and difficult to decide. For non-cirrhotic HBeAg-positive patients receiving NA therapy who have achieved persistent virological response, biochemical response, and serological response (HBeAg loss or seroconversion), discontinuation of NA therapy may be considered if the therapeutic responses persist during the consolidation treatment. However, the time of consolidation treatment varies, for at least 12 months in the 2018 AASLD [2] guidelines and for at least 3 years in Chinese CHB treatment guidelines [17]. However, due to the potential risk of virus relapse after NA withdrawal, close post-NA monitoring is still needed. For non-cirrhotic HBeAg-negative patients, the duration of anti-HBV treatment is unclear as the rate of HBV relapse is high in these patients, and therefore the long-term antiviral treatment is required. For both HBeAg-positive and HBeAg-negative patients with cirrhosis, NAs should be treated indefinitely.

HBsAg loss, with or without seroconversion to anti-HBs, termed as "functional cure," is considered as the optimal treatment endpoint for both HBeAg-positive and HBeAg-negative CHB patients. As serum HBsAg levels parallel the expression of cccDNA (the viral persistence reservoir), HBsAg loss represents a complete suppression HBV, low risk of HBV recurrence, and an improved long-term outcome. However, cirrhosis and HCC may still occur in patients with HBsAg loss [126, 127]. This is because HBV is still not completely eradicated due to the persistence of cccDNA in the liver and the integration of HBV DNA into host genome even in patients with HBsAg loss or seroconversion. Thus, for patients who stop antiviral therapy based on the endpoint of the HBsAg loss, close monitoring of HBV DNA, ALT levels, and disease progression is extremely necessary.

In addition to HBsAg quantification, other noninvasive serological markers are being developed to guide the antiviral efficacy and the duration or endpoint of therapy: hepatitis B core-related antigen (HBcrAg) and circulating HBV RNA [1].

Both HBcrAg and circulating HBV RNA levels correlate well with the intrahepatic cccDNA levels and may be potential predictive biomarkers to monitor the safe discontinuation of NA therapy [128–130].

3 Developing New Drugs for HBV Treatment

Current antiviral drugs can sufficiently suppress the serum HBV DNA, achieving complete virological response in the majority of CHB patients and thus reducing the morbidity and mortality of HBV-related liver diseases. The combination of Peg-IFN-α with NA either simultaneously or sequentially can enhance the rate of functional cure of CHB (HBsAg loss or seroconversion), but the benefits are mainly limited to a relatively small proportion of patients (≈10%), especially in those with low baseline HBsAg level and on-treatment HBsAg response. The ultimate treatment goal for CHB is to cure HBV infection with the elimination of all forms of potentially replicating HBV, which is hardly achievable with current antiviral therapy due to the persistence of cccDNA (the HBV transcriptional template) in the hepatocyte nucleus [2]. Therefore, novel therapeutic drugs are needed either targeting different steps of HBV life cycle or modulating the host immune system (Fig. 1).

3.1 New Drugs Targeting HBV Life Cycle

3.1.1 HBV Entry Inhibitors

Entry is the first step for HBV infecting hepatocytes. NTCP has been identified as a special functional receptor for HBV entry into hepatocytes [131]. Therefore, entry inhibitors have been proposed as promising agents for protecting uninfected hepatocytes. New drugs targeting viral entry receptor NTCP including Myrcludex B (phase II) and cyclosporin A (in vitro) are being developed and investigated.

Myrcludex B is a synthetic lipopeptide derived from HBV preS1 domain. Binding to NTCP, Myrcludex B not only effectively prevents HBV spread among intrahepatic cells but may also hinder the amplification of intrahepatic cccDNA pool in infected hepatocytes [132]. In a phase I clinical trial, Myrcludex B showed excellent tolerability up to high doses (up to 20 mg intravenously and 10 mg subcutaneously), and the pharmacologic properties followed a two-compartment target-mediated drug disposition model [133]. The results of a phase II clinical trial showed that 10 mg Myrcludex B had more potency of antiviral activity than lower doses, while no noteworthy change of HBsAg concentrations was observed [134]. As Myrcludex B can block the infection of new hepatocytes [132], it may be quite attractive for use in the liver transplantation setting to prevent reinfection of transplanted liver.

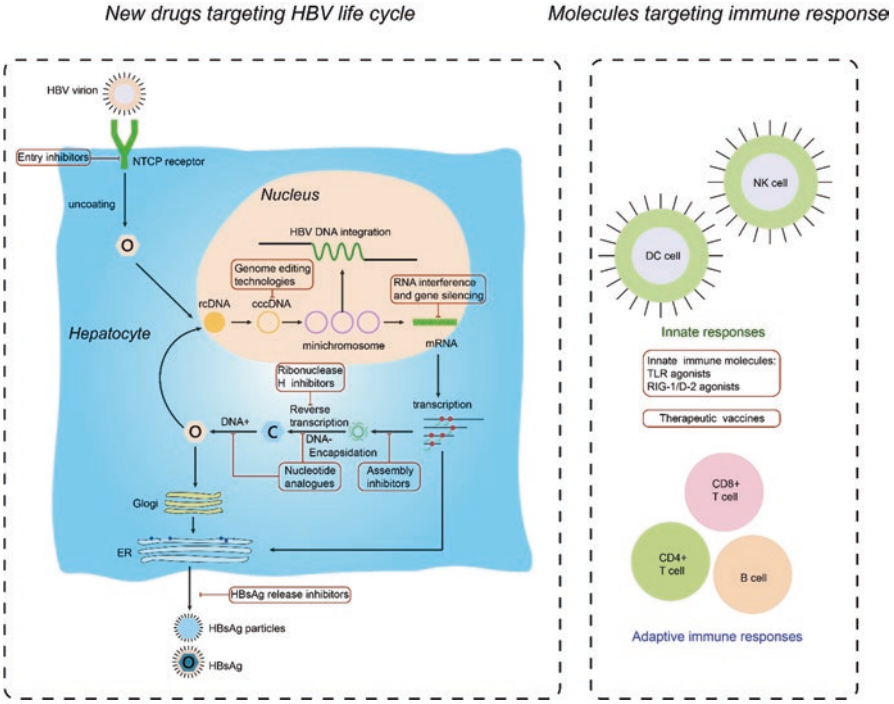

Fig. 1 Development of new anti-HBV drugs either targeting HBV life cycle or modulating host immune response

Left panel: the HBV life cycle including viral entry, trafficking, cccDNA formation, transcription, encapsidation, replication, capsid assembly, and viral secretion is shown. The development of new drugs targeting different steps of HBV life cycle is shown in red box: entry inhibitors such as Myrcludex B, inhibition of cccDNA formation by genome-editing technologies such as TALENs and CRISPR/Cas9, mRNA degradation or translational suppression by RNA interference, assembly inhibitors, ribonuclease H inhibitors, and HBsAg release inhibitors

Right panel: immunomodulators such as TLR agonists, RIG-1/NOD-2 agonists, and therapeutic vaccines can be used to enhance the innate and adaptive immune responses to control HBV infection. Abbreviations: NTCP, sodium taurocholate cotransporting polypeptide; cccDNA, covalently closed circular DNA; rcDNA, relaxed circular DNA; ER, endoplasmic reticulum; TLR, Toll-like receptor; RIG-1, retinoic acid-inducible gene 1; NOD-2, nucleotide-binding oligomerization domain protein 2

Cyclosporin A (CsA), a cyclic non-ribosomal peptide, is usually used as an immunosuppressant in organ transplantation. It has been reported that CsA and its analogues can potentially inhibit the transporter activity of NTCP, thereby blocking HBV entry into hepatocytes [135, 136]. However, it has been found that CsA and its analogues impair sodium-dependent bile acid uptake, thus inducing various adverse events [137]. To identify new compounds that inhibit HBV entry without affecting the NTCP-dependent bile acid uptake, Shimura and his colleagues recently characterized some CsA derivatives and found that SCY450 and SCY995 did not impair

the bile acid uptake and were effective in inhibiting different HBV genotypes and relevant ETV-resistant HBV isolate [138]. Nevertheless, current studies about CsA and its derivatives mostly focus on in vitro experiments; future in vivo studies using animal models and clinical trials are needed.

3.1.2 Therapeutic Approaches Targeting HBV cccDNA

HBV cccDNA is the viral transcription and replication template, and thus its elimination within the hepatocytes is essential for the cure of CHB. Although current anti-HBV therapies such as the use of ETV, TDF, or TAF can potently suppress the HBV DNA to undetectable level, they have little effect on the level and activity of cccDNA within the infected hepatocytes. Therefore, novel therapeutic approaches directly targeting HBV cccDNA are necessary to completely eradicate persistent HBV infections.

APOBEC3 cytidine deaminases are important innate host antiviral factors. It has been reported that the APOBEC3B upregulation triggered by the activation of LTBR can inhibit HBV replication during reverse transcription and degrade cccDNA in the nucleus [139–141]. The inhibition of HBV replication through the activation of the LTBR/APOBEC3 pathway in HBV-infected hepatocytes was recently demonstrated in cell and murine models by using engineered non-lytic T cells expressing HBV-specific T-cell receptors [142]. However, by comparing the intrahepatic cccDNA levels with the expression levels of LTBR and APOBEC3 in the chronically HBV-infected liver biopsy tissues, the activation of the LTBR/APOBEC3 pathway was found to have no major impact on HBV cccDNA metabolism [143]. Future studies are needed to test whether LTBR agonists can degrade cccDNA through the activation of APOBEC3.

Genome-editing technologies including transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) system, which are designed to target specific DNA sequences, represent highly promising therapeutic tools to achieve the ultimate goal of curing CHB [144–146]. TALENs comprise a nonspecific Fok1 nuclease domain fused to a customizable sequence-specific DNA-binding domain: transcription activator-like effectors (TALEs) derived from the plant pathogen *Xanthomonas* [147]. The DNA-binding domain can be easily engineered to target and disrupt essentially any specific DNA sequence. It has been reported that TALENs targeting HBV-specific sites within the viral genome led to targeted disruption of approximately 31% of cccDNA in HepG2.2.15 cells and the reduced viral replication in HBV replication murine model without evident toxicity [148]. Despite encouraging results showing the utilization of TALENs against cccDNA, the safe and efficient delivery of the therapeutic transgenes to the infected hepatocytes and the potential off-target effects must be addressed before reaching the clinic. For example, as HBV DNA sequences are frequently integrated into the host genome, what deleterious effects would happen if the designed TALENs cleaved HBV DNA at these sites of HBV integration [149, 150].

CRISPR/Cas system is derived from the acquired immune system of bacteria and archaea against invading foreign DNA via RNA-guided DNA cleavage. This system can be used flexibly by designing sgRNA to any DNA sequences and thus is more easily customizable than TALENs [145]. Recently, several studies have demonstrated that CRISPR/Cas system is able to disrupt or inactivate HBV cccDNA and integrated HBV genomes [151–153]. Li et al. showed that HBV-specific CRISPR/Cas system mediated removal of the full-length integrated HBV DNA and the disruption of HBV cccDNA in a stable HBV cell line [154]. Moreover, recent characterization of smaller *Staphylococcus aureus* Cas9 (SaCas9) has led to the successful package of the derived CRISPR-SaCas9 system into the adeno-associated virus (AAV) type 8 vector [155]. It was shown that the AAV-delivered CRISPR-SaCas9 could efficiently reduce serum HBsAg and HBeAg in HBV transgenic mice during 58-day continuous observation after vein injection [155]. Very recently, Kostyushev et al. showed that CRISPR/Cas9 system from *Streptococcus pyogenes* (*Sp*) and *Streptococcus thermophilus* (*St*) targeting conserved regions of the HBV genome resulted in degradation of over 90% of HBV cccDNA by 6 days post-transfection. Although deep sequencing revealed that *St*-CRISPR/Cas9 had no effect on the host genome, the *Sp*-CRISPR/Cas9 induced off-target mutagenesis [156].

There are challenging issues need to be solved before utilizing CRISPR/cas9 technology to eliminate HBV cccDNA [157]. Firstly, the risk of the intrinsic off-target effects of CRISPR/Cas9 needs to be eliminated. Ideally, the targeted sequences need to be well-conserved among virus isolates and contain nonhomologous sequences in the human genomes, thus avoiding the off-target effects [158, 159]. Secondly, the efficacy of in vivo delivery of the CRISPR/Cas system into hepatocytes needs to be improved. More efficient viral vectors including AAV or nonviral vectors including lipid-like nanoparticles to deliver CRISPR/Cas9 into the hepatocytes need to be explored [160]. Thirdly, reliable and convenient assays for high-throughput quantification of HBV cccDNA are needed. HBV cccDNA levels in HBV-infected hepatocytes are extremely low; although reverse transcription-polymerase chain reaction (RT-PCR) or Southern blot procedures are currently used in basic research studies, these methods are not completely reliable and are also time-consuming and labor-intensive [161–163]. Therefore, a reliable and efficient assay for cccDNA quantification is necessary for the examination of the CRISPR/Cas9 effect on cccDNA.

3.1.3 RNA Interference

RNA interference (RNAi) is a process by which small interfering RNA molecules of 21–25 nucleotides induce gene silencing at the posttranscriptional level to down-regulate the expression of targeted genes. RNAi-mediated inhibition of gene expression and protein production using synthetic small interfering RNAs (siRNAs) has become a tool in antiviral gene therapy [164–166]. In the past, a major barrier for the clinical application of RNAi was the lack of safe and effective delivery vehicle. Recent developments in RNAi technology have overcome the delivery challenge

[166, 167]. The 2018 FDA approval of the first siRNA therapeutic ONPATTRO™ (patisiran) for the treatment of transthyretin-mediated amyloidosis represents an important milestone in the field of RNAi-based therapies.

The potential of RNAi application in CHB treatment has been demonstrated in murine models [168] and infected chimpanzees [169]. Currently, a phase II clinical trial with the RNAi-based agent ARC-520 (developed by Arrowhead Research Corporation) has been conducted in CHB patients [169]. The RNAi ARC-520 was shown to be safe and well-tolerated and significantly decreased HBsAg levels in treatment-naïve HBeAg-positive CHB patients. However, the HBsAg levels were reduced less significantly in HBeAg-negative or long-term NA treatment-experienced CHB patients. This phenomenon is explained by the finding that HBsAg is expressed not only from the episomal cccDNA mini-chromosome but also from transcripts arising from HBV DNA integrated into the host genome, which is the dominant source in HBeAg-negative patients [169], suggesting that ARC-520 only targets the cccDNA-derived pgRNA rather than the integrated HBV DNA. To overcome the limited efficacy of ARC-520 in HBeAg-negative patients, ARO-HBV (JNJ-3839, targeting two sources of HBsAg) has been developed and assessed by Arrowhead [170]. The results obtained from a phase II trial were announced at the 2019 annual AASLD meeting that monthly usage of ARO-HBV could effectively reduce all viral products, including HBV DNA, HBV RNA, HBeAg, HBcrAg, and HBsAg [171].

AB-729 developed by Arbutus is another RNAi therapeutic targeted to hepatocyte using a novel conjugated N-acetylgalactosamine (GalNAc) delivery technology. It acts on all HBV RNA transcripts, enabling the suppression of all viral antigens, including HBsAg. Preclinical study has demonstrated the anti-HBV activity of AB-729 in vitro [172]. AB-729 phase Ia/Ib clinical trial (AB-729-001) is a single- and multiple-dose clinical trial to investigate the safety, tolerance, pharmacokinetics, and pharmacodynamics of AB-729 subcutaneously administered to healthy subjects and CHB patients.

In addition to the abovementioned RNAi molecules, other RNAi-based agents currently under development are summarized in Table 5.

3.1.4 Capsid Assembly Inhibitors/Modulators

HBV capsid has multiple functions in HBV life cycle, including genome packaging, reverse transcription, and intracellular trafficking, making it an excellent target for development of new antiviral therapy [173]. Small-molecule compounds targeting core protein or capsid, also termed as core assembly modulators (CAMs), can interfere with pgRNA encapsidation, HBV DNA replication by misdirecting or accelerating the formation of capsid-like structures [174]. Based on their different effects on the capsid assembly, CAMs can be categorized into two classes: the type I CAMs, represented by heteroaryldihydropyrimidine (HAP), function to misdirect the formation of aberrant or non-capsid structures; the type II CAMs, represented

Table 5 Developing new drugs for HBV treatment

Developing new drugs for HBV treatment	Category	Mechanism of action	Compound	Development status
New drugs targeting HBV life cycle	Entry inhibitors	Targeting viral entry receptor NTCP	Myrcludex B	II
			Cyclosporin A	Preclinical
	Therapeutic approaches targeting HBV cccDNA	Knockout of HBV cccDNA	ZFNs and TALENs	Preclinical
	RNA interference	Targeting viral sequences and inducing mRNA degradation or translational suppression	ARC-520	II
			ARO-HBV	I/II
			RG6004	I/II
			AB-729	I
			Vir-2218 (ALN-HBV)	Preclinical
			BB-103	Preclinical
			Lunar-HBV	Preclinical
	Capsid assembly inhibitor/ modulator	Inhibiting HBV replication by causing destabilization of viral nucleocapsid	GLS4	II
			JNJ-6379	III
			NVR 3-778	IIa
			ABI-H0731	II
			Bay 41-4109	I
			AB-506	I
			ABI-H2158	I
			RG7907	I
			QL-007	I
			GLP-26	Preclinical
			CB-HBV-001	Preclinical
			ABI-H3773	Preclinical
	New nucleoside/ nucleotide analogues	New nucleoside/ nucleotide analogues	Besifovir	III
			CMX157	IIa
	Ribonuclease H inhibitors	Blocking the production of the plus-polarity DNA strand and leading to the section of biologically inert viral genomes	α-Hydroxytropolones	Preclinical
			N-Hydroxyisoquinolinediones	Preclinical
			N-Hydroxypyridinediones	Preclinical
	HBsAg release inhibitors	Inhibiting the release of HBsAg	REP 2139	II
			REP 2165	II

(continued)

Table 5 (continued)

Developing new drugs for HBV treatment	Category	Mechanism of action	Compound	Development status
Molecules targeting immune response	TLR-7 and TLR-8 agonists	Leading to the production of endogenous cytokines such as IFN to control the virus	RO7020531	II
			GS-9688	II
			GS-9620	Preclinical
	RIG-1/NOD-2 agonists	Eliminate infected hepatocytes, help noninfected hepatocytes establish an antiviral state	SB 9200	II
	Programmed death-1 inhibitors	Blockade PD-1 pathway, promote the proliferation of HBV-specific T cells	PD-1 inhibitors	II
Therapeutic vaccine	Therapeutic vaccine	Stimulating CD_4 and CD_8 T-cell response	HBsAg-HBIG complexes	III
			INO-1800	I
			HB-110	I
			GS-4774	II
			TG-1050	I
			AIC 649	I
			HeP T cell	I

by phenylpropenamides (PPAs) and sulfamoylbenzamides (SBAs), function to accelerate the formation of morphologically intact empty capsids [175, 176].

Antiviral profiling study with BAY41-4109 (HAP) and JNJ-632 (SBA) in primary human hepatocytes has revealed that CAMs not only inhibit HBV replication but also HBV RNA transcription and antigen production, suggesting that CAMs have a dual mechanism of action, inhibiting early and late steps of the viral life cycle [177, 178]. A very recent antiviral profiling study with another CAM molecule NVR 3-778 (SBA) also showed potent anti-HBV activity with a mean EC_{50} of 0.40 μM in HepG2.2.15 cells, and the combination of NVR 3-778 with NAs in vitro resulted in synergistic antiviral activity [174]. Similarly, the potent anti-HBV activity of NVR 3-778 was also shown in a mouse model with HBV-infected humanized liver [179]. NVR 3-778 and Peg-IFN-α in combination showed higher antiviral activity than each compound alone or ETV in the mouse model [179]. In a phase I

clinical study, the combination of NVR 3-778 with Peg-IFN-α led to more reduction of HBV DNA and RNA than monotherapy in HBeAg-positive CHB patients [180]. NVR 3-778 is now in phase II clinical trial.

Other potent CAMs that have also proceeded to clinical trials include JNJ-6379 (SBA) in phase III [181], GLS4 (HAP) in phase II [182], and ABI-H0731 (the first-generation core protein allosteric modifier, CpAM) in phase II (Table 5) [183, 184]. Very recently, through high-throughput screening of an Asinex small-molecule library containing approximately 20,000 compounds, 8 novel structurally distinct CAMs including BA-53038B have been identified [185].

3.1.5 New Nucleoside/Nucleotide Analogues

Current highly potent and low-resistant NAs (ETV, TDF, and TAF) can effectively suppress the serum HBV DNA to undetectable level in the majority of CHB patients. However, if a new NA with even greater inhibition of intrahepatic DNA replication can be developed, it may provide rescue therapy to CHB patients with poor response or drug resistance to current first-line NAs. Several new NAs are currently under different clinical phases of development.

Besifovir (LB80380/BSV), a novel acyclic nucleotide phosphonate with a similar chemical structure to tenofovir, was approved by the Korean Ministry of Food and Drug Safety in 2017 for CHB treatment. The antiviral efficacy of BSV was demonstrated to be similar with that of ETV in a phase IIb multicenter randomized trial [186]. However, the side effect of L-carnitine depletion occurred in 94.1% (64/75) patients receiving BSV treatment [186]. Recently, a phase III clinical trial was conducted in Korea to compare the antiviral efficacy and safety of BSV and TDF in 197 CHB patients [187]. Patients were randomly assigned to groups receiving BSV (150 mg, n = 99) or TDF (300 mg, n = 98) for 48 weeks. After 48 weeks, BSV group continued BSV treatment, whereas the TDF group switched to BSV treatment for an additional 48 weeks. The results showed that at week 48, the rates of virologic responses (HBV DNA <69 IU/mL or 400 copies/ml) were 80.9% and 84.9% in the BSV group and TDF group, respectively. At week 96, 87.2% of patients in the BSV-BSV and 85.7% of patients in the TDF-BSV group achieved virologic response. Thus, the 1- and 2-year treatment outcome of CHB patients with BSV was comparable to that of TDF. There were no BSV-related drug resistance mutations, osteoporosis, or renal toxicity. However, to secure a niche in the field of anti-HBV medicine, the safety and efficacy of BSV from long-term follow-up study is needed [187].

Another new NA CMX157 is currently under phase II clinical trial [184, 188] (Table 5).

3.1.6 Ribonuclease H Inhibitors

In HBV life cycle, the viral pgRNA is encapsidated by the core antigen and is reverse-transcribed by the viral polymerase to minus-strand DNA. During the minus-strand DNA elongation, the viral pgRNA is degraded by ribonuclease H

(RNaseH) to permit the synthesis of plus-strand DNA [189]. Mature capsid particles are either secreted from the cell as virions or cycled back to the nucleus to amplify and/or replenish the cccDNA pool. Inhibitors targeting the RNaseH activity would truncate the minus-strand DNA and block the synthesis of plus-strand DNA, thus blocking the release of infectious virions and the amplification and/or replenishment of the cccDNA pool [190]. Recent low-throughput anti-HBV RNaseH screening pipeline has led to the identification of several chemical classes of potential HBV RNaseH inhibitors including α-hydroxytropolones (α-HT), *N*-hydroxyisoquinolinediones (HID), and N-hydroxypyridinediones (HPD) [191, 192]. These RNaseH inhibitors are promising candidates for developing new anti-HBV drugs and could be used in combination with existing anti-HBV drugs or other novel antivirals under development to improve the functional cure of CHB in the future.

3.1.7 HBsAg Release Inhibitors

HBsAg, the most abundant circulating viral antigen, has been reported to contribute to T-cell tolerance and exhaustion, leading to the attenuation of host immune response [167]. Thus, inhibition of HBsAg release may help to restore the HBV-specific T-cell-mediated immune response. Nucleic acid polymers (NAPs), the phosphorothioated oligonucleotides, have been shown to block the assembly of subviral particles (the primary source of circulating HBsAg), thus inhibiting the release of HBsAg from infected hepatocytes [173, 193]. Recent clinical studies have demonstrated that treatment with the NAPs REP 2139 or its analogue REP 2165 leads to the loss of HBsAg in the majority of CHB patients, regardless of HBeAg status or the presence of HDV coinfection [194–197]. In the open-label, nonrandomized, phase 2 clinical study (REP 301 study), 12 HBeAg-negative, HBV-/HDV-coinfected patients treated with REP 2139-Ca in combination with Peg-IFN-α resulted in 5 patients achieving HBsAg loss and 7 patients achieving HDV RNA negative 1 year after the termination of treatment [194]. Very recently, the final follow-up data from the REP 401 study aiming to assess the safety and efficacy of REP 2139-Mg or REP 2165-Mg (250 mg iv qW) in combination with TDF (300 mg PO qD) and Peg-IFN-α (180 μg SC qW) in 40 HBeAg-negative CHB patients were reported by Replicor Inc. (http://replicor.com/, as of August 2019). The data showed that 40% of participants achieved functional cure of HBV. Meta-analysis of all HBeAg-negative patients in the REP 301 and the REP 401 studies revealed that the extent of transaminase flare activity correlated with significant HBsAg reductions from baseline and greater chance of achieving functional cure. Transitioning REP 2139-Mg from IV to SC administration is expected to improve tolerability, thus enabling higher frequency of administration and further improvement of HBsAg loss. The safety and efficacy of 48 weeks of subcutaneously administered REP 2139-Mg in combination with TDF and Peg-IFN-α against HBV and HDV infections is planned to be assessed in upcoming REP 501 trial (http://replicor.com/, as of August 2019).

3.2 New Agents Modulating Host Immune Response

Host immune responses including innate and adaptive immune response play important roles in the control of chronic HBV infection. Based on the immunopathogenesis of HBV infection, modulating innate or adaptive immunity or both in combination with other direct antiviral drugs to control HBV infection may provide new strategies for CHB treatment. The immunomodulating therapeutic agents under development include TLR-7 and TLR-8 agonists, retinoic acid-inducible gene 1 (RIG-I)/nucleotide-binding oligomerization domain protein 2 (NOD-2) agonists, and programmed death-1 (PD-1) inhibitors, therapeutic vaccines, and others.

3.2.1 TLR-7 and TLR-8 Agonists

Toll-like receptors serve as the first-line defense against invading pathogens [198]. Activation of TLR may help to fight against HBV. TLR agonists (TLR-7 and TLR-8) can induce endogenous interferon production and innate responses, leading to induction of ISGs and other signaling cascades to inhibit HBV replication [199–201]. Currently, the TLR-7 agonists (GS9620, RO7020531) and TLR-8 agonist (GS-9688) are under different phases of clinical trials.

The TLR-7 agonist GS-9620 was shown to induce sustained reduction of HBV viral load and serum HBsAg levels mainly via a type I IFN-α-dependent mechanism in the human hepatocytes [202] and woodchuck [203] and chimpanzee models of CHB [204]. However, in a recent Italian study enrolling 28 CHB patients with HBV suppression (tested negative for HBeAg) by NA therapy, 12-week administration of different doses of GS-9620 (oral, once weekly) appeared to increase T-cell and NK-cell responses, but had no significant effect on HBsAg levels in the enrolled CHB patients [205]. Similarly, in a phase II, double-blind, randomized, placebo-controlled study enrolling 162 CHB patients who are virally suppressed by NA treatment, the administration of GS-9620 resulted in dose-dependent pharmacodynamic induction of ISG15, but had no significant effect on the systemic induction of IFN-α expression and no significant effect on the levels of HBsAg [206]. RO7020531, another TLR-7 agonist, when used in combination with a capsid assembly modulator RO7049389, was reported to significantly reduce the levels of HBV DNA and HBsAg in HBV replication mouse model [207]. At the 2019 APASL annual meeting, RO7020531 was reported to be safe and well-tolerated in healthy volunteers and could induce the increase of IFN-α-related cytokine and ISGs [208]. Further evaluation of the TLR-7 agonist in combination with anti-HBV drugs on the reduction of HBV replication, HBsAg, and cccDNA levels in treating CHB patients is needed.

The TLR-8 agonist GS-9688 was shown to induce sustained efficacy and HBsAg serological conversion in the CHB woodchuck model [209]. A randomized, blind, placebo-controlled study showed that GS-9688 was well-tolerated in CHB patients [210]. Currently, GS-9688 is undergoing phase II clinical trials.

3.2.2 RIG-I/NOD-2 Agonists

RIG-I and NOD-2 are the pattern-recognition receptors recognizing signature patterns of foreign RNA, resulting in the activation of the IFN-α signaling pathway and the subsequent induction of ISGs and pro-inflammatory cytokines [211, 212]. Recently, it was reported that the 5′-ε region of HBV-derived pgRNA is recognized by RIG-I, resulting in the predominant production of type III, but not type I, IFNs in human primary hepatocytes. In addition, the RIG-I was also found to counteract the interaction of HBV polymerase with the 5′-ε region of pgRNA in an RNA-binding dependent manner, resulting in the suppression of HBV replication [212].

The RIG-I agonist SB 9200 (inarigivir) is a novel oral modulator of innate immunity [213]. Inarigivir has been investigated in a phase II multicenter clinical trial (the ACHIEVE study) enrolling 80 treatment-naïve non-cirrhotic CHB patients. The enrolled patients were randomized 4:1 to receive ascending doses of inarigivir (25 mg, 50 mg, 100 mg, and 200 mg) or placebo for 12 weeks, followed by a switch to 300 mg TDF daily for a further 12 weeks. At the 2018 APASL annual meeting, the first cohort inarigivir 25 mg was reported to have good safety and significant antiviral effects on HBV replication [214]. At the 2019 EASL annual meeting, the final results of the ACHIEVE study were reported [215]. It was demonstrated that the reductions of HBV DNA and HBV RNA were observed in both HBeAg-positive and HBeAg-negative patients in a dose-dependent manner, while the extent of HBV RNA reduction was greater in HBeAg-negative patients. The HBsAg response (defined as >0.5 $\log_{10}$ reduction at either 12 or 24 weeks) was seen in 22% of patients, but the HBsAg decline was not dose-dependent. Further investigations of inarigivir at doses of up to 400mg daily in combination with TDF or added to NA-suppressed CHB patients are underway [215].

3.2.3 Programmed Death-1 (PD-1) Inhibitors

Adaptive immune responses are essential in obtaining successful control of viral infection, and T-cell responses are the important contributors [216]. However, virus-specific T cells are exhausted in chronic HBV infection, which is one of the major barriers to eliminating the virus. In the course of chronic HBV infection, overexpressed inhibitory receptors on T cells are associated with dysfunctional T-cell responses. Programmed death receptor 1 (PD-1), the most highly expressed inhibitory receptor on HBV-specific T cells, together with the increased expression of PD-L1 (PD-1's ligand) in HBV-infected hepatocytes likely contributes to the exhaustion of T cells and the high HBV replication levels in CHB patients [217–220]. Thus, blockade of PD-1/PD-L1 pathway would promote the proliferation of HBV-specific T cells, thus restoring the function of T cells to control HBV [217, 221].

Reversing "T-cell exhaustion" through the blockade of the PD-1/PD-L1 pathway to improve specific anti-HBV T-cell responses has been proven in in vitro, HBV mouse and woodchuck models [222, 223]. Very recently, the safety and immunologic efficacy of the PD-1 inhibitor nivolumab were investigated in a phase Ib study

enrolling 24 NA-suppressed HBeAg-negative CHB patients [224]. The enrolled patients were treated with either single dose of nivolumab at 0.1 mg/kg (n = 2) or 0.3 mg/kg (n = 12) or two doses of nivolumab 0.3 mg/kg (at baseline and at week 4) in combination with 40 yeast units of the HBV therapeutic vaccine GS-4774 (n = 10). Twelve weeks after the administration of nivolumab, no significant reduction of HBsAg level was observed in the two patients receiving nivolumab at 0.1 mg/kg. Of the 22 patients who received 0.3 mg/kg nivolumab with or without GS-4774, 20 (91%) had significant HBsAg decline from baseline. One out of the ten patients receiving the combination of nivolumab plus GS-4774 achieved sustained HBsAg loss. Thus, this pilot study supports the inclusion of PD-1/PD-L1 blockade in future combination strategies toward functional cure of chronic HBV infection [224].

In addition to above mentioned immunomodulators, other kinds of immunomodulators including IFN-λ [225], IL-12 [226–228], IL-21 [229, 230], and IL-8 [231] have also been reported to play a role in anti-HBV immunity.

3.2.4 Therapeutic Vaccines

Since the naturally cured HBV infection is accompanied by immune reconstitution [232], stimulation of HBV-specific B- and T-cell immunity by therapeutic vaccination represents a potential approach to overcome the immune tolerance in CHB patients [232]. Different categories of therapeutic vaccines (including protein-based, DNA-based, and vector-based vaccines) have been developed and investigated in both animal models and humans [233, 234]. Of note, most of the therapeutic vaccines in clinical trials are being investigated in combination with current antiviral drugs.

Protein-Based Vaccines

Protein vaccines include subunit vaccines and antigen-antibody complex vaccines. In an open-label and controlled clinical study, 195 HBeAg-positive CHB patients were randomized to receive 12 doses of AS02B-adjuvanted HBsAg vaccine plus LAM daily or LAM daily alone for 52 weeks [235]. However, disappointingly, the combined administration of vaccine and LAM did not demonstrate superior clinical efficacy in HBeAg-positive CHB patients as compared to LAM therapy alone. To increase the antigen-based immune therapy for CHB, a vaccine formulation containing both HBsAg and HBcAg (designated as HeberNasvac) was developed [236]. In a recent open-label phase III study, 160 CHB patients were randomized 1:1 to receive 10 doses of HeberNasvac (100 μg HBsAg and 100 μg HBcAg per dose) via nasal spray or 48 subcutaneous injections of Peg-IFN-α (180 μg per dose) [237]. The HBeAg loss was found to be more frequent in the HeberNasvac group as compared to the Peg-IFN-α group, but the antiviral effect was comparable in the two groups.

The antigen-antibody (HBsAg-HBIG) complex therapeutic vaccine with alum as adjuvant showed promising results in a double-blind, placebo-controlled, phase IIb

clinical trial [238], but results from a phase III clinical trial enrolling 450 CHB patients treated with alum-adjuvanted HBsAg-HBIG or alum alone were disappointing as no significant difference in reduction of HBV DNA level and normalization of liver function was observed in the two groups [239]. The unfavorable results from the therapeutic protein vaccines are most likely due to the fact that those vaccines preferentially induce antibody but not cytotoxic T-cell responses [233].

DNA-Based Vaccines

DNA-based vaccines encoding HBV envelope proteins were designed to induce HBV-specific T cells. INO-1800, a multi-antigen DNA vaccine encoding HBsAg and a consensus sequence of HBcAg, is now in phase I clinical trial administered with or without INO-9112 encoding human IL-12 in 90 CHB patients either on ETV or TDF treatment [240]. HB-110, another multi-antigen DNA vaccine encoding HBsAg, PreS1Ag, HBcAg, HBV polymerase, and human IL-12, was recently investigated in a phase I clinical trial [241]. The enrolled 27 ADV-treated CHB patients were randomized to receive a combination of HB-110 plus ADV or ADV alone. The results showed that the HB-110 add-on to ADV did not show greater T-cell responses and HBeAg loss than ADV alone [241]. Both INO-1800 and HB-110 vaccines need to be administered via in vivo electroporation to enhance vaccine antigen expression and immunogenicity, and the purpose of adding IL-12 as an adjuvant was aimed to rescue the antiviral function of exhausted HBV-specific T cells.

Vector-Based Vaccines

The currently studied vector-based therapeutic vaccines mainly include the yeast-based vaccine GS-4774 [242, 243] and the adenovirus-based vaccine TG1050 [244, 245].

GS-4774 is a recombinant, heat-killed, *Saccharomyces cerevisiae* yeast-based vaccine expressing HBV-specific antigens including HBsAg, HBcAg, and HBx. In a randomized, open-label, phase II study of GS-4774, 178 non-cirrhotic CHB patients who were virally suppressed by NAs were randomized 1:2:2:2 to receive NA alone or NA plus GS-4774 at the doses of 2, 10, or 40 yeast units subcutaneously every 4 weeks until week 20 [243]. It was shown that GS-4774 was safe and well-tolerated but did not provide significant reductions in serum HBsAg in those treatment-experienced CHB patients. In another phase II study, the safety and efficacy of GS-4774 alone or in combination with TDF were investigated in 195 treatment-naïve CHB patients [242]. Although GS-4774 in combination with TDF was able to induce a strong immunomodulatory effect (the increased production of IFN-γ, TNF-α, and IL-2 by CD8+ T cell), it did not reduce the HBsAg levels in those treatment-naïve CHB patients, either, suggesting that GS-4774 might be used in combination with other antiviral agents to boost the anti-HBV immune response [242].

TG1050 consists of a non-replicative adenovirus 5 vector encoding a unique large fusion protein composed of a modified HBV core, a modified HBV polymerase, and selected domains of the envelope proteins [193]. Injection of TG1050 was able to induce a robust T-cell response and to exert an antiviral effect in HBV-naïve and HBV-persistent mouse models [244]. The safety and efficacy of TG1050 in CHB patients under NAs were assessed in a phase Ib clinical trial [245]. TG1050 was found to have a good safety profile and capable of inducing HBV-specific cellular immune response. Further clinical evaluation of TG1050 in combination with other anti-HBV agents is needed [245].

4 Conclusions

In conclusion, over the past 20 years, tremendous progress has been made in the understanding of HBV pathogenesis and the treatment of CHB. The advent of NAs has made CHB an easily treatable disease. The current anti-HBV therapy with potent and high genetic barrier NAs (ETV, TDF, and TAF) can suppress the viral replication to undetectable level in the majority of CHB patients, preventing the progression of CHB to cirrhosis and markedly decreasing the rates of HBV-related HCC. The combination of NAs and Peg-IFN-α even makes the functional cure of CHB possible in selected patients with low baseline HBsAg level and on-treatment HBsAg response to Peg-IFN-α. To increase the rate of functional cure of CHB, a combination of the existing anti-HBV drugs and one or more of the abovementioned new antiviral agents, either the direct antiviral drugs targeting the different steps of HBV life cycle or the indirect antiviral drugs modulating the host immune responses, will be necessary. With the concerted efforts of basic research scientists and clinical experts from both professional societies and pharmaceutical companies, the ultimate eradication of HBV infection is likely to be achieved in the foreseeable future.

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References

1. European Association for the Study of the Liver (2017) Electronic address eee, European Association for the Study of the L. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 67(2):370–398
2. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH et al (2016) AASLD guidelines for treatment of chronic hepatitis B. Hepatology (Baltimore, MD) 63(1):261–283

3. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ et al (2006) Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 130(3):678–686
4. Chen CF, Lee WC, Yang HI, Chang HC, Jen CL, Iloeje UH et al (2011) Changes in serum levels of HBV DNA and alanine aminotransferase determine risk for hepatocellular carcinoma. Gastroenterology 141(4).:1240–8, 8 e1–2
5. Sinn DH, Lee J, Goo J, Kim K, Gwak GY, Paik YH et al (2015) Hepatocellular carcinoma risk in chronic hepatitis B virus-infected compensated cirrhosis patients with low viral load. Hepatology 62(3):694–701
6. Grossi G, Vigano M, Loglio A, Lampertico P (2017) Hepatitis B virus long-term impact of antiviral therapy nucleot(s)ide analogues (NUCs). Liver Int 37(Suppl 1):45–51
7. Wu CY, Lin JT, Ho HJ, Su CW, Lee TY, Wang SY et al (2014) Association of nucleos(t)ide analogue therapy with reduced risk of hepatocellular carcinoma in patients with chronic hepatitis B: a nationwide cohort study. Gastroenterology 147(1):143–51 e5
8. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H et al (2004) Lamivudine for patients with chronic hepatitis B and advanced liver disease. New Engl J Med 351(15):1521–1531
9. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN et al (2006) Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 295(1):65–73
10. Seto WK, Hui AJ, Wong VW, Wong GL, Liu KS, Lai CL et al (2015) Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative chronic hepatitis B: a multicentre prospective study. Gut 64(4):667–672
11. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM et al (2018) Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology (Baltimore, MD) 67(4):1560–1599
12. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ et al (2016) Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 10(1):1–98
13. EASL (2017) 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 67(2):370–398
14. Zhang J, Wen XY, Gao RP (2019) Hepatitis B virus-related liver cirrhosis complicated with dermatomyositis: a case report. World J Clin Cases 7(10):1206–1212
15. Nemoto M, Nishioka K, Fukuoka J, Aoshima M (2019) Hepatitis B virus-associated vasculitis: a case report of multiple cavitary masses in the lung mimicking granulomatous polyangiitis. Intern Med. https://doi.org/10.2169/internalmedicine.3012-19
16. Wong GL (2018) Management of chronic hepatitis B patients in immunetolerant phase: what latest guidelines recommend. Clin Mol Hepatol 24(2):108–113
17. Hou J, Wang G, Wang F, Cheng J, Ren H, Zhuang H et al (2017) Guideline of prevention and treatment for chronic hepatitis B (2015 update). J Clin Transl Hepatol 5(4):297–318
18. Menendez-Arias L, Alvarez M, Pacheco B (2014) Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance. Curr Opin Virol 8:1–9
19. Koumbi L (2015) Current and future antiviral drug therapies of hepatitis B chronic infection. World J Hepatol 7(8):1030–1040
20. Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY et al (2000) Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. Gastroenterology 119(1):172–180
21. Ayoub WS, Keeffe EB (2011) Review article: current antiviral therapy of chronic hepatitis B. Aliment Pharmacol Ther 34(10):1145–1158
22. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML et al (2003) Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 348(9):808–816
23. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M et al (2003) Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med 348(9):800–807
24. Salpini R, Alteri C, Cento V, Pollicita M, Micheli V, Gubertini G et al (2013) Snapshot on drug-resistance rate and profiles in patients with chronic hepatitis B receiving nucleos(t)ide analogues in clinical practice. J Med Virol 85(6):996–1004

25. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M et al (2006) Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 131(6):1743–1751
26. Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL et al (2009) 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology 136(2):486–495
27. Han GR, Cao MK, Zhao W, Jiang HX, Wang CM, Bai SF et al (2011) A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for the prevention of perinatal transmission of hepatitis B virus infection. J Hepatol 55(6):1215–1221
28. Han GR, Jiang HX, Yue X, Ding Y, Wang CM, Wang GJ et al (2015) Efficacy and safety of telbivudine treatment: an open-label, prospective study in pregnant women for the prevention of perinatal transmission of hepatitis B virus infection. J Viral Hepat 22(9):754–762
29. Marcellin P, Chang TT, Lim SG, Sievert W, Tong M, Arterburn S et al (2008) Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology (Baltimore, MD) 48(3):750–758
30. Seto WK, Lai CL, Fung J, Wong DK, Yuen JC, Hung IF et al (2011) Significance of HBV DNA levels at 12 weeks of telbivudine treatment and the 3 years treatment outcome. J Hepatol 55(3):522–528
31. Kim KH, Kim ND, Seong BL (2010) Discovery and development of anti-HBV agents and their resistance. Molecules 15(9):5878–5908
32. Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L et al (2004) Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to Lamivudine. Antimicrob Agents Chemother 48(9):3498–3507
33. Van Rompay KK, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K, Cihlar T et al (2008) Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. Antimicrob Agents Chemother 52(9):3144–3160
34. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM et al (2002) Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 40(6):1331–1333
35. Magalhaes-Costa P, Matos L, Barreiro P, Chagas C (2015) Fanconi syndrome and chronic renal failure in a chronic hepatitis B monoinfected patient treated with tenofovir. Rev Esp Enferm Dig 107(8):512–514
36. Hsu YC, Wei MT, Nguyen MH (2017) Tenofovir alafenamide as compared to tenofovir disoproxil fumarate in the management of chronic hepatitis B with recent trends in patient demographics. Expert Rev Gastroenterol Hepatol 11(11):999–1008
37. Yang HC, Kao JH (2014) Persistence of hepatitis B virus covalently closed circular DNA in hepatocytes: molecular mechanisms and clinical significance. Emerg Microb Infect 3(9):e64
38. Dill MT, Makowska Z, Trincucci G, Gruber AJ, Vogt JE, Filipowicz M et al (2014) Pegylated IFN-alpha regulates hepatic gene expression through transient Jak/STAT activation. J Clin Invest 124(4):1568–1581
39. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC et al (2006) A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 354(10):1001–1010
40. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z et al (2006) Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 354(10):1011–1020
41. Arends P, Sonneveld MJ, Zoutendijk R, Carey I, Brown A, Fasano M et al (2015) Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians. Gut 64(8):1289–1295
42. Luo J, Li X, Wu Y, Lin G, Pang Y, Zhang X et al (2013) Efficacy of entecavir treatment for up to 5 years in nucleos(t)ide-naive chronic hepatitis B patients in real life. Int J Med Sci 10(4):427–433

43. Maklad S, Reyad EM, William EA, Abouzeid A (2018) Efficacy and safety of entecavir 0.5 mg in treating naive chronic hepatitis B virus patients in Egypt: five years of real life experience. Gastroenterol Res 11(2):138–144
44. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z et al (2008) Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 359(23):2442–2455
45. Marcellin P, Zoulim F, Hezode C, Causse X, Roche B, Truchi R et al (2016) Effectiveness and safety of tenofovir disoproxil fumarate in chronic hepatitis B: a 3-year, prospective, real-world study in France. Dig Dis Sci 61(10):3072–3083
46. Buti M, Gane E, Seto WK, Chan HL, Chuang WL, Stepanova T et al (2016) Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of patients with HBeAg-negative chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol 1(3):196–206
47. Agarwal K, Brunetto M, Seto WK, Lim YS, Fung S, Marcellin P et al (2018) 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. J Hepatol 68(4):672–681
48. Nguyen NH, Trinh HN, Nguyen TT, Do ST, Tran P, Nguyen HA et al (2015) Safety and efficacy of entecavir in adefovir-experienced patients. J Gastroenterol Hepatol 30(1):43–50
49. Zoulim F, Locarnini S (2009) Hepatitis B virus resistance to nucleos(t)ide analogues. Gastroenterology 137(5):1593–608 e1-2
50. Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J et al (2009) Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. Hepatology 49(5):1503–1514
51. Deng XL, Li QL, Guo JJ (2013) Dynamics of lamivudine-resistant hepatitis B virus strains in patients with entecavir rescue therapy. Virus Genes 47(1):1–9
52. Yuen MF, Lai CL (2011) Treatment of chronic hepatitis B: evolution over two decades. J Gastroenterol Hepatol 26(Suppl 1):138–143
53. Petersen J, Heyne R, Mauss S, Schlaak J, Schiffelholz W, Eisenbach C et al (2016) Effectiveness and safety of tenofovir disoproxil fumarate in chronic hepatitis B: A 3-year prospective field practice study in Germany. Dig Dis Sci 61(10):3061–3071
54. Chung GE, Cho EJ, Lee JH, Yoo JJ, Lee M, Cho Y et al (2017) Tenofovir has inferior efficacy in adefovir-experienced chronic hepatitis B patients compared to nucleos(t)ide-naive patients. Clin Mol Hepatol 23(1):66–73
55. Park JY, Kim CW, Bae SH, Jung KS, Kim HY, Yoon SK et al (2016) Entecavir plus tenofovir combination therapy in patients with multidrug-resistant chronic hepatitis B: results of a multicentre, prospective study. Liver Int 36(8):1108–1115
56. Lee YB, Lee JH, Lee DH, Cho H, Ahn H, Choi WM et al (2014) Efficacy of entecavir-tenofovir combination therapy for chronic hepatitis B patients with multidrug-resistant strains. Antimicrob Agents Chemother 58(11):6710–6716
57. Konerman MA, Lok AS (2016) Interferon treatment for hepatitis B. Clin Liver Dis 20(4):645–665
58. European Association for the Study of the L (2012) EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 57(1):167–185
59. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G et al (2005) Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 352(26):2682–2695
60. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R et al (2004) Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 351(12):1206–1217
61. Liaw YF, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ et al (2012) Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 6(3):531–561
62. Li SY, Li H, Xiong YL, Liu F, Peng ML, Zhang DZ et al (2017) Peginterferon is preferable to entecavir for prevention of unfavourable events in patients with HBeAg-positive chronic hepatitis B: a five-year observational cohort study. J Viral Hepat 24(Suppl 1):12–20

63. Stelma F, van der Ree MH, Jansen L, Peters MW, Janssen HLA, Zaaijer HL et al (2017) HBsAg loss after peginterferon-nucleotide combination treatment in chronic hepatitis B patients: 5 years of follow-up. J Viral Hepat 24(12):1107–1113
64. Marcellin P, Wursthorn K, Wedemeyer H, Chuang WL, Lau G, Avila C et al (2015) Telbivudine plus pegylated interferon alfa-2a in a randomized study in chronic hepatitis B is associated with an unexpected high rate of peripheral neuropathy. J Hepatol 62(1):41–47
65. Marcellin P, Ahn SH, Chuang WL, Hui AJ, Tabak F, Mehta R et al (2016) Predictors of response to tenofovir disoproxil fumarate plus peginterferon alfa-2a combination therapy for chronic hepatitis B. Aliment Pharmacol Ther 44(9):957–966
66. Ahn SH, Marcellin P, Ma X, Caruntu FA, Tak WY, Elkhashab M et al (2018) Hepatitis B surface antigen loss with tenofovir disoproxil fumarate plus peginterferon alfa-2a: week 120 analysis. Dig Dis Sci 63(12):3487–3497
67. Marcellin P, Ahn SH, Ma X, Caruntu FA, Tak WY, Elkashab M et al (2016) Combination of tenofovir disoproxil fumarate and peginterferon alpha-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B. Gastroenterology 150(1):134–44 e10
68. de Niet A, Jansen L, Stelma F, Willemse SB, Kuiken SD, Weijer S et al (2017) Peg-interferon plus nucleotide analogue treatment versus no treatment in patients with chronic hepatitis B with a low viral load: a randomised controlled, open-label trial. Lancet Gastroenterol Hepatol 2(8):576–584
69. Bourliere M, Rabiega P, Ganne-Carrie N, Serfaty L, Marcellin P, Barthe Y et al (2017) Effect on HBs antigen clearance of addition of pegylated interferon alfa-2a to nucleos(t)ide analogue therapy versus nucleos(t)ide analogue therapy alone in patients with HBe antigen-negative chronic hepatitis B and sustained undetectable plasma hepatitis B virus DNA: a randomised, controlled, open-label trial. Lancet Gastroenterol Hepatol 2(3):177–188
70. Ning Q, Han M, Sun Y, Jiang J, Tan D, Hou J et al (2014) Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). J Hepatol 61(4):777–784
71. Hu P, Shang J, Zhang W, Gong G, Li Y, Chen X et al (2018) HBsAg loss with Peg-interferon alfa-2a in hepatitis B patients with partial response to nucleos(t)ide analog: new switch study. J Clin Transl Hepatol 6(1):25–34
72. Brouwer WP, Xie Q, Sonneveld MJ, Zhang N, Zhang Q, Tabak F et al (2015) Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: a multicenter randomized trial (ARES study). Hepatology (Baltimore, MD) 61(5):1512–1522
73. van Campenhout MJH, Brouwer WP, Xie Q, Guo S, Chi H, Qi X et al (2019) Long-term follow-up of patients treated with entecavir and peginterferon add-on therapy for HBeAg-positive chronic hepatitis B infection: ARES long-term follow-up. J Viral Hepat 26(1):109–117
74. Kim CH, Um SH, Seo YS, Jung JY, Kim JD, Yim HJ et al (2012) Prognosis of hepatitis B-related liver cirrhosis in the era of oral nucleos(t)ide analog antiviral agents. J Gastroenterol Hepatol 27(10):1589–1595
75. Shin SK, Kim JH, Park H, Kwon OS, Lee HJ, Yeon JE et al (2015) Improvement of liver function and non-invasive fibrosis markers in hepatitis B virus-associated cirrhosis: 2 years of entecavir treatment. J Gastroenterol Hepatol 30(12):1775–1781
76. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM et al (2013) Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 381(9865):468–475
77. Shim JH, Lee HC, Kim KM, Lim YS, Chung YH, Lee YS et al (2010) Efficacy of entecavir in treatment-naive patients with hepatitis B virus-related decompensated cirrhosis. J Hepatol 52(2):176–182
78. Lee SK, Song MJ, Kim SH, Lee BS, Lee TH, Kang YW et al (2017) Safety and efficacy of tenofovir in chronic hepatitis B-related decompensated cirrhosis. World J Gastroenterol 23(13):2396–2403
79. Papatheodoridis GV, Idilman R, Dalekos GN, Buti M, Chi H, van Boemmel F et al (2017) The risk of hepatocellular carcinoma decreases after the first 5 years of entecavir or tenofovir in Caucasians with chronic hepatitis B. Hepatology 66(5):1444–1453

80. Wong GL, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT et al (2013) Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. Hepatology 58(5):1537–1547
81. Shim JJ, Oh CH, Kim JW, Lee CK, Kim BH (2017) Liver cirrhosis stages and the incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving antiviral therapy. Scand J Gastroenterol 52(9):1029–1036
82. Yang Y, Wen F, Li J, Zhang P, Yan W, Hao P et al (2015) A high baseline HBV load and antiviral therapy affect the survival of patients with advanced HBV-related HCC treated with sorafenib. Liver Int 35(9):2147–2154
83. Dan JQ, Zhang YJ, Huang JT, Chen MS, Gao HJ, Peng ZW et al (2013) Hepatitis B virus reactivation after radiofrequency ablation or hepatic resection for HBV-related small hepatocellular carcinoma: a retrospective study. Eur J Surg Oncol 39(8):865–872
84. Zhang H, Zhou Y, Yuan G, Zhou G, Yang D, Zhou Y (2015) Antiviral therapy improves the survival rate and decreases recurrences and fatalities in liver cancer patients following curative resection: a meta-analysis. Mol Clin Oncol 3(6):1239–1247
85. Fontana RJ, Ellerbe C, Durkalski VE, Rangnekar A, Reddy RK, Stravitz T et al (2015) Two-year outcomes in initial survivors with acute liver failure: results from a prospective, multicentre study. Liver Int 35(2):370–380
86. Chinese Society of Hepatology CMA, Chinese Society of Infectious Diseases CMA, Hou JL, lai W (2015) The guideline of prevention and treatment for chronic hepatitis B: a 2015 update. Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology 23(12):888–905
87. Zhang X, Liu L, Zhang M, Gao S, Du Y, An Y et al (2015) The efficacy and safety of entecavir in patients with chronic hepatitis B- associated liver failure: a meta-analysis. Ann Hepatol 14(2):150–160
88. Wan YM, Li YH, Xu ZY, Wu HM, Xu Y, Wu XN et al (2019) Tenofovir versus entecavir for the treatment of acute-on-chronic liver failure due to reactivation of chronic hepatitis B with genotypes B and C. J Clin Gastroenterol 53(4):e171–e1e7
89. Yuen MF (2015) Anti-viral therapy in hepatitis B virus reactivation with acute-on-chronic liver failure. Hepatol Int 9(3):373–377
90. Ishigami M, Ogura Y, Hirooka Y, Goto H (2015) Change of strategies and future perspectives against hepatitis B virus recurrence after liver transplantation. World J Gastroenterol 21(36):10290–10298
91. Marzano A, Gaia S, Ghisetti V, Carenzi S, Premoli A, Debernardi-Venon W et al (2005) Viral load at the time of liver transplantation and risk of hepatitis B virus recurrence. Liver Trans 11(4):402–409
92. Protzer-Knolle U, Naumann U, Bartenschlager R, Berg T, Hopf U, Meyer zum Buschenfelde KH et al (1998) Hepatitis B virus with antigenically altered hepatitis B surface antigen is selected by high-dose hepatitis B immune globulin after liver transplantation. Hepatology 27(1):254–263
93. European Association for the Study of the Liver (2016) Electronic address eee. EASL Clinical Practice Guidelines: liver transplantation. J Hepatol 64(2):433–485
94. Radhakrishnan K, Chi A, Quan DJ, Roberts JP, Terrault NA (2017) Short course of postoperative hepatitis B immunoglobulin plus antivirals prevents reinfection of liver transplant recipients. Transplantation 101(9):2079–2082
95. Cholongitas E, Goulis I, Antoniadis N, Fouzas I, Imvrios G, Papanikolaou V et al (2014) New nucleos(t)ide analogue monoprophylaxis after cessation of hepatitis B immunoglobulin is effective against hepatitis B recurrence. Transpl Int 27(10):1022–1028
96. Fung J, Wong T, Chok K, Chan A, Cheung TT, Dai JW et al (2017) Long-term outcomes of entecavir monotherapy for chronic hepatitis B after liver transplantation: results up to 8 years. Hepatology 66(4):1036–1044
97. Xu X, Chen J, Wei Q, Liu ZK, Yang Z, Zhang M et al (2019) Clinical practice guidelines on liver transplantation for hepatocellular carcinoma in China (2018 edition). Hepatobiliary Pancreat Dis Int 18(4):307–312

98. Roche B, Samuel D (2011) The difficulties of managing severe hepatitis B virus reactivation. Liv Int 31(Suppl 1):104–110
99. Hoofnagle JH (2009) Reactivation of hepatitis B. Hepatology 49(5 Suppl):S156–S165
100. Bessone F, Dirchwolf M (2016) Management of hepatitis B reactivation in immunosuppressed patients: an update on current recommendations. World J Hepatol 8(8):385–394
101. Hsu C, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL et al (2014) Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. Hepatology 59(6):2092–2100
102. WHO (2017) Global hepatitis report, 2017. World Health Organization, Geneva
103. Indolfi GEP, Dusheiko G, Siberry G, Chang MH, Thorne C et al (2019) Hepatitis B virus infection in children and adolescents. Lancet Gastroenterol Hepatol
104. Sokal EM, Paganelli M, Wirth S, Socha P, Vajro P, Lacaille F et al (2013) Management of chronic hepatitis B in childhood: ESPGHAN clinical practice guidelines: consensus of an expert panel on behalf of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. J Hepatol 59(4):814–829
105. Takano T, Tajiri H, Etani Y, Miyoshi Y, Tanaka Y, Brooks S (2015) Natural history of chronic hepatitis B virus infection in childhood and efficacy of interferon therapy. Scand J Gastroenterol 50(7):892–899
106. Sokal EM, Conjeevaram HS, Roberts EA, Alvarez F, Bern EM, Goyens P et al (1998) Interferon alfa therapy for chronic hepatitis B in children: a multinational randomized controlled trial. Gastroenterology 114(5):988–995
107. Kasirga E (2015) Lamivudine resistance in children with chronic hepatitis B. World J Hepatol 7(6):896–902
108. Pawlowska M, Domagalski K, Smok B, Rajewski P, Wietlicka-Piszcz M, Halota W et al (2016) Continuous up to 4 years entecavir treatment of HBV-infected adolescents – a longitudinal study in real life. PLoS One 11(9):e0163691
109. Murray KF, Szenborn L, Wysocki J, Rossi S, Corsa AC, Dinh P et al (2012) Randomized, placebo-controlled trial of tenofovir disoproxil fumarate in adolescents with chronic hepatitis B. Hepatology 56(6):2018–2026
110. Zhou K, Terrault N (2017) Management of hepatitis B in special populations. Best Pract Res Clin Gastroenterol 31(3):311–320
111. Xu WM, Cui YT, Wang L, Yang H, Liang ZQ, Li XM et al (2009) Lamivudine in late pregnancy to prevent perinatal transmission of hepatitis B virus infection: a multicentre, randomized, double-blind, placebo-controlled study. J Viral Hepat 16(2):94–103
112. Pan CQ, Han GR, Jiang HX, Zhao W, Cao MK, Wang CM et al (2012) Telbivudine prevents vertical transmission from HBeAg-positive women with chronic hepatitis B. Clin Gastroenterol Hepatol 10(5):520–526
113. Lin Y, Liu Y, Ding G, Touqui L, Wang W, Xu N et al (2018) Efficacy of tenofovir in preventing perinatal transmission of HBV infection in pregnant women with high viral loads. Sci Rep 8(1):15514
114. Yi W, Pan CQ, Hao J, Hu Y, Liu M, Li L et al (2014) Risk of vertical transmission of hepatitis B after amniocentesis in HBs antigen-positive mothers. J Hepatol 60(3):523–529
115. Wu Q, Huang H, Sun X, Pan M, He Y, Tan S et al (2015) Telbivudine prevents vertical transmission of hepatitis B virus from women with high viral loads: a prospective long-term study. Clin Gastroenterol Hepatol 13(6):1170–1176
116. Pan CQ, Duan Z, Dai E, Zhang S, Han G, Wang Y et al (2016) Tenofovir to prevent hepatitis B transmission in mothers with high viral load. N Engl J Med 374(24):2324–2334
117. Wang M, Bian Q, Zhu Y, Pang Q, Chang L, Li R et al (2019) Real-world study of tenofovir disoproxil fumarate to prevent hepatitis B transmission in mothers with high viral load. Aliment Pharmacol Ther 49(2):211–217
118. Zampino R, Pisaturo MA, Cirillo G, Marrone A, Macera M, Rinaldi L et al (2015) Hepatocellular carcinoma in chronic HBV-HCV co-infection is correlated to fibrosis and disease duration. Ann Hepatol 14(1):75–82

119. Pol S, Haour G, Fontaine H, Dorival C, Petrov-Sanchez V, Bourliere M et al (2017) The negative impact of HBV/HCV coinfection on cirrhosis and its consequences. Aliment Pharmacol Ther 46(11–12):1054–1060
120. Holmes JA, Yu ML, Chung RT (2017) Hepatitis B reactivation during or after direct acting antiviral therapy – implication for susceptible individuals. Expert Opin Drug Saf 16(6):651–672
121. Sagnelli E, Sagnelli C, Macera M, Pisaturo M, Coppola N (2017) An update on the treatment options for HBV/HCV coinfection. Expert Opin Pharmacother 18(16):1691–1702
122. European Association for the Study of the Liver (2018) Electronic address eee, European Association for the Study of the L. EASL Recommendations on Treatment of Hepatitis C 2018. J Hepatol 69(2):461–511
123. Konopnicki D, Mocroft A, de Wit S, Antunes F, Ledergerber B, Katlama C et al (2005) Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. AIDS 19(6):593–601
124. Thio CL (2009) Hepatitis B and human immunodeficiency virus coinfection. Hepatology 49(5 Suppl):S138–S145
125. Gunthard HF, Aberg JA, Eron JJ, Hoy JF, Telenti A, Benson CA et al (2014) Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society-USA Panel. JAMA 312(4):410–425
126. Liu F, Wang XW, Chen L, Hu P, Ren H, Hu HD (2016) Systematic review with meta-analysis: development of hepatocellular carcinoma in chronic hepatitis B patients with hepatitis B surface antigen seroclearance. Aliment Pharmacol Ther 43(12):1253–1261
127. Kim GA, Lee HC, Kim MJ, Ha Y, Park EJ, An J et al (2015) Incidence of hepatocellular carcinoma after HBsAg seroclearance in chronic hepatitis B patients: a need for surveillance. J Hepatol 62(5):1092–1099
128. Wong DK, Seto WK, Cheung KS, Chong CK, Huang FY, Fung J et al (2017) Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA. Liver Int 37(7):995–1001
129. Suzuki F, Miyakoshi H, Kobayashi M, Kumada H (2009) Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol 81(1):27–33
130. Giersch K, Allweiss L, Volz T, Dandri M, Lutgehetmann M (2017) Serum HBV pgRNA as a clinical marker for cccDNA activity. J Hepatol 66(2):460–462
131. Yan H, Zhong GC, Xu GW, He WH, Jing ZY, Gao ZC et al (2012) Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. elife 1
132. Volz T, Allweiss L, Ben MM, Warlich M, Lohse AW, Pollok JM et al (2013) The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. J Hepatol 58(5):861–867
133. Blank A, Markert C, Hohmann N, Carls A, Mikus G, Lehr T et al (2016) First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. J Hepatol 65(3):483–489
134. Late-breaking abstracts (2014) Hepatology (Baltimore, MD) 60(6):1267A–1290A
135. Nkongolo S, Ni Y, Lempp FA, Kaufman C, Lindner T, Esser-Nobis K et al (2014) Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. J Hepatol 60(4):723–731
136. Watashi K, Sluder A, Daito T, Matsunaga S, Ryo A, Nagamori S et al (2014) Cyclosporin A and its analogs inhibit hepatitis B virus entry into cultured hepatocytes through targeting a membrane transporter, sodium taurocholate cotransporting polypeptide (NTCP). Hepatology (Baltimore, MD) 59(5):1726–1737
137. Vaz FM, Paulusma CC, Huidekoper H, de Ru M, Lim C, Koster J et al (2015) Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. Hepatology (Baltimore, MD) 61(1):260–267
138. Shimura S, Watashi K, Fukano K, Peel M, Sluder A, Kawai F et al (2017) Cyclosporin derivatives inhibit hepatitis B virus entry without interfering with NTCP transporter activity. J Hepatol 66(4):685–692

139. Janahi EM, McGarvey MJ (2013) The inhibition of hepatitis B virus by APOBEC cytidine deaminases. J Viral Hepat 20(12):821–828
140. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X et al (2014) Specific and non-hepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science (New York, NY) 343(6176):1221–1228
141. Chen Y, Hu J, Cai X, Huang Y, Zhou X, Tu Z et al (2018) APOBEC3B edits HBV DNA and inhibits HBV replication during reverse transcription. Antivir Res 149:16–25
142. Koh S, Kah J, Tham CYL, Yang N, Ceccarello E, Chia A et al (2018) Nonlytic Lymphocytes Engineered to Express Virus-Specific T-Cell Receptors Limit HBV Infection by Activating APOBEC3. Gastroenterology 155(1):180–93.e6
143. Meier MA, Suslov A, Ketterer S, Heim MH, Wieland SF (2017) Hepatitis B virus covalently closed circular DNA homeostasis is independent of the lymphotoxin pathway during chronic HBV infection. J Viral Hepat 24(8):662–671
144. Gaj T, Gersbach CA, Barbas CF 3rd (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31(7):397–405
145. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32(4):347–355
146. Schiffer JT, Aubert M, Weber ND, Mintzer E, Stone D, Jerome KR (2012) Targeted DNA mutagenesis for the cure of chronic viral infections. J Virol 86(17):8920–8936
147. Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14(1):49–55
148. Bloom K, Ely A, Mussolino C, Cathomen T, Arbuthnot P (2013) Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. Mol Ther 21(10):1889–1897
149. Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M et al (2008) Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell 31(2):294–301
150. Holkers M, Maggio I, Liu J, Janssen JM, Miselli F, Mussolino C et al (2013) Differential integrity of TALE nuclease genes following adenoviral and lentiviral vector gene transfer into human cells. Nucleic Acids Res 41(5):e63
151. Kennedy EM, Kornepati AV, Cullen BR (2015) Targeting hepatitis B virus cccDNA using CRISPR/Cas9. Antivir Res 123:188–192
152. Lin SR, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC et al (2014) The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. Molecular therapy Nucleic acids 3:e186
153. Dong C, Qu L, Wang H, Wei L, Dong Y, Xiong S (2015) Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. Antivir Res 118:110–117
154. Li H, Sheng C, Wang S, Yang L, Liang Y, Huang Y et al (2017) Removal of integrated hepatitis B virus DNA using CRISPR-Cas9. Front Cell Infect Microbiol 7:91
155. Li H, Sheng C, Liu H, Wang S, Zhao J, Yang L et al (2018) Inhibition of HBV expression in HBV transgenic mice using AAV-delivered CRISPR-SaCas9. Front Immunol 9:2080
156. Kostyushev D, Kostyusheva A, Brezgin S, Zarifyan D, Utkina A, Goptar I et al (2019) Suppressing the NHEJ pathway by DNA-PKcs inhibitor NU7026 prevents degradation of HBV cccDNA cleaved by CRISPR/Cas9. Sci Rep 9(1):1847
157. Yang HC, Chen PJ (2018) The potential and challenges of CRISPR-Cas in eradication of hepatitis B virus covalently closed circular DNA. Virus Res 244:304–310
158. Choi YM, Lee SY, Kim BJ (2019) Naturally occurring hepatitis B virus mutations leading to endoplasmic reticulum stress and their contribution to the progression of hepatocellular carcinoma. Int J Mol Sci 20(3)
159. Sung FY, Lan CY, Huang CJ, Lin CL, Liu CJ, Chen PJ et al (2016) Progressive accumulation of mutations in the hepatitis B virus genome and its impact on time to diagnosis of hepatocellular carcinoma. Hepatology (Baltimore, MD) 64(3):720–731
160. Jiang C, Mei M, Li B, Zhu X, Zu W, Tian Y et al (2017) A non-viral CRISPR/Cas9 delivery system for therapeutically targeting HBV DNA and pcsk9 in vivo. Cell Res 27(3):440–443

161. Zhang Y, Mao R, Guo H, Zhang J (2017) Detection of HBV cccDNA methylation from clinical samples by bisulfite sequencing and methylation-specific PCR. Methods Mol Biol 1540:73–84
162. Wei HS, Dong QM, Zhuang H, Song SJ, Liu ZY, Cheng J (2006) Establishing a fluorescence quantitative polymerase chain reaction for detection of HBV cccDNA in serum. Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology 14(1):56–58
163. Lucifora J, Salvetti A, Marniquet X, Mailly L, Testoni B, Fusil F et al (2017) Detection of the hepatitis B virus (HBV) covalently-closed-circular DNA (cccDNA) in mice transduced with a recombinant AAV-HBV vector. Antivir Res 145:14–19
164. Maepa MB, Roelofse I, Ely A, Arbuthnot P (2015) Progress and prospects of anti-HBV gene therapy development. Int J Mol Sci 16(8):17589–17610
165. Thi EP, Dhillon AP, Ardzinski A, Bidirici-Ertekin L, Cobarrubias KD, Cuconati A et al (2019) ARB-1740, a RNA interference therapeutic for chronic hepatitis B infection. ACS Infect Dis 5(5):725–737
166. Gish RG, Yuen MF, Chan HL, Given BD, Lai CL, Locarnini SA et al (2015) Synthetic RNAi triggers and their use in chronic hepatitis B therapies with curative intent. Antivir Res 121:97–108
167. Brahmania M, Feld J, Arif A, Janssen HL (2016) New therapeutic agents for chronic hepatitis B. Lancet Infect Dis 16(2):e10–e21
168. McCaffrey AP, Nakai H, Pandey K, Huang Z, Salazar FH, Xu H et al (2003) Inhibition of hepatitis B virus in mice by RNA interference. Nat Biotechnol 21(6):639–644
169. Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D et al (2017) RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med 9(409)
170. Hamilton J (2018) Overcoming the challenges of RNAi-based therapy: an interview with James Hamilton. Ther Deliv 9(7):511–513
171. Edward Gane SAL, Lim TH, Simone Strasser WS, Cheng W, Alexander Thompson BG, Schluep T, James Hamilton GC, Wong D, Christian Schwabe KJ, Ferrari C, Ching-Lung Lai RGG, Yuen M-F (2019) RNA interference (RNAi) in chronic hepatitis B (CHB): data from Phase 2 study with JNJ-3989. Hepatol Int 13(Suppl 1):S1–S266
172. Lee ACH, Thi EP, Cuconati A, Ardzinski A, Holland R, Huang H et al (2019) Function and drug combination studies in cell culture models for AB-729, a subcutaneously administered siRNA investigational agent for chronic hepatitis B infection. J Hepatol 70:E471–E
173. Peters MG, Locarnini S (2017) New direct-acting antiviral agents and immunomodulators for hepatitis B virus infection. Gastroenterol Hepatol 13(6):348–356
174. Lam AM, Espiritu C, Vogel R, Ren S, Lau V, Kelly M et al (2019) Preclinical characterization of NVR 3-778, a first-in-class capsid assembly modulator against hepatitis B virus. Antimicrob Agents Chemother 63:1
175. Cole AG (2016) Modulators of HBV capsid assembly as an approach to treating hepatitis B virus infection. Curr Opin Pharmacol 30:131–137
176. Lahlali T, Berke JM, Vergauwen K, Foca A, Vandyck K, Pauwels F et al (2018) Novel potent capsid assembly modulators regulate multiple steps of the hepatitis B virus life cycle. Antimicrob Agents Chemother 62(10)
177. Berke JM, Dehertogh P, Vergauwen K, van Damme E, Raboisson P, Pauwels F et al (2016) Capsid assembly modulator JNJ-56136379 prevents de novo infection of primary human hepatocytes with hepatitis B virus. Hepatology (Baltimore, MD) 64:124a-a
178. Berke JM, Dehertogh P, Vergauwen K, Van Damme E, Mostmans W, Vandyck K et al (2017) Capsid assembly modulators have a dual mechanism of action in primary human hepatocytes infected with hepatitis B virus. Antimicrob Agents Chemother 61(8)
179. Klumpp K, Shimada T, Allweiss L, Volz T, Lutgehetmann M, Hartman G et al (2018) Efficacy of NVR 3-778, alone and in combination with pegylated interferon, vs entecavir in uPA/SCID mice with humanized livers and HBV infection. Gastroenterology 154(3):652–62.e8

180. Yuen MF, Gane EJ, Kim DJ, Weilert F, Yuen Chan HL, Lalezari J et al (2019) Antiviral activity, safety, and pharmacokinetics of capsid assembly modulator NVR 3-778 in patients with chronic HBV infection. Gastroenterology 156(5):1392–1403.e7
181. F. Zoulim JZY JJV et al Safety, pharmacokinetics and antiviral activity of a novel hepatitis B virus capsid assembly modulator, JNJ-56136379, in patients with chronic hepatitis B. APASL2019.
182. Zhang H ZX, Chen H 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. AASLD 2018 Abstract LB-13.
183. Ma XL LJ et al (2019) 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. EASL 2019 Abs LBO-06: Hepatology
184. Feng S, Gao L, Han X, Hu T, Hu Y, Liu H et al (2018) Discovery of small molecule therapeutics for treatment of chronic HBV infection. ACS Infect Dis 4(3):257–277
185. Zhang X, Cheng J, Ma J, Hu Z, Wu S, Hwang N et al (2019) Discovery of novel hepatitis B virus nucleocapsid assembly inhibitors. ACS Infect Dis 5(5):759–768
186. Lai CL, Ahn SH, Lee KS, Um SH, Cho M, Yoon SK et al (2014) Phase IIb multicentred randomised trial of besifovir (LB80380) versus entecavir in Asian patients with chronic hepatitis B. Gut 63(6):996–1004
187. Ahn SH, Kim W, Jung YK, Yang JM, Jang JY, Kweon YO et al (2019) Efficacy and safety of besifovir dipivoxil maleate compared with tenofovir disoproxil fumarate in treatment of chronic hepatitis B virus infection. Clin Gastroenterol Hepatol 17(9):1850–9.e4
188. Painter GR, Almond MR, Trost LC, Lampert BM, Neyts J, De Clercq E et al (2007) Evaluation of hexadecyloxypropyl-9-R-[2-(Phosphonomethoxy)propyl]- adenine, CMX157, as a potential treatment for human immunodeficiency virus type 1 and hepatitis B virus infections. Antimicrob Agents Chemother 51(10):3505–3509
189. Tavis JE, Lomonosova E (2015) The hepatitis B virus ribonuclease H as a drug target. Antivir Res 118:132–138
190. Hu Y, Cheng X, Cao F, Huang A, Tavis JE (2013) beta-Thujaplicinol inhibits hepatitis B virus replication by blocking the viral ribonuclease H activity. Antivir Res 99(3):221–229
191. Cai CW, Lomonosova E, Moran EA, Cheng X, Patel KB, Bailly F et al (2014) Hepatitis B virus replication is blocked by a 2-hydroxyisoquinoline-1,3(2H,4H)-dione (HID) inhibitor of the viral ribonuclease H activity. Antivir Res 108:48–55
192. Edwards TC, Lomonosova E, Patel JA, Li Q, Villa JA, Gupta AK et al (2017) Inhibition of hepatitis B virus replication by N-hydroxyisoquinolinediones and related polyoxygenated heterocycles. Antivir Res 143:205–217
193. Blanchet M, Sinnathamby V, Vaillant A, Labonte P (2019) Inhibition of HBsAg secretion by nucleic acid polymers in HepG2.2.15cells. Antivir Res 164:97–105
194. Bazinet M, Pantea V, Cebotarescu V, Cojuhari L, Jimbei P, Albrecht J et al (2017) Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and REP 301-LTF): a non-randomised, open-label, phase 2 trial. Lancet Gastroenterol Hepatol 2(12):877–889
195. Al-Mahtab M, Bazinet M, Vaillant A (2016) Safety and efficacy of nucleic acid polymers in monotherapy and combined with immunotherapy in treatment-naive Bangladeshi patients with HBeAg+ chronic hepatitis B infection. PLoS One 11(6):e0156667
196. Usman Z, Mijocevic H, Karimzadeh H, Daumer M, Mamun AM, Bazinet M et al (2019) Kinetics of hepatitis B surface antigen quasispecies during REP 2139-Ca therapy in HBeAg positive chronic HBV infection. J Viral Hepat
197. Vaillant A (2019) REP 2139: antiviral mechanisms and applications in achieving functional control of HBV and HDV infection. ACS Infect Dis 5(5):675–687
198. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2(8):675–680
199. Lang T, Lo C, Skinner N, Locarnini S, Visvanathan K, Mansell A (2011) The hepatitis B e antigen (HBeAg) targets and suppresses activation of the toll-like receptor signaling pathway. J Hepatol 55(4):762–769

200. Rehermann B, Nascimbeni M (2005) Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 5(3):215–229
201. Jo J, Tan AT, Ussher JE, Sandalova E, Tang XZ, Tan-Garcia A et al (2014) Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. PLoS Pathog 10(6):e1004210
202. Niu C, Li L, Daffis S, Lucifora J, Bonnin M, Maadadi S et al (2018) Toll-like receptor 7 agonist GS-9620 induces prolonged inhibition of HBV via a type I interferon-dependent mechanism. J Hepatol 68(5):922–931
203. Menne S, Tumas DB, Liu KH, Thampi L, AlDeghaither D, Baldwin BH et al (2015) Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. J Hepatol 62(6):1237–1245
204. Lanford RE, Guerra B, Chavez D, Giavedoni L, Hodara VL, Brasky KM, et al. (2013) GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 144(7):1508-17, 1517.e1–10
205. Boni C, Vecchi A, Rossi M, Laccabue D, Giuberti T, Alfieri A et al (2018) TLR7 agonist increases responses of hepatitis B virus-specific T cells and natural killer cells in patients with chronic hepatitis B treated with nucleos(T)ide analogues. Gastroenterology 154(6):1764–77.e7
206. Janssen HLA, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen AH et al (2018) Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. J Hepatol 68(3):431–440
207. L. DaiY.YuL.GuJ.ZhaoL.ZhuH.YunY.JiW.ZhuJ.YoungL.Gao. Combination treatment of a TLR7 agonist RO7020531and a capsid assembly modulator RO7049389 achieved sustainable viral load suppression and HBsAg loss in an AAV-HBV mouse model. J Hepatol 2018;68(Supplement 1): S17-SS8.
208. Andrea Luk JFG, Folitar I, Triyatni M, Glavini K, Zhao L, Upmanyu R, Racek T, Hoppe S, Fok B, Zhu Y, Jin Y, Jiang Q (2019) A single and multiple ascending dose study of Toll-like receptor 7 (TLR 7) agonist (RO7020531) in Chinese healthy subjects. Hepatol Int 13(Suppl 1):s39
209. Daffis S, Chamberlain J, Zheng J, Santos R, Rowe W, Mish M et al (2017) Sustained efficacy and surface antigen seroconversion in the woodchuck model of chronic hepatitis B with the selective toll-like receptor 8 agonist GS-9688. J Hepatol 66(1):S692–S6S3
210. Gane EJ, Kim HJ, Visvanathan K, Kim YJ, Nguyen A-H, Reyes M et al (2019) A randomized, placebo-controlled, blinded phase 1b study evaluating the oral TLR8 agonist GS-9688 in patients with chronic hepatitis B (CHB). Hepatol Int 13(Suppl 1):S53
211. Jones M, Cunningham ME, Wing P, DeSilva S, Challa R, Sheri A et al (2017) SB 9200, a novel agonist of innate immunity, shows potent antiviral activity against resistant HCV variants. J Med Virol 89(9):1620–1628
212. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S et al (2015) The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity 42(1):123–132
213. Dawood A, Abdul Basit S, Jayaraj M, Gish RG (2017) Drugs in development for hepatitis B. Drugs 77(12):1263–1280
214. Macfarlane C, Locarnini S, Jackson K, Walsh R, Edwards R et al (2018) Inarigivir: a novel RIG-I agonist for chronic hepatitis B. Hepatol Int 12(Suppl 2):S264
215. Yuen M-F, Chen C-Y, Liu C-J, Jeng RW-J, Elkhashab M, Coffin C et al (2019) Ascending dose cohort study of inarigivir – a novel RIG I agonist in chronic HBV patients: final results of the ACHIEVE trial. J Hepatol 70(1 Supplement):e47–ee8
216. Cho H, Kang H, Lee HH, Kim CW (2017) Programmed cell death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) in viral hepatitis. Int J Mol Sci 18(7)
217. Peng G, Li S, Wu W, Tan X, Chen Y, Chen Z (2008) PD-1 upregulation is associated with HBV-specific T cell dysfunction in chronic hepatitis B patients. Mol Immunol 45(4):963–970

218. Kassel R, Cruise MW, Iezzoni JC, Taylor NA, Pruett TL, Hahn YS (2009) Chronically inflamed livers up-regulate expression of inhibitory B7 family members. Hepatology (Baltimore, MD) 50(5):1625–1637
219. Zhang WJ, Peng CH, Zheng SS (2013) Programmed death 1 and programmed death ligand 1 expressions in patients with chronic hepatitis B. Hepatobiliary Pancreat Dis Int 12(4):394–399
220. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH et al (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439(7077):682–687
221. Fisicaro P, Valdatta C, Massari M, Loggi E, Biasini E, Sacchelli L, et al (2010) Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. Gastroenterology 138(2):682–93, 693.e1–4.
222. Zhang E, Zhang X, Liu J, Wang B, Tian Y, Kosinska AD et al (2011) The expression of PD-1 ligands and their involvement in regulation of T cell functions in acute and chronic woodchuck hepatitis virus infection. PLoS One 6(10):e26196
223. Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X et al (2014) Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. PLoS Pathog 10(1):e1003856
224. Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM et al (2019) Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: a pilot study. J Hepatol
225. Pagliaccetti NE, Chu EN, Bolen CR, Kleinstein SH, Robek MD (2010) Lambda and alpha interferons inhibit hepatitis B virus replication through a common molecular mechanism but with different in vivo activities. Virology 401(2):197–206
226. Schurich A, Pallett LJ, Lubowiecki M, Singh HD, Gill US, Kennedy PT et al (2013) The third signal cytokine IL-12 rescues the anti-viral function of exhausted HBV-specific CD8 T cells. PLoS Pathog 9(3):e1003208
227. Zeng Z, Kong X, Li F, Wei H, Sun R, Tian Z (2013) IL-12-based vaccination therapy reverses liver-induced systemic tolerance in a mouse model of hepatitis B virus carrier. J Immunol (Baltimore, MD: 1950) 191(8):4184–4193
228. Carreno V, Zeuzem S, Hopf U, Marcellin P, Cooksley WG, Fevery J et al (2000) A phase I/II study of recombinant human interleukin-12 in patients with chronic hepatitis B. J Hepatol 32(2):317–324
229. Tang L, Chen C, Gao X, Zhang W, Yan X, Zhou Y et al (2019) Interleukin 21 reinvigorates the antiviral activity of hepatitis B virus (HBV)-specific CD8+ T cells in chronic HBV infection. J Infect Dis 219(5):750–759
230. Li HJ, Kang FB, Li BS, Yang XY, Zhang YG, Sun DX (2015) Interleukin-21 inhibits HBV replication in vitro. Antivir Ther 20(6):583–590
231. Tsuge M, Hiraga N, Zhang Y, Yamashita M, Sato O, Oka N et al (2018) Endoplasmic reticulum-mediated induction of interleukin-8 occurs by hepatitis B virus infection and contributes to suppression of interferon responsiveness in human hepatocytes. Virology 525:48–61
232. Lobaina Y, Michel ML (2017) Chronic hepatitis B: immunological profile and current therapeutic vaccines in clinical trials. Vaccine 35(18):2308–2314
233. Kosinska AD, Bauer T, Protzer U (2017) Therapeutic vaccination for chronic hepatitis B. Curr Opin Virol 23:75–81
234. Dembek C, Protzer U, Roggendorf M (2018) Overcoming immune tolerance in chronic hepatitis B by therapeutic vaccination. Curr Opin Virol 30:58–67
235. Vandepapeliere P, Lau GK, Leroux-Roels G, Horsmans Y, Gane E, Tawandee T et al (2007) 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 25(51):8585–8597
236. Lobaina Y, Palenzuela D, Pichardo D, Muzio V, Guillen G, Aguilar JC (2005) Immunological characterization of two hepatitis B core antigen variants and their immunoenhancing effect on co-delivered hepatitis B surface antigen. Mol Immunol 42(3):289–294

237. Fernandez G, A LS, Jerez E, L EA, Freyre F, J AA et al (2018) Five-year follow-up of chronic hepatitis B patients immunized by nasal route with the therapeutic vaccine HeberNasvac. Euro J Hepato-Gastroenterol 8(2):133–139
238. Xu DZ, Huang KL, Zhao K, Xu LF, Shi N, Yuan ZH et al (2005) Vaccination with recombinant HBsAg-HBIG complex in healthy adults. Vaccine 23(20):2658–2664
239. Xu DZ, Wang XY, Shen XL, Gong GZ, Ren H, Guo LM et al (2013) Results of a phase III clinical trial with an HBsAg-HBIG immunogenic complex therapeutic vaccine for chronic hepatitis B patients: experiences and findings. J Hepatol 59(3):450–456
240. Obeng-Adjei N, Hutnick NA, Yan J, Chu JS, Myles DJ, Morrow MP et al (2013) 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 20(12):652–662
241. Yoon SK, Seo YB, Im SJ, Bae SH, Song MJ, You CR et al (2015) Safety and immunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. Liver Int 35(3):805–815
242. Boni C, Janssen HLA, Rossi M, Yoon SK, Vecchi A, Barili V et al (2019) Combined GS-4774 and tenofovir therapy can improve HBV-specific T-cell responses in patients with chronic hepatitis. Gastroenterology 157(1):227–41.e7
243. Lok AS, Pan CQ, Han SH, Trinh HN, Fessel WJ, Rodell T et al (2016) Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B. J Hepatol 65(3):509–516
244. Martin P, Dubois C, Jacquier E, Dion S, Mancini-Bourgine M, Godon O et al (2015) TG1050, an immunotherapeutic to treat chronic hepatitis B, induces robust T cells and exerts an antiviral effect in HBV-persistent mice. Gut 64(12):1961–1971
245. Zoulim F, Fournier C, Habersetzer F, Sprinzl M, Pol S, Coffin CS et al (2019) Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B Patients: a phase 1b placebo-controlled trial. Hum Vaccin Immunother. https://doi.org/10.1080/21645515.2019.1651141