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Current Opinion in Virology

Hepatitis-B virus: replication cycle, targets, and antiviral approaches

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An estimated 257 million people are chronic carriers of hepatitis-B virus (HBV) infection, which resulted in around 1 million deaths, mainly due to hepatocellular carcinoma (HCC). Long-term nucleotide analog treatment of HBV infection is associated with favorable prognosis, no disease progression, and a reduction of HCC risk, but lifelong treatments are required. A better understanding of HBV replication cycle and the host immune response will likely improve the identification of new targets for drug development. Studies are ongoing to determine if it is possible to successfully combine direct-acting antivirals (DAA) with an immunomodulatory therapy to allow increased cure rates. This review will start with summarizing the HBV replication cycle, recall current treatments, and then discuss potential targets and antiviral approaches in development to optimistically reach the HBV cure.

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Introduction

An estimated 257 million people are living with hepatitis-B virus (HBV) infection, defined as testing positive for the hepatitis-B surface antigen (HBsAg). In 2015, HBV infection resulted in 887 000 deaths, mainly from complications, including end-stage liver disease and hepatocellular carcinoma (HCC) [1]. The chronic infection is, in general, asymptomatic (70–90% of cases) and is detected only in late stages [2]. Although a protective vaccine has excellent efficacy and safety, the implementation of vaccination campaigns varies worldwide [3]. Pegylated-interferon (PEG-IFN) and nucleos(t)ide analogs (NAs) are two treatment approaches approved and available that suppress HBV replication and slow disease progression. However, these treatments do not lead to a cure (defined by HBsAg loss) [4]. Therefore, understanding HBV replication cycle is important for drug development to identify targets. The main aim of therapy is to obtain a virological cure, that is, clearance of covalently closed circular DNA (cccDNA), or at least a functional cure defined as HBsAg loss. There is major hope to stop therapy following successful HBsAg loss in some patients. Studies are ongoing to determine if it is possible to successfully combine direct-acting antivirals (DAA) with an immunomodulatory therapy to allow more patients to cure. This review will start with summarizing the HBV replication cycle, recall current treatments, and then discuss the different targets, antiviral, and immunomodulatory approaches in development.

Natural history

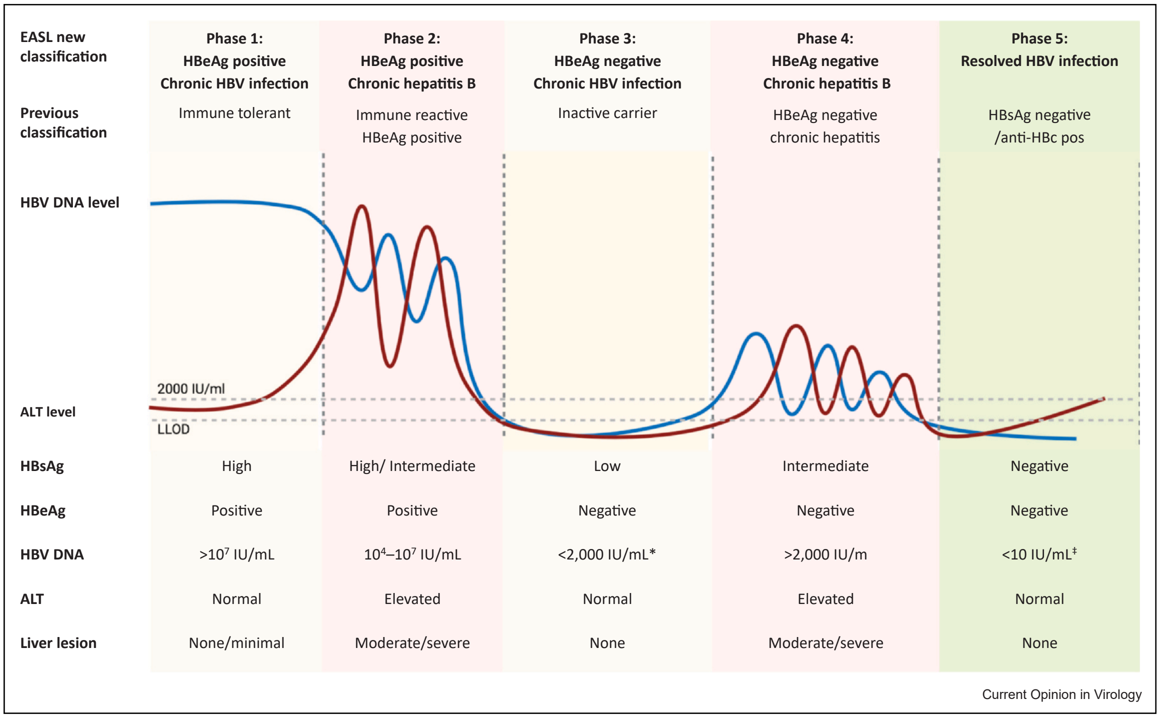
Chronic hepatitis B (CHB) can lead to cirrhosis and HCC. Fibrosis is an important prognosis factor ; therefore, it is important to stage fibrosis to prioritize treatement. Fibrosis progression is associated with advanced age and comorbidities such as diabetes or obesity, and exogenous factors (alcohol, HIV coinfections, medications, etc.). Fibrosis regression in patients with HBV infection is achievable by antiviral [5]. It has been also demonstrated that treatment also reduced the risk of HCC [6]. Two different techniques are used to determine the stage of fibrosis: noninvasive tests based on serological markers or on elasticity (Fibroscan), despite their difficulties to differentiate mild from moderate fibrosis; and histological analysis. Patients have been categorized according to different patterns and prognosis, considering HBe status, levels of transaminases, and levels of viremia [6] (Figure 1).

Extrahepatic manifestations of HBV infection have been well-described, such as vasculitis, glomerulonephritis, and cutaneous lesions [6]. Other manifestations have been more recently reported such as hematological malignancies. It is important to diagnose all these extrahepatic manifestations since they are associated with important morbidity and mortality. The underlying mechanisms include HBV-

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
CAM	capsid assembly modulator
cccDNA	covalently closed circular DNA
CHB	chronic hepatitis B
ETV	entecavir
HBsAg	Hepatitis B surface antigens
HBV	hepatitis B virus
HBx	viral protein X
HCC	hepato-cellular carcinoma
hNTCP	human sodium taurocholate cotransporting polypeptide
IFNα	interferon alpha
NA	nucleoside analogue
NAPs	nucleic acid polymers
ORF	open reading frames
PEG-IFN	pegylated interferon
TAF	tenofovir alafenamide
TDF	tenofovir disoproxil fumarate
TFV	tenofovir

Figure 1



EASL new classification	Phase 1: HBeAg positive Chronic HBV infection	Phase 2: HBeAg positive Chronic hepatitis B	Phase 3: HBeAg negative Chronic HBV infection	Phase 4: HBeAg negative Chronic hepatitis B	Phase 5: Resolved HBV infection
Previous classification	Immune tolerant	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative chronic hepatitis	HBsAg negative /anti-HBc pos
HBsAg	High	High/ Intermediate	Low	Intermediate	Negative
HBeAg	Positive	Positive	Negative	Negative	Negative
HBV DNA	>10^7 IU/mL	10^4–10^7 IU/mL	<2,000 IU/mL*	>2,000 IU/m	<10 IU/mL‡
ALT	Normal	Elevated	Normal	Elevated	Normal
Liver lesion	None/minimal	Moderate/severe	None	Moderate/severe	None

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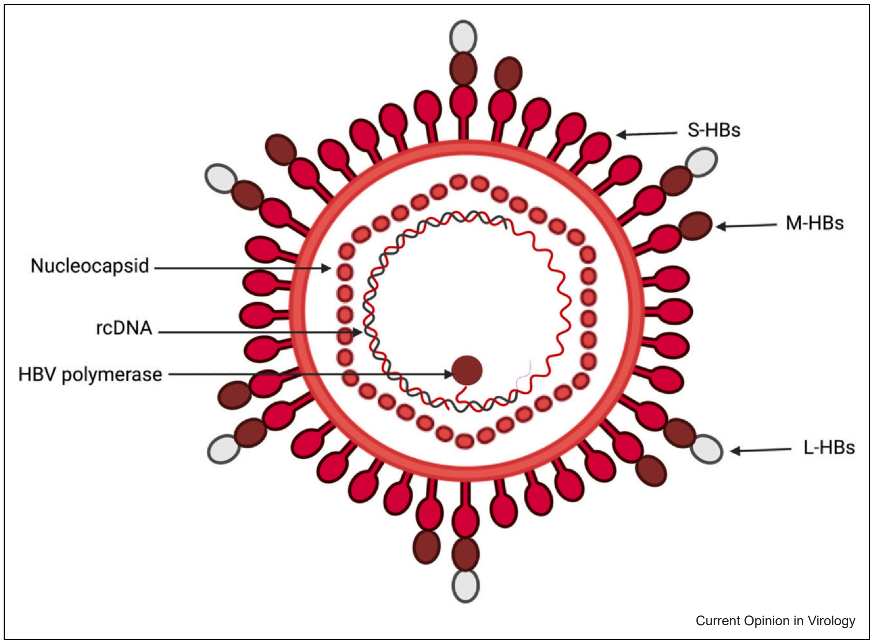
The natural history of chronic HBV infection is described by five distinct phases. First, the ‘HBeAg-positive chronic HBV infection’ phase known previously as immune-tolerant phase consisting of high HBV DNA levels, HBeAg positivity, and normal ALT levels with none-to-minimal liver lesion. The second phase ‘HBeAg-positive CHB’, immune-reactive HBeAg positive, is characterized by high levels of HBV DNA and ALT and moderate-to-severe liver necroinflammation. Most patients can achieve HBeAg seroconversion and enter the third phase, ‘HBeAg-negative chronic HBV infection’, with positive anti-HBe, low (< 2000 IU/mL) HBV DNA levels, and normal ALT levels. Other patients can progress to the fourth phase, ‘HBeAg-negative CHB’ (reactivation), characterized by moderate-to-high levels of serum HBV DNA and ALT. The fifth phase, the HBsAg-negative phase also known as functional cure, is characterized by serum- negative HBsAg with or without anti-HBs.

induced inflammation. Suppression of HBV replication usually improves these extrahepatic manifestations [7].

Hepatitis-B virus structure

HBV belongs to the Hepadnaviridae family, a type of hepatotropic DNA viruses [8]. HBV was isolated in the serum of infected patients, and it usually takes different forms, the virions or the Dane particles. There are empty virions (spherical or elongated), which are noninfectious particles that lack DNA. On the other hand, the Dane particles are the infectious ones (Figure 2a). To note that, in the serum of infected subjects, the noninfectious particles or empty virions are found 10 000–1000 000 times more than Dane particles [9].

Figure 2a



Dane particle structure. Dane particles measure 44 nm in diameter and consist of a lipoprotein envelope and a nucleocapsid. The viral envelope consists of a bilayer of lipids, originating from the membrane of the host cells, and covered by viral surface proteins. About 42-nm HBV virion consists of a DNA-containing nucleocapsid surrounded by a lipoprotein envelope comprising the L, M, and S-surface proteins. The viral envelope is a bilayer of lipids, originating from the membrane of the host cells, in which are embedded viral surface proteins. It contains an icosahedral nucleocapsid 27 nm in diameter. The protein capsid protects the viral genome, composed of a (-) and a (+) strand of DNA, and dependent viral RNA/DNA polymerase is covalently linked to the (-) strand of DNA. Also, in the capsid are cellular protein kinases, chaperones, and oligoribonucleotides linked to the 3′ end of the (+) DNA strand.

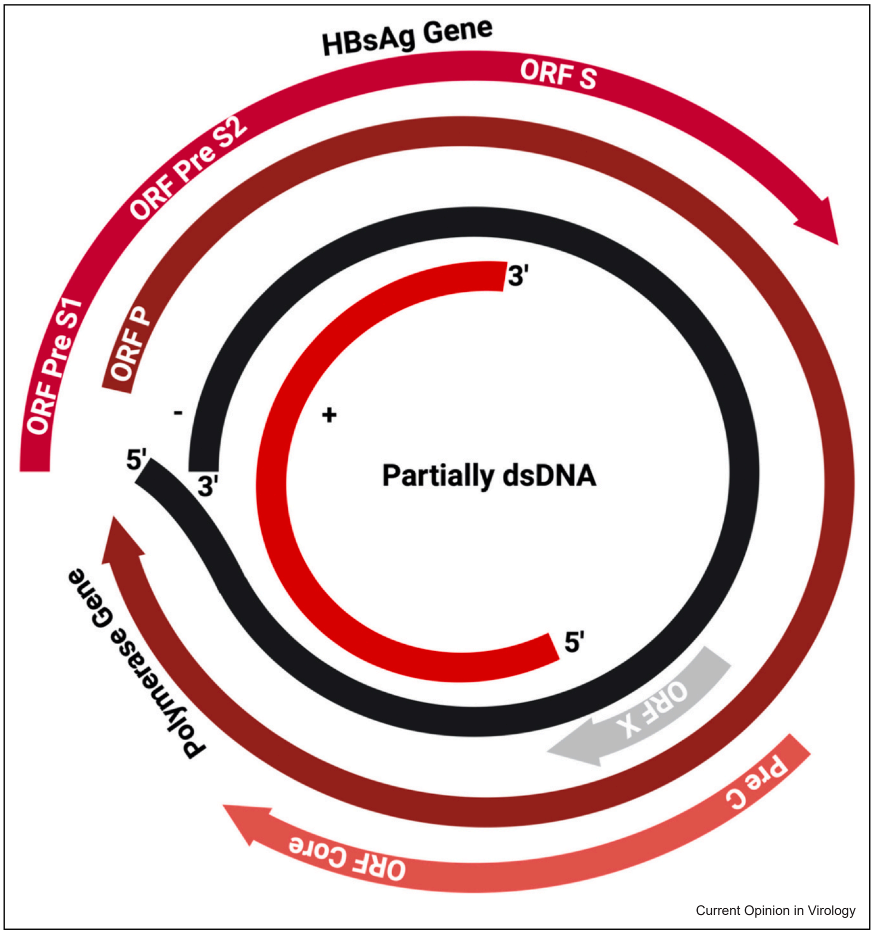
Dane particles measure 44 nm in diameter and consist of a lipoprotein envelope and a nucleocapsid. The particles contain an icosahedral nucleocapsid that is 27 nm in diameter and protects the viral genome. Inside the capsid, there is a (-) and a (+) strand of DNA, in addition to dependent viral RNA/DNA polymerase covalently linked to the (-) strand of DNA [11,12].

The HBV genome is one of the smallest-known genomes among DNA viruses consisting of only 3200 nucleotides (nt). The DNA of the virion is in a relaxed circular partially double-stranded form, composed of a long full (-) strand, and a short (+) strand (Figure 1b). The long strand is the coding strand and is of constant length. At its 5′ end, this DNA strand has a short terminal redundancy of nine bases, which overlaps the 3′ end, and therefore has a short sequence interruption called a ‘gap’ at this point. The viral polymerase is covalently linked via a phosphodiester bridge at the 5′ end of the (-) strand. The short, noncoding strand has a well-defined 5′ end, which carries an 18-base oligonucleotide, but a variable 3′ end. Its length is, therefore, not constant and represents 30–85% of the size of the (-) strand. The maintenance of the circular shape is ensured by the pairing of the 5′ ends of each strand over a length of 200 nt, called the cohesive region. In addition, the genome contains two repeated sequences of 10–12 bases on either side of the gap, called DR1 and DR2, which are essential for the replication of the virus [10]. Despite its small size, HBV DNA carries a very large amount of information, with the long strand possessing coding sequences in all three transcriptional reading frames (8).

There are four open-reading frames (ORF) (Figure 2b). The S ORF contains three transcription initiation codons and therefore codes for three surface proteins: the S gene codes for HBsAg or major protein S (small protein: S), the preS2/S gene codes for the medium protein preS2 (medium protein: M), and the pre-S1/preS2/S gene encodes the large protein preS1 (large protein: L) [10].

The ORF C encodes the core proteins or capsid proteins. A first initiation codon allows the synthesis of a signal sequence (from the preC gene) necessary for the translocation of the HBe protein in the endoplasmic reticulum (ER) and its secretion in the plasma. In the absence of this signal sequence (transcription begins at the second initiation codon), the HBc protein is synthesized. It is not excreted in the plasma and assembles to form the viral capsid. The longest ORF P, encodes the viral polymerase. It covers 80% of the genome and therefore partially or totally overlaps all the other ORFs

Figure 2b



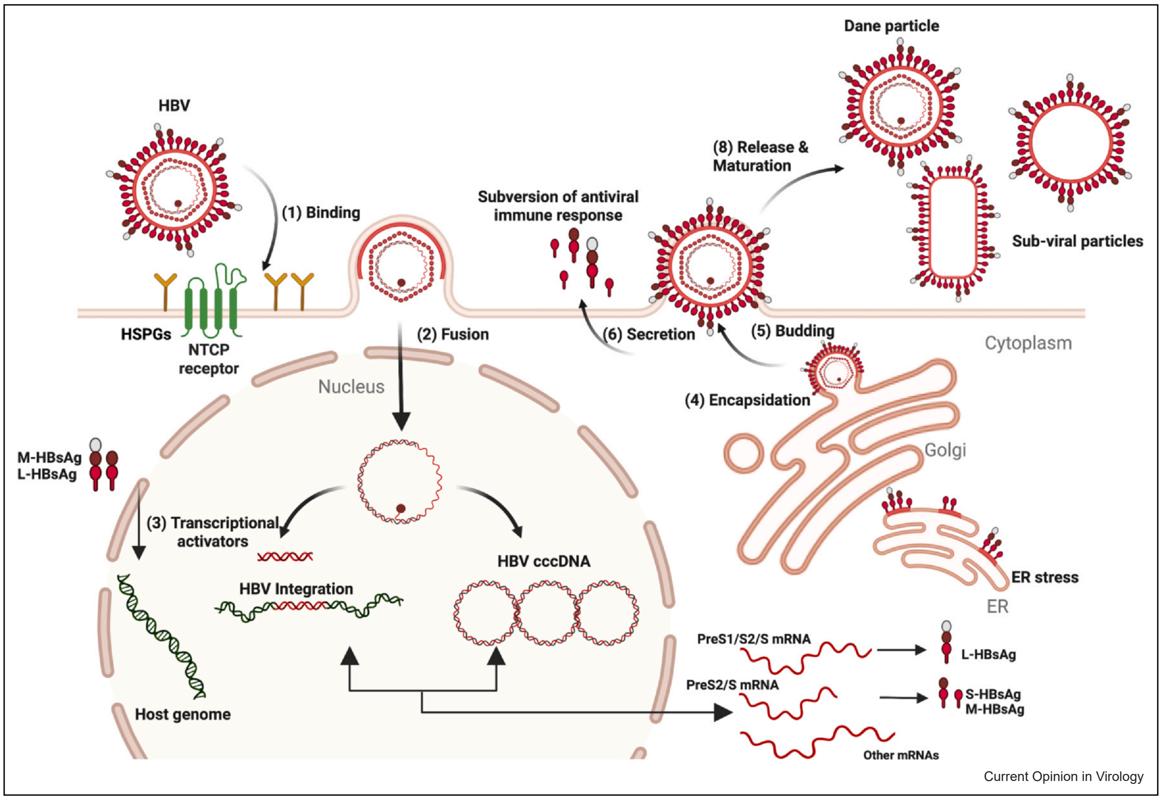
Genomic organization of HBV. The four ORFs are represented by different colors. The S reading frame, which delimits the preS1, preS2 regions, and the S gene, encodes the three envelope proteins (L, M, and S). The C reading frame with the preC region and the C gene encodes the capsid protein HBc and the HBe protein. The P reading frame encodes DNA polymerase. The X reading frame encodes the X protein. The complete negative-sense (-) and incomplete positive-sense (+) strand of the viral genome is indicated, as well as the viral polymerase.

[13,14]. The smaller ORF encodes a trans-activator protein X.

Hepatitis-B virus replication cycle

The HBV multiplication cycle takes place both in the cytoplasm and in the nucleus of hepatocytes. Viral infection begins with the attachment of the particle to the surface of hepatocytes (Figure 3). The virion initially interacts with heparan sulfate (HSPG), a family of complex polysaccharides, on the surface of hepatocytes, with low affinity. This step allows a strong interaction of HBV virion and sodium taurocholate cotransporting polypeptide (NTCP) receptor (sodium taurocholate co-transporting polypeptide), a transporter of bile acids expressed in the liver, via the pre-S1 domain of the L protein [12]. Iwamoto et al. have shown that epidermal growth factor receptor also facilitates this process [13]. The entry of HBV is independent of pH, which suggests the fusion of its envelope with the cell membrane. During this step, the nucleocapsid containing the relaxed circular form of DNA (rcDNA) is released into the cytoplasm and transported to the nucleus due to the presence of nuclear localization signal located on C-terminal domain, which is highly conserved between all genotypes. The viral genome is thus released into the nucleoplasm [14]. The released circular DNA will be converted into cccDNA, an episomal form serving as a transcriptional matrix for the messenger RNA (mRNA) synthesis by the RNA polymerase II [15]. Different mRNAs will be transcribed: subgenomic mRNAs encoding structural and regulatory proteins and a pregenomic RNA (pgRNA) [16]. The pgRNA is encapsulated with the viral polymerase [15]. It serves as a template for transcription within the nucleocapsid via a complex process, similar to retroviruses. The replication process involves a unique mechanism: a reverse transcription step and three intramolecular transpositions. After synthesis of the negative and positive polarity strands,

Figure 3



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The HBV replication cycle. HBV entry (1) into hepatocytes is mediated by the interaction between HBsAg and the low-specific HSPG and high-specific NTCP receptors on the plasma membrane of differentiated hepatocytes that lead to endocytosis. Nucleocapsid is transported to the nucleus (2) where the partially double-stranded HBV DNA genome is released and converted by cellular factors to cccDNA (3), a template for transcription of all viral RNAs. HBV RNAs are exported in the cytoplasm for protein translation. pgRNA is then selectively packaged into newly formed nucleocapsids (4), where it is reverse-transcribed to rcDNA. Nucleocapsids can be enveloped to form a mature virion and released from cells or transported to the nucleus to reload cccDNA pool (5). If reverse transcription is interrupted, virions containing pgRNA are released from cells (6). Linear genomes have a high tendency to integrate into host DNA; viral genome integration is not required for viral genome replication, but serves as a template for viral RNA and protein production.

the nucleocapsid contains a partially double-stranded relaxed circular DNA that can either acquire an envelope via the endoplasmic reticulum and/or the intermediate compartment to be then secreted outside the hepatocytes or return to the nucleus to increase the cccDNA pool. The enveloped viral particles are then excreted by exocytosis, allowing infection of neighboring cells [17].

Candidates for current treatments

Drug strategies under consideration aim to target each step of the HBV replication cycle. There are experimental treatments designed to block viral entry into the cell, and others that should prevent the release of viral proteins from infected cells. There are drug candidates directed at viral-particle assembly and replication, and others geared at gumming up expression of viral-protein-encoding genes. Immune-activating agents are also considered.

Which patients should be treated?

Several guidelines have provided guidelines regarding treatments [18–20]. With current therapies, all patients with HBeAg-positive or -negative CHB, defined by HBV DNA more than 2000 IU/mL, alanine aminotransferase (ALT) above the upper limit of normal, or at least moderate liver necroinflammation or fibrosis, are candidates for treatment. Furthermore, patients with detectable HBV DNA and cirrhosis (compensated or not), and those with a family history of HCC, are also candidates for therapy. Patients with detectable HBV DNA and extra-hepatic manifestations are also candidates for therapy, irrespectively of the severity of the liver disease [7].

Which patients should not be treated?

Patients with HBeAg-negative chronic HBV infection, previously termed inactive carriers, are characterized by the presence of serum antibodies to HBeAg (anti-HBe), undetectable or low HBV DNA (< 2000 IU/mL), and normal ALT. Since these patients have favorable prognosis, they are not candidates for treatment. However, they need lifelong follow-up, as there is a risk of reactivation, and they are at risk of developing extrahepatic manifestations. Concerns regarding HBV DNA integration have been raised, and relatively abundant integrant-derived viral RNAs have been reported in tissue harvested from chronic HBV carriers [10]. In the first step of drug development, these patients might not be a priority for therapy. However, a short-duration HBV treatment with high efficacy and favorable safety could be a treatment for all HBV-infected individuals.

Patients with HBeAg-positive chronic HBV infection, previously termed immune-tolerants, are characterized by the presence of serum antigens to HBeAg (HBeAg), high HBV DNA (> 10^5 IU/mL), and normal ALT. These patients have also favorable prognosis and are not current candidates for treatment. However, there is a recent debate about treating these patients to decrease the risk of HBV integration and HCC risk. An algorithm is proposed to manage patients with chronic HBV infection or hepatitis (Figure 4).

Current therapies for chronic hepatitis B

Current approved treatments include PEG-IFN or NA (Table 1) [10]. PEG-IFN has the advantage of achieving a sustained virological response after a limited course of treatment, in approximately 20% of patients. The limitations of PEG-IFN are the mild efficacy and its side effects and poor tolerability. HBV DNA undetectability with long-term lamivudine has been associated with a reduction in the incidence of HCC [6]. Among analogs, three additional drugs are recommended, with high efficacy, favorable tolerability, and no or rare resistance: tenofovir (TDF), tenofovir alafenamide (TAF), and entecavir (ETV).

Future strategies

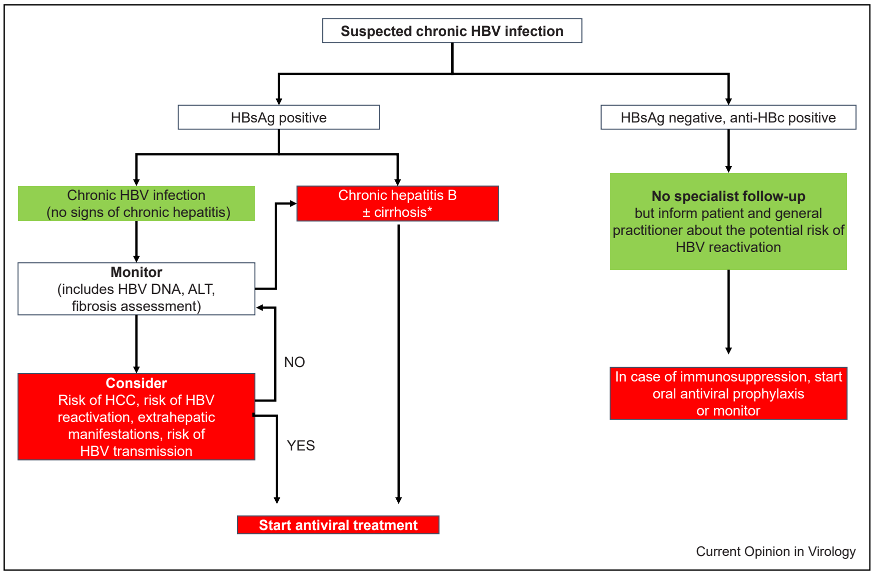
Current treatments for CHB efficiently control virus replication and improve prognosis, but do not lead to complete viral clearance. Thus, a lifelong treatment is usually required for patients with CHB [14]. HBsAg loss has been associated with a favorable prognosis and is the ideal goal for therapy [21,22].

Many strategies with DAA are ongoing to achieve cccDNA decrease and/or HBsAg loss (Figure 5). These strategies may be effective if combined with NA or immunomodulatory therapies. The mode of actions, targets, and drugs are listed in Table 1. The ideal combination and duration of treatment remains to be identified.

Entry inhibitors

In 2016, Li W et al. have shown that NTCP receptor acts as a functional receptor of HBV and HDV. The entry inhibitors can block the infection at its earliest step so that they can prevent the establishment of viral genomes in naive or regenerating host cells [23]. For instance, Bulevirtide (Hepcludex® [Gilead]) is a synthetic lipopeptide that is derived from pre-S1 domain of HBV envelope protein. Bulevirtide contains the NTCP-binding pre-S1 domain of HBV envelope protein, therefore, it competitively binds to NTCP, the major functional receptor for

Figure 4



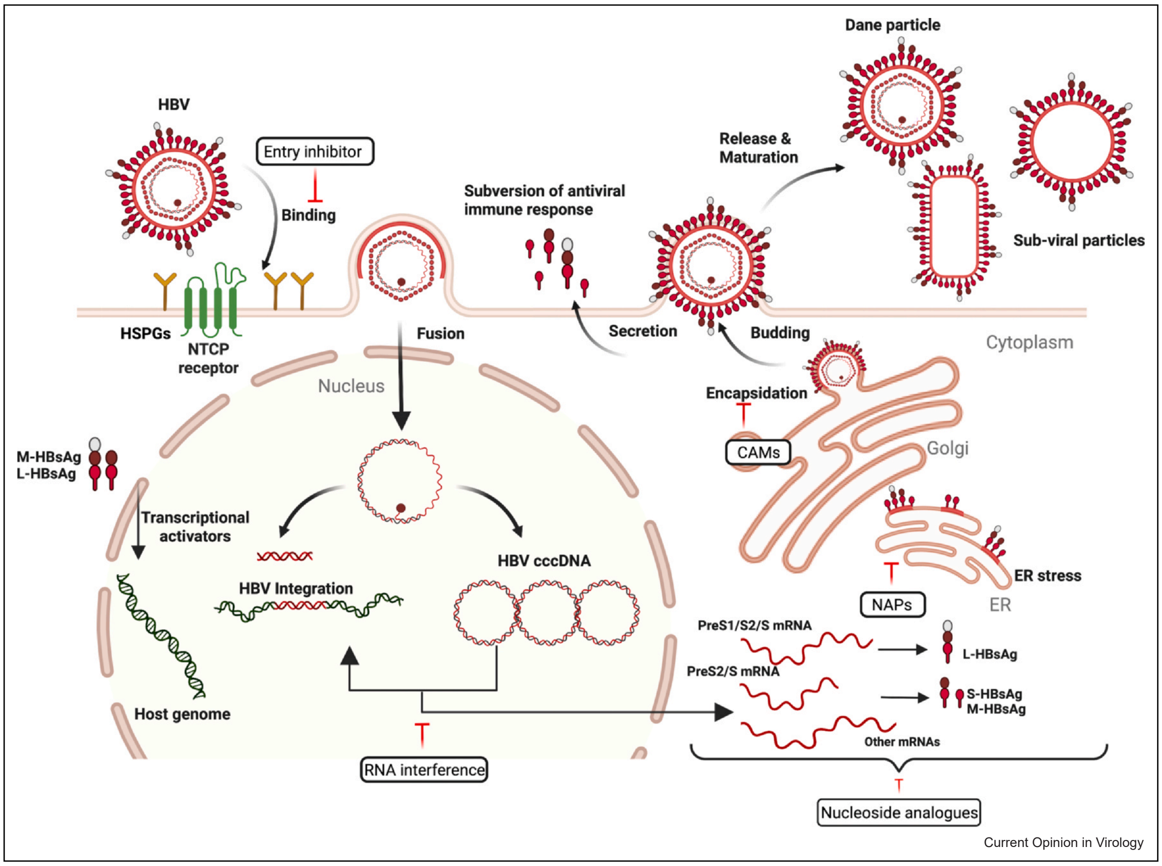
Chronic HBV infection Management Algorithm.

Table 1

Current treatment strategies for chronic hepatitis.

Features	Peg-IFN	ETV, TDF, and TAF
Route of administration	Subcutaneous injections	Oral
Treatment duration	48 weeks	Long-term
Tolerability	Low	High
Strategy	Long-term immune response	Inhibition of viral replication
Level of viral suppression	Moderate	High
Effect on HBeAg loss	Moderate	Low in the first year, moderate over long-term
Effect on HBsAg levels	Variable	Minimal to none
Risk of viral resistance	No	Minimal to none

Figure 5



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Treatment targets in the viral replication cycle. Entry inhibitor treatments block the bile acid transporter NTCP receptor to prevent HBV binding and infection of hepatocytes. NA inhibit HBV replication and slow down or stop disease progression. CAMs inhibit viral replication by interfering with HBV capsid assembly. The NAP blocks the assembly of SVPs in hepatocytes. This effect blocks the release of HBsAg and reduces intracellular HBsAg. RNAi is an ASO that targets HBV RNAs, HBV mRNA, and pgRNA, to reduce the levels of viral proteins.

HBV entry into hepatocytes, as a result inhibiting attachment of HBV to NTCP. In early clinical trials with Bulevirtide therapy, no HBsAg decrease was observed.

Capsid assembly modulators

The HBV core protein plays a major role in several steps of the viral cycle, including assembly of nucleocapsids, subcellular trafficking of the HBV genome, and maintenance of the nuclear cccDNA pool, therefore it represents an attractive target for new therapies [24]. Several capsid assembly modulators (CAMs) have been evaluated in clinical trials (phases I and II), such as RO7049389 [25], GLS4-JHS [24], ABI-H0731 [26], JNJ-6379 [27], and AB-836 [28]. Short-term results have shown several of these compounds to be safe and well-tolerated and demonstrated antiviral activity on treatment. However, several of the first studies with the first generation of CAM have shown a mild HBsAg decline with CAM ± NA. Second and third generation from this CAM family will have to improve HB loss and seroconversion. The future role of CAMs is still to be defined. We hope that future CAMs with more potent inhibition of HBV replication may either allow more patients to achieve a maintained HB seroconversion or will allow more patients to stop therapy. In vitro-resistant variants to the capsid have been described, and some patients may develop resistance [29].

Activated site polymerase inhibitor

ATI-2173 is a liver-targeted phosphoramidate prodrug of clevudine that is in phase-2 clinical development. ATI-2173 actively binds to and distorts the HBV polymerase enzyme's active site to disrupt all aspects of polymerase activity (protein priming, chain elongation, and DNA synthesis) (31). Delay in post-treatment HBV DNA rebound has been observed, similarly as in previous clevudine studies. Irrespective of the doses, all had virologic rebound 24 weeks after stopping treatment. ATI-2173 has been placed on clinical hold by the Food and Drug Administration.

Hepatitis-B surface antigen secretion inhibitors

It is believed that the excess production of HBsAg, resulting in the secretion of subviral noninfectious particles, plays an important role in immune evasion by inducing exhaustion. In 2020, Bazinet et al. identified a nucleic acid polymer (NAP) that inhibits the assembly and secretion of HBV subviral particles (SVPs) [30]. NAPs such as REP 2139 and REP 2165 block the assembly of SVPs, preventing the release of HBsAg and allowing its clearance and restoration of functional immune control of infection. NAPs have shown promising results in terms of efficacy and safety in a small number of HBV-infected patients. Further data are required to confirm these interesting preliminary results.

Inhibitors of covalently closed circular DNA

Chronicity is believed to be the consequence of persistent cccDNA inside the nucleus. Current treatments do not cure HBV infection because they do not eliminate the cccDNA. There is hope for new therapeutic strategies that focus on inhibiting its formation or removing HBV cccDNA through silencing or eliminating it from infected cells. Several drugs that target cccDNA are in development. Gorsuch et al. have described a potentially curative approach using a highly specific engineered ARCUS nuclease (ARCUS-POL) targeting the HBV genome [31], where they showed a rescued HBsAg expression, circulating surface antigen. This paper presents a gene-editing approach for elimination of cccDNA aiming toward cure. Gene-editing approach will have to demonstrate excellent safety before starting clinical trials in patients with CHB. There remain some issues with off-target risks.

RNA interference

RNA interference (RNAi) by small-interfering RNA (siRNA) and antisense oligonucleotide (ASO) has been evaluated in early phases of drug development (phases I and II) for patients with CHB. RNAi targets post-transcriptional mRNAs and pgRNAs to reduce HBV antigen production and viral replication. This family of drugs may have two activities: a DAA effect but also an immune restoration role (i.e. by reducing viral antigens and limiting overwhelming of the host immune response against HBV). Phase- I/-II trials on siRNAs have shown to be safe and well-tolerated. Within this drug family, we can find JNJ-3989, AB-729, and bepirovirsen [32].

Interim results from the randomized phase-2b B-Clear study were presented recently [32]. Bepirovirsen, a naked ASO, appears to be a potent antiviral, but post-treatment follow-up will be needed to determine durability off-treatment. We have to better understand if ALT elevations/serious adverse events reflect restored innate immune response. Future studies may combine 24 weeks bepirovirsen with other novel agents, in which resultant therapy is a likely approach.

In the REEF-1 study, dose–response relationship between JNJ-3989 (siRNA) dose and reduction in all viral markers studied was observed [33], with more significant reductions in naive HBeAg+ patients. Interestingly, a notable stability or further decline in viral markers were observed after treatment discontinuation, despite a slow increase in HBsAg. Why there is more sustained suppression of the other viral markers compared with HBsAg? In the REEF-2 study, HBeAg– patients were treated with JNJ-3989 (siRNA) + JNJ-6379 (CAM) + NA or Placebo, for 48 weeks. The treatment led to virological suppression in patients without advanced fibrosis, but did not lead to HBsAg loss 24 weeks after stopping all treatments [33]. JNJ-6379 does not have a side effect — would the outcome have been different with another CAM? Why is there no further decrease of HBsAg after 24 weeks (already seen in REEF-1), incomplete target engagement? Potential antagonizing effect? How much of the HBsAg in this group of patients is from integrated DNA? Is an 'Inactive carrier state' or 'partial cure' the new aim?

Toll-like receptors-7/8 agonists

So far, antivirals were not able to induce functional cure and immune control of the infection, in most patients. Therefore, immunomodulatory drugs have been designed and tested in order to boost or restore immune responses against HBV. Toll-like receptors (TLR) are innate immune sensors expressed mostly by myeloid cells (dendritic cells, monocytes, and macrophages). Preclinical data using TLR agonists showed important remodeling of the liver immune microenvironment, associated with pro-inflammatory cytokine release and increased innate and adaptive immune cell activation [34–36]. Small-molecule agonists of TLR-7 (GS-9620, RO6864018) and TLR8 (GS-9688) are currently being tested in phase-1 and -2 clinical trials. While a surrogate of immune activation (induction of interferon-stimulated gene 15) was reported in most CHB patients treated with GS-9620, this was not accompanied with a significant loss of HBsAG levels [37,38]. Novel innate immunity-activating agents are to be tested.

Checkpoint inhibitors

CHB associates with severe T-cell dysfunction [39,40]. Immune checkpoint inhibitors (ICI) aim at re-invigorating exhausted T cells by targeting T-cell-inhibitory receptors (i.e. Programmed death protein 1), or its ligands (i.e. programmed death-ligand 1). ICIs have been tested in several clinical trials in order to treat patients with HBV-related HCC [41–43]. Although promising results were obtained in phases 1 and 2 as second-line treatment, a randomized phase-3 study did not show a significant clinical benefit of anti-PD-1 as a first-line treatment. The lack of efficacy of anti-PD-1 in this type of indication could be due to a profound exhaustion of tumor-infiltrating T-cell responses and the development of epigenetic scarring that prevents their functional recovery, as recently described in patients with chronic HCV infection [44,45].

Therapeutic vaccination

Several therapeutic vaccines (combined or not with ICI or antiviral molecules) have been tested or are currently under investigation in clinical trials. Many different formulations and immunization routes have been tested, but so far, none of these vaccines were able to successfully induce functional cure, despite improved HBV viral parameters and HBV-specific immune responses [46]. Among the recent vaccine candidates, TG-1050 is an adenoviral vector vaccine that has already been tested in phase-Ib clinical trial [27]: in patients treated with NA, a decrease in HBsAg of more than 0.2 log10 IU/mL occurred in 19% of vaccinees (n = 7) against 8% in non-vaccinated individuals (n = 3), one year after vaccination. Other vaccine candidates are being tested in phase-1 and -2 trials. While results are pending for many of them, the persistence and depth of exhaustion mechanisms, together with limited vaccine immunogenicity, are the current limits of therapeutic vaccination strategies.

Cellular therapies and infusion with immune compounds

Cell therapies by adoptive transfer of TCR-engineered T cells (TCR-T), which are genetically modified T-cell-targeting HBV epitopes, may constitute a promising approach for the treatment of HBV and HBV-associated HCC. Two first-in-human proof-of-concept clinical trials have shown encouraging results for three liver transplant patients with metastases from their primary HCC tumor [47,48]. These patients received 1–40 infusions of TCR-T. No side effects related to the treatment were observed. Despite the advanced stages of the cancers, two patients showed clinical signs of response to the treatment. In particular, in patients treated with CD8+ T lymphocytes directed against HBsAg, a proliferation of therapeutic T lymphocytes was observed (up to 2% of total circulating CD8+ T cells), as well as a 90% reduction in circulating HBsAg. A single-center (SAFE-T-HBV), single-arm, open-label study is currently ongoing to determine the safety of T cells genetically engineered to transiently express a HBV-specific T cell receptor (HBV/TCR mRNA) [49] on patients with HBV-induced HCC and for whom conventional treatments have failed. Recent results showed that HBV/TCR LT mRNAs were well-tolerated, inducing no serious systemic inflammatory events in 8 treated patients. In addition, reduction in the tumor lesions or absence of progression was observed in 3 patients [50]. Together, these results confirmed the safety of engineered T-cell therapies and suggest a strong therapeutic potential for the treatment of end-stage HCC induced by CHB. In addition to TCR-T, infused soluble T cell receptor (IMC-I109V) as well as broadly neutralizing anti-HB antibodies have entered clinical trial evaluations. The tolerability and efficacy of these immune-mediated therapeutics will need to be fine-balanced.

Conclusion

There is a hope to cure HBV infection. Several drugs, with different modes of action, are evaluated in clinical trials. The selection of the best synergistic combinations will be important. Furthermore, selection of the ideal patient's profile according to the mode of action of the investigational drug will be interesting to increase response rates. Earlier, Zhang et al. have shown that regardless of the ALT levels, treating all HBV patients, from 18 to 80 years old, NA treatment may protect them from complications [51]. This study has also shown that the mortality level due to HBV complication can be decreased by 65% by 2030–2050. In the future HBV cure strategy, there remains a role for NA, at least in the short term. In many combination trials, NAs are considered because of their safety, potent efficacy in inhibiting HBV DNA replication, negligible risk of drug resistance, and given orally, once daily. Furthermore, NA can result in functional cure in selected patients: those with more than three years of suppressed HBV DNA on NA monotherapy with end-of-treatment HBsAg at lower levels (< 100 IU/mL). PEG-IFN may be also considered in the short term. Peg-IFN treatment can result in HBsAg loss. Limited course of Peg-IFN monotherapy (48 weeks) can result in durable off-treatment HBsAg loss in ~11% of patients overall. In addition to monotherapy, many trials are studying it in combination with new therapies. De novo combination of Peg-IFN and NA, and Peg-IFN switch from NA or add-on to NA in selected virally suppressed patients may increase HBsAg loss compared with continuing NA. A short duration (12–24 weeks) may be considered within selected patients, in combination with investigational therapies to increase HBsAg loss. Future combinations with DAA and immune modulators are promising and may be feasible in some patients. Since immune-mediated elimination of HBV is often associated with liver flares, the safety of these therapies must be carefully examined. Hepatitis delta virus is a small RNA virus that needs HBsAg for its envelope, for entry into hepatocytes, and secretion. Hepatitis delta virus infection is the most severe form of viral hepatitis, with a high risk of developing cirrhosis and HCC. It will be also important to cure hepatitis delta virus infection [52,53].

Potential competing interests

NN, PT and AM: no conflict of interest. Tarik Asselah is a speaker and investigator for Antios Therapeutics, AbbVie, Eiger Bio-Pharmaceuticals, Enyo Pharma, Gilead Sciences, GSK, Janssen, and Roche.

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CRediT authorship contribution statement

TA designed and supervised the paper. All the authors contributed to the drafting of the review, the critical revision of the paper, and the final approval of the version.

Data Availability

Review NA.

Declaration of Competing Interest

None.

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