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Towards HBV curative therapies

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Abstract

Tremendous progress has been made over the last 2 decades to discover and develop approaches to control hepatitis B virus (HBV) infections and to prevent the development of hepatocellular carcinoma using various interferons and small molecules as antiviral agents. However, none of these agents have significant impact on eliminating HBV from infected cells. Currently the emphasis is on silencing or eliminating cccDNA, which could lead to a cure for HBV. Various approaches are being developed including the development of capsid effectors, CRISPR/Cas9, TALENS, siRNA, entry and secretion inhibitors, as well as immunological approaches. It is very likely that a combination of these modalities will need to be employed to successfully eliminate HBV or prevent virus rebound on discontinuation of therapy. In the next 5 years clinical data will emerge which will provide insight on the safety and feasibility of these approaches and if they can be applied to eradicate HBV infections globally. In this review, we summarize current treatments and we highlight and examine recent therapeutic strategies that are currently being evaluated at the preclinical and clinical stage.

Keywords

antiviral agents; cccDNA; HBV cure; immunotherapy

1 | INTRODUCTION

Chronic HBV infection affects around 240 million people worldwide (Figure 1A) and long-term risks such as cirrhosis and hepatocellular carcinoma (HCC) account for approximately 600 000 deaths annually.¹ HCC is one of the most frequent cancers in Africa and Asia and fibrosis is the most important prognostic predictor of survival.² Despite the availability of several FDA-approved drugs (Figure 1B), continuous treatment is necessary for virus replication control. Therefore, current HBV research and development programmes aim to achieve a curative strategy that either eliminates or permanently silences HBV infection.

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CONFLICTS OF INTEREST
Raymond Schinazi is the founder and major shareholder of Cocrystal Pharma, Inc. Tarik Asselah is a speaker and investigator for BMS, Janssen, Gilead, Roche and Merck. Maryam Ehteshami and Leda Bassit have no conflicts of interest to declare.

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This review will discuss approved therapies and will review novel therapeutic agents currently in development.

2 | HBV REPLICATION CYCLE AND THERAPEUTIC TARGETS

HBV belongs to the hepadnaviridae family of viruses. Similar to other related species such as duck hepatitis virus and woodchuck hepatitis virus, the relatively small genome of HBV consists of a 3.2 kb partially double-stranded DNA, referred to as relaxed circular DNA (rcDNA). HBV infects hepatocytes through a recently identified human sodium taurocholate cotransporting polypeptide (hNTCP) receptor. Upon fusion with the host membrane, the rcDNA-containing nucleocapsid is released into the cytoplasm and travels to the nucleus (Figure 2). Once inside, rcDNA is converted to a highly stable episomal DNA known as covalently closed circular DNA (cccDNA) via the host DNA repair machinery molecule. cccDNA is the transcriptional template for subsequent virus gene expression and generation of pregenomic RNA (pgRNA). HBV DNA is also found integrated into the host chromosome. Integration of viral DNA does not appear to play a direct role in virus replication. Instead, it is thought that HBV DNA integration may render the cellular environment more permissive to virus replication through modulating gene expression. It also likely plays an important role in hepatocellular carcinogenesis (reviewed in Ref. 7).

Gene organization of cccDNA is uniquely intricate whereby several overlapping open reading frames (ORFs) code for 7 viral proteins: pgRNA serves as the template for viral polymerase-mediated reverse transcription and subsequent synthesis of rcDNA. Another gene product of HBV is the core protein, also known as hepatitis B core antigen (HBcAg), which forms the viral nucleocapsid. Precore protein (hepatitis B e antigen, HBeAg), is a proteolytically processed viral protein that is often secreted from the infected cells and can serve as a marker for disease stage. Viral surface proteins, S, M and L (named based on their small, medium and large sizes respectively) are coded from the S gene whereby S is translated from S mRNA, M is translated from preS2 + S mRNA and L is translated from preS1 + PreS2 + S mRNA. All surface proteins of different length are collectively referred to as HBsAg. Finally, cccDNA also codes for viral protein X (HBx), a nonstructural protein that likely acts as a transcriptional transactivator and plays a role in regulating viral gene expression.

Once all viral proteins are synthesized and the rcDNA-containing nucleocapsid is formed, it can travel through the cellular secretion pathway and be released as an enveloped and infectious virion. Alternatively, the nucleocapsid can cycle back to the nucleus intracellularly, whereby the recently synthesized rcDNA serves to replenish the cccDNA pool. In this way, cccDNA can be maintained even in the absence of observable viremia. Furthermore, cccDNA can remain dormant for a long time and only become transcriptionally activated years or decades after the initial infection. Although several therapeutic strategies have been developed against HBV replication, targeting or eliminating cccDNA, as an inert, yet highly stable mini-chromosome has proven challenging. In this way, cccDNA elimination, or at the very least, transcriptional control, lies at the heart of the quest for a cure for chronic hepatitis B.
In theory, any step of the virus replication cycle can be a target for antiviral therapeutics (Figure 2). The most successful target so far has been the reverse transcription activity of *pol*. Interferon (IFN) treatment has also shown modest success in inhibiting virus replication. Other areas of therapeutic research include targeting of the core/capsid protein, mRNA transcription and cccDNA stability and formation. In addition to gene expression targeting technologies such as CRISPR/Cas9 and small interfering RNA (siRNA), several immune-modulatory strategies that aim to enhance both the innate and adaptive response to CHB infection are also under investigation. The following sections will give a more detailed account of these novel strategies in development and their mechanism of action.

3 | APPROVED THERAPIES FOR TREATMENT OF HBV INFECTION

The goal of CHB therapy is to improve survival by preventing risk of cirrhosis and end-stage liver disease, and to improve quality of life. Several therapies have been approved for CHB infection (Figure 1B). Historically, under long-term lamivudine (LAM) treatment, HBV DNA suppression leads to reduction in cirrhosis decompensation and HCC (Figure 1C). Thus, achieving HBV DNA clearance allows for reduction in necro-inflammation and reduction in the risk of fibrosis progression. There are currently 2 principal therapeutic strategies approved for both HBe antigen-positive (HBeAg+ve) and antigen-negative (HBeAg-ve) patients (Table 1). This includes a finite treatment course of interferon alpha (IFN-α)/pegylated interferon (PEG-IFN) or, a long-term maintenance treatment with nucleoside analogues (NAs).

A 1-year treatment with PEG-IFN offers the potential for immune control of HBV infection, with higher rates of HBeAg seroconversion and the possibility of viral suppression after stopping treatment, with loss of hepatitis B surface antigen (HBsAg) in a significant proportion of patients who maintain undetectable HBV DNA (Figure 3). However, PEG-IFN is administered by subcutaneous injection and is associated with poor tolerability and a risk of depression. Furthermore, PEG-IFN is contraindicated in subjects with decompensated cirrhosis, in persons with autoimmune disease and during pregnancy.

NAs suppress HBV replication via direct antiviral activity. Usually, tolerability is good and compliance to treatment appears adequate. Entecavir (ETV) and tenofovir disoproximal fumarate (TDF) are potent HBV inhibitors with a high barrier to resistance and should be used as first-line monotherapies. The chemical structures of various HBV reverse transcriptase inhibitors are depicted in Figure 1B. ETV is a nucleoside analogue that disrupts DNA elongation as a delayed chain-terminator, whereas tenofovir (TFV) is a nucleotide (adenosine) analogue that causes immediate chain termination when incorporated into HBV DNA during reverse transcription. ETV is unusual in that it is a D-enantiomer of guanosine, and this in part accounts for its tighter binding in the active site of the enzyme as part of the mechanism of interference. Normal nucleotides can be incorporated following ETV at the 3′ hydroxyl; nevertheless, chain termination occurs shortly thereafter because of structural disruptions to the enzyme, so it is an indirect (or delayed) chain terminator, in contrast to the other agents that are used to treat HBV. TFV, in contrast, lacks a 2-deoxyribose (cyclic) moiety including the 3′ hydroxyl group, and therefore once incorporated, it terminates polymerization. For TFV, the innovation is the use of the acyclic...
phosphonate group, which achieves 2 functions: (a) this group is resistant to cellular esterases that otherwise might serve to remove the phosphate group to form a nucleoside derivative, and (b) in contrast to other nucleoside analogues, this stable alteration circumvents the need for the cellular addition of the alpha phosphate, which is the rate-limiting step in the synthesis of 2′-deoxynucleoside triphosphates, the active substrates for reverse transcriptase.

The phosphonate group of TFV differs from a normal phosphate group in that a carbon is directly linked to the phosphorus. For a phosphate group, the phosphorus would be directly linked to an oxygen atom in place of the carbon atom in the TFV diagram above. The phosphonate is referred to as acyclic because in naturally occurring nucleotides, the phosphate group is linked to a sugar moiety (cyclic, ribose or deoxyribose), whereas this is absent in this drug. The nucleoside analogues used for HBV treatment are cyclic nucleosides (ETV, lamivudine [LAM] and telbivudine). Adefovir (ADV), in spite of its different characteristics, differs from TFV only by the lack of the methyl group (Figure 1B).

More than 95% of persons treated with the highly potent TDF and ETV achieve virological clearance. NAs are administered orally, and tolerance is good. The safety of these drugs over lifelong therapy remains to be established. Regarding the risk of drug resistance, although common in the past with earlier, less potent NAs such as LAM and ADV, resistance has become extremely rare with TDF and ETV. Long-term clinical data up to 6 years and beyond are emerging for the newer NAs that are providing reassuring data on their efficacy and safety. There are large data that long-term blockage of HBV replication by the most potent drugs (ETV and TDF) results in an improved long-term survival with a decreased risk of progression to cirrhosis, end-stage liver disease and HCC. Furthermore, a study analysing liver histology in persons treated with TDF for 5 years demonstrated fibrosis regression in the majority of patients and also cirrhosis reversal (Figure 4).\textsuperscript{22,23} In addition, the cirrhosis reversal was observed \textit{during} treatment in 75% of patients with cirrhosis, probably associated with a decreased risk of HCC and improved survival. Real-world data with ETV and TDF in routine clinical practice are confirming the favourable safety and excellent efficacy of these therapies.

4 | TENOFOVIR ALAFENAMIDE (TAF)

TAF, as a single-agent treatment, has been developed for CHB. It is a novel prodrug of TFV with potent antiviral activity against HBV. The drug is more stable in plasma with more than 90% reduction in circulating TFV level compared to TDF.\textsuperscript{24} The dose is once-daily, oral, 25 mg tablet. Chemical structures of TAF is provided in Figure 1B. In the phase III clinical trials,\textsuperscript{25–27} treatment with TAF through 72 weeks demonstrated: viral suppression (HBV DNA < 29 IU/mL) similar to TDF; higher rates of alanine aminotransferase (ALT) normalization; no resistance development in either treatment group at Week 48; rates of HBeAg loss and seroconversion similar to TDF in Study 110; good tolerance in HBeAg-negative and -positive subjects; treatment-emergent AEs similar to TDF; significantly less declines in hip and spine bone mineral density (BMD) compared to TDF with improved bone biomarkers; and significantly smaller decreases in estimated glomerular filtration rate.
by Cockcroft-Gault (eGFR\textsubscript{CG}) compared to TDF, with improved markers of renal tubular function (Figure 5).

5 | RECENT DEVELOPMENTS REGARDING APPROVED THERAPIES

5.1 | Combination therapy (TDF plus PEG-IFN)

In a recent study, HBsAg loss was evaluated in patients receiving the combination of TDF and PEG-IFN for a finite duration.\textsuperscript{28} In an open-label, active-controlled study, 740 patients with CHB were randomly assigned to receive TDF plus PEG-IFN for 48 weeks (group A), TDF plus PEG-IFN for 16 weeks followed by TDF for 32 weeks (group B), TDF for 120 weeks (group C) or PEG-IFN for 48 weeks (group D). At week 72, 9.1% of subjects in group A had HBsAg loss compared with 2.8% of subjects in group B, none of the subjects in group C and 2.8% of subjects in group D. A significantly higher proportion of subjects in group A had HBsAg loss than in group C (\(P < .001\)) or group D (\(P = .003\); Figure 6). However, the proportions of subjects with HBsAg loss did not differ significantly between group B and group C (\(P = .47\)) or group D (\(P = .88\)). HBsAg loss in group A occurred in HBeAg-positive and HBeAg-negative patients with all major viral genotypes. Finally, a significantly greater proportion of subjects receiving TDF plus PEG-IFN for 48 weeks had HBsAg loss than those receiving TDF or PEG-IFN alone.

6 | NOVEL THERAPEUTIC STRATEGIES IN DEVELOPMENT

6.1 | Entry inhibitors

The discovery of human sodium taurocholate cotransporting polypeptide (hNTCP) as the cellular receptor that mediates HBV fusion has facilitated drug development efforts with regard to inhibition of HBV entry. Myrcludex B represents the most advanced therapeutic agent that targets hNTCP-mediated HBV entry into the cell (Table 2). It is a synthetic 47 amino acid, N-myristoylated lipopeptide that is derived from the amino acid sequence of L surface protein. By binding to this receptor in a competitive manner, Myrcludex B excludes viral HBsAg binding, and thus inhibiting virus entry.\textsuperscript{29} Although Mycludex B showed positive effects on virus control and intracellular cccDNA accumulation,\textsuperscript{29} some serious adverse events were observed in phase I clinical trials with healthy volunteers.\textsuperscript{30} Myrcludex B has also shown promise for the treatment of hepatitis D virus, which uses the same hNTCP receptor as HBV.\textsuperscript{31} Another molecule of interest for inhibition of HBV entry is irbesartan.\textsuperscript{32} Originally, this molecule was developed against hypertension and received FDA approval in 1997. Because irbesartan interferes with hNTCP receptors, it was also shown to be able to inhibit HBV virus entry in HepG2 cell culture.\textsuperscript{33} It remains to be determined whether the addition of entry inhibitors to the current arsenal of HBV therapeutics would provide any additional benefits, since at least in theory, this class of inhibitors should be able to protect uninfected cells from HBV entry and subsequent establishment of HBV cccDNA pool.\textsuperscript{34}

6.2 | RNA interference

RNA interference is a relatively novel strategy that targets virus replication at the level of gene expression (Table 2). Briefly, short RNA molecules are designed to target specific viral
mRNA sequences. Upon entry into the cell, they hybridize to viral mRNA and the resulting double-stranded RNA is targeted for degradation.\textsuperscript{35} ARC-520 represents a leading siRNA molecule that when conjugated with cholesterol, can be delivered to the liver upon intravenous administration. Experiments on chimpanzees showed that ARC-520 is highly effective at reducing circulating HBV DNA levels as well as serum HBeAg and HBsAg.\textsuperscript{35,36} ARC-520 has shown to be relatively well tolerated in a phase I clinical trial, although some hypersensitivity reactions were observed. Therefore, the authors recommend future co-administration with antihistamines.\textsuperscript{37} Based on these promising results, ARC-520 has advanced to phase II trials. However, these trials were recently terminated because of formulation toxicities.\textsuperscript{38} Interestingly, a recent study has identified a novel small molecule inhibitor that can reduce viral messenger RNA (mRNA) and pgRNA expression.\textsuperscript{39} Despite strong evidence that RG7834 does not function in as RNAi, it is not yet clear how this therapeutic agent can suppress HBV transcription in a virus-specific manner. As well, it has been shown that RG7834 can indeed reduce levels of viral antigens in the humanized mouse model, although it is still not clear whether this small molecule would also reduce HBsAg levels expressed from integrated HBV DNA as seen in individuals with chronic hepatitis B.

6.3 | Gene-editing strategies

Recent advancement in novel sequence-specific nuclease technology has led to the development of several novel therapeutic strategies against HBV (Table 2). These include zinc finger nucleases, TALENs (transcription activator-like effector nucleases) and, CRISPR/Cas9 systems, which through viral gene editing, have shown some success in reducing viral DNA and cccDNA levels in both cell culture and animal models.\textsuperscript{40-42} Most antiviral gene-editing therapeutics act as mutagenic agents that, upon cell entry, cause DNA damage at sequence-specific sites within cccDNA. DNA repair via non-homologous recombination at these sites is error-prone and this in turn, results in generation of insertion/deletions within cccDNA.\textsuperscript{43}

Zinc finger nucleases have successfully been used in tissue culture to reduce HBV levels.\textsuperscript{44-47} For example Weber et al developed a zinc finger nuclease that targeted the core and pol regions of cccDNA. Using an adeno-associated virus (AAV) delivery system, they showed that HBV production from HepAD38 cells could be significantly controlled upon zinc finger nuclease delivery. However, they were unable to show specific mutations within cccDNA in a reproducible manner.\textsuperscript{41} The efficacy of TALENs in reducing HBV levels in cell culture was first demonstrated by Bloom et al in 2013 whereby targeting the S and C regions in cccDNA resulted in disruption of 35\% of cccDNA molecules in the HepG2 cell line.\textsuperscript{48} In agreement with these findings, it was demonstrated that TALENs could also reduce the circulation of viral particle equivalents in the murine hydrodynamic injection model\textsuperscript{48} and cause reductions in secreted HBeAg levels.\textsuperscript{49} Use of CRISPR/Cas9 for treatment of HBV was first evaluated in 2014.\textsuperscript{42} Through the simultaneous administration of multiple small guiding RNAs specific for various regions of the HBV genome, several studies have demonstrated successful inhibition of virus replication and production of viral markers such as HBsAg.\textsuperscript{50-53} One study also showed a marked decrease in cccDNA levels in HepAD38. This reduction was associated with rampant presence of mutations within viral DNA.\textsuperscript{40} Overall, preclinical studies with gene-editing technologies have been promising. However,
several challenges still remain. For one, in vivo delivery methods that would maximize efficacy and minimize immune response to therapeutic gene editors are still being developed. There is also a concern for viral escape mutants as a result of treatment with sequence-specific zinc finger nucleases, TALENs or CRISPR/Cas9 systems. Finally, adverse events need to be carefully investigated with these agents, both in terms of non-specific cleavage of host chromosomes, and chromosome destabilization as a result of cleavage of viral DNA integrants. Despite these concerns, some gene-editing agents such as EBT106 (Excision Biotherapeutics) are cautiously advancing towards clinical development (https://www.excisionbio.com Biotherapeutics 2017, posting date. [Online]).

6.4 | Secretion inhibitors

In addition to infectious virion secretion, a large number of incomplete viral particles are released from infected hepatocytes. These include spherical and filamentous particles made up of HBsAg, also known as Australia antigen. It is hypothesized that these particles are released in copious amounts to induce immune tolerance and exhaustion. Based on this, several inhibitors such as REP 2139 and REP 2165 have been developed that inhibit the secretion of HBsAg containing subparticles (Table 2). Phase I clinical trials have shown positive results with regard to safety, tolerability and control of HBV viraemia. Based on these findings, Phase II clinical trials are underway.

6.5 | Polymerase inhibitors

Currently, nucleoside analogue inhibitors are the backbone of chronic HBV treatment. As discussed elsewhere, when administered as monotherapy, nucleoside analogues are quite successful at suppressing viral replication, but do not eliminate the virus from the human body. There are currently several nucleoside analogue inhibitors in development that aim to provide therapeutic, pharmacological and tolerability improvements on the existing drug regimens. These include CMX-157, a novel prodrug of TAF and MIV-210, a nucleoside analogue with antiviral activity in woodchucks. Another novel acyclic nucleoside analogue is besifovir (LB80380), which in comparison to entecavir, was shown to achieve similar therapeutic results in treatment-naïve chronic hepatitis B patients. It remains to be seen whether these novel nucleoside analogues, as mono or combination therapy, provide substantial advantages over current FDA-approved therapies.

6.6 | Immunomodulators

An important characteristic of chronic hepatitis B is the development of immune exhaustion as a result of continuous exposure of T cells to high levels of viral antigens. Overexpression of several inhibitory receptors such as PD1 and CTLA-4 has been shown to be associated with T-cell exhaustion. As such, it has been hypothesized that small molecules that inhibit PD-1 expression, may result in T-cell reactivation, and subsequent mounting of an efficient immune response that may be able to eliminate chronic hepatitis B. It is worth noting that most immune checkpoint inhibitors have the potential to be used in any number of diseases where immune reactivation is desired. These include important human diseases such as melanoma, lymphoma and other cancers. A list of current PD-1 and other immune checkpoint inhibitors is provided in Table 2.
Recent developments with regard to toll-like receptor (TLR) agonists have also been shown to be effective in modulating the innate immune response against HBV. Activation of virus-specific TLRs can lead to production of IFN-α and -β, both of which play important roles with regard to suppression of virus replication (See review 59). Specifically, TLR-7 plays a critical role in activating the immune response within the liver environment without causing systemic inflammation. Stimulation of TLR-8 as a result of viral infection was shown to result in IFN-γ production and subsequent virus control. At the same time, it was found that TLR-8 function was impaired in patients with chronic HBV infection. Based on this, several therapeutic strategies have been developed to harness the innate immune response in response to chronic hepatitis B. GS-9620 is a TLR-7 agonist that showed promising results in animal studies with chimpanzee and woodchuck models. Treatment with GS-9620 resulted in reduction in circulating HBV DNA and woodchuck surface antigen. Conversely, when GS-9620 was administered to patients with chronic hepatitis B in a phase II trial, it was found that the T cell response was improved in a transient manner, but no significant reduction in HBsAg levels was detected. It was hypothesized that variations in dosing may account for this discrepancy.

Development of therapeutic vaccines represents another recent strategy against chronic hepatitis B. It is hypothesized that the stimulation of T cells with HBV antigens may result in the generation of an effective and HBV-specific adaptive immune response that may eliminate the chronic infection. Based on this, several therapeutic vaccines are currently being evaluated in the clinic. Examples include GS-4774, which consists of highly immunogenic recombinant HBCAg, HBsAg and HBx epitopes, ABX-203 consisting of HBCAg and HBsAg epitopes, and INO-1800 consisting of DNA plasmids coding for HBCAg and HBsAg. Other therapeutic vaccines currently in clinical trials are listed in Table 2. Despite promising outcomes of recent immune therapies, several potential challenges still remain. For one, manipulation of the innate or adaptive immune response may be accompanied by related adverse events in either a local, or systemic manner. This was most acutely observed in recent clinical trials involving PD-1 agonists and other immune-modulatory therapies whereby the liver and the endocrine system were negatively affected.

6.7 | Inhibitors of cccDNA formation

The major strategy to “cure” HBV is to silence or eliminate cccDNA from the hepatocyte nucleus. cccDNA is the main element of viral replication cycle as it serves as the template for transcription of all viral RNAs including pgRNA, and subsequently the progeny HBV DNA genomes are formed. cccDNA is either originated from new incoming virions or amplified from encapsidated rcDNA from the hepatocyte cytoplasm. Because hepatocytes have a long half-life (>6-months or even years), the half-life of cccDNA is also relatively long; therefore, elimination of cccDNA by hepatocyte turnover is not a major means of clearance. Reverse transcriptase nucleoside analogue (NA) inhibitors have a negligible effect on cccDNA formation, stability or amplification, and therefore HBV commonly rebounds after cessation of treatment with NA. In addition, the presence of HBsAg in serum of chronic hepatitis B (CHB) individuals with undetectable levels of HBV DNA after or during NA therapy strongly suggest that this protein is transcribed either from cccDNA or
integrated HBV DNA in the nucleus of hepatocytes. Enormous advances in understanding the HBV replication cycle have facilitated the development of novel antiviral therapies targeting multiple steps of viral replication with numerous mechanistic actions. Both in vitro and in vivo models for testing these novel antiviral therapies have also significantly improved and mutually open a new era for extinction of hepatitis B chronic infection.\(^7^4\)

Several in vitro studies demonstrate that it is possible to degrade cccDNA in the nucleus of hepatocytes with minimal hepatotoxicity effect. As discussed above, programmable RNA-guided DNA endonucleases, including CRISPR/Cas9 system have shown in both cell and mouse models the potential to serve as effective tool for the depletion of the cccDNA pool in HBV-infected hepatocytes (Table 2).\(^4^0,4^4,5^2\) Moreover, up-regulation of APOBEC3A/B deaminase by several cytokines including IFN-\(\alpha\), IFN-\(\gamma\), tumour necrosis factor-\(\alpha\) and lymphotoxin-\(\beta\) receptor agonist can cause partial degradation of cccDNA without hepatotoxicity.\(^7^5\) These targets aim to prevent cccDNA formation by damaging or destroying it. However, more research needs to be done regarding off-target effects of gene-editing applications or the in vivo delivery efficiency before they advance to humans.

6.8 | Silencing cccDNA with epigenetic drugs

Viral cccDNA is a mini-chromosome that is inherently stable and silencing its activity by epigenetic modifications with epigenetic drugs (epidrugs) may represent a novel class of antivirals for treatment of chronic hepatitis B. HBx binds to cccDNA and plays an important role on activation of viral gene transcription, leading to high level of virus replication. In contrast, inhibition of HBx binding to cccDNA leads to suppression of its transcription and virus replication by modifying the epigenetic regulation of cccDNA function with host restriction factors, including Smc5/6 complex and protein arginine methyltransferase PRMT1. HBV core inhibitors targeting cccDNA-bound HBc may potentially alter the epigenetics of cccDNA and in combination with HBx inhibitors could in theory lead to a stronger synergistic effect on cccDNA transcriptional silencing and HBV replication.\(^7^6\)

6.9 | Nucleocapsid assembly inhibitors or modulators

Novel direct acting core assembly inhibitors or modulators have an important impact in HBV replication cycle. They can interfere with several steps of the HBV replication cycle including capsid assembly, reverse transcription, as well as pgRNA and polymerase protein packaging. These molecules may also affect intracellular trafficking of relaxed circular DNA in the nucleus, virus assembly by disrupting its interaction with envelope proteins, and core binding to cccDNA.\(^7^7\) Two main classes of core assembly modulators have been developed, including class I heteroaarylpyrimidines (HAP), and class II phenylpropenimides (PP) or sulfamoylbenzamide (SBA) and derivatives (Table 2). NVR 3–778 is a SBA compound developed by Novira (later acquired by Johnson & Johnson, New Brunswick, NJ, USA) that is in phase Ia clinical trial (NCT02112799 & NCT02401737), and has shown synergistic effect when in combination with PEG-IFN. Evaluation of NVR 3–788 with PEG-IFN in mice with humanized liver confirmed inhibition of both HBV DNA replication and HBV RNA production.\(^7^8\) Recent in vitro studies with JNJ-632 (SBA) or Bay41–409 (HAP) compounds in human primary hepatocytes showed that both compounds inhibited early and late steps of viral replication by (a) preventing formation of cccDNA, and (b) reducing

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intracellular HBV RNA levels and HBe and HBs antigens production when these compounds were added at the time of infection. Clinical studies are being conducted with 3 other potent core assembly modulators, including morphothiadine mesilate GLS4 (HAP) in phase II (HEC Pharm/Sunshie, Guangdong, China-CFDA), JNJ56136379 in phase I (JnJ Janssen, New Brunswick, NJ, USA - NCT02662712), and the first-generation Core protein Allosteric Modifier (CpAM) in phase I Assembly Biosciences (NCT02662712). New improved second generation of CpAMs that induce formation of aberrant capsids were recently developed that show favourable preclinical profile in inhibiting 4 HBV markers, including secretion of HBeAg and HBsAg, pgRNA and cccDNA (Table 2). A new generation of capsid assembly inhibitor AB-506 (Arbutus Biopharma, Burnaby, BC, Canada) was developed that increases thermal stability to core protein compared to first-generation inhibitors, and it also inhibits pgRNA encapsidation in HepAD38 cells and demonstrates potent in vivo activity in mouse model. AB-423 (Arbutus Biopharma) is another inhibitor of capsid assembly that has demonstrated potent inhibition of HBV replication by blocking pgRNA encapsidation. This compound is in phase I clinical study (Table 2).

7 | WHO TO TREAT: THE IMPORTANCE OF FIBROSIS AS PROGNOSIS FACTOR

Fibrosis is the most important prognosis predictor of survival. Therefore, subjects at risk of fibrosis progression or with advanced fibrosis may be prioritized for treatment (Table 3). Several new markers have been developed to assess fibrosis. For instance, our group in France developed a simple scoring system to determine the severity of fibrosis in persons with genotypes B or C HBV infection who are HBeAg-positive. We developed 2 prediction scoring systems (PSs). PS1 analysed data on HBV genotype (B vs C), patient age (<30 vs ≥30 years), level of hepatitis B surface antigen (≤17 500 vs >17 500 IU/mL) and level of alanine aminotransferase (<3-fold vs >3-fold the upper limit of normal). PS2 analysed data on only age and level of hepatitis B surface antigen. Our system differentiated persons with no or mild fibrosis (F0-F1) from those with marked or severe (F2-F4) fibrosis with a high positive predictive value (PPV). The high level of specificity for the identification of non-severe fibrosis (F0-F2) limits the risk of overlooking patients with severe fibrosis (F3-F4).

In another study, the expression of 13 fibrosis-related microRNAs (miRNAs; miR-20a, miR-21, miR-27a, miR-27b, miR-29a, miR-29c, miR-92a, miR-122, miR-146a, miR-155, miR-221, miR-222 and miR-224) was analysed in 194 serums and 177 liver biopsies of patients with either CHB or chronic hepatitis C (CHC) to develop models to diagnose advanced fibrosis and cirrhosis (Metavir F3-F4). In CHB subjects, the model (serum miR-122, serum miR-222, platelet count and alkaline phosphatase) was more accurate than APRI and FIB-4 to discriminate in between mild and moderate fibrosis (F1-F2) and F3-F4 (AUC of CHB model: 0.85 vs APRI: 0.70 and FIB-4: 0.81). In CHC persons, the model (hepatic miR-122, hepatic miR-224, platelet count, albumin and alanine aminotransferase) was more accurate than both APRI and FIB-4 to discriminate in between patients with F3-F4 and F1-F2 (AUC of the CHC model = 0.93 vs APRI: 0.86 and FIB-4: 0.79). Most of the
miRNAs tested were differentially expressed in patients with CHB and CHC. In particular, serum miR-122 was 28-fold higher in patients with CHB than in those with CHC. Both CHB and CHC models may help for the diagnosis of advanced fibrosis and cirrhosis (F3-F4).

8 | HBSAG QUANTIFICATION: A NEW TOOL FOR HBV MONITORING

Quantifying HBsAg is certainly an important new tool for predicting the severity of disease and distinguishing inactive carriers from persons with HBeAg-negative chronic active hepatitis. In this way, HBsAg measurement helps tailor follow-up and treatment management. In addition, a decline in quantitative HBsAg during therapy is a strong predictor of SVR after PEG-IFN therapy and of the probability of HBsAg loss, which is the ultimate goal of therapy. In patients treated with nucleoside analogues, a decline in HBsAg levels is also a predictor of HBsAg loss, allowing therapy to be discontinued. A low HBsAg level is predictive of favourable response. Baseline HBsAg levels are reliable predictors of sustained response and several data confirm this result. Baseline HBsAg levels are significantly lower in patients who achieve an SVR than in non-responders, both in HBeAg-positive and HBeAg-negative patients.

9 | CONCLUSIONS

The recent success in eliminating hepatitis C virus infection from the liver using safe and potent combinations of direct antiviral agents has raised hopes that this will also be possible for HBV infections. Lifetime use of current anti-HBV agents can decrease and suppress the HBV replication to undetectable levels, but it does not produce a cure. To have a sustained clinical cure, the discovery and development of novel combined modalities that prevent virus rebound on discontinuation of therapy is needed. There is also a need to silence or eliminate cccDNA, and decrease or eliminate HBsAg, HBeAg, pgRNA and possibly other HBV markers to undetectable levels. In addition, restoring or increasing the host immune response should lead to a functional and perhaps an absolute cure. Once these persons are cured, theoretically they can be vaccinated against HBV to prevent reinfection. Only then, we will have all the tools necessary to eradicate HBV globally.

Acknowledgments

Funding information

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Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>adeno-associated virus</td>
</tr>
<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>cccDNA</td>
<td>covalently closed circular DNA</td>
</tr>
<tr>
<td>CHB</td>
<td>chronic hepatitis B</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;CG&lt;/sub&gt;</td>
<td>estimated glomerular filtration rate, Cockcroft-Gault method</td>
</tr>
<tr>
<td>ETV</td>
<td>entecavir</td>
</tr>
<tr>
<td>HAP</td>
<td>heteroarylpurimidines</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Hepatitis B e antigen</td>
</tr>
<tr>
<td>nucleocapsid</td>
<td>precore protein</td>
</tr>
<tr>
<td>HBeAg-ve</td>
<td>antigen-negative</td>
</tr>
<tr>
<td>HBeAg+ve</td>
<td>HBe antigen-positive</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
</tr>
<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
</tr>
<tr>
<td>HBx</td>
<td>viral protein X</td>
</tr>
<tr>
<td>HCC</td>
<td>hepatocellular carcinoma</td>
</tr>
<tr>
<td>hNTCP</td>
<td>human sodium taurocholate cotransporting polypeptide</td>
</tr>
<tr>
<td>IFN-α</td>
<td>interferon alpha</td>
</tr>
<tr>
<td>LAM</td>
<td>lamivudine</td>
</tr>
<tr>
<td>LdT</td>
<td>telbivudine</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NA</td>
<td>nucleoside analogue</td>
</tr>
<tr>
<td>NI</td>
<td>nucleoside inhibitors</td>
</tr>
<tr>
<td>NNI</td>
<td>non-nucleoside inhibitors</td>
</tr>
<tr>
<td>ORF</td>
<td>open reading frames</td>
</tr>
<tr>
<td>PEG-IFN</td>
<td>pegylated interferon</td>
</tr>
<tr>
<td>pgRNA</td>
<td>pregenomic RNA</td>
</tr>
<tr>
<td>PP</td>
<td>phenylpropenimides</td>
</tr>
<tr>
<td>QD</td>
<td>once daily</td>
</tr>
<tr>
<td>rcDNA</td>
<td>relaxed circular DNA</td>
</tr>
<tr>
<td>SBA</td>
<td>sulfamoylbenzamide</td>
</tr>
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</table>
siRNA  small interfering RNA
TAF  tenofovir alafenamide
TDF  tenofovir disoproxil fumarate
TFV  tenofovir
TLR  toll-like receptor

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88. Agarwal K, Cheng W, Sievert W, et al. Bi-weekly dosing of ARB-1467 LNP siRNA in HBeAg negative, virally suppressed patients with chronic HBV infection leads to deeper declines in HBsAg and potential association with IL28b. The Liver Meeting, Washington, DC, USA; 2017 Abstract LB-17.


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Key points

- Chronic hepatitis B affects an estimated 240 million people worldwide.
- Current treatment includes the use of nucleoside analogue inhibitors and interferons which can control virus replication but are not curative.
- Several novel therapeutic strategies are in development. These include immunotherapy, gene-editing technology and small molecule inhibitors that target the viral capsid and cccDNA.
- These novel therapies aim to provide a functional cure for chronic hepatitis B.
FIGURE 1.
Global distribution of HBV and current therapies. (A) Worldwide distribution of chronic HBV infection. (B) Approved therapies for chronic HBV infection: ETV (entecavir), PEG-IFN (pegylated-interferon), TDF (tenofovir), LAM (lamivudine), LdT (telbivudine), TAF (tenofovir alafenamide). (C) Long-term lamivudine therapy leads to reduction in cirrhosis decompensation and HCC.\textsuperscript{13,14}
FIGURE 2.
Schematic representation of mechanisms of HBV replication and inhibition. The virus replication begins with the attachment of the virion to hepatocyte cell surface receptor hNTCP. This step can be blocked with entry inhibitors such as myrcludex B. Upon entry, the virion is released in the cytoplasm and the nucleocapsid travels to the nucleus where rcDNA enters the nucleus and cccDNA is formed. Novel cccDNA formation, or maintenance of already formed cccDNA can theoretically be disrupted with small molecule inhibitors or gene-editing technology such as CRISPR/Cas9. Viral mRNA and pregenomic RNA are transcribed from cccDNA. Inhibition of transcription suppresses viral gene expression. HBV DNA also gets integrated into the host chromosome. Secretion inhibitors can prevent the release of HBsAg, which can be independent of the virus replication cycle. Capsid effectors/inhibitors can mislead proper nucleocapsid formation while nucleoside analogues inhibit reverse transcription inside the nucleocapsid. Secretion inhibitors can subsequently inhibit the release of enveloped virions. Finally, immunomodulators such as therapeutic vaccines and TLR agonists can enhance the immune response to chronic hepatitis B.
FIGURE 3.
HBsAg loss after therapy. Loss of HBsAg is the most reliable indicator to measure a functional cure of hepatitis B. In HBeAg(+) patients, HBsAg loss was around 11% with Peg-IFN after 4 y, and around 10% after 5 y of TDF. In HBeAg(−) patients, HBsAg loss was still around 11% with Peg-IFN. However, no HBsAg loss was observed after 2 y of TDF or ETV treatment.\textsuperscript{15–17,22,23}
FIGURE 4.
Fibrosis regression under TDF. Advanced liver fibrosis and particularly cirrhosis were previously considered to be largely irreversible. Data from small studies suggest this may be possible. The histological benefits of 5 y of TDF therapy in the largest study of paired liver biopsies in HBV over 5 y of therapy are shown. The histogram shows that the proportion of patients with Ishak stages 0–2 increased over the study period, whereas, in contrast, the proportion with Ishak stages 4–6 decreased, thus dramatically illustrating a marked overall decrease in liver scarring for this cohort.\textsuperscript{22,23}
FIGURE 5.
Efficacy of TAF in phase III clinical trials. In the phase III clinical trials, in both HBeAg-positive and HBeAg-negative chronic hepatitis B, treatment with TAF through 72 wk demonstrated comparable viral suppression (HBV DNA < 29 IU/mL) to TDF and improved rates of ALT normalization.\textsuperscript{25,26}
Combination therapy with TDF and PEG-IFN. At week 72, 9.1% of subjects in group A had HBsAg loss compared with 2.8% of subjects in group B, none of the subjects in group C and 2.8% of subjects in group D. A significantly higher proportion of subjects in group A had HBsAg loss than in group C ($P < .001$) or group D ($P = .003$). However, the proportions of subjects with HBsAg loss did not differ significantly between group B and group C ($P = .47$) or group D ($P = .88$).
# TABLE 1
Advantages and disadvantages of PEG-IFN and nucleoside analogues

<table>
<thead>
<tr>
<th></th>
<th>Peg-IFN</th>
<th>Nucleoside analogues</th>
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</thead>
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<tr>
<td><strong>Advantages</strong></td>
<td>Finite duration</td>
<td>High efficacy</td>
</tr>
<tr>
<td></td>
<td>Higher rates of HBs loss and/or seroconversion</td>
<td>Favourable tolerability</td>
</tr>
<tr>
<td></td>
<td>No resistance</td>
<td>Oral administration</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Poor tolerability</td>
<td>Long life duration</td>
</tr>
<tr>
<td></td>
<td>Moderate efficacy</td>
<td>Unknown long-term toxicity</td>
</tr>
<tr>
<td></td>
<td>Risk of adverse events</td>
<td>Costs</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous injection</td>
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# TABLE 2

Summary of recent anti-HBV antiviral agents in clinical and preclinical development

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Antiviral agent</th>
<th>Status</th>
<th>Developer</th>
<th>Comments and references</th>
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<tr>
<td>Entry inhibitors</td>
<td>Myrcludex B</td>
<td>Phase II</td>
<td>MYR GmbH/Hepatera</td>
<td>Bogomolov et al (2016)77</td>
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<tr>
<td>Inhibitors of viral mRNA</td>
<td>ALN-HBV</td>
<td>Phase I-II</td>
<td>Alnylam</td>
<td>siRNA</td>
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<td>ARC-520</td>
<td>Terminated</td>
<td>Arrowhead Pharmaceuticals</td>
<td>siRNA</td>
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<td></td>
<td>ARB-1467</td>
<td>Phase II</td>
<td>Arbutus Biopharma</td>
<td>siRNA88,89</td>
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<td></td>
<td>ARB-1740</td>
<td>Preclinical</td>
<td>Arbutus Biopharma</td>
<td>siRNA90</td>
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<td></td>
<td>RO7020922 (RG7834)</td>
<td>Phase I</td>
<td>Roche</td>
<td></td>
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<tr>
<td></td>
<td>Ionis HBVRx (GSK3228836)</td>
<td>Phase I</td>
<td>Ionis Pharma/GSK</td>
<td>Antisense molecule</td>
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<td></td>
<td>IONIS-HBVLRx (GSK33389404)</td>
<td>Phase I</td>
<td>Ionis Pharma/GSK</td>
<td>Antisense molecule91</td>
</tr>
<tr>
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<td>AB-452</td>
<td>Preclinical</td>
<td>Arbutus Biopharma</td>
<td>RNA destabilizer92</td>
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<tr>
<td>Gene editing</td>
<td>EBT106</td>
<td>Preclinical</td>
<td>Excision Biotherapeutics</td>
<td>CRISPR/Cas9 system93</td>
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<td></td>
<td>Anti-HBV sgRNA</td>
<td>Preclinical</td>
<td>CRISPR/Cas9 system, Kennedy et al (2015)47</td>
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<td></td>
<td>Zinc finger nuclease</td>
<td>Preclinical</td>
<td>Weber et al (2014)51</td>
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<tr>
<td>HBsAg secretion inhibitor</td>
<td>REP2139 &amp; REP2165</td>
<td>Phase II</td>
<td>Replicor</td>
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<td>BM601</td>
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<td>Bazinet et al (2017)55,56</td>
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<td>LB80380 (besifovir, besivo)</td>
<td>Phase III/Approved in S. Korea</td>
<td>Ilong Pharmaceutical</td>
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<td>Capsid assembly inhibitors/ modulators</td>
<td>GLS-4</td>
<td>Phase II</td>
<td>HEC Pharm, Sunshine</td>
<td>China-CFDA</td>
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<td></td>
<td>NVR 3–778</td>
<td>Phase Ia</td>
<td>Novira Pharmaceuticals/ Janssen</td>
<td>Klunpp et al (2017)78</td>
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<td>BAY41–4109</td>
<td>Phase I</td>
<td>Berke et al (2017)79</td>
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<td></td>
<td>AB-423</td>
<td>Phase I</td>
<td>Arbutus Biopharma</td>
<td>Eley et al AASLD (2017)99</td>
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<td>Drug class</td>
<td>Antiviral agent</td>
<td>Status</td>
<td>Developer</td>
<td>Comments and references</td>
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<tr>
<td>cccDNA inhibitor</td>
<td>AB-506</td>
<td>IND enabling</td>
<td>Arbutus Biopharma</td>
<td>Mani et al AASLD (2017)&lt;sup&gt;10&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Disubstituted sulphonamides</td>
<td>Preclinical</td>
<td></td>
<td>Cai et al (2012)&lt;sup&gt;100&lt;/sup&gt;</td>
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<tr>
<td>Therapeutic vaccine</td>
<td>GS-4774</td>
<td>Phase II</td>
<td>Globimmune</td>
<td>Recombinant X, S &amp; C epitopes&lt;sup&gt;101&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>ABX-203</td>
<td>Phase II/III</td>
<td>Abivax</td>
<td>Recombinant S &amp; C epitopes</td>
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<td></td>
<td>TG-1050</td>
<td>Phase I</td>
<td>Transgene</td>
<td>Non-replicative adenovirus. C, pol &amp; S epitopes. Levin (2017)&lt;sup&gt;102&lt;/sup&gt;</td>
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<td></td>
<td>INO-1800</td>
<td>Phase I</td>
<td>Inovio</td>
<td>DNA plasmids encoding S &amp; C</td>
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<tr>
<td>TLR agonists</td>
<td>GS-9620</td>
<td>Phase II</td>
<td>Gilead Sciences</td>
<td>TLR-7 agonist. Boni et al (2016)&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RO6864018 (RG7795)</td>
<td>Phase II</td>
<td>Roche</td>
<td>TLR-7 agonist</td>
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<tr>
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<td>GS-9688</td>
<td>Phase I</td>
<td>Gilead Sciences</td>
<td>TLR-8 agonist</td>
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<tr>
<td>Immuno-modulators</td>
<td>PD-1/ PDL-2 mAb</td>
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<td>Merck Sharp and Dohme; Bristol-Myers Squibb</td>
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<td>CTLA-4 mAb</td>
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<td></td>
<td>SB9200 (Iranigivir)</td>
<td>Phase II</td>
<td>Spring Bank Pharmaceuticals</td>
<td>RIG-I and NOD-2 agonist&lt;sup&gt;103&lt;/sup&gt;</td>
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<sup>a</sup>X = HBx, S = HBsAg, C = HBcAg.
TABLE 3

Who to treat?

<table>
<thead>
<tr>
<th>Phase</th>
<th>Immune tolerant</th>
<th>HBeAg-positive CHB</th>
<th>Inactive carrier</th>
<th>HBeAg-negative CHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg status</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ALT</td>
<td>Normal</td>
<td>Elevated</td>
<td>Normal</td>
<td>Elevated (fluctuating)</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>Very high &gt; 200,000 IU/mL</td>
<td>&gt;2000 IU/mL</td>
<td>&lt;2000 IU/mL</td>
<td>&gt;2000 IU/mL (fluctuating)</td>
</tr>
<tr>
<td>Histology</td>
<td>Normal or mild inflammation &amp; mild fibrosis</td>
<td>Inflammation &amp; fibrosis: moderate to severe</td>
<td>Normal or mild inflammation</td>
<td>Inflammation and fibrosis: moderate to severe</td>
</tr>
<tr>
<td>Disease progression</td>
<td>Low</td>
<td>Moderate to high</td>
<td>No, very low</td>
<td>Moderate to high</td>
</tr>
<tr>
<td>Treatment</td>
<td>Not indicated</td>
<td>Indicated</td>
<td>Not indicated</td>
<td>Indicated</td>
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