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Progress Report

# Chronic hepatitis B: Are we close to a cure?

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ABSTRACT

Approximately 300 million people worldwide are persistently infected with the hepatitis B virus and are at risk of developing hepatocellular carcinoma and liver cirrhosis, which can progress to end-stage liver disease. Despite the effectiveness of the current vaccination policy, the prevalence of the disease remains high, and the burden for health services is considerable. The currently available antiviral strategies are either poorly effective or only effective for non-curative suppression of viral replication. Recent efforts have been focused on improving the cure rate for chronic hepatitis B and developing strategies to eliminate infected cells.

Several approaches are under evaluation, and these include targeting the virus at different stages of its life cycle and boosting the antiviral immune response. This article reviews these latest approaches and comments on their feasibility and potential translation into clinical applications.



## 1. Introduction

Hepatitis B virus (HBV) infection is a major global health problem, despite the availability of effective vaccine prophylaxis. According to the latest World Health Organization (WHO) reports, an estimated 240–280 million people are chronic hepatitis B (CHB) carriers, among whom the disease occurs with a very high burden, as approximately 1 million people die every year from CHB-related disease [1,2].

Dramatic improvements in the efficacy of the treatment of chronic hepatitis C were made possible by the availability of highly potent direct antiviral agents, which created an expectation of similar results being achieved in chronic HBV.

Despite the availability of highly effective direct antiviral agents for HBV for the last 17 years, a cure cannot be achieved in most cases because of the peculiar features of this virus.

In fact, the viral life cycle of HBV involves the formation of particularly stable episomal minichromosomes, covalently closed circular DNA (cccDNA) molecules, which serve as a template for transcription and a reservoir for future replication cycles [3,4]. Furthermore, the HBV genome is able to integrate into the host genome, thus reinforcing viral antigen production and favouring HBV oncogenesis [5].

The inability to arrest this complex replicative machinery leads to the persistence of viral antigen production, which, in turn, progressively exacerbates the functional failure of the immune response; the immune response represents the most effective tool for viral control [6].

A CHB "cure" can be defined at different levels. Basically, the most desirable end point is the elimination of both the viraemia (HBV-DNA) and the viral surface antigen (HBsAg), followed by seroconversion to anti-HBsAg (anti-HBs) antibodies [7]. This condition is largely satisfactory because it is associated with a substantial improvement of outcomes and a reduced risk of developing complications, at least in non-cirrhotic patients [8–10]. A complete cure, however, would only be accomplished by elimination of cccDNA from infected hepatocytes, which represents definite viral eradication and ensures protection from the risk of reactivation in the case of immunosuppression [11] (Fig. 1).

However, both of these endpoints still represent a challenge because they are not adequately met by current therapies. Therefore, clinicians must rely on a surrogate but more realistic end point, which is the induction of sustained virological remission [1,12].

The current research aimed at designing new strategies for HBV "elimination" focuses on the following two main assumptions derived from the known mechanisms underlying viral persistence: (a) The need to target the virus directly and/or (b) the need to restore an effective immune response.

## 2. Current HBV therapies

Two different therapeutic approaches are currently available for patients with CHB: (1) a finite antiviral and immunomodulatory

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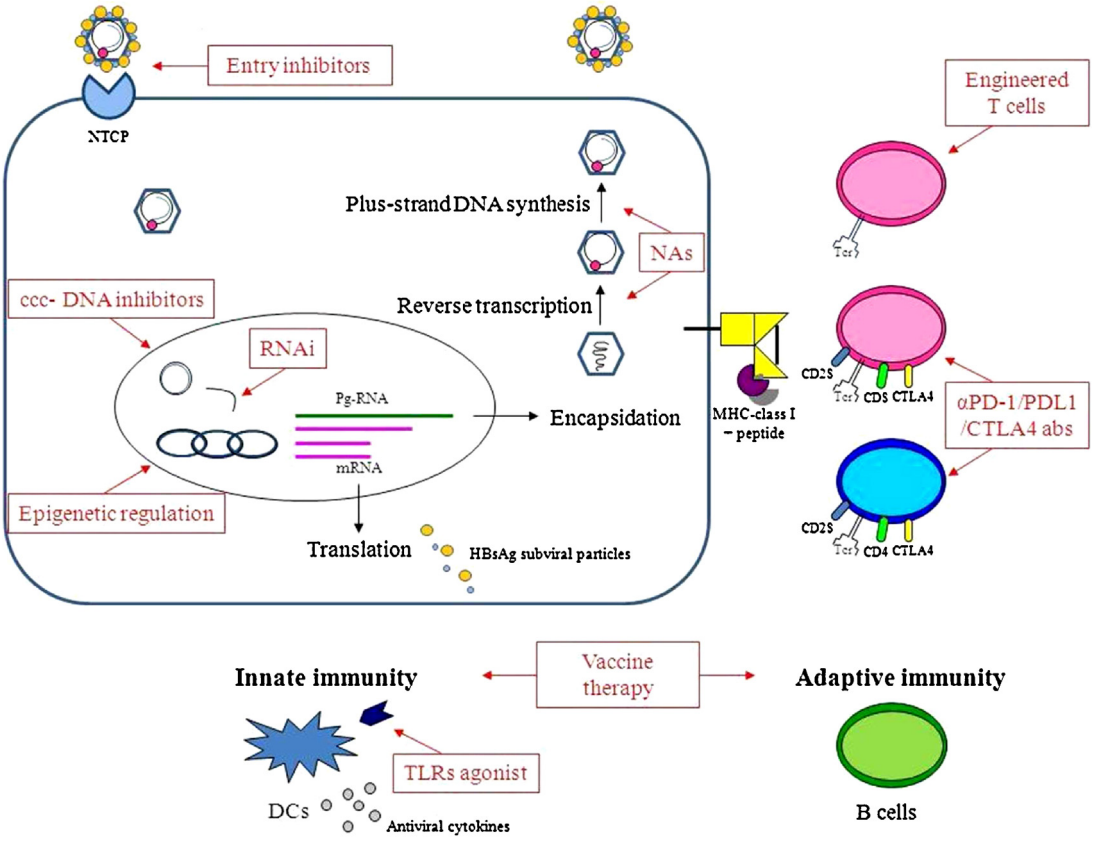


**Fig. 1.** Schematic representation of the potential curative approaches for chronic hepatitis B. NCTP, sodium taurocholate cotransporting polypeptide (HBV receptor); NAs, nucleos(t)ide analogues; DCs, dendritic cells; TLRs, Toll-Like Receptors; RNAi, RNA interference; TcR, T cell receptor.

treatment with interferon-α; and (2) an indefinite treatment with nucleos(t)ide analogues (NAs), which can successfully achieve non-curative suppression of viral replication [1] (Fig. 2).

Treatment with pegylated interferon-α (PEG-IFNα2a) can have a curative effect mediated by viral inhibition and an enhancement of the host immune response. Unfortunately, a curative effect of PEG-IFNα2a is observed in fewer than 10% of patients, regardless of HBeAg status [13,14].

A better definition of the factors that predict the response, such as HBsAg levels, viral genotypes, and HBeAg levels for the HBeAg-positive population, and validation of the stopping rules will most likely result in better selection of the patients to be treated, which, in turn, will lead to higher rates of treatment response [15]. However, these optimization attempts would lead to improved effectiveness of PEG-IFN therapy based on the exclusion of patients with unfavourable features; consequently, this strategy would not impact the overall cure rates for CHB.

Since the introduction of lamivudine in 1998, four other agents with progressively higher antiviral activity and a more efficient genetic barrier to drug resistance have been approved. These drugs successfully achieve satisfactory HBV DNA suppression rates, which are stably maintained with third-generation NAs (entecavir and tenofovir), at least based on the data acquired to date [16,17] (Fig. 2).

NAs inhibit HBV DNA synthesis via a competitive interaction with the natural substrates of the HBV polymerase; however, they do not interfere with cccDNA formation (Fig. 1). As a consequence, in most patients, HBV replication rebounds after antiviral therapy is discontinued.

Based on these findings, is it possible to achieve significant CHB cure rates with these agents? The currently available data would suggest otherwise: HBsAg seroclearance is considered a rare event during NA therapy; this event is observed in only 0.5–1% of all treated patients per year. The rate of decline of serum HBsAg levels is such that, according to mathematical models, a complete

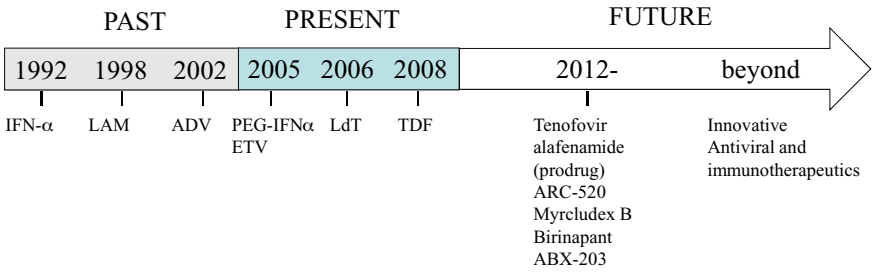


**Fig. 2.** Timeline of milestones in chronic hepatitis B treatment. The new drugs at a more advanced stage of development (phase III) are listed as "future". LAM, lamivudine; ADV, adefovir dipivoxil; ETV, entecavir; LdT, telbivudine; TDF, tenofovir disoproxil fumarate.

elimination could theoretically be achieved in a time frame of 20–30 years [18,19].

This effect could be expected for both HBeAg-positive and HBeAg-negative CHB. In fact, although the rate of decline of HBsAg levels is more pronounced in HBeAg-positive patients, the baseline HBsAg levels are usually higher in HBeAg-positive than in HBeAg-negative patients.

This approach could improve in the future, once adequate data on the use of the most potent NAs are available and the therapeutic strategies are optimized through the use of new tools for monitoring treatment outcomes [20].

Certainly, prolonged viral remission is the *sine qua non* for the design of new treatment possibilities, because although remission typically occurs slowly, it triggers the necessary mechanisms leading to the end of viral control. In support of this hypothesis, cccDNA levels significantly decrease after NA therapy [11,21], and prolonged NA-induced viral remission is able to restore the function of HBV-specific T cells [22].

Based on this hypothesis, combination treatments capable of boosting anti-HBV reactivity while steadily suppressing viral replication are expected to accelerate the decline in HBsAg levels. Thus, the administration of PEG-IFNα2a, once complete suppression of HBV replication has been obtained with NAs, represents the newest therapeutic approach currently being evaluated [23,24].

## 3. New approaches

### 3.1. The viral target

As described earlier, treatment of CHB requires the elimination or molecular inactivation of the intrahepatic cccDNA pool. Whether this approach is clinically achievable remains an open question; at present, preclinical studies are evaluating a number of compounds (restriction enzymes and sulfonamide inhibitors) with highly promising results [25,26]. The recent finding that IFNα and members of the TNFα family induce cccDNA degradation in a specific (without affecting the host genome) and non-cytopathic fashion seems to be an important milestone [27]. However, the potent antiviral activity of IFNα shown in this model does not fit with the CHB cure rate observed for PEG-IFNα in clinical practice. One can speculate that larger amounts of IFNα (similar to the levels used in these highly complex in vitro models) are required to achieve cccDNA degradation or that the formulation and manner of administration of IFNα currently used in clinical practice exploits only a minimal portion of its biological potential. This hypothesis is supported by the finding that the delivery of IFNα to HBV-infected liver cells via HBV-positive T-cell receptor-like antibodies can induce an effective and robust response, thus setting up a delivery system that is potentially suitable for other cytokines [28]. Taken together, these findings suggest that cytokines with antiviral activity could continue to represent the backbone of CHB therapy once their use is optimized.

A possible alternative to cccDNA elimination is either its direct functional inactivation or the prevention of its transcription (Fig. 1). The most intriguing approaches in this field involve epigenetic modifications [29]. Furthermore, RNA interference (RNAi) has been shown to potently block viral mRNA [30,31]. However, the possibility of translating these proofs of concept into the clinical arena currently is still distant.

Another stage of the viral life cycle that could represent a target of therapy is viral capsid assembly and, ultimately, virion secretion. Along this line, a series of compounds have been investigated, including heteroarylpyrimidines (HAPs) and phenylpropenamides; however, they appear to still be in the preclinical stage of development. Regarding viral secretion, recent data have reported the effectiveness of alisporivir in reducing HBV replication and HBsAg secretion in in vitro models [32].

A novel approach that is currently under investigation in cancer and infectious diseases is targeting of IAP (inhibitor of apoptosis protein), with the goal of inducing apoptosis in virally infected cells. After encouraging results from in vitro and animal models, an antagonist of the IAP protein, birinapant, is currently in a phase I clinical trial in patients with chronic HBV.

An approach that is potentially closer to clinical translation entails interfering with the earliest stage of the viral cell cycle by blocking viral entry.

Studies of HBV vaccination [32] and the optimal coverage required to provide sterilizing immunity to HBV as well as studies of the use of passive prophylaxis to prevent graft reinfection after liver transplantation for CHB-related disease [33] have confirmed that anti-HBsAg contains a neutralizing antibody component.

Further studies have identified the pre-S1 domain of the HBV-L protein to be the essential component of viral infectivity; its critical role is due to the interaction between HBsAg and receptors on the hepatocyte surface [34,35]. These results have recently been corroborated by the identification of a bile salt transporter in hepatocytes (NTCP) that functions as a high-affinity receptor for HBV and HDV [36].

The confirmation that HBV exhibits a selective tissue-specific (and species-specific) tropism strongly suggests, once again, that HBV infection is an ideal candidate for entry inhibitor compounds (Fig. 1 and Table 1). Based on these assumptions, fine mapping of the L protein sequence, which is critical for HBV infectivity, led to the selection of a myristoylated peptide containing amino acids 2 to 48 of the L protein (Myrcludex B), which is currently in clinical development. After validation in in vitro studies [37], Myrcludex B was shown to completely abrogate the infection [38] at low doses and to inhibit viral spread in already-established infections, thus preventing the infection of naïve hepatocytes [39].

Myrcludex B has been proven to be highly selective in targeting hepatocytes, has passed acute and long-term toxicity studies, and is currently in phase II clinical trials in chronically HBV/HDV-infected patients (Table 1).

Although expectations for these impending results are high, some remarks should be made. The significance of this approach appears to be immediately relevant in selected settings, such as preventing graft reinfection after liver transplantation or limiting the viral spread in acute hepatitis.

However, it is more difficult to foresee its efficacy in the context of well-established CHB, which is known to be a highly efficient infection; at the chronic stage, virtually all hepatocytes are infected.

Certainly, to be effective in CHB, this approach must rely on hepatocyte turnover, which, in turn, is dependent on the ability of the host immune response to clear the infected hepatocytes.

Considering the natural half-life of hepatocytes and the absence of a system to reinforce the immune-mediated turnover, a significant beneficial effect of this approach in CHB is not predictable, and important safety concerns must be considered.

### 3.2. Immunotherapeutic approaches

Spontaneous HBsAg seroclearance after the acute phase, which is observed in more than 90% of patients who encounter the virus as adults, occurs through the development of a robust innate or adaptive immune response. Conversely, CHB results from an inability to mount a sufficiently strong immune reaction, which, over time, is worsened by the persistent exposure to high amounts of viral antigens, ultimately leading to a complete functional exhaustion or deletion of the HBV(+) T cells [6,40].

Thus, mimicking the immune events that occur during self-limiting HBV infection represents a feasible approach to treating

**Table 1**
Ongoing clinical trials (partial source: www.clinicaltrials.gov) or recently completed clinical trials evaluating new compounds (alone or in association with already approved CHB drugs).

| Class | Compound | Target | Status of development |
|---|---|---|---|
| Peptide | εPA-44 | CHB | Phase II |
| Therapeutic vaccine | Double-plasmid DNA vaccine | HBeAg-positive CHB | Phase II |
| Cytokines/immunostimulating | IFN-γ[a] | CHB | Phase II |
| Therapeutic vaccine | Naked DNA vaccine (pCMVS2.S) | CHB | Phase I/II |
| Therapeutic vaccine | ABX 203 (HBs and HBc antigens) | CHB | Phase II/II |
| Vaccine/immunological/growth factors | HBIG + GM-CSF + HBV Vaccine | HBeAg-negative CHB | Phase I/II |
| Therapeutic vaccine/immunostimulating | GS-4774 (engineered multi-genotype) | CHB | Phase II |
| Immunostimulating | GS-9620 (TLR-7 agonist) | CHB | Phase I/II |
| Cytokines/antivirals | Pegylated Interferon Lambda | HBeAg-positive CHB | Phase II |
| Peptide/entry inhibitors | Myrcludex B (preS1-derived lipopeptide) | CHB | Phase II |
| Immunostimulating | Thymosin-α | CHB (cirrhosis) | Phase IV |
| Apoptosis inhibitor | Birinapant (IAP antagonist) | CHB | Phase I |
| Antivirals | NVR 3-778 (core inhibitor) | CHB | Phase Ia |
| Viral transcription inhibitors | ARC-520 (siRNAs) | CHB | Phase II |

CHB, chronic hepatitis B; HBIG, hepatitis B immune globulin; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon-gamma; HBeAg, hepatitis e antigen.

[a] Updated information not available; unknown study progress.

CHB. This strategy involves restoration of proper immune function, which is lost at the chronic stage [41], and it represents the most widely explored strategy to date.

Attempts to cure CHB by therapeutic vaccination have produced disappointing results, at least with the first formulations. The main drawback of this strategy is most likely that it aims to target and boost anti-envelope specificities, which have been proven to target the subset of HBV-positive cells that are more rare in the circulating compartment of patients with CHB [42,43]. To overcome these limitations, other strategies are exploring the use of different antigens to elicit a wider and multispecific response, while also making use of advanced technologies (Table 1). In particular, the core antigen is the most frequent specificity in CHB and it is also positively correlated with better control of infection [42]. Therefore, the therapeutic potential of vaccines that target the core protein is currently under evaluation on the basis of encouraging results obtained in the preclinical phase [44].

Currently, these data are limited to animal models; therefore, their translation into CHB therapy will most likely require more time [45–57].

More direct and sophisticated approaches involve cell-therapy strategies, which are based on the use of different types of engineered T cells. These T cells are genetically modified to express a predefined antiviral specificity; the rationale for this procedure is to replace or reinforce the pool of absent or functionally weak host T cells with a new pool of perfectly functional T cells, while also targeting the immunodominant epitopes associated with viral control [48,49].

These types of procedures, albeit innovative, have already reached the clinical setting, and they are under evaluation, in particular, in the context of malignant disease or virus-associated malignant disease [50,51].

The appeal of using this immunotherapy is immediately evident: manipulation of these cells facilitates the treatment tailoring to the patient (considering his or her HLA status and providing an antiviral specificity that is completely missing). Furthermore, with the progressively increasing use of this approach in the oncohaematological field, we are gaining confidence in the idea of exploiting this technological progress.

Thus, this strategy could lead to a cure for CHB. However, several points must be addressed to establish its effectiveness and safety in the setting of CHB: first, very strong activation of T-cell-mediated killing can result in hepatitis flares of unknown consequence; second, it must demonstrated that the engineered T cells do not undergo the same mechanisms of functional exhaustion as natural T cells due to circulating antigens and given the pro-immunotolerant environment of the liver.

Another potential strategy to boost the antiviral T cell response expected to produce results involves manipulation of the inhibitory receptors that belong to the extended CD28/CTLA-4 family of T-cell regulators (Fig. 1). The rationale for the use of this approach is based on repeated observations that these molecules, PD-1 in particular, are highly expressed on both peripheral and intrahepatic HBV-specific T cells and that manipulation of these pathways, via either the stimulatory or the inhibitory receptors, can restore antiviral function [52–54]. According to these data, the expression of inhibitory receptors has become a measure of functional weakness, which is responsible for the lack of immune control.

Once again, knowledge gained from their pioneering use in the treatment of malignancy will benefit their potential use in CHB, as cancer and chronic viral infections share the common mechanism of using immune checkpoint pathways to strongly downregulate T-cell activation [55].

This approach started with ipilimumab, which was approved by the Food and Drug Administration (FDA) in 2011 for advanced melanoma, and other checkpoint agents continue to be developed, including PD-1 and PD-L1 inhibitors, which are currently being tested alone or in combination with different formulations across multiple tumour types [56,57]. Research in this specific field currently represents a very innovative topic, according to the clinicaltrials.gov registry.

In terms of viral hepatitis, no data are available for the treatment of CHB; however, the anti-PD1 antibody was evaluated in the treatment of patients with chronic hepatitis C, with apparently modest therapeutic results. However, the clinical significance of this study is limited due to the small patient population and, more importantly, the use of a single dose of anti-PD1 [58].

The development of this approach as a cure for CHB is particularly attractive given its high biological potential and the advantages over other immunotherapy strategies, such as the engineered T cells described earlier; this approach does not require a demanding and invasive procedure, and it boosts the preexisting response, thereby eliminating the risk of cross-reactivity or side effects from genetic modification (i.e., lymphoproliferative disorders). However, major safety concerns arise from manipulation of the patterns of physiological immune checkpoints, such as the possibility of developing autoimmune manifestations; therefore, an attempt to disrupt the physiological tolerance to render the mechanism effective against antiviral reactivity requires careful evaluation before implementing this approach in clinical practice.

In addition to immunotherapy based on manipulation/stimulation of adaptive immunity, recent studies have focused on the pathways of innate immunity, which play a key role in the control of infection. Activation of the innate immune response also stimulates adaptive immunity; therefore, this strategy is expected to have wider effects on the immune control of CHB.

To translate this basic rationale to a clinical cure for CHB, recent studies have attempted to trigger the innate response by targeting Toll-like receptors (TLRs), which are pattern-recognition receptors (PRRs) capable of recognizing conserved pathogen-associated molecular patterns (PAMPs).

Downregulation of the expression of TLRs on HBV-infected hepatocytes has been shown to be a possible mechanism of immune evasion [59]; the use of TLR agonists could therefore reestablish proper immune function [60]. Recently, a TLR7 agonist (GS-9620) was developed, and it demonstrated sustained reduction of viraemia and antigenemia in animal models of CHB [61,62].

GS-9620 is a new therapeutic strategy approaching clinical use, as it is in clinical phase I/II studies (Table 1).

## 4. Conclusion

The unsuccessful CHB cure rates achieved with the current treatments make the development of new therapeutics mandatory. The development of new antiviral therapies, combined with the excellent results obtained with the vaccination policy, could lead to the control of HBV infection and possibly eradication in the near future.

Multiple efforts at different levels are under way to achieve this goal; some efforts are focused on HBV virology, whereas others are evaluating the feasibility of boosting the immune response.

CHB therapy appears unchanged when compared with the remarkable improvement in patients with chronic hepatitis C, as it continues to rely on the same strategies of a decade ago (interferon and nucleos(t)ide analogues). However, on examining these advances in depth, this statement is not completely true; these agents have been progressively optimized, and they will further improve in the near future. At the same time, significant advancements have been made in the understanding of HBV virology and the features of immunological impairment. All of these findings drive researchers to develop new strategies, while also learning from other models of viral infection and the newest cancer therapies.

The primary end point of the more recent strategies currently under evaluation is to cure CHB. However, the CHB model is a highly complex model of infection, and the development of new strategies will face some important issues.

A simplification of these problems includes the need to be highly selective, for approaches that directly target the virus; instead, for approaches that target the host immune response, it is necessary to avoid the severe side effects associated with the disruption of immunotolerance. Further complicating this scenario, ideally, these two distinct approaches, if used in combination, require an additive or even synergistic activity against HBV. The rationale of a multi-target approach, which is already under evaluation in current trials, is given by multiple, specular evidences. First, the restoration of immune response involves a stage of complete or partial inhibition of viral replication, in turn decreasing antigenemia. On the other hand, virological inhibition should be able to prevent infection of new cells, but complete "clearance" of infected hepatocytes cannot be achieved unless successful T-cell-mediated elimination takes place. Both of these issues make "curing CHB in 100% of patients" an ongoing challenge, and the therapeutic translation of new knowledge is not achievable in the short-term.

However, many milestones in understanding the biology of HBV have been achieved in recent years, which means that we are currently experiencing a new trend in acquisition of knowledge in the field. Once these new studies are completed in the coming few years, a completely new scenario will be at hand.

## Conflict of interest

None declared.

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