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# Chronic Hepatitis B therapies: challenges and opportunities

✍Julio Cesar Aguilar-Rubido[1], Maarten Alexander Abraham van de Klundert[2], Marie-Louise Michel[3]

[1]Department of Biomedical Research, Centro de Ingeniería Genética y Biotecnología, CIGB Ave. 31 entre 158 y 190, Cubanacán, Playa, CP 11600, La Habana, Cuba
[2]Technical University of Munich, Munich, Germany
[3]Institut Pasteur, Paris, France
✍julio.aguilar.abroad@gmail.com; julio.aguilar@cigb.edu.cu

**ABSTRACT**

Despite advances in prevention and treatment, chronic hepatitis B (CHB) remains a global public health concern. The WHO has recently raised a campaign to control the increasing mortality trend related to viral hepatitis. The cases of hepatocellular carcinoma and liver cirrhosis resulting from CHB disease progression accounts for more than half of total fatalities. Although the efficacy of current treatments in controlling hepatitis B virus (HBV) replication is high, the absence of sustained antiviral effect after treatment discontinuation and their low effect on HBsAg/HBeAg seroconversion are known limitations of approved therapies. In addition, the use of long lasting therapies increases the costs of nucleos(t)ide analogue (NUC) treatments and limits their epidemiological effectiveness. The recent understanding of the immunology and physiology of CHB have led to the development of several innovative products. Their clinical implementation will be required to accomplish WHO goals in terms of morbidity and mortality during the next decade. The present document reviews the major limitations of approved therapies, including the works presented at the most recent meetings of the major societies for the study of the liver as well as the pre-clinical and clinical development of innovative therapies. More specifically, strategies including therapeutic vaccines under development are analyzed, together with some of the challenges and opportunities faced for the introduction of therapeutic vaccination against CHB.

*Keywords: chronic hepatitis B, hepatitis C, therapy, nucleot(s)ide analogues, therapeutic vaccine, interferon, HeberNasvac®*

**RESUMEN**

**Terapias contra la hepatitis B crónica: desafíos y oportunidades.** La hepatitis B crónica (HBC) es un problema de salud global, a pesar de los avances en su prevención y tratamiento. Los casos de carcinoma hepotocelular y cirrosis hepática causados por la progresión de la enfermedad abarcan más de la mitad de todos los fallecimientos. La Organización Mundial de la Salud (OMS) inició una campaña para el control de la mortalidad producto de las hepatitis virales. La ausencia de un efecto antiviral sostenido después del cese del tratamiento y su impacto en la seroconversión a los antígenos de superficie (HBsAg) y e (HBeAg) del virus de la hepatitis B (HBV), se mantienen como limitaciones de las terapias aprobadas, a pesar de la alta eficacia de los tratamientos actuales para el control de la replicación del HBV. Además, la implementación de terapias de larga duración con análogos de nucleós(t)idos (NUC) incrementa los costos del tratamiento y limita su eficacia epidemiológica. La comprensión reciente de la inmunología y la fisiología de la HBC ha permitido el desarrollo de varias terapias innovadoras. Su implementación requerirá que se alcancen los objetivos trazados por la OMS en términos de morbilidad y mortalidad durante la próxima década. En este artículo se revisan las principales terapias aprobadas para el tratamiento de la HBC, ¡unto a los trabajos presentados en los congresos más recientes de las principales sociedades para el estudio del hígado, así como los desarrollos preclínicos y clínicos de terapias innovadoras. Además, se discuten las estrategias vacunales terapéuticas contra la HBC en desarrollo y algunos de sus desafíos y oportunidades.

*Palabras clave:* hepatisis B crónica, hepatitis C, análogos de nucleót(s)idos, vacunas terapéuticas, interferón, HeberNasvac®

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## Introduction

Mortality caused by viral hepatitis is on the rise, contrary to what is seen for HIV, tuberculosis, and malaria [1]. The Global Hepatitis Report 2017 issued by the World Health Organization (WHO) indicates that viral hepatitis represents a major public health challenge. More than one third of the world population show serological markers of past or current infection with the hepatitis B virus (HBV). The estimates of current carriers of the virus are in the range of 248 up to 257 million, approximately 3.5 % of the world population [1, 2]. Globally, an estimate of 1.34 million people died from viral hepatitis in 2015. The total mortality due to viral hepatitis is comparable to tuberculosis, and higher than those caused by the human immunodeficiency virus (HIV) or malaria. In contrast to the mortality caused by HIV, malaria and tuberculosis, the mortality due to viral hepatitis is on the rise.

HBV infection is the cause of approximately 60 % of the mortality generated by viral hepatitis [1]. CHB infection commonly results in progressive hepatic disease, which leads to liver cirrhosis and cancer in up to 25 % of the carrier patients. Approximately 0.9 million deaths are directly attributable to HBV infection every year, 90 % of these casualties as a

1. WHO. Global Hepatitis Report 2017. Geneva: World Health Organization; 2017.

consequence of liver cirrhosis (LC) or hepatocellular carcinoma (HCC) [1]. HBV infected persons may develop sequelae like oesophageal varices with digestive bleedings, ascites and splenomegaly. HBV infection can cause life-threatening acute-on-chronic liver failure (ACLF) after disease reactivation, for instance in the context of irregular medication with nucleos(t)ide analogues (NUCs), or the discontinuation of NUC treatment. Overall, an important proportion of CHB patients experience a dramatic fall in their quality of life, and are at risk of dying as a direct consequence of the infection [1, 2].

The current CHB therapies have a high efficacy in controlling the HBV replication. However, the effectiveness of these treatments in routine practice has been questioned by several studies and reviews. This statu quo demands a critical revision of recent clinical trial results, an implication of such results in recommendations for CHB therapy. This process should involve experts, international organizations, policy makers, regulators and even politicians. The final goal should be to limit the number of new infections, and to optimise treatment to reduce the mortality in already infected people [1].

Therefore, this review article is aimed to present the state of the art in the field of CHB therapies, exposing the limitations of currently approved products as revealed in recent meetings and publications. Moreover, results of innovative therapies are discussed, with special emphasis in those under clinical development. Particularly, therapeutic vaccination is addressed as a feasible strategy, discussing the immunological setting and recent results of two large studies assessing therapeutic vaccination as monotherapy and in combination with antiviral treatment.

## Current therapies for chronic hepatitis B

Peginterferon (PegIFN) and nucleos(t)ide analogues (NUCs) are the recommended drugs for CHB infection in most countries. PegIFN reduces viral replication by stimulating the innate immune response and offers the advantage of higher sustained response rates at the price of considerable side effects and high costs. NUCs interfere with the viral polymerase, preventing viral replication, and can be administered orally to efficiently suppress HBV viremia. Otherwise, even prolonged treatment with NUCs (>5 years) only rarely provokes a sustained virological response. Virological relapse is generalized after therapy discontinuation, a process hampered by potential side effects in a proportion of patients that develop ALT increases, leading to hepatic decompensation in some of them. Therefore, a quasi-eternal therapy is recommended in most patients [3-5].

The products for treating CHB as well as the treatment recommendations are in a constant process of improvement. All major associations for the study of the liver publish their guidelines and recommendations, which are continuously updated. Guidelines are based on variables such as serum HBV DNA levels, ALT elevation, and liver tissue histology [3-5]. Indication for treatment also considers age, health status, family history of HCC or LC and extrahepatic manifestations.

Recently, noninvasive techniques to measure liver fibrosis are being increasingly applied to take the decision of starting treatment or during disease management. Most international guidelines recommend to initiate treatment in patients with HBV DNA levels above 2000 IU/mL (>10 000 copies/mL), and in patients with sign of hepatitis, such as elevated ALT levels or moderate to severe liver damage demonstrated by liver histology or non-invasive tools (liver elastography or serologic algorithms such as fibrotest) [3-5].

### The efficacy and safety of treatments

As currently available therapies are unable to completely eliminate viral infection from the liver, their main goal of their use is to halt the disease progression to LC or HCC. The degree of progression cannot be assessed directly and therefore clinical decisions are based on surrogate markers. The expected results of current treatments are based in the control or change in secondary variables: the viral reduction or suppression, the ALT normalization and, in a lower proportion, the changes in HBeAg/HBsAg serology. The expected therapeutic effect of antivirals and PegIFN treatments on the secondary variables of efficacy is described in table 1.

### Side effects of current therapies

In general, NUCs are better tolerated than IFN-based treatments. However, long term application of tenofovir difumarate (TDF) can cause renal dysfunction and bone demineralization. It is expected that tenofovir alafenamide (TAF) will develop less side effects. Another risk of NUC therapy is the decompensation after treatment discontinuation or due to irregular medication. The risk of decompensation hampers the safety and benefits of therapy cessation after a certain number of years or when certain clinical endpoints have been met. The safety limitations of current CHB treatments are summarized in table 2 [6-8].

#### *The effect of tenofovir on bone mineral density*

The results of two Phase III studies on the effect of tenofovir di-fumarate (TDF) and the new tenofovir derivate (TAF) became available in 2017. A total of 1289 patients from these two trials were randomized

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7. Bristol-Myers Squibb Company. Package Insert Baraclude, (Entecavir): tablets for oral use, oral solution. 2015 [cited 2018 Oct 4]. Available from: https://packageinserts.bms.com/pi/pi_baraclude.pdf

8. Gilead Sciences, Inc. VIREAD® (tenofovir disoproxil fumarate) tablets, for oral use, powder for oral use. 2018 [cited: 2018 Oct 4]. Available from: http://www.gilead.com/~/media/files/pdfs/medicines/liver-disease/viread/viread_pi.pdf.

Table 1. General scenery of the efficacy data from antivirals and Pegylated Interferon (PegIFN)*

| Variable | Antivirals | PegIFN |
|---|---|---|
| Antiviral effect on treatment (<300 copies/mL)** | 90-100 % | 30-50 % HBeAg+<br>50-80 % HBeAg– |
| Antiviral effect after treatment stop (24 weeks follow-up; <300 copies/mL)** | 0-20 % | 0-10 % HBeAg+<br>10-25 % HBeAg– |
| HBeAg loss** | 10-25 %<br>24 weeks post-treatment | 20-40 %<br>24 weeks post-treatment |
| HBeAg seroconversion** | 10-20 %<br>24 weeks post-treatment | 20-30 %<br>24 weeks post-treatment |
| HBsAg loss** | 0-5 % after 5 years of treatmenttreatment | 5-10 % after 5 years of treatment |
| ALT normalization | >90 % after 3 months and during treatment | 40-70 % at the end of treatment |

*There is variability depending on the characteristics of the patients and the viral genotype; however these data reflect the current limitations of widely approved therapies in relation to efficacy.
** Depending on baseline levels and population under treatment.
*** Depending on viral genotype.

2:1 to TAF 25 mg QD or TDF 300 mg QD, each with matching placebo, and treated for 96 weeks. Dual energy X-ray absorptiometry (DXA) scans were performed throughout the first 48 weeks. Patients were evaluated for overall change in bone mineral density (BMD). Changes in BMD were further assessed in patients at high risk for bone density loss: female gender, Asian race, older age (> 50 years), and underlying renal disease (GFR < 90 mL/min). The mean (SD) % changes in hip BMD from baseline at week 48 for the TAF arm was –0.16 (2.24 %) and for the TDF arm was –1.86 % (2.45 %). For the spine, mean (SD) % change at week 48 was –0.57 % (2.91 %) in the TAF arm and –2.37 % (3.20 %) in the TDF arm. Subjects with > 3 % decline in hip and spine BMD were significantly greater in TDF treated patients (27 and 38 %) compared to TAF treated patients (8 and 20 %). The percentage of patients with > 3 % decline in hip and spine BMD was relatively consistent among TAF treated patients across baseline osteoporosis risk categories. In contrast, patients treated with TDF showed higher rates of > 3 % BMD decline in hip and spine in high-risk groups than in low-risk groups [9].

The difference in BMD decline between TAF and TDF was more pronounced in patients with multiple risk factors, with 10 % of TAF-treated patients experiencing > 3 % decline in hip BMD regardless of number of risk factors. In contrast, 20 % of TDF-treated patients with 2 risk factors had a > 3 % hip BMD decline while patients with 3 or 4 risk factors had 41 and 58 % of individuals with > 3 % hip BMD decline at Week 48. A similar trend was seen with changes in spine BMD decline. The only baseline predictor consistent for having a < 3 % hip and spine BMD decline at week 48 was treatment with TAF. The authors concluded that the changes in BMD over time and in proportion of patients with > 3 % BMD decline in hip and spine demonstrate significant safety benefits of TAF compared to TDF. The safety benefits of TAF are most pronounced in high risk populations [9]. In general, from this comparison was clear the important effect of TDF in the reduction of the bone mineral density. The use of TAF induced a lower number of these side effects compared to TDF.

### *On the renal toxicity of nucleotide analogues*

Long-term treatment with adefovir (ADV) and TDF has been associated with renal toxicity, as compared to treatment with nucleoside analogue ETV in patients with CHB, according to the work of the Department of Internal Medicine and Liver Research Institute at the Seoul National University College of Medicine. Long-term renal effects of ADV experienced TDF treated patients was compared to ETV treated patients. In this retrospective single center study, authors selected 87 patients who were treated with ADV and subsequent TDF from June 2008 to Dec 2013. Patients were matched by treatment duration: ADV plus TDF (ADV + TDF group) with ETV treated patients, and treatment duration of TDF group with ETV treated patients. Nucleotide analogues (ADV, TDF) showed significant decrease in GFR compared to ETV, and TDF showed significant hypophosphatemia development compared to ETV [10].

Table 2. General scenery of the efficacy data from antivirals and Pegylated Interferon (PegIFN)

| Product type | Adverse events (reported and included in product inserts) |
|---|---|
| Interferon-based therapies | Severe psychiatric adverse reactions including: Depressions, suicidal ideation, suicide, relapse of drug dependence and drug overdose. |
| | ALT increases with increase in bilirubin or evidence of hepatic decompensation. |
| | Flu-like syndrome, other causes of persistent fever must be ruled out, particularly severe infections (bacterial, viral, fungal) have been reported during treatment. |
| | Neutropenia, decreases in white blood cell (WBC) and neutrophil count. |
| | Pulmonary symptoms, including dyspnoea, pulmonary infiltrates, pneumonia, and pneumonitis, including fatality. |
| | Risk of exacerbation of autoimmune disease. |
| | Hyperglycaemia, hypoglycaemia and diabetes mellitus. |
| | Serious, acute hypersensitivity reactions (e.g. urticaria, angioedema, broncho-constriction, and anaphylaxis) are rarely detected. |
| | Cardiovascular events such as hypertension, supraventricular arrhythmias, congestive heart failure, chest pain and myocardial infarction. |
| | Retinopathies including retinal haemorrhages, cotton wool spots, papilloedema, optic neuropathy and retinal artery or vein obstruction, which may result in loss of vision. |
| | Dizziness, confusion, somnolence, or fatigue. |
| Antivirals | Severe acute exacerbation of hepatitis B after uncontrolled cessation of treatment. |
| | Viread: new or worsening renal impairment: include acute renal failure and Fanconi syndrome. Creatinine clearance should be assessed before initiating treatment. |
| | Lactic acidosis/severe hepatomegaly with steatosis: treatment should stop in patients who develop symptoms or suggestive laboratory findings |
| | Decreases in bone mineral density (BMD): assessment of BMD is required in patients with a history of pathologic fracture or other risk factors for osteoporosis. |

## Efficacy limitations of current therapies

Large cohort studies that became available in 2017 and 2018 highlighted important limitations of currently available therapies in terms of efficacy.

### *Long-term effect of current therapies in preventing LC and HCC*

PegIFN, ETV and TDF(TAF) are the recommended products for first-line therapy of CHB infection by major associations for the study of the liver. Although these therapies efficiently suppress HBV replication, the effectiveness of these products in preventing LC and HCC has been controversial. A large, observational, open-label, prospective cohort study of HBeAg-positive CHB patients who received PegIFN or ETV therapy was presented during the APASL 2017 meeting. Cumulative incidences of progression to LC and HCC were calculated with respect to treatment type, directly comparing the effects of PegIFN and the ETV. Based on the international model for prediction called 'REACH-B model', Chinese experts compared the observed incidence of LC and HCC with the expected incidence in each group according to this model. The authors observed that PegIFN treated patients showed a lower cumulative incidence of LC and HCC than ETV treated ones. A lower cumulative incidence of HCC was observed in PegIFN treated patients than predicted by the model. There was no significant difference in the cumulative HCC incidence between the observed and the predicted cases for ETV treated patients, demonstrating a comparatively

9. Seto WK, Asahina Y, Brown TT, Peng CY, Stanciu C, Abdurakhmanov D, *et al*. Improved bone safety of Tenofovir Alafenamide compared to Tenofovir Disoproxil Fumarate over 2 years in patients with chronic HBV Infection. Clin Gastroenterol Hepatol. 2018.S1542-3565(18):30633-5.

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superior long-term outcome of PegIFN treatment [11]. In a smaller study, Wrankle and colleagues [12] observed limited effectiveness of antivirals in CHB patients coinfected with Hepatitis Delta Virus (HDV). They showed that PegIFN treatment was more efficient in preventing liver progression and death related to CHB. The results of these studies evidenced a limited effect of NUCs on the main clinical variables, the most relevant outcome of CHB treatment.

### *Liver failure due to irregular medication with NUCs*

ACLF is characterized by a rapid disease progression and high mortality. In most Asian countries, hepatitis B causes 70-80 % of all cases of ACLF, indicating that it is a serious threat to public health. Most HBV-related ACLF cases result from irregular medication with NUCs, as recently revealed by a large cohort study [13].

The study of Zheng *et al*. followed a cohort of 1118 patients with HBV-related ACLF that were admitted to nine hospitals in China between January 2005 and December 2015. Up to 761 patients with CHB and 357 patients with HBV related LC were divided into six groups by different predisposing factors: irregular medication of NUCs (IMNA), HBV reactivation (HBVR), infection, use of hepatotoxic drugs, alcohol, and others. In CHB patients, 8.94 % of ACLF cases were caused by IMNA. The rate of improvement of IMNA-related cases was the lowest, only 50 %. IMNA, hepatic encephalopathy, and hepatorenal syndrome were independent risk factors for developing ACLF. In HBV-related ACLF patients with LC, there was 19.33 % of cases caused by IMNA and the improvement rate of IMNA was also the lowest, only 37.68 %. Multiple-factor analysis showed IMNA, unrelated infections, hepatic encephalopathy, hepatorenal syndrome are independent risk factors for developing ACLF [13]. The authors recommend paying more attention to patient's adherence to NUC treatment because irregular and uncontrolled interruptions may exacerbate the disease and lead to HBV-related ACLF in an important proportion of patients. In our opinion, this is the most complete study assessing the risks of irregular treatment of CHB with NUCs. The study emphasizes the importance of FDA warnings against uncontrolled treatment discontinuation (Table 2).

## Chronic hepatitis B treatment: new developments

A wave of novel treatments approaches appear at the horizon of CHB therapy. Many of these new approaches are now in different phases of clinical testing and they have been eclipsed by the results in the field of treatments for chronic hepatitis C. Some of the most important compounds are described below, which are summarized in tables 3 and 4, this last on therapeutic vaccine candidates that were clinically evaluated in the past 3 years.

### Cell-based immunotherapy

Adoptive T-cell therapy intends to restore antiviral T-cell immunity, to clear chronic viral infections or eradicate tumors expressing specific (viral or endogenous) antigens. Such a therapeutic approach has been developed for the treatment of CHB by the Technical University of Munich (TUM) [32]. First, they identified T-cell receptors (TCRs) specific for HBV S-derived peptides (S20 and S172), or for a core-derived peptide (C18) from T cells of patients with acute and resolved HBV infection. Subsequently, these HBV-specific TCRs were used to engraft human T cells by retroviral transduction. It was demonstrated that HBV-specific TCR engrafted CD8+ and CD4+ T cells recognized low concentrations of cognate peptide presented on HBV replicating cells. Upon recognition of their cognate peptide, TCR-grafted T cells secreted IFN gamma, TNF alpha, and IL2. The engrafted T cells could specifically kill hepatoma cells expressing HBV antigens from an integrated HBV genome, as well as HBV-infected cells in vitro. HBV-specific TCRs also mediated elimination of HBV by CD4+ T cells only, and when expressed on T cells from patients with CHB [32].

Once tested in SCID mice repopulated with HLA-A*02-positive primary human hepatocytes and infected with HBV, TCR-redirected HLA-matched T cells could efficiently and specifically target infected hepatocytes in the liver. Intrahepatic analyses revealed a strong reduction of cccDNA loads and other markers of HBV replication [32].

### RNA interference therapy

RNA interference (RNAi) can be employed to induce the degradation of specific RNA targets, such as viral (m) RNA transcripts. One of such RNAi-based drug, ARC-520 (ARC), targets HBV mRNA and has been reported safe and effective for the treatment of CHB. Prolonged therapy with an ARC-520 injection in treatment-naïve, HBeAg positive and negative CHB patients resulted in significant reductions of HBs antigen [17]. In a recent clinical trial, a total of 8 CHB (5 HBeAg–, 3 HBeAg+) received up to 12 doses of 4 mg/kg ARC once every 4 weeks with daily ETV simultaneous treatment. The patients were administered with ETV for 34 to 44 weeks after a single dose of ARC and before receiving the first ARC dose of the multi-dose extension. This product was well tolerated when dosed every 4 weeks. A single dose of ARC together with ETV resulted in reduction of HBsAg up to 44 weeks. Multiple doses of ARC resulted in an additional reduction in HBsAg in all CHB patients; HBeAg-positive CHB showed a larger HBsAg multilog reduction. It was suggested that the delayed onset of HBsAg reduction in HBeAg-negative CHB may be an indirect effect due to the reduction of other viral proteins [17].

### *HBV core assembly modulator*

HBV core assembly modulators have been developed to disrupt HBcAg multimerization, and thereby formation of core particles, HBV RNA encapsidation, and ultimately viral replication. The safety, tolerability, pharmacokinetics and antiviral activity of AL-3778, a first-in-class, orally administered HBV core assembly modulator, was recently studied alone and in combination with PegIFN [33]. Safety and efficacy were assessed in HBeAg positive non-cirrhotic CHB patients with HBV DNA > 20 000 IU/mL and elevated ALT. All study groups were treated for

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Table 3. General scenery of the efficacy data from antivirals and Pegylated Interferon (PegIFN)*

| Product code/ Composition | Company/ Clinical trials | Stage of development | Main Results [Ref.] |
|---|---|---|---|
| GS-7340 (TAF) NUC Tenofovir alafenamide, a nucleotide, pro-drug of tenofovir | Gilead NCT01940471, NCT01940341 & NCT01671787] | In registration process & registered in several countries | Highly bioavailable, stable in plasma and also suitable for the efficient delivery of TDF (active form) to hepatocytes and lymphoid tissues reducing systemic exposure of TDF. TAF is demonstrating less nephrotoxicity and bone demineralization effect compared to TDF. Similar efficacy compared to TDF in phase III clinical trials [13] |
| Besifovir Acyclic nucleotide analogue | IDong Pharmaceuticals NCT01937806 | Phase IIb | Besifovir had the same antiviral property as compared to entecavir over 96 weeks of treatment for chronic hepatitis B patients. Besifovir was well tolerated and also had a good clinical safety profile [14] |
| Synthetic N-acylated pre-S1 polypeptide | MYR GmbH | Phase II for HBV & HDV | Entry inhibitor inactivating the hepatitis B virus (HBV) and hepatitis D virus (HDV) receptor sodium taurocholate co-transporting polypeptide (NTCP). After 24 weeks of treatment with myrcludex B and/ or pegylated interferon α-2a, HDV RNA decreased in all patients with chronic hepatitis B and D. Two of eight patients which received either myrcludex B or pegylated interferon α-2a, became negative for HDV RNA, and five of seven patients who received both drugs at the same time became negative. The drug was well tolerated [15] |
| CRV431 A non-immuno-suppressive derivative of Cyclosporine A (CsA) | ContraVir Pharmaceuticals Inc. | Cytochrome P450-mediated Phase I *in vitro* metabolism | Cytochrome P450-mediated Phase I in vitro metabolism of CRV431 was studied using selective chemical inhibition and recombinant human enzymes. Additionally, the metabolic profile of CRV431 in human, rat, and monkey liver microsomes. It is anticipated that the drug–drug interaction potential between CRV431 and the NUCs used in CHB treatment would be minimal [16] |
| ARC-520 / 521 SiRNA able to target viral RNA | Arrowhead NCT02604212 NCT02604199 | Phase II | RNAi-based drug that targets HBV mRNA. ARC has been reported safe and effective for the treatment of CHB. Prolonged RNAi therapy with ARC-520 injection in treatment naïve, HBeAg positive and negative CHB patients resulted in significant reductions of HBs antigen. Well tolerated product when dosed every 4 weeks. A single dose of ARC together with ETV resulted in reduction of HBsAg up to 44 weeks. Multiple doses of ARC resulted in an additional reduction in HBsAg in all CHB patients; HBeAg-positive CHB showed a larger HBsAg multi-log reduction. It was suggested that the delayed onset of HBsAg reduction in HBeAg-negative CHB may be an indirect effect due to the reduction of other viral proteins [17] |
| REP-2139 Nucleic acid polymers | Replicor NCT02565719 NCT02233075 | Phase II | Strong serum HBsAg reductions in study vs control group. More pronounced ALT increases in patients with higher sHBsAg reductions. NAP therapy + add-on PegIFN evidenced efficacy in patients with HBeAg (-) chronic HBV/HDV co-infection. Most patients (4/5) with HBsAg loss at 24 weeks follow-up are HBsAg, HDV RNA & HBV DNA neg at 1 year follow-up [18-21] |
| GS-9620 TLR7 agonist | Gilead Science NCT02166047 NCT02579382 | Phase II | GS-9620 is a drug currently being tested in clinical trials for the treatment of chronic hepatitis B virus (HBV) infection. GS-9620 has previously been shown to suppress HBV in various animal models. GS-9620 does not directly activate antiviral pathways in human liver cells, but can induce prolonged suppression of HBV via induction of interferon. It is suggested that other parts of the immune response also play an important role in the antiviral response to GS-9620 [22] |

28 days, followed by an off-treatment period of 28 days. Patients were randomized to receive AL-3778 or matching placebo at doses of 100, 200, 400, 600 and 1000 mg, or to receive treatment with PegIFN in combination with AL-3778 (600 mg), or PegIFN plus placebo. Dose-related HBV DNA and HBV RNA reductions were observed, but no statistically significant changes in HBV serology parameters were observed after 28 days of therapy with AL-3778. The largest mean HBV DNA reduction was observed with the 600 mg AL-3778/PegIFN combination (1.97 log IU/mL), which was greater than the reduction in patients treated with AL-3778 alone (1.72 log10) or PegIFN alone (1.06 log10).

After 28 days of treatment, mean HBV RNA (log10 copies/mL) changes in sera from baseline were 0.00 in untreated, –0.73 in PegIFN treated, –0.82 in 600-mg BD AL-3778 treated and –1.5 in 600-mg BD AL-3778/PegIFN combination treated patients [33]. The reduction of serum HBV RNA is consistent with the novel mechanism of action of AL3778, to disrupt HBV RNA encapsidation [33]. AL-3778 was well tolerated with mainly Grade 1 and 2, transient AEs. The authors reported one case of nonlife threatening rash SAE related to the administration of the product. Dose-related HBV DNA reductions and HBV RNA reductions were observed, with evidence of additive antiviral effects in combination with PegIFN [33].

## Secretion and entry inhibitors

Replicor's nucleic acid polymers (NAPs) are based on phosphorothioated nucleic acid polymers which, based on their nucleotide sequence specifically, interact with proteins that could interfere with HBsAg secretion [18-21]. It was shown that the application of NAPs in combination with PegIFN and TDF was beneficial in patients with HBeAg negative chronic HBV infection. Significantly higher ALT flares correlated with HBsAg suppressions suggesting that application of NAPs substantially improves the efficacy of PegIFN. Although the efficiency of NAPs in reducing CHB morbidity is still to be assessed, the results of multiple studies are encouraging [18-21].

Another HBV entry inhibitor, Myrcludex B, has been shown safe for use in patients and can reduce viral replication [15]. Nevertheless, this compound does not target the HBV cccDNA, and its application does not lead to HBV clearance. Its seems to be effective in suppressing viremia in HDV coinfected patients as well (Table 3).

## Therapeutic vaccination as monotherapy

A therapeutic vaccine to treat CHB patients, HeberNasvac®, was registered after two decades of research and development in the field of therapeutic vaccination, by the Cuban National Regulatory Agency (CECMED). This regulatory agency approved the Sanitary Registration of HeberNasvac® to the Center

23. Altimmune Inc. Product pipeline. 2018 [2018 Oct 4]. Available from: https://altimmune.com/pipeline/#hepTcell

24. Boni C, Janssen HLA, Rossi M, Yoon SK, Vecchi A, Barili V, *et al*. Combined GS-4774 and Tenofovir therapy can improve HBV-specific T-cell responses in patients with chronic hepatitis. Gastroenterology. 2019;157(1):227-41.e7.

25. Lok AS, Pan CQ, Han SH, Trinh HN, Fessel WJ, Rodell T, *et al*. Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B. J Hepatol. 2016;65(3):509-16.

26. Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, *et al*. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study. J Hepatol. 2019;71(5):900-7.

27. Al-Mahtab M, Akbar SM, Aguilar JC, Uddin MH, Khan MS, Rahman S. Therapeutic potential of a combined hepatitis B virus surface and core antigen vaccine in patients with chronic hepatitis B. Hepatol Int. 2013;7(4):981-9.

28. Wedemeyer H, Hui AJ, Sukeepaisarnjaroen W, Tangkijvanich P, Su WW, Nieto GE, *et al*. Therapeutic vaccination of chronic hepatitis B patients with ABX203 (NASVAC) to prevent relapse after stopping NUCs: contrasting timing rebound between tenofovir and entecavir. J Hepatol. 2017;66:S101.

Table 4. Summary of the state of the art of major clinical developments of the last three years (2016-2018) for the treatment of chronic hepatitis B*

| Product code/ Composition | Company/ Clinical trial index | Study design and main details | Results at the end of 2018 [Ref.] |
|---|---|---|---|
| FP-02.2 (HepTCell) Long peptide comprising CD4 and CD8 T-cell epitopes. IC31® Adjuvant based in depot effect and TLR9 targeting was used in part of the study groups. | Altimmune NCT02496897 | A phase I, Randomized, Double-blind, Placebo-controlled, Multi-center, Ascending-dose Trial to Evaluate the Safety, Tolerability and Immunogenicity of Vaccine. A total of 40 patients received one or two dose levels of HepTcell, with and without IC31. 20 patients received placebo or IC31 alone. | All patients received 3 injections, 28 days apart and were followed by 6 months after the final dose. The phase I trial was completed in 2018. All dose combinations were well tolerated and in the adjuvanted groups, T cell responses against HBV markedly increased over baseline compared to placebo [23]. |
| GS-4774 Recombinant yeast antigen expressing X, Env, Core proteins | Gilead NCT02174276 | A phase II, Randomized, Open-Label Study to Evaluate the Safety and Efficacy of GS-4774 in Combination With Tenofovir Disoproxil Fumarate (TDF) for the Treatment of Subjects With Chronic Hepatitis B and Who Are Currently Not on Treatment. Participants were randomized to receive TDF alone or GS-4774 plus TDF for 20 weeks. After Week 20, GS-4774 was discontinued. All participants continued on TDF. | T cell function increases with a prevalent effect on CD8 T cells specific for pol, then for env, core and HBx. However, this immune response seems to be insufficient to induce a significant HBsAg reduction between the group treated with NUC vs. the group treated with the combination of NUC and GS-4774 [24]. |
| | NCT01943799 | A phase II, Randomized, Open-Label Study to Evaluate the Safety and Efficacy of GS-4774 for the Treatment of Virally-Suppressed Subjects With Chronic Hepatitis B. | GS-4774 was well tolerated, but did not provide significant reductions in serum HBsAg in virally suppressed patients with chronic hepatitis B [26][25] . |
| | Not indexed | A phase I exploratory study evaluating anti-PD-1 treatment with or without GS-4774 in HBeAg negative chronic hepatitis B patients. The study enrolled virally suppressed HBeAg negative patients without advanced fibrosis. | There was a significant decline in HBsAg in patients taking antivirals and receiving anti PD1 treatment. No added benefit of administering GS-4774 [26]. |
| HeberNasvac® (ABX203) Recombinant antigens containing HBsAg and HBcAg | Center for Genetic Engineering and Biotechnology & CRO Ltd. | A phase III, treatment controlled, open and randomized clinical trial comparing HeberNasvac vs PegIFN treatment. HeberNasvac was administered in treatment naïve patients as a monotherapy. A cycle of five IN administrations was followed by a cycle of five IN/SC immunizations using 100 μg per Ag/route. | Significant superiority of HeberNasvac in term of sustained antiviral effect (frequency and mean) 24 and 48 weeks after the end of each treatment. Significantly better safety profile and evidences of higher serological responses [27]. |
| | Center for Genetic Engineering and Biotechnology & Abivax | A phase IIb trial with HeberNasvac administered in patients treated with antivirals. A cycle of five IN administrations was followed by a cycle of five IN/SC immunizations using 100 μg per Ag/route. Antiviral treatment continued up to one month after the end of vaccinations. | HeberNasvac administration was safe when vaccination was combined with antivirals. Vaccination of CHB patients under HBV suppression did not prevent viral relapse after stopping NUCs. The study revealed unexpected relapse timing between TDF and ETV [28] |
| INO-1800 DNA plasmids encoding HBsAg and HBcAg | Inovio Pharmaceuticals NCT02431312 | A phase I, open-label study to evaluate the safety, tolerability, and immunogenicity of dose combinations of INO-1800 (DNA plasmids encoding HBsAg and HBcAg) and INO-9112 (DNA plasmid encoding human interleukin 12) delivered by electroporation in 90 patients treated with NUCs. | Phase 1 clinical trial demonstrated immune response results with INO-1800 was safe, well tolerated and showed ability to activate and expand CD8+ killer T cells, meeting the objectives of the clinical trial [29]. |
| TG1050 Adenovirus serotype 5 vector encoding a large fusion protein (Truncated Core, modified Pol, and two Env domains) | Transgene NCT02428400 | A phase I/Ib, double-blind, randomized, placebo-controlled study conducted in CHB patients. Single and Multiple Injections of the Therapeutic Vaccine TG1050 in NUC-Suppressed Chronic Hepatitis B (CHB) Patients. All patients were receiving antiviral treatment with either TDF or ETV for at least two years, and have their HBV infection well-controlled. | Safety established following single dose (SD) and multiple doses (MD) administration of TG1050 in CHB patients under NUC therapy. TG1050 induces a robust and HBV-specific cell-mediated immune response in patients with CHB [30]. |

*The trend of vaccinating patients under antiviral treatment is evident and should be carefully revisited.

for Genetic Engineering and Biotechnology. This therapeutic vaccine consists of virus like particles (VLPs) of purified recombinant HBsAg and HBcAg, and it is administered by nasal and subcutaneous routes. This product was presented during the 2017 and 2018 congresses of the Asia Pacific Association for the Study of the Liver (APASL), where the authors presented a compilation of non-clinical and clinical pharmacology data [34]. Several regulatory agencies are currently evaluating the possibility of granting Sanitary Registration to this novel product.

### *HeberNasvac® pharmacology*

A group of pharmacological studies in animal models were performed in Cuba, in collaboration with the Institut Pasteur, France, and at the Ehime University in Matsuyama, Japan. The preclinical immunogenicity studies, developed in normal Balb/c mice as well as in AAV-HBV-transduced (HBV-persistent) and HBsAg-transgenic mice, supported the selection of the optimal formulation, the antigen doses and proportions, as well as the routes of administration [34-37].

HBsAg transgenic and adeno-associated virus-HBV transfected mice, in the background of humanized HLA, were used as models, to evaluate the capacity of the nasal route of immunization to generate systemic and especially liver immune responses. The application of HeberNasvac® induced CD4+ and CD8+ T-cell responses, and the secretion of pro-inflammatory cytokines involved in viral control and disease resolution [37]. Furthermore, the immunogenicity studies in the AAV-HBV model of CHB infection demonstrated the effect of nasal immunization, in contrast to subcutaneous (SC) immunization, on the

29. Inovio's DNA Immunotherapy Demonstrates Immune Response Results Key in Treating Chronic Hepatitis B Infection. 2018 Mar 14 [cited: [2018 Oct 4]. Available from: http://ir.inovio.com/news-and-media/news/press-release-details/2018/Inovios-DNA-Immunotherapy -Demonstrates-Immune-Response-Results-Key-in-Treating-Chronic-Hepatitis-B-infection/default.aspx.

30. Zoulim F, Fournier C, Habersetzer F, Sprinzl M, Pol S, Coffin CS, *et al*. Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial. Hum Vaccin Immunother. 2019:1-12.

31. Zheng Y, Chen S, Li S, Xu Y, Li X, Zhao H, *et al*. The percentage and severity of HBV-related acute-on-chronic liver failure patients result from irregular medication of nucleos(t)ide analogues [Abstract]. Hepatol Int. 2017;11(Suppl 1):S97.

homing of virus specific effector CD4 T cells to the liver [37].

### *HeberNasvac®: Main clinical developments*

Several clinical trials evaluated the safety and efficacy of HeberNasvac® as monotherapy, three of them in CHB patients and one in healthy volunteers. In general, HeberNasvac® vaccination was safe and induced strong antiviral and serological responses [27, 38]. The most important study of HeberNasvac® as monotherapy was the treatment controlled and randomized phase III clinical trial conducted to evaluate the efficacy and safety of this product in CHB patients in comparison with PegIFN treatment [39].

The phase III trial was designed for 160 CHB patients randomized in two groups (1:1). Both, HBeAg positive or negative patients with history of altered transaminases or moderate fibrosis/histological activity index were enrolled. In the first cycle, the patients received five administrations of the formulation by IN route every two weeks. A second cycle of five administrations started one month after the first cycle. The second cycle encompassed 5 administrations of equal doses by the IN route and 5 subcutaneous injection given simultaneously. A dose of 100 µg of each antigen (100 µg of HBsAg and 100 µg of HBcAg) was used by each route [39].

Considering the efficacy, both the intention to treat and per protocol analysis, a significantly higher proportion of vaccinated patients with HBV DNA below 250 copies/mL at the end of 24 weeks of treatment-free follow up was found, as compared to the proportion of patients in the same conditions 24 weeks after the end of PegIFN treatment. After therapeutic vaccination with HeberNasvac®, patients developed a two to five times increase of ALT, resembling immune activation against HBV antigen expressing hepatocytes, which was followed by a reduction in the viral load. The ALT flares did not result in clinical symptoms, and became normalized again at the end of HeberNasvac® treatment [39]. In line with a restoration of antiviral responses, a higher proportion of HBeAg loss and seroconversion for HeberNasvac®-treated HBeAg positive patients was observed at the end of follow-up.

Regarding safety, no serious or severe adverse events (AE) were detected after immunization by nasal and/or subcutaneous routes with HeberNasvac®. The more frequent AE were similar in nature for both products. The number of different AE, their frequency, intensity and duration were lower in the group treated with HeberNasvac® as compared to PegIFN.

## Therapeutic vaccination as a part of combined therapies

### *Therapeutic vaccination in combination with RNA interference and antivirals*

A promising approach to lower the HBV antigen load using RNAi prior to therapeutic vaccination was developed at the Technical University of Munich. The siRNAs were conjugated to N-Acetylgalactosamine (GalNAc), allowing efficient and specific delivery in hepatocytes. Michler *et al.* evaluated the capacity of GalNAc coupled siRNA's directed against HBV RNA to suppress HBV gene expression in a transgenic mouse model [40]. Subsequently, they investigated the recovery of HBV-specific B- and T cell responses, both spontaneously, and after therapeutic vaccination [40].

Highly viremic HBV transgenic mice were treated with: 1) nucleoside analogue ETV, 2) shRNA-expressing Adeno-Associated Virus vector (AAV-shHBV) or 3) GalNAc-conjugated siRNAs to target HBV mRNA and decrease HBsAg concentration in blood. Subsequently, mice were therapeutically vaccinated with a HBcAg/HBsAg protein prime- and a Modified Vaccinia Ankara virus (MVA)-boost immunization to stimulate adaptive immunity.

The treatment with ETV strongly reduced HBV DNA by 4 log10, but antigen levels remained unchanged. Monthly subcutaneous injections of GalNAc-siRNAs, as well as treatment with AAV-shHBV, efficiently suppressed HBsAg and HBV DNA in serum by 2 log10, and HBeAg by 1 log10. The heterologous prime-boost vaccination induced B-cell immunity and anti-HBs-seroconversion in all animals, but HBV-specific CD8 T cell responses were only seen in animals with lower antigen titers after siRNA/shRNA pretreatment [40]. The siRNA treatment followed by therapeutic vaccination showed an additive effect cumulating in >4 log10 reductions of HBsAg and HBV DNA in serum, to undetectable levels, compared to pretreatment levels.

The duration of siRNA pretreatment (3, 6 or 8 weeks) prior to therapeutic vaccination treatment correlated with increasing HBV-specific CD8 T cell responses. The best treatment scheme resulted in a >5 log10 reduction of HBsAg to undetectable levels in all treated animals. Thus, this combinatorial approach using RNAi and vaccination therapy for hepatitis B allowed reconstitution of HBV-specific T cell responses and suppression of HBV to undetectable levels in a preclinical mouse model of CHB [40].

### *Therapeutic vaccination in combination with anti-PD-1 treatment and antivirals*

Nivolumab is a monoclonal antibody used to treat cancer by blocking a negative regulator of T-cell activation and response, thus allowing the immune system to attack the tumor [26]. This antibody blocking the immune checkpoint inhibitor PD-1 has also been tested in the treatment of CHB [26]. A phase 1 study evaluating Nivolumab treatment with or without a therapeutic vaccine candidate GS-4774 was recently concluded. The yeast-based T-cell vaccine GS-4774, has shown to be immunogenic in mouse models and healthy volunteers but with no clinical efficacy in CHB patients. The combination of both immunotherapeutic strategies was designed to increase HBV-specific T-cell frequency and to restore their functionality aimed at inducing a durable control of HBV [26].

The phase I exploratory study enrolled virally suppressed HBeAg negative patients without advanced fibrosis. Patients received either single dose of Nivolumab, or received 40 Yeast Units GS-4774 at baseline, and at Week 4 prior to a single dose of Nivolumab. The primary endpoint was change in HBsAg 12 weeks after receiving Nivolumab. No grade 3 or 4 adverse events or serious AEs were detected.

A significant decline in HBsAg levels, as compared to baseline, was found in the group treated with

32. Wisskirchen K, Kah J, Malo A, Asen T, Volz T, Allweiss L, *et al*. T cell receptor grafting allows virological control of Hepatitis B virus infection. J Clin Invest. 2019; 129(7):2932-2945.

33. Kennedy W, Yuen MF, Kim DJ, Weilert F, Chan HL, Lalezari J, *et al*. Safety, tolerability, pharmacokinetics and antiviral activity of AL-3778, a first-in-class, HBV core assembly modulator alone and in combination with peginterferon-alpha 2A, in treatment naive HBeAg-positive chronic hepatitis B patients [Abstract]. Hepatol Int. 2017;11(Suppl 1):S7.

34. Aguilar JC, Lobaina Y. Immunotherapy for chronic hepatitis B using HBsAg-based vaccine formulations: From preventive commercial vaccines to therapeutic approach. Euroasian J Hepatogastroenterol. 2014 Jul-Dec;4(2):92-7.

35. Aguilar JC, Lobaina Y, Muzio V, García D, Pentón E, Iglesias E, *et al*. Development of a nasal vaccine for chronic hepatitis B infection that uses the ability of hepatitis B core antigen to stimulate a strong Th1 response against hepatitis B surface antigen. Immunol Cell Biol. 2004;82(5):539-46.

36. Lobaina Y, Trujillo H, García D, Gambe A, Chacon Y, Blanco A, *et al*. The effect of the parenteral route of administration on the immune response to simultaneous nasal and parenteral immunizations using a new HBV therapeutic vaccine candidate. Viral Immunol. 2010;23(5):521-9.

37. Bourgine M, Crabe S, Lobaina Y, Guillen G, Aguilar JC, Michel ML. Nasal route favors the induction of CD4(+) T cell responses in the liver of HBV-carrier mice immunized with a recombinant hepatitis B surface- and core-based therapeutic vaccine. Antiviral Res. 2018;153:23-32.

38. Betancourt AA, Delgado CA, Estévez ZC, Martínez JC, Ríos GV, Aureoles-Roselló SR, *et al*. Phase I clinical trial in healthy adults of a nasal vaccine candidate containing recombinant hepatitis B surface and core antigens. Int J Infect Dis. 2007;11(5):394-401.

39. Al Mahtab M, Akbar SMF, Aguilar JC, Guillen G, Penton E, Tuero A, *et al*. Treatment of chronic hepatitis B naïve patients with a therapeutic vaccine containing HBs and HBc antigens (a randomized, open and treatment controlled phase III clinical trial). PLoS One. 2018;13(8):e0201236.

40. Michler T, Kosinska A, Bunse T, Heikenwälder M, Grimm D, Milstein S, *et al*. Preclinical study of a combinatorial RNAi/vaccination therapy as a potential cure for chronic hepatitis B [Abstract]. J Hepatol. 2017;66:S112.

Nivolumab alone, and one patient seroconverted from HBsAg to anti-HBsAg. Therapeutic vaccination did not increase the effectiveness of Nivolumab. In general, a single dose of Nivolumab up to 0.3 mg/kg was well tolerated in virally suppressed HBeAg negative CHB infected patients. There was a significant decline in HBsAg in patients receiving anti PD1 treatment with no added benefit of GS-4774 administration. Noteworthy, during this study, patients were under treatment with NUCs [26].

### *Therapeutic vaccine (GS-4774) in combination with NUCs*

The modulatory effect of GS-4774 on HBV-specific T cell responses in treatment-naive, HBeAg-negative CHB patients was recently studied [24]. A total of 12 HBeAg negative, viremic, genotype D-infected CHB patients received 6 vaccine doses, one per month, in combination with TDF, as part of a larger study. A total of 26 chronic HBeAg-negative, genotype D-infected patients treated with the antiviral alone served as controls. HBV-specific T cell responses towards HBV peptides were studied before, during, and after vaccine therapy by IFN-γ ELISPOT and intracellular cytokine staining for IFN-γ, TNF-α, IL-2 and CD107 degranulation.

While all patients normalized ALT and experienced a decline in HBV-DNA, none had a significant HBsAg decline. *Ex vivo* IFN-γ ELISPOT responses were significantly improved upon HBV core peptide stimulation at week 48 compared to baseline. Following *in vitro* expansion, a significant increase in the percentage of HBV-specific IFN-γ and IL2 producing T cells was detected at weeks 24 and 48. This functional improvement was predominantly sustained by CD8+ T cells, which showed also an increased production of TNF-α. A simultaneous improvement of more than one T cell function with double and triple cytokine-secreting HBV-specific T cells was detected in 11 of 12 patients. It was concluded that GS-4774 combined with TDF can improve the T cell function with a prevalent effect on CD8 T cells specific for pol, then for env, core and HBx. However, according to the authors, this immune response seems to be insufficient to induce a significant HBsAg reduction between the group treated with NUC vs. the group treated with the combination of NUC and GS-4774 [24].

### *Therapeutic vaccine HeberNasvac® in combination with NUCs*

A group of hepatologists and scientists from Europe and Asia, sponsored by the French company ABIVAX assessed HeberNasvac®, also called ABX203, in virally suppressed patients [28]. A Phase IIb trial was conducted in Asian countries. The therapeutic vaccination using HeberNasvac® was first developed as a monotherapy for patients that were not using antiviral treatment. Moreover, it was tested in a limited number of interferon-experienced patients. In this case, HeberNasvac® was evaluated in patients under long time virological suppression.

Both, as a monotherapy, or in combination with antivirals, HeberNasvac® was administered via IN during a priming cycle of five administrations of 100 µg of each antigen per dose. Then, it was followed by a cycle of five simultaneous intranasal (IN) & subcutaneous (SC) immunizations using the same dose per administration route (200 µg of each antigen HBsAg and HBcAg in total per immunization day). Antiviral treatment continued up to one month after the end of vaccinations.

A total of 276 HBeAg negative non-cirrhotic patients that were under NUC treatment for more than 2 years (HBV-DNA negative and normal ALT levels) were randomized to continue the treatment with NUCs during 24 weeks in combination with HeberNasvac® conventional schedule (n = 184) vs. treatment with NUCs only (n = 92). After 24 weeks, antiviral therapy was stopped in all patients, 4 weeks after end of vaccination. The primary end-point of the study was the percentage of subjects who maintained HBV-DNA levels <40 IU/mL, 24 weeks after NUC treatment discontinuation [28].

The patients included in the trial had a mean age of 50 years, ongoing therapy with NUCs during 4.78 ± 2.37 years at the start of vaccinations, were mainly Asian (94 %), male (72 %) and 57 % had HBsAg levels of >1000 IU/mL at baseline. ABX203 vaccination was safe and well tolerated with only 2.2 % SAEs in both treatment arms (not drug related). The primary endpoint was reached by 6.9 % of vaccinated patients and 11.7 % of those receiving NUCs only (p = 0.20). Similarly, the authors reported no differences between the study groups in the percentage of patients with normal ALT and AST values (74 % vs. 80 %), HBV-DNA <2000 IU/ml with ALT <2×ULN (31 % vs. 41 %) and HBsAg declines. Anti-HBs and anti-HBe humoral immune responses were not induced by ABX203. Strikingly, however, viral rebound (HBV-DNA >2000 IU/mL) occurred much earlier in patients treated with TDF (>70 % by week 12) vs. ETV (<10 % by week 12), irrespective of ABX203 treatment and without impacting outcomes [28]. This prospective randomized HBV therapeutic vaccine study was also the largest prospective study stopping NUCs and showed that ABX203 did not prevent viral relapse after stopping NUCs. Also, it revealed unexpected relapse timing between TDF and ETV.

## Pros and cons of therapeutic vaccination of virally suppressed patients

Alternative treatments for CHB are subject of intense research worldwide, with vaccination among the most studied. The rationale of vaccination under viral suppression is based on the observation that a decrease in HBV load seems to precede the detection of HBV specific T-cell responses, both in patients resolving natural infections, and in those undergoing HBeAg seroconversion during chronic infection. Thus, a reduction in HBV load by antiviral chemotherapy may increase the responsiveness of HBV-specific T cells, which are hyporesponsive in cases of persistent HBV infection or viral antigen stimulation (reviewed in [41]).

Regarding the combination of therapeutic vaccines and antivirals, there are also few aspects that need closer consideration: HBV-specific T cells are detectable during the first few months of lamivudine treatment [42] and this restoration of T-cell activity is partial, transient and does not lead to an increase in HBeAg seroconversion [43]. In the case of ABX203,

41. Michel ML, Deng Q, Mancini-Bourgine M. Therapeutic vaccines and immune-based therapies for the treatment of chronic hepatitis B: perspectives and challenges. J Hepatol. 2011;54(6):1286-96.

42. Boni C, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, *et al*. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest 1998;102:968-75.

43. Boni C, Penna A, Bertoletti A, Lamonaca V, Rapti I, Missale G, *et al*. Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. J Hepatol. 2003;39:595-605.

the product was evaluated in patients under strict antiviral control for several years [28]. Other important trials have evaluated different vaccine candidates in similar conditions without satisfactory results in terms of virological control after treatment discontinuation [25, 44, 45].

Taking into account the immunology of the liver, there are some theoretical disadvantages from immunizing patients under long-term antiviral treatment. Essentially, the induced immune cells should exert their function in the liver. However, the role of an anti-inflammatory environment in the liver has been reinforced, as evidenced by the sustained reduction in ALT levels in most patients under antiviral treatment [45-47]. In parallel, HBV replication is suppressed to undetectable levels in most patients under treatment. It has been described that the hepatocytes only express HLA class II under non-physiological pro-inflammatory conditions [48-50]. In fact, inflammatory mediators or the HBV infection itself have been proposed as eliciting agents [50], coincident with its absence in this setting.

Overall, the elimination of the virus and the subsequent normalization of ALT during long term antiviral therapy reduce the inflammatory mediators, and consequently the expression of HLA class II and the related CD4 T helper activity. The number of intracellular peptides to be presented on the groove of HLA molecules also decreases. A link between the intracellular expression of antigens and the low viral replication has been previously reported [51]. The fact that most immunotherapeutic products are studied under viral suppression deserves a critical revision from the immunological point of view.

### New guidelines on treatment discontinuation and its impact on therapeutic vaccination

New recommendations introduced by the EASL have been presented at the EASL International Liver Congresses of 2018. The guidelines recommend antiviral treatment discontinuation under strict monitoring in non-cirrhotic HBeAg-negative patients with long time virological suppression and low fibrosis [52, 53]. This novel recommendation opens the possibility to new scenarios for evaluating therapeutic vaccines [3]. The recommendations of treatment discontinuation in HBeAg negative patients have been discussed exhaustively during the 2018 International Liver Congress held in Paris. The ALT increases in patients with low levels of fibrosis has been considered favorable, because it is likely to reflect the reactivation of an antiviral immune response, which may that lead to HBsAg elimination in 20 to 40% of patients in the 3 years after treatment discontinuation. On the other hand, patients continuing treatment with NUCs evidenced no reduction in their serum HBsAg levels [54-57].

Altogether, the natural reactivation of the immune response after antiviral treatment cessation represents a solid and effective factor that may further potentiate the vaccine induced-immune response. Vaccination during or after stopping NUCs may further enhance off-therapy viral control in a synergistic manner. With the better understanding of antiviral rebound dynamic corresponding to ETV and TDF as well as their related ALT flares, the dynamic of immune reactivation post-treatment cessation may be predicted and optimized as part of the immunotherapeutic process. Further studies in this setting are required in order to target the important number of CHB patients currently under antiviral treatment for several years.

## Concluding remarks

The quest for an effective, safe and definitive treatment for CHB remains an important challenge. Recent studies conducted in China followed CHB patients under treatment for a decade or more. A large and long lasting study confirmed the significant effect of PegIFN in preventing LC and HCC development; but this effect was not confirmed for patients treated with ETV [9]. Moreover, irregular medication with NUCs was responsible of approximately 20 % of all cases of acute on chronic liver failure (ACLF) developed in cirrhotic patients, and near 10 % of ACLF in the case of CHB patients without cirrhosis. To further complicate this picture, in both sceneries (CHB and cirrhotic patients), the irregular medication with NUCs induced the most severe form of liver failure as compared to other etiological causes. These recent findings evidenced that the most frequently used treatment, the NUCs, have very important limitations in their implementation during routine medical practice. Other renal manifestations and bone issues have been described and it is expected that TAF will be able to reduce their impact.

In developing and underdeveloped countries, where the CHB disease is more prevalent and governments are unable to provide CHB treatments, informative campaigns should be reinforced in support of regular medication with NUCs. Otherwise, the pharmacological and epidemiological impact of these products may be lost due to product misuse. The WHO objective of controlling the increasing mortality of viral hepatitis may be at risk.

New products appear in the horizon that represents a hope in front of the present reality. Therapeutic vaccination as monotherapy has reached the registration of the first product in the countries of origin (Cuba and Bangladesh). However, challenges are expected to come for therapeutic vaccination as in patients under viral suppression. Current recommendations of the major societies for the study of the liver clearly advice on antiviral treatment cessation. In 2017, the ILC edition held in Amsterdam proposed recommendations for stopping antiviral treatment for European HBeAg negative patients under antiviral treatment under strict follow-up. This opens a space for evaluating therapeutic vaccines after antiviral treatment.

The therapeutic elimination of the HBV is complex, due to the frequent HBV DNA integrations in host genome. The multiple mechanisms of tolerance induction that prevents the recovery of the required multifunctional, potent and multiantigenic Th1-like response for controlling viral infection further complicates this scenario. The clearance of cccDNA is now the main objective of many novel therapies and combined treatments. The accomplishment of WHO goals regarding the control of viral hepatitis by 2030 is challenging because at present CHB contributes to near 70 % of the mortality and mortality in on the rise.

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In order to control CHB, it will be necessary to implement a large number of preventive, diagnostic and therapeutic actions. The progressive approval of therapeutic vaccines for CHB and its successful testing may provide an alternative for life-long NUC treatment or reactogenic PegIFN. Furthermore, optimization studies will be required for patients under antiviral treatment, for the best possible allocation of immunotherapy.

## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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