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Hepatitis: A Global Health Concern

Chapter 2

Chronic Hepatitis B Treatment: *Statu Quo* and New Developments

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Abstract

The state of the art in the field of CHB treatment reveals several limitations of currently approved therapies. Fortunately, the recent understanding of the immunology and physiology of chronic hepatitis B infection is leading to several innovative therapeutic strategies for chronic hepatitis B. Novel therapies support the global efforts by the World Health Organization in order to prevent disease progression and mortality by liver cirrhosis and hepatocellular carcinoma as a result of viral hepatitis, a disease with a mortality trend on the rise worldwide. The results of the most advanced products for chronic hepatitis B treatment will be considered in the present revision of the *statu quo* of therapies. Special attention is given to therapeutic vaccination. The main pharmacological and clinical trials as well as the notorious case of therapeutic vaccination in patients with viral suppression as the result of combined treatment with antivirals. These areas of research deserve in deep analysis and discussion. The products in the more advanced clinical status will be highlighted as well as the recent registration of a novel therapeutic vaccine.

Keywords: ILC 2017; chronic hepatitis B; hepatitis C; therapy; nucleot(s)ide analogues; therapeutic vaccine.

1. Introduction

The Global Hepatitis Report issued by the World Health Organization in 2017 shows that viral hepatitis still represents a major public health challenge. More than one third of the

World population has been infected by the Hepatitis B Virus (HBV). The estimates of chronic carriers of the virus are in the range of 248 up to 257 million, approximately the 3.5% of the World population [1,2]. With 1.34 million deaths by 2015, the mortality of viral hepatitis is comparable to tuberculosis, and higher than those caused by the human immunodeficiency virus (HIV) or malaria. However, while mortality from HIV, tuberculosis, and malaria is now declining, mortality caused by viral hepatitis is on the rise [1].

The Hepatitis B Virus (HBV) infection is responsible of approximately 65-70% of the mortality generated by viral hepatitis [1]. The long-term sustained HBV chronic replication becomes a progressive hepatic disease that leads to liver cirrhosis and cancer in up to 25% of carrier patients. Almost 0.9 million deaths are produced by the different forms of presentation and progression of HBV infection every year. Almost 90% of HBV-related casualties are the consequence of liver cirrhosis (LC) or hepatocellular carcinoma (HCC). It is important to remind that a significant number of patients suffer important sequelae like oesophageal varices with digestive bleedings, ascites, splenomegaly and also the episodes of acute on chronic liver failure (ACLF) after HBV reactivation, non-related infections, after taking other drugs or caused by irregular medication with NUCs. In summary, an important proportion of CHB patients experience a dramatic fall in their quality of life and eventually death related to the disease in approximately 25% of patients according to natural history [1,2].

The present *statu quo* demands the constant revision of current products and recommendations for CHB treatment. The state of the art should be considered by experts, international organizations, policy makers, regulators and even politicians, in order to optimize the present recommendations and ensure the adequate treatment as well as treatment adherence. The main objective should be to contain disease progression and consequently limit the expansion in mortality that the World is currently witnessing.

This chapter aims at revisiting the state of the art in the field of CHB treatment, exposing the limitations of currently approved therapies as revealed in recent meetings and publications as well as discussing the results of the most advanced products undergoing clinical evaluation. Specifically, we focus also on the results of the most advanced therapeutic vaccines, discussing their main pharmacological and clinical trials as well as the notorious case of therapeutic vaccination in patients with viral suppression as the result of combined treatment with antivirals.

2. Chronic Hepatitis B Treatments: Statu quo

Peginterferon (PegIFN) and nucleos(t)ide analogues (NUCs) are the widely approved treatments for CHB infection. Both have considerable advantages and limitations. PegIFN offers the advantage of higher sustained response rates at the price of considerable side effects and high costs. NUCs offer a relatively easy daily oral dosing, and viral suppression for prolonged treatment duration. However, relapse is common after therapy discontinuation and

quasi-eternal therapy therefore necessary in most patients. Prolonged treatment with NUCs may marginally enhance chances of virologic and serologic response at the cost of potential side effects [3-5].

The products for treating CHB as well as the treatment recommendations have been improved continuously. For decades, the major associations for the study of the liver have released their recommendations with subsequent periodical updates. In general, doctors follow variables such as serum HBV DNA levels, ALT elevation and histologic changes of liver tissue [3-5]. Indication for treatment also considers age, health status, family history of HCC or LC and extrahepatic manifestations.

The main international guidelines recommend to initiate treatment in patients with HBV DNA levels above 2,000 IU/mL (>10,000 copies/mL) and also with sign of on going hepatitis (elevated ALT levels or liverfibrosis demonstrated by liver histology or non-invasive tools such as liver elastography or serologic algorithms suchas fibrotest [3-5].

The description of the *statu quo* 2017 should also take into account HeberNasvac, a therapeutic vaccine co-developed as a novel treatment for CHB patients by an international team from Cuba, Bangladesh and Japan. HeberNasvac, an example of South-South and South-North cooperation has received the sanitary registration in the countries were the most important clinical trials have been conducted, Cuba and Bangladesh and it has been tested in several other territories including eight different countries in Asia.

2.1. The efficacy and safety of current treatments

Complete eradication of HBV is a rare event after treatment. That's why the main goal of therapy is to halt the progression of liver inflammation to advanced fibrosis, LC or HCC. These outcomes are not evident until after decades of infection, thus surrogates measures are pursued during treatment. A summary of the most important variables of efficacy for both NUCs and PegIFN based treatments is described in **Table 1**. 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.

In addition to the efficacy limitations of current treatments, a large number of safety limitations have been described. In general, the treatment with NUCs has been regarded as safer and better tolerated than IFN-based treatments, however renal manifestations and bone demineralization have been described for long term treatments with NUCs, as well as the risk of decompensation after treatment discontinuation or irregular medication. It is expected that treatment with the novel drug Tenofovir Alafenamide (TAF) will be able to reduce the incidence of such pathologies. Nevertheless, there is a trend to consider antiviral interruption or

cessation after certain number of years. More information regarding the efficacy and safety limitations of current CHB treatments can be found in the specific Product Inserts and it is also summarized in **Table 2** [6-8].

2.2. Recent relevant limitations revealed after large and long lasting studies

In the present section we will focus in the novel information arising during 2017, considered relevant in terms of treatment efficacy and safety. Several results have shown the compilation of the last decade or more of experience in large number of patients.

2.2.1. Long-term effect in preventing LC and HCC

Although PegIFN and the NUCs are currently recommended products for first-line therapy of CHB infection by major associations for the study of the liver, the long-term effect of these products in preventing LC and HCC has been controversial. The studies directly comparing the long-term outcomes of these two types of treatments were absent. In the last decade, a large, observational, open-label, prospective cohort study of HBeAg-positive CHB patients who received PegIFN or ETV therapy was carried out by Chinese scientists and presented during the meeting of the Asia Pacific Association for the Study of the Liver (APASL 2017). Cumulative incidences of unfavorable events (progression to LC and HCC) were calculated with respect to treatment type. Based on the REACH-B model, Chinese experts analyzed the incidence in these two groups, and compared the observed incidence of LC and HCC with the expected incidence in each group.

PegIFN treated patients showed a lower cumulative incidences of unfavorable events and cirrhosis than ETV treated ones. Univariate/multivariate exploration indicated that the type of treatment was associated with the occurrence of unfavorable events in patients with CHB infection. Based on the REACH-B model, a lower cumulative incidence of HCC was observed in PegIFN treated patients than predicted cases based on the REACH-B model. On the other hand, there was no significant difference of the cumulative HCC incidence between the observed and the predicted cases in the ETV treated patients, demonstrating a comparatively superior effect in the case of PegIFN treatment [9].

The value of these results highlights the real contribution of the immunomodulatory therapies to the control of CHB disease progression. Limiting the progression of the disease is the most relevant and preferred consequence of CHB treatment. These results in CHB patients, linked to the recently published data from Wranke and colleagues [10] showing the limited effect of antivirals in patients coinfected with Hepatitis Delta in contrast to PegIFN treatment should have a positive impact in the use of PegIFN as first line treatment for CHB therapy.

2.2.2. Irregular medication with NUCs as a relevant cause of ACLF

The acute-on-chronic liver failure (ACLF) is one of the most challenging health problems worldwide, characterized by its rapid progression and high mortality. In most Asian countries, hepatitis B causes 70-80% of all etiologies of ACLF, so HBV-related ACLF is a serious public health. An important percentage and severity of HBV-related ACLF patients result from irregular medication with NUCs as recently revealed by a large and long lasting study conducted in China [11].

The study focused on patients with HBV-related ACLF. From a total of 1118 subjects admitted to nine hospitals in China from January 2005 to December 2015. 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, drug, alcohol, others. The percentage and improvement rate of HBV-related ACLF induced by different predisposing factors were appraised by statistical analyses. In HBV-related ACLF patients with CHB, the percentage of cases caused by IMNA reached 8.94 %. The rate of improvement of IMNA derived cases was the lowest, only 50%.Multiple-factor analysis shows IMNA, hepatic encephalopathy, hepatorenal syndrome were independent risk factors. In HBV-related ACLF patients with LC, the percentage of cases caused by IMNA was 19.33%, and the improvement rate of IMNA was also the lowest, only 37.68%. Multiple-factor analysis shows IMNA, infection, hepatic encephalopathy, hepatorenal syndrome are independent risk factors for developing ACLF [11].

In summary, the percentage of cases caused by irregular medication with NUCs was almost 20% for LC patients and approximately the half in CHB patients. The severity of the liver failure was higher in the case of IMNA compared to other etiologies. Authors recommend paying more attention to patient's adherence to NUC treatment because frequent 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 evidencing the effect of irregular medication with NUCs in connection with FDA warnings against uncontrolled treatment discontinuation see **(Table 2)**.

2.2.3. The effect of tenofovir on bone mineral density

Some doubts remain regarding the association of TDF with the appearance of osteoporosis. The data presented in relation with two Phase III studies involving TDF and the new product Tenofovir Alafenamide (TAF), further clarified the effect of these antivirals on bone mineral density.

A total of 1289 patients from two phase III trials were randomized 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 from baseline and by proportion of patients with > 3% decline 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 percentage of changes [mean (SD)] 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, the percentage of changes 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 [12].

The difference between TAF and TDF were more pronounced in patients with multiple risk factors, with TAF treated patients having 10 % of 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 patients 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 [12]. In summary, from this comparison was clear the important effect of TDF in the reduction of the bone mineral density, even when some authors claimed that this was the effect of comorbidities. The use of TAF induced a lower number of these side effects compared to TDF, demonstrating superior safety.

2.2.4. On the renal toxicity of nucleotide analogues

The long-term nucleotide analogue treatment (adefovir (ADV) and TDF) increase renal toxicities compared to the nucleoside analogue ETV treatment in patients with CHB, according to the work developed by 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 com-

pared to ETV. A long term study needs to be performed in this population [13].

3. CHB Treatment: New Developments

A wave of novel treatments approaches appear in the horizon of CHB therapy. These new developments have been eclipsed by the spectacular results in the field of treatments for chronic hepatitis C. Many of these approaches are now under clinical testing and their validation in patients is expected in the near future. A novel therapeutic vaccine (HeberNasvac) has been registered for the first time to treat a chronic infectious disease, in this case the Chronic Hepatitis B.

3.1. Cell-based immunotherapy

Adoptive T-cell therapy of CHB or HCC intends to restore antiviral T-cell immunity to clear the infection or control HBV-derived tumor growth. This novel strategy is being developed by the Technical University of Munich (TUM) and it has been focused in the use of adoptive T-cell therapy for the treatment of CHB [14]. A group of 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 have been identified. HBV-specific TCRs were used to engraft human T cells by retroviral transduction. Subsequently, 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 were shown to kill hepatoma cells expressing HBV antigens from an integrated HBV genome, as well as HBV-infected cells. HBV-specific TCRs also mediated elimination of HBV when expressed on CD4+ T cells only, and when expressed on T cells from patients with CHB [14].

TCR-redirected T cells could efficiently target infected hepatocytes in the liver when transferred into SCID mice repopulated with HLA-A*02-matched primary human hepatocytes and infected with HBV. After 5 days, ALT levels were moderately increased. Intrahepatic analyses revealed a strong reduction of cccDNA loads and other markers of HBV replication. The authors proposed TCR-transduced T cells with high functional avidity for adoptive T-cell therapy of CHB [14]. Interestingly, these results suggests that TCR-engrafted T cells could also be employed to eliminate HCC expressing HBV antigens from integrated HBV genome fragments, as is often the case in HBV-related HCC.

3.2. RNA interference therapy

RNA interference (RNAi) is an effective antiviral approach which targets the viral transcripts. The use of ARC-520 (ARC), an RNAi drug, targets cccDNA-derived mRNA in CHB patients and has previously reported safety and antiviral activity in CHB patients. Prolonged

RNAi therapy with ARC-520 injection in treatment naïve, HBeAg positive and negative patients with chronic HBV resulted in significant reductions of HBs antigen [15]. In a recent clinical trial, a total of 8 CHB (5 HBeAg-neg, 3 HBeAg-pos) received up to 12 doses of 4 mg/kg ARC once every 4 weeks with daily ETV simultaneous treatment. The patients received ETV for 34 to 44 weeks after a single dose of ARC before receiving the first ARC dose of the multi-dose extension. All CHB had viral DNA undetectable throughout the 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; HBeAg-positive CHB showed a larger HBsAg multi-log reduction. Results are consistent with previous findings in chimps showing more cccDNA-driven antigen production in naïve HBeAg-pos and a higher fraction of integrated DNA in HBeAg-neg. It was suggested that the delayed onset of HBsAg reduction in HBeAg-neg CHB may be an indirect effect due to the reduction of other viral proteins [15].

3.3. HBV core assembly modulator

HBV core assembly modulator has been designed to disrupt the HBV RNA encapsidation in the HBcAg. The data on safety, tolerability, pharmacokinetics and antiviral activity of AL-3778, a first-in-class, and orally administered HBV core assembly modulator was studied alone and in combination with PegIFN [16]. Safety and efficacy were assessed in HBeAg(+) non-cirrhotic CHB patients with HBV DNA > 20 000 IU/mL and elevated ALT. All study groups were treated for 28 days and followed off-treatment for 28 days. Patients were randomized to receive AL-3778 or matching placebo at doses of 100, 200, 400, 600 and 1000 mg and also to receive separate treatment arms 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 dosing. Changes in HBsAg levels were negligible, as expected from the short treatment duration. 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 AL 3778 alone (1.72 log10) or PegIFN alone (1.06 log10). After 28 days' treatment, mean HBV RNA (log10 copies/ml) changes 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 [16].

In summary, AL-3778 was well tolerated with mainly Grade 1 and 2, transient AEs. There was a 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. Reduction of serum HBV RNA is consistent with the novel mechanism of action of AL3778, to disrupt efficient HBV RNA encapsidation [16].

3.4. HBsAg secretion inhibitors

The Nucleic Acid Polymers (NAPs) have been designed to reduce serum HBsAg concentration, aiming to improve the efficacy of immunotherapy through a functional control of chronic HBV infection. It has been recently presented the preliminary results of an ongoing trial assessing the effect of NAPs combined with TDF & PegIFN therapies in CHB-HBeAg(-) patients [21].

The data obtained up to 2017 confirmed the tolerability and efficacy of NAPs when used in combination with PegIFN and TDF in patients with HBeAg negative chronic HBV infection. The significant ALT flares observed in those with the higher HBsAg suppressions appear to be therapeutic in nature and suggest that NAP-mediated HBsAg clearance substantially improves the efficacy of PegIFN in this patient population. It is still pending to understand the sustained off therapy effect of this novel treatment, however the results are encouraging [21].

NAP monotherapy achieved 2-7 log reductions of serum HBsAg accompanied by 3-9 log reductions in serum HBV DNA and the appearance of anti-HBs. Direct PCR and deep sequencing analysis to study the "a" determinant region during REP 2139 therapy was performed to explore the potential role of mutations in the HBsAg response observed during NAP therapy [22]. Deep and direct sequencing revealed that no mutations were present in the "a" determinant region during NAP therapy any of the 12 studied patients. In the 9 responder patients, 18 different mutations were observed, all outside the "a "determinant, confirming that HBsAg reductions observed are not due to the evolution of HBsAg variants undetectable by standard HBsAg assays. These studies further validate the hypothesis of the functional control of HBV infection by NAP treatment [22].

The intracellular delivery of NAPs by electroporation resulted in post-entry antiviral effects against HBV infection in vitro. The authors consider that this antiviral effect of NAPs involves a post-transcriptional mechanism that interferes with the release of HBsAg into the supernatant. These results are in agreement with the published antiviral effects of NAPs in the DHBV model and confirm that NAPs act in human HBV infection by blocking the release of HBsAg from infected hepatocytes [23].

NAP monotherapy led to a mono- or bi-phasic HBV viral load decline and complex HBsAg inhibition patterns in 9 of 12 patients, with anti-HBs seroconversion in 6 of those 9. Kinetic analysis of the 1st HBsAg decline phase indicates a mean HBsAg half-life of 5.3 ± 3.2 days, which is strikingly shorter than estimated under approved medications, e.g., lamivudine (half-life = 38 d), suggesting REP2139 inhibits HBsAg release from infected hepatocytes [24].

3.5. Therapeutic vaccination as monotherapy

After two decades of research and development in the field of therapeutic vaccination, Cuban National Regulatory Agency (CECMED) approved the Sanitary Registration of HeberNasvac® to the Center for Genetic Engineering and Biotechnology.HeberNasvac®, a therapeutic vaccine to treat CHB patients is administered by nasal and subcutaneous routes and encompasses the HBsAg and HBcAg purified as recombinant VLPs. This product was presented during APASL 2017 meeting in Shanghai, where the authors compiled the data of non-clinical and clinical pharmacology of HeberNasvac® [21]. Other regulatory agencies are analyzing the possibility of granting Sanitary Registration to this novel product.

3.5.1. HeberNasvac®: Non-clinical pharmacology in summary

A group of pharmacological studies in animal models were developed in Cuba, and also in collaboration with Pasteur Institute, Paris, France, and Ehime University in Matsuyama, Japan. The Clinical trials were conducted in Cuba and also in Bangladesh. The preclinical immunogenicity studies, developed in normal Balb/c mice as well as in transfected and transgenic mice, supported the selection of the optimal formulation, the antigen doses and proportions, as well as the routes of administration [22,23].

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. HeberNasvac generated CD4(+) and CD8(+) T-cell responses and induced pro-inflammatory cytokines involved in viral control and disease resolution [23,24]. The immunogenicity studies in the AAV model of CHB infection demonstrated the effect of nasal immunization in the homing of virus specific effector CD4 T cells to the liver in contrast to SC immunization.

3.5.2. 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 [25,26]. The most important study of HeberNasvac® as monotherapy was the treatment controlled, and randomized phase III clinical trial conducted with the objective of evaluating the efficacy and safety of this product in CHB patients in comparison with PegIFN treatment [27].

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 admin-

istrations 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 [27].

Regarding safety, no serious or severe adverse events (AE) were detected after immunization by nasal and/or subcutaneous routes. The more frequent AE were similar in nature for both products. The number of different AE, their frequency, intensity and duration were much more reduced in the group treated with HeberNasvac® compared to PegIFN. Considering efficacy, both the intention to treat and per protocol analysis showed 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 compared to the proportion of patients in the same conditions 24 weeks after the end of PegIFN treatment. After HeberNasvac® immunization, patients developed a homogeneous, generalized and two to five times increase of ALT resembling immune activation, followed by a viral load reduction. Such not clinically symptomatic flares lead to a generalized normalization of ALT values at the end of HeberNasvac® treatment [27]. Serological evaluations evidenced a higher proportion of HBeAg loss and seroconversion for HeberNasvac®-treated HBeAg positive patients at the end of follow-up.

3.6. Therapeutic Vaccination as a part of Combined Therapies

3.6.1. Therapeutic vaccination in combination with RNA interference and antivirals

Michler and coworkers presented a promising approach to control HBV replication and lower antigen load using RNAi. Stabilized and liver-targeted siRNAs were evaluated in their capacity to suppress HBV gene expression and allow recovery of HBV-specific B- and T cell responses,-both spontaneously and after therapeutic vaccination. The optimal time point of vaccination was determined by comparing different durations of antigen suppression [28].

Highly viremic HBV transgenic mice were treated with: 1/ nucleoside analogue ETV to decrease HBV DNA, 2/ an shRNA-expressing Adeno-Associated Virus vector (AAV-shHBV) or N-Acetylgalactosamine (GalNAc)-conjugated siRNAs to target cccDNA and decrease HBsAg and 3/ therapeutic vaccination with HBcAg / HBsAg protein prime vaccination and a Modified Vaccinia Ankara virus (MVA)-boost immunization to stimulate adaptive immunity.

ETV strongly reduced HBV DNA by 4 $\log_{10}$ but antigen levels remained unchanged. Monthly subcutaneous injections of GalNAc-siRNAs as well as AAV-shHBV efficiently suppressed HBsAg and HBV DNA in serum by 2 $\log_{10}$ and HBeAg by 1 $\log_{10}$. 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. The siRNA treatment followed by therapeutic vaccination

showed an additive effect cumulating in >4 log_{10} reductions of HBsAg and HBV DNA in serum compared to pretreatment levels [28].

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. This kind of combinatorial approach using RNAi and vaccination therapy for hepatitis B allows reconstitution of HBV-specific T cell responses and suppression of HBV to undetectable levels in a preclinical mouse model of CHB [28]. The approach presented by Michler and coworkers deserve clinical translation.

3.6.2. Therapeutic vaccination in combination with anti-PD-1 treatment and antivirals

A phase1 study evaluating anti-PD-1 treatment with or without GS-4774 in HBeAg negative CHB patients was concluded in 2017. The combination of both immunotherapeutic strategies was designed to increase HBV-specific T-cell frequency and activity aimed at inducing a durable control of HBV. Nivolumab was used as the inhibitor of the immune checkpoint receptor PD-1 [29].

This phase 1 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 single dose of Nivolumab. The primary endpoint was change in HBsAg 12 weeks after Nivolumab dosing. Patients were also assessed for safety and immunologic changes, including receptor occupancy, flow cytometry, and in vitro responses by ELISpot. As a result of the study, no grade 3 or 4 adverse events or serious AEs were detected.

Significant decline in HBsAg levels compared to baseline was found in the group treated with nivolumab alone. No difference was observed due to the use of the vaccine in terms of HBsAg decline. One patient evidenced DNA clearance and HBsAg seroconversion in the group treated with the inhibitor alone. In summary, single dose 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. It is important to highlight that in the present setting the patients have been pre-treated with NUCs [29].

3.6.3. Therapeutic vaccines (GS-4774) in combination with NUCs

A yeast-based T-cell vaccine containing HBV core, surface and X proteins GS-4774 has shown to be immunogenic in mouse models and healthy volunteers. The modulatory effect of GS-4774 on HBV-specific T cell responses in treatment-naive, HBeAg-negative CHB patients

was recently studied [30]. 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.

The HBV-specific T cell responses were studied before, during and after vaccine therapy both ex vivo (IFN-γElispot) and after 10 days *in vitro* expansion (intracellular cytokine staining for IFN-γ, TNF-α, IL-2 and CD107 degranulation) in the presence of peptides covering the overall HBV proteome or control HBV-unrelated peptides. Immunological data were assessed in relation to HBsAg/HBV-DNA/ALT decline.

While all patients normalized ALT and have HBV-DNA suppressed, 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 week 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 difference in HBsAg reduction between the group treated with NUC vs. the group treated with the combination of NUC and GS-4774 [30].

3.6.4. HeberNasvac in combination with NUCs

A group of hepatologists and scientists from Europe and Asia, sponsored by the French company ABIVAX assessed HeberNasvac in virally suppressed patients [31]. A Phase IIb trial was conducted in Asian countries. The therapeutic vaccination using HeberNasvac® was developed as a monotherapy for patients that were not using antiviral treatment and, in addition, it has been tested also in a limited number of patients with previous interferon treatment and unsatisfactory response. HeberNasvac® has shown superior efficacy compared to PegIFN in first line therapy of CHB. The study presented at ILC2017 was the first evaluation of this product under conditions of strict virological suppression for at least one year and a mean of antiviral treatment of more than 4 years.

During this trial, HeberNasvac® was administered intranasally during a priming cycle of five administrations of 100 μg of each antigen per dose, followed by a cycle of five subcutaneous/intranasal immunizations using the same dose per administration route (200 μg of each antigen HBsAg and HBcAg in total *per* immunization day cycle. Antiviral treatment continued up to one month after the end of vaccinations. The presented study assessed ABX203 vacci-

nation of HBeAg(-) CHB patients under antiviral treatment for several years, evaluating the capacity of this treatment to prevent relapse after stopping antiviral therapy with NUCs.

A total of 276 HBeAg(-) non-cirrhotic patients who had been treated for at least 2 years with NUCs and who were HBV-DNA negative with normal ALT levels were randomized to continue the treatment with NUCs during 24 weeks in combination with ABX203 administering 5 intranasal administrations every 2nd week followed by a second cycle of 5 intranasal/subcutaneous booster administrations one month later (n = 184) vs. treatment with NUCs only (n = 92). After 24 weeks, antiviral therapy was stopped in all patients. The patients were followed for 24 weeks –or in case they reach 10'000 copies/mL reinserted in antiviral treatment. The primary end-point of the study was the percentage of subjects who maintained HBV-DNA levels <40 IU/ml 24 weeks after stopping NUCs [31].

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 NA only (p = 0.20). Similarly, authors report 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 <2xULN (31% vs. 41%) and HBsAg declines. 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 (figures) and without impacting outcomes [31]. This prospective randomized HBV therapeutic vaccine study and also the largest prospective study stopping NUCs showed that ABX203 did not prevent viral relapse after stopping NUCs. Also, it revealed unexpected relapse timing difference between TDF and ETV.

Future studies will be planned to investigate if alternative vaccine regimens (e.g. vaccination after stopping NUCs) may induce off-therapy viral control. As a result of this trial it is now better understood the dynamic of antiviral rebound, -consequently the dynamic of immune reactivation post treatment can be expected to be more delayed in patients receiving ETV. In addition, the study also evidenced the safety of this novel therapeutic vaccine[31].

3.6.5. Therapeutic vaccination of patients undergoing treatment with NUCs, a critical revision

Alternative treatments for CHB are subject of intense research worldwide. One of the most studied alternatives has been the therapeutic vaccination. As summarized in the present report, important clinical trials combining therapeutic vaccination and antiviral treatments have failed in their attempt to reach the study endpoints [30,31].

The rationale favoring of vaccination under viral suppression is based in 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 displaying flare-ups of hepatitis associated with HBeAg seroconversion during chronic infection. Also, the reduction in HBV load by antiviral chemotherapy may, therefore, increase the responsiveness of HBV-specific T cells, which are hyporesponsive in cases of persistent HBV or viral antigen stimulation [Reviewed in 32].

Against the combination of therapeutic vaccines and antivirals there are also few aspects that need consideration: HBV-specific T cells are detectable during the first few months of lamivudine treatment [33] and this restoration of T-cell activity is partial and transient and does not lead to an increase in HBeAg seroconversion [34]. In the case of ABX203, the product was evaluated in patients under strict antiviral control for several years [31]. Other important trials have evaluated different vaccine candidates in similar conditions without satisfactory results in terms of virological control after treatment discontinuation [35-37].

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 response need to migrate to the liver to exert their function, however, the liver is under non-inflammatory conditions evidenced by the sustained reduction in ALT levels in most patients under antiviral treatment by the week 12 of treatment [37-39], paralleling the reduction of HBV DNA levels. Important publications support that hepatocytes express HLA class II in non-physiological conditions [40-42]. Inflammatory mediators or the HBV infection itself have been proposed as eliciting agents [42]. The elimination of the virus and the normalization of ALT during long term antiviral therapy further reduce the inflammatory mediators, consequently the expression of HLA class II and the CD4 T helper activity. On the other hand, the reduction of the replication has been linked to a lower intracellular expression of viral antigens, mainly cytoplasmic HBcAg. It has been demonstrated that the control of the replication can be predicted by the low intracellular expression of HBcAg [43]. Taken together, in the virally suppressed patients it is expected a reduced intracellular expression of viral antigens, absence of HLA class II expression and reduction in the presentation of viral peptides to vaccine-induced T cells by both HLA class I and II.

3.6.6. New opportunities in the field of therapeutic vaccination

New opportunities appeared in 2017, specifically the updated guidelines of the EASL introduced novel recommendations in relation with treatment cessation in HBeAg negative patients under antiviral treatment that may open a window of future research in the field of therapeutic vaccination after treatment cessation. Specifically, it is now accepted by EASL guidelines that antiviral treatment can be discontinued in non-cirrhotic HBeAg-negative pa-

tients after consolidation of antiviral achievements and also under strict evaluation. This novel scenario provided by the 2017 version of the EASL CHB management guidelines favor the evaluation of therapeutic vaccines in a completely new and promising immunological environment [3]. The recommendations of treatment discontinuation in HBeAg negative patients were also based in the detected increase of the anti HBV immune response after NUC cessation as a consequence of the viral rebound. Such ALT increases in patients with controlled level of fibrosis and under strict assessment are considered benign in nature, with an important relation with HBsAg elimination in around 20 to 40% of HBsAg elimination at a long time follow-up. Patients continuing treatment with NUCs evidenced no reduction in their serum HBsAg levels [44-47].

A second opportunity appears for therapeutic vaccination after antiviral treatment cessation: the natural reactivation of the immune response represents a solid and effective factor that may further potentiate the vaccine induced immune response. The EASL 2017 guidelines also recommend to delay the reintroduction of patients back to NUC treatment until completing the analysis of more than one time point, ideally this period should be from 6 to 12 months. This recommendation creates a gap of time for the coexistence of the immune response generated by the therapeutic vaccine with the HBV produced in hepatocytes and presented in the newly elicited HLA molecules. The objective of future clinical trials in this future scenario post cessation should be to significantly increase this naturally induced 30% HBsAg loss and generate a robust anti HBsAg seroconversion on time.

4. Conclusion

According to the World Hepatitis Report 2017, CHB is responsible for most cases of HCC and LC and in consequence, is the main source of mortality among viral hepatitis [1]. 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; however this effect was not confirmed for patients treated with ETV [9]. In addition, 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 [11]. These recent findings evidenced that the most used treatment, the antivirals, have very important limitations in their post marketing studies. 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 target of controlling the increasing mortality of viral hepatitis found in the last decade 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, new challenges are still in the route of therapeutic vaccination as this is the case of their use in patients under viral suppression. This is not a minor issue considering that part of the World population that has been detected as HBV positive and require treatment is using one of the registered antiviral drugs or their approved generics. However, the current recommendations of the major societies for the study of the liver have clear recommendations nowadays regarding antiviral treatment cessation. This year, the 2017 edition of the ILC held in Amsterdam proposed recommendations for stopping antiviral treatment for European HBeAg negative patients under antiviral treatment under strict follow-up. This recommendation opened a new horizon for therapeutic vaccination after antiviral treatment, however, this scenario requires clinical optimization before implementation in order to further reinforce the naturally induced immunity after antiviral treatment cessation.

The scenario in CHB patients is more complex due to the HBV DNA integrative capacity and also the multiple mechanisms of tolerance induction that prevents the recovery of the required multifunctional, potent and multiantigenic Th1-like response for controlling viral infection. The clearance of cccDNA is now the main objective of many novel therapies and combined treatments. Although many therapies are slowly reaching this goal, it is still far from being considered as a solved problem. In addition, it is still a matter of discussion how these strategies will be implemented considering the increasing regulatory environments in terms of safety and the costs in clinical investigation needed to push forward these strategies, considering that part of the patients are unaware of their conditions, another section of the patient's pool will not evolve to serious conditions and another part will not have the money to cover their treatment. All these variables together complicates the scenario for the accomplishment of WHO goals regarding the control of viral hepatitis by 2030, considering that CHB contributes to near 70% of the mortality and this variable is increasing.

In order to control CHB, it will be necessary to implement a large number of preventive, diagnostic and therapeutic actions. The Sanitary Registration granted to HeberNasvac®, the first therapeutic vaccine approved for a chronic infectious disease, represents a finite, safe and effective alternative for the treatment of CHB patients and it was registered by the first time in its country of origin (Cuba) where it is being introduced in a large number of HBsAg-positive patients. The registration was granted based in the significant superiority of HeberNasvac®

monotherapy in terms of safety and efficacy variables compared to PegIFN treatment.

The introduction of HeberNasvac® in CHB patients should be carefully followed, supported and assessed by WHO. This product could represent a valuable tool to accomplish WHO objective of eliminating viral hepatitis as a health problem by 2030, as proposed in the Hepatitis Global Report 2017 [1]. Poor countries and developing nations from BRICS cannot escape from the misuse of current antiviral treatments, producing the most severe form of ACLF and responsible of the 10 and 20% of such liver failures in CHB and cirrhotic patients, respectively. In the countries where quasi-eternal therapies cannot be provided to patients in order to ensure their regular medication with NUCs, the approval of a novel, finite and effective treatment constitutes promising news.

5. Tables

Table 1: General scenery of the efficacy data from antivirals and PegIFN considering more than 20 publications and book chapters. 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.

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

*Depending on baseline levels & population under treatment. ¥ Depending on viral genotype

Table 2: Current therapies: adverse events in summary considering product inserts and publications.

Product	Adverse events in summary (considering product inserts) and reports
Interferon-based therapies	Severe psychiatric adverse reactions including: depression, 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 with alfa interferons.
	Neutropenia, decreases in white blood cell (WBC) count and absolute neutrophil count.
	Pulmonary symptoms, including dyspnoea, pulmonary infiltrates, pneumonia, and pneumonitis, including fatality.
	Risk of exacerbation of autoimmune disease.
	Hyperglycaemia, hypoglycaemia and diabetes mellitus have been observed in patients treated with alfa interferons.
	Serious, acute hypersensitivity reactions (e.g. urticara, angioedema, broncho-constriction, anaphylaxis) rarely detected.
	Cardiovascular events such as hypertension, supraventricular arrhythmias, congestive heart failure, chest pain and myocardial infarction have been associated with interferon therapy.
	Retinopathy including retinal haemorrhages, cotton wool spots, papilloedema, optic neuropathy and retinal artery or vein obstruction, which may result in loss of vision, have been reported.
	Some patients develop dizziness, confusion, somnolence, or fatigue should be cautioned to avoid driving or operating machinery.
Antivirals	Severe acute exacerbation of hepatitis B after uncontrolled cessation of treatment.
	New onset or worsening renal impairment: Can include acute renal failure and Fanconi syndrome. Creatinine clearance should be assessed before initiating treatment with VIREAD.
	Lactic acidosis/severe hepatomegaly with steatosis: treatment should stop in patients who develop symptoms or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity
	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 or bone loss.

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6. Package Insert PEGASYS® (peginterferon alfa-2a) Hoffmann-La Roche Inc.

7. Package Insert Baraclude, (Entecavir): tablets for oral use, oral solution.

8. VIREAD® (tenofovirdisoproxil fumarate) tablets, for oral use, powder for oral use.

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