Current treatments for chronic hepatitis B virus infections
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Over 240 million people worldwide are chronically infected with hepatitis B virus (HBV) and although a prophylactic vaccine and effective antiviral therapies are available, no cure exists. Curative regimens are urgently needed because up to one million deaths per year are caused by HBV-related liver cancer and end-stage liver disease. HBV is an hepatotrophic virus which belongs to the Hepadnaviridae family and replicates its DNA genome via a reverse transcriptase mechanism. Effective therapies have been developed for chronic hepatitis B (CHB) infection in the last two decades; their results are discussed in this review as well as future perspectives.

Antiviral drugs and their mode of action
Interferon-alpha (IFNα) and its pegylated form (Peg-IFNα), and 5 other drugs that belong to the class of nucleos(t)ide analogs (NUCs), have been approved for this indication in most parts of the world [2–7]. IFNα is an innate immunity cytokine that induces the expression of genes (collectively named as interferon stimulated genes; ISGs) encoding intracellular or secreted proteins with direct or indirect antiviral properties, and promotes the differentiation/activation of immune cells [8,9]. In the HBV setting, the IFNα antiviral activity result from a complex mode of action including the activation of natural killer (NK)/NKT cells, inhibition of viral genome transcription via epigenetic regulation of HBV covalently closed circular DNA (cccDNA), destabilization of viral nucleocapsid, but also, as recently suggested, degradation of cccDNA via the activation of APOBEC3A in infected cells [10–13]. Overall, its antiviral effect in CHB patients remains modest and the reason for this remains largely unknown but could include an insufficient delivery to the infected liver, a refractoriness of infected hepatocytes to IFNα signaling, or other mechanisms.

NUCs directly inhibit the reverse transcriptase activity of the HBV polymerase. The approved NUCs include lamivudine (LMV), a deoxy cytidine analog with an unnatural l-conformation, and the related l-nucleoside, telbivudine (LdT; β-L-thymidine). A second group, the acyclic phosphonates, which include adefovir dipivoxil (ADV), a prodrug for the acyclic 2′-deoxy adenosine monophosphate analog adefovir, and the structurally similar tenofovir disoproxil fumarate (TDF). A third group contains a d-cyclopentane sugar moiety and has the most potent anti-HBV drug discovered to date, the deoxy guanosine analog entecavir (ETV). This structural classification of NUCs is useful clinically because it helps predict pathways of drug resistance [14,15]. In chronically HBV-infected hepatocytes, NUCs inhibit the viral polymerase activity resulting in a decreased production of virions, a reduced recycling of viral nucleocapsids to the nucleus of infected cells, and theoretically a decline of viral cccDNA, although the latter can only be observed after many years of treatment [2,14,15]. NUCs do not inhibit the de novo formation of cccDNA in newly infected cells, implying that persistent residual viremia during antiviral
therapy can lead to infection of new hepatocytes and re-establishment of viral cccDNA reservoir REF. Furthermore, the lack of complete inhibition of viral DNA synthesis may also lead to the maintenance of the cccDNA pool via the recycling of viral nucleocapsids to the nucleus of infected cells REF. A decrease of the total amount of intrahepatic cccDNA can be observed during long-term therapy as a consequence of, firstly, the inhibition of the intracellular recycling pathway, secondly, decreased rate of infection of new cells, thirdly, dilution of cccDNA via hepatocyte turn-over, as cccDNA may be lost through cell division [16–20].

A simplified view of the mode of actions of the approved antiviral agents in the HBV life cycle is shown in Figure 1.

**Goals of therapy and treatment end-points**

The goal of therapy for CHB is to improve the quality of life and survival by preventing or delaying progression of the disease toward cirrhosis, decompensated cirrhosis, and HCC. This goal can be achieved if HBV replication is suppressed in a sustained manner. It is accompanied by a reduction in histological inflammatory activity of CHB and decreased risk of developing cirrhosis and HCC, particularly in non-cirrhotic patients [3,6,7,21]. Several recent studies in large cohorts have shown that the risk of HCC development is significantly decreased by successful antiviral therapy compared to untreated historical patient cohorts, but is not abated [22–25]. Chronic HBV infection cannot be completely eradicated due to the persistence of cccDNA in the nucleus of infected
hepatocytes [19,26–28], which explains HBV reactivation, for instance in patients who receive immunosuppressive therapy or chemotherapy [29,30]. Thus therapy has at least to ensure a degree of viral suppression that will lead to biochemical remission, histological improvement and prevention of complications. The ideal end-point is HBsAg loss (i.e. HBsAg seroclearance) associated with anti-HBs antibody (i.e. HBsAb) seroconversion, as it would allow treatment cessation. However; it is achieved only in a minority of treated patients with the available anti-HBV agents [2,3,5–7].

Treatment indications

The natural history of chronic HBV infection is usually classified in four phases. These phases are not obligatory consecutive over time and include: firstly, immune tolerance phase or low inflammatory phase associated with high viral load and normal transaminase levels, secondly, the immune active phase associated with high viral load, elevated liver enzymes, and immunohistochemical signs of necro-inflammation in the liver; patients can be either HBeAg positive or negative, thirdly, the inactive carrier state with very low viral load and normal liver enzyme levels, fourthly, the HBsAg clearance phase where HBsAg becomes negative, possibly with anti-HBsAb seroconversion, undetectable HBV DNA in serum and normal liver function tests. The indications for treatment are generally the same for both HBeAg-positive and HBeAg-negative CHB. This is based mainly on the combination of three criteria: firstly, serum HBV DNA levels, secondly, serum ALT levels, thirdly, and the severity of liver disease.

The international clinical practice guidelines from AASLD, EASL and APASL recommend that patients with immune — active CHB should be considered for treatment when they have HBV DNA levels above 2000 IU/mL for HBeAg-negative patients or above 20 000 IU/mL for HBeAg-positive patients, serum ALT levels above the upper limit of normal (ULN), and moderate to severe active necro-inflammation and/or at least moderate liver fibrosis severity [2,3,5–7,21].

Indications for treatment may also take into account age, health status, family history of cirrhosis or HCC, extrahepatic manifestations of the disease, as well as concurrent medications. For instance, inactive carriers receiving chemotherapy or other immune suppressant treatments need to receive pre-emptive antiviral therapy to prevent viral reactivation; several studies have demonstrated the efficacy of NUCs in these situations [31,32]. HIV co-infected patients should be treated with HAART including a high barrier to resistance NUC active on both HIV and HBV, that is, TDF. Currently, ‘Immune-tolerant’ patients are not considered for antiviral therapy by most guidelines, despite their persisting high viral load. Pregnant women with high viral >200 000 UI/mL are now considered for antiviral therapy with TDF to decrease viral load at the time of delivery and increase the success rate of prevention of mother to child transmission in association with hepatitis B immune globulins (HBIG) and vaccine administration in the newborns; indeed, in such situation of highly viremic mothers, the classic immune prophylaxis based on HBIG and vaccine in newborns fails in approximately 10–20% of cases while it fails in less than 5% when mothers receive antiviral treatment [33–35]. Treatment of children presenting criteria of immune-active disease is now recommended by international liver societies [36,37].

Results of antiviral therapy

The results of antiviral therapy have been summarized recently in several international clinical practice guidelines and reviews [6,21,38].

The use of peg-IFN in HBeAg positive patients with CHB allows to obtain viral suppression in 10–40% of patients, with an HBeAg seroconversion rate of approximately 30–35% which is accompanied by normalization of ALT levels in 35–50% patients. HBsAg loss can be observed in approximately 5% of patients 6 months after treatment cessation and 10% at 3 years post-treatment.

In HBeAg positive patients, the use of NUCs with a high barrier to resistance, that is, entecavir or TDF, allows to achieve viral suppression in substantially higher number of patients (approximately 60–75% of patients after 3 years of therapy). HBeAg seroconversion rate remains low (approximately 20%) despite better viral suppression. ALT levels normalize in 75–80% of patients. HBsAg loss is observed in up to 12% after 7 years of continuous of TDF therapy [39].

In HBeAg negative patients, peg-IFN administration achieves HBV DNA suppression in approx. 20–40% of patients, normalization of ALT levels in 60% patients and HBsAg loss is observed in 6% of patients 3 years after treatment cessation. The use of NUCs with high barrier to resistance achieves viral suppression in 90% of patients, with a similar number of patients normalizing ALT levels. HBsAg loss is rarely observed (<1%) with both Entecavir and TDF despite long term administration [39].

Improvement of liver histology has been observed in patients treated with peg-IFN as well as in patients on continuous NUC therapy with Entecavir or TDF [40–43]. Control of viral replication has been associated with decreased rate of progression of liver disease toward cirrhosis, decompensation of cirrhosis, or HCC [44–48].

The therapeutic efficacy of these treatments can be affected by factors such as the development of adverse effects, poor patient compliance, previous treatment with suboptimal regimens, infection with drug resistant viral
strains, inadequate drug exposure because of pharmacologic properties of particular drug(s) and individual genetic variation [2,3,5–7,14,49].

The results are summarized in Table 1.

Safety is an issue mainly in patients receiving IFN based therapy. The most common side effects are flu-like symptoms after IFN injections, neutropenia and/or thrombopenia, auto-immune syndromes, infections especially in cirrhotics, and depression, a common side effect with IFN. With NUCs, safety is significantly less often an issue. Nevertheless, TDF administration can be associated with kidney function disorders and kidney tubulopathy. Both Entecavir and TDF dose should be adapted to kidney function. Entecavir cannot be administered during pregnancy.

Management of antiviral drug resistance
Good adherence to anti-HBV therapies is important for maintaining maximal suppression of HBV replication and decreasing the likelihood of resistance emergence [14,15]. Investigation of adherence to NUC therapy in patients with CHB has shown that nearly 40% may not be fully adherent; this significantly impacts on the rates of viral suppression [50]. The rapidity of selection of drug resistant mutants depends on the barrier to resistance of the administered NUC, treatment history as sequential therapy with low barrier to resistance drugs favors the development of resistance, the replication fitness of the variants and selective advantage in the viral quasi-species, and the replication space for virus spreading. Cross-resistance is defined as resistance to drugs to which a virus has never been exposed as a result of changes that have been selected for by the use of another drug [14,15]. The initial drug choice and subsequent rescue therapies should be based on the knowledge of cross-resistance, so that the second agent has a different resistance profile to the initial failing agent. This is particularly important since drug resistant mutants that have been selected by previous treatments are thought to be archived in viral cccDNA reservoirs in the liver [14,15]. The add-on strategy with NUCs having complementary cross-resistance profiles is mandatory when using drugs with a low barrier to resistance. However, although there is a strong virologic rationale for an add-on strategy with a complementary drug to prevent the emergence of multi-drug resistant strains and raise the barrier to resistance, the current trend is to recommend a switch to a complementary drug having a high barrier to resistance such as TDF [51,52]. The basic understanding of antiviral drug resistance, and treatment management have been recently reviewed in detail [15,53].

Can we do more than viral suppression today?
Combination of currently approved drugs
As peg-IFN and antivirals have different mechanisms of action, it has been hypothesized that combining the 2 drug classes could improve rates of HBsAg loss [11]. Several trials have evaluated combination treatment with peg-IFN and oral antivirals for patients with CHB, but the results are inconclusive. Despite the observation that combination of peg-IFN with Lamivudine or Telbivudine showed a higher on-treatment virological response, it did not show a higher rate of sustained off-treatment virological or serological response [5,54–56]. None of these trials were designed to evaluate serum HBsAg loss as a primary end point. A recent study compared the efficacy and safety of TDF and peg-IFN combination therapy for 48 weeks, with TDF for 120 weeks and pegIFN alone for 48 weeks in patients with CHB. A combination regimen involving a short duration (16 weeks) of peg-IFN was also evaluated to explore whether a peginterferon-sparing regimen could affect rates of HBsAg loss. At week seventy-two, 9% of subjects receiving the combination for 48 weeks had HBsAg loss compared with 3% of subjects in patients who received a shorter duration of combined peg-IFN, none of the subjects in the TDF monotherapy arm, and 3% of subjects in the peg-IFN monotherapy group [57]. Overall, less than 10% of good candidates lost HBsAg. Based on these findings and other studies of peg-IFN alone in HBV, patients with genotype A have the best chance of responding to IFN based therapies. Furthermore, those with very active liver inflammation evidenced by an ALT of >10 times the upper limit of normal (ULN) were found in this study (and other studies) to have a higher chance of responding. Currently, this type of combination is not recommended by clinical practice guidelines. Another recent study looked at the effect of adding peg-IFN to Entecavir and showed that Peg-IFN add-on led to significantly more decline in serum HBsAg, HBcAg, and

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<td><strong>Efficacy of approved and preferred antiviral therapies in treatment naive CHB patients (adapted from AASLD and EASL clinical guidelines)</strong></td>
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HBV DNA; however, the rate of HBsAg loss was not reported [58].

There are only very few studies of de novo combination of NUCs with high barrier to resistance in treatment naïve patients. One randomized trial compared the efficacy and safety of ETV monotherapy with those of a combination of ETV and TDF [59]. At week 96, comparable proportions of patients in each study arm achieved the primary end point of a level of HBV DNA <50 IU/mL. Among HBeAg-positive patients, a greater proportion given combination therapy achieved levels of HBV DNA <50 IU/mL than those given ETV alone (80.4% vs 69.8%). However, this difference was observed only in patients with baseline levels of HBV DNA ≥10(8) IU/mL (79% vs 62%) and not in those with baseline levels of HBV DNA <10(8) IU/mL (83% in both arms). Rates of HBeAg loss and HBeAg seroconversion were comparable between groups. The combination therapy could provide an incremental benefit to HBeAg-positive patients with baseline levels of HBV DNA ≥10(8) IU/mL [59]. In the same trial, mean HBsAg changes from baseline at Weeks 12, 48, and 96 were more pronounced in HBeAg-positive than in HBeAg-negative patients, in patients with genotype A than in those with genotypes C or D, and in patients with elevated baseline ALT, but were similar between treatment groups and between patients of different age categories. Overall, during the study observation time, the kinetics of HBsAg decline were slow even in the group of patients who received the combination of TDF and ETV [60]. Another recent trial evaluated the effects of single therapy with TDF and combination therapy with TDF and Emtricitabine in immune-tolerant patients [61]. At week 192, 55% of patients in the TDF + placebo group and 76% of patients in the TDF + Emtricitabine group had levels of HBV DNA <69 IU/mL. No patients were found to have viral resistance to therapy. The rate of HBeAg seroconversion was low and no patient had loss of hepatitis B surface antigen. In multivariate analysis, female sex and TDF + Emtricitabine treatment were associated with a favorable response. It was therefore concluded that in this patient population with high viral load the combination of TDF and Emtricitabine provided better viral suppression than TDF alone [61]. Although the results of these two trials showed that combination of NUCs could induce a more profound viral suppression, this strategy is not yet recommended by international guidelines.

**Stopping NUC therapy**

Several reasons prompted clinicians to stop prolonged NUC administration after achieving long-term viral suppression. The first was to determine whether long-term viral suppression (associated with HBeAg seroconversion in previously HBeAg positive patients or not in the HBeAg negative CHB patients), could lead to HBV specific immune restoration that would allow treatment cessation and leave patients in a status of inactive carrier. Many studies have addressed this question but were mainly disappointing as most patients presented a relapse of viral replication often associated with an increase in ALT levels and sometimes with severe flares. Therefore, most clinical practice guidelines do not recommend this strategy [21,38]. A recent, sophisticated immunological study performed at the time of NUC cessation suggested that patients who maintained a control of viral replication and did not present flares after treatment cessation had the most vigorous HBV specific T cells (Kennedy et al., AASLD abstract 2015). However, this type of analysis will be challenging to implement in clinical practice as a guide for treatment management.

Another reason to attempt stopping NUC strategies was to investigate whether a relapse of viral replication followed by an ALT exacerbation could trigger HBV specific immunity to clear infected hepatocytes and induce HBsAg clearance. This came from the pioneering study from Hadziyiannis et al, who observed 33 HBeAg-negative patients with CHB, undetectable serum HBV DNA, and normal levels of aminotransferases after 4–5 years treatment with adefovir dipivoxil [62]. During the first few months of the post-discontinuation period, all patients experienced virological and 25 (76%) had biochemical relapse. During the follow-up period, 18 patients (55%) who had discontinued antiviral therapy achieved sustained response with HBV DNA level <2000 IU/L and persistently normal level of ALT. Among these, 13 (39%) cleared HBsAg. Fifteen patients (45%) with virological and/or biochemical relapse were re-treated with oral antiviral agents (11 during the first 18 months and 4 after the third year) [62]. A recent study investigated the biochemical, serologic and virologic effects in patients who received antiviral treatment for at least 8 years with HBV DNA <29 IU/mL and subsequently stopped TDF for 24 weeks (Buti et al., AASLD Abstract 2015). 41 patients completed the 24 weeks follow-up. Among them, 32 had a virological relapse >2000 U/L, 25 had elevated ALT at the end of observation, 15 (27%) presented peak ALT >5 x ULN. Only 11 maintained HBV DNA <2000 IU/mL and normal ALT levels, and 3 out of the 41 (7%) patients lost HBsAg (Buti et al., AASLD abstract 2015). Overall, all these results suggest that NUC interruption should not be performed outside of clinical trials and careful post-treatment monitoring as antiviral rescue may be needed in many patients. It is also clearly contra-indicated in cirrhotic patients.

**Prevention of HCC — should patients with mild liver disease be treated?**

Current international treatment guidelines recommend delaying therapy until patients show clear signs of active liver disease extending over several months, including persistent ALT elevations and, when biopsies are available, evidence of inflammation and/or fibrosis [3,6,7,21].

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Application of the guidelines can block the progression of fibrosis and cirrhosis and may reduce the rate of progression to HCC [22–25]. Several clinical trials, cohort studies, and meta-analysis showed that antiviral therapy of CHB can decrease the major complications of chronic infection, including decompensation of cirrhosis and HCC development [47,48,63,64]. There is still a debate regarding the stage of the disease in which antiviral therapy with NUCs provides the best benefit in terms of HCC prevention and/or delay. These differences in results or interpretation could be due to the duration of observation on treatment and bias in patients selection for these studies with heterogeneity in terms of ethnicity, duration of infection, viral genotypes, and co-factors of HCC (alcohol, aflatoxin, tobacco, metabolic syndrome, etc.) [44,65,66]. It is important to remember that in all studies the HCC incidence in these patients treated with NUCs was significantly decreased but not eliminated [22–25,66]. Another important concern is that HCC risk factors in HBV carriers are not well understood. Current thinking favors the notion that the HCC risk begins, in the vast majority of cases, with the immune reactive phase, but there is no proof that it does not begin much earlier [44,67]. The current information on long-term antiviral treatment efficacy and safety allows to consider earlier treatment intervention in patients with chronic HBV infection. A change in treatment practices is much more feasible now as much better drugs have become available with a better antiviral potency and a higher barrier to resistance. It is interesting to see that the results of the first clinical trial of NUCs in immune tolerant patients has recently been published [68] and showed a significant drop in viremia levels in the majority of patients, although no HBsAg seroconversion occurred and the impact on HCC development could not be determined due to the short duration of follow-up. In theory, it would be best to initiate NUC treatment as early as possible, to suppress viral replication and thereby decrease the integration events and molecular damage in infected hepatocytes [67], as this was demonstrated for HIV infection with the treatment of HIV infected patients with high CD4 count despite a high viral load. When biopsies are available, attempts should be made to establish hepatocyte infection levels and identify low-level inflammatory activity and/or fibrosis. The presence of some degree of inflammatory activity associated with lower levels of HBV DNA in serum (less than 8 log 10 IU/mL, but higher than 4 log 10 IU/mL) would suggest a high level of accumulated hepatocyte damage/change even in the absence of other indicators of histological change, and treatment would seem strongly warranted [67]. This is also supported by the observation that HBV specific T cell functions are conserved in patients in the so-called ‘immune tolerance’ phase [69]. An additional, unappreciated, cause of clonal hepatocyte repopulation occurs in non-cirrhotic liver as well [70]. Immune killing of infected hepatocytes is the strongest known pressure on the infected hepatocyte population in the non-cirrhotic liver and, analogous to cirrhosis, should lead to emergence of HBV resistant hepatocytes that are able to avoid immune killing. Indeed, most analyses of long-term carriers suggest that 50% or more of hepatocytes no longer support HBV infection and/or support much reduced levels of replication [71].

**Perspectives**

Currently the rate of ‘functional cure’ of infection defined by HBsAg clearance and HBsAb seroconversion, despite the lack of complete cccDNA eradication [72], remains very low with NUCs and peg-IFN. There is therefore a need for more effective antiviral treatments to increase the rate of HBsAg seroconversion allowing treatment cessation in more patients. Furthermore, the loss of HBsAg may also enhance the beneficial effect of current treatments on the prevention of HCC.

To define new therapeutic options and head toward treatments with finite duration, it is therefore necessary to develop new molecules acting on novel targets to set true combination therapies [72]. These novel approaches are discussed in the following paper by Leverero et al. exact reference to be added by the publisher; it is in the same issue of the journal!

**References**


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