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REVIEW ARTICLE

Optimal therapy for chronic hepatitis B: hepatitis B virus combination therapy?

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Keywords
PEGIFN – combination therapy – NUC – cccDNA – HBsAg – HBsAg loss

Abbreviations
ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; ETV, entecavir; HBV, hepatitis B virus; NAs, nucleos(t)ide analogues; PEG-IFN, pegylated interferon alpha.

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Abstract

Currently available antiviral treatment for chronic hepatitis B can be divided into two classes of therapeutic agents: pegylated interferon alpha (PEG-IFN) and nucleos(t)ide analogues (NAs). The major advantages of NAs are good tolerance and potent antiviral activity associated with high rates of on-treatment response to therapy. The advantages of PEG-IFN include a finite course of treatment, the absence of drug resistance, and an opportunity to obtain a durable post-treatment response to therapy. The use of these two antiviral agents with different mechanisms of action in combination is theoretically an attractive approach for treatment, either simultaneously, as sequential combination therapy (add-on), or even as an immediate switch from one agent to the other. Different NAs have also been combined in certain clinical situations. At present, several studies have confirmed certain virological advantages to combination therapies, but pivotal prospective studies demonstrating long-term clinical benefit to patients are still missing. Therefore, combination treatment, especially with PEG-IFN plus NAs, is not indicated and was not recommended by the European Association for the Study of the Liver Clinical Practice Guidelines written in 2012, while the guidelines for the use of combination NAs is limited to very few clinical situations.

Key points

- NAs and PEG-IFN may have additive or even synergistic effects.
- Better understanding of the association of quantitative HBsAg with cccDNA, the viral matrix, will help to guide combination therapies.
- The ability to suppress intrahepatic viral spreading was more pronounced with combination PEG-IFN plus NA in studies with CHB patients.
- Robust prospective studies to determine the role of combination therapy are not yet available although a first study is ongoing.
- The optimal mode of combining PEG-IFN with NAs is still unknown (dual therapy, add-on or switch).
- Due to the very limited amount of data there are no recommendations for the use of combination therapy in European Association for the Study of the Liver Clinical Practice Guidelines of 2012.
- The European Association for the Study of the Liver considers combination therapy in CHB as a still unmet need and is supporting further assessment of safety and efficacy

The European Association for the Study of the Liver (EASL) considers combination therapy in CHB as a still unmet need and is supporting further assessment of safety and efficacy. Up to date, there is no clear data to support combination therapy in clinical practice. The purpose of this paper is to give an overview of the recent studies in CHB combination therapy and aims at helping in the understanding of the association of quantitative HBsAg with the viral matrix, cccDNA, which is important to help guiding future combination therapies.

cccDNA: a template for HBV transcription

Prerequisite for HBsAg loss and a clinical cure of hepatitis B virus (HBV) infection is understanding the association between HBsAg and covalently closed circular DNA (cccDNA). cccDNA is the viral minichromosome that serves as the template of HBV transcription in the nucleus of infected hepatocytes, enables maintenance of chronic HBV infection and is mostly responsible for failure of viral clearance and relapse of viral activity after antiviral therapy, thus limiting the possibility of achiev-

ing a clinical cure (2). Although NAs potently suppress viral replication and prevent disease progression in most patients, they must be administered long term (potentially for life, especially in HBeAg negative patients), because they do not directly target and thus affect the viral reservoir cccDNA in hepatocytes. In contrast, patients on PEG-IFN appear to have a higher chance of achieving clearance of HBsAg compared to patients on NAs. HBsAg loss is considered to be the closest outcome to a cure of HBV and is associated with improved survival (3). Nevertheless, even if HBsAg loss occurs more frequently on PEG-IFN than on NA based therapy, this clinical outcome is still rare with rates of 3–7% in large clinical trials (1). Response rates are influenced by a number of factors, including baseline viral load, alanine aminotransferase (ALT) levels, HBV genotype, the patient's age and treatment duration. In addition, clinical use of PEG-IFN is limited by significant side-effects. The fact that no single currently available medication can induce both potent HBV DNA suppression and high rates of HBeAg, and HBsAg clearance has prompted interest in combination therapy with agents having additive or in theory even synergistic effects.

In the past few years, we have learnt that quantifying HBsAg proteins in daily practice may be a useful tool to predict the outcome of PEG-IFN therapy after 12 weeks of administration (4).

These quantitative assays also provide insight into the viral transcriptional and replicative activity of CHB (3). Basically, the total amount of HBsAg proteins secreted into the serum of chronically infected HBV patients can be seen as equivalent to the transcriptional activity of cccDNA. An important point for the clinical understanding of chronic HBV infection is that experimental infection studies have shown that cccDNA can be formed not only from incoming virions but also from newly synthesized nucleocapsids, which, instead of being enveloped and secreted into the blood, are transported into the nucleus to ensure accumulation and maintenance of the cccDNA pool (4, 5). Based on this, multiple rounds of infection are not needed to establish a cccDNA pool in infected hepatocytes. Lower intrahepatic cccDNA loads are generally identified in human liver biopsies obtained from chronically HBV-infected patients (median 0.1–1 cccDNA copy/cell) (6–9) compared to animal systems, suggesting that different viral and host mechanisms control cccDNA dynamics and cccDNA pool size in infected human hepatocytes. Recent research studies investigating novel small antiviral compounds for antiviral therapies in the future has revealed the intriguing possibility of epigenetic regulation of cccDNA contents (12).

Hepatitis B virus NAs inhibit viral polymerase and do not directly affect cccDNA activity. Various *in vitro* and *in vivo* studies suggest that the cccDNA minichromosome is very stable in quiescent hepatocytes (13, 15). Thus, the significant decrease in cccDNA levels (approximately 1 $\log_{10}$ reduction) that generally occurs after 1 year of therapy with polymerase inhibitors is thought to be because of insufficient recycling of viral nucleocapsids to the nucleus as a result of strong inhibition of viral DNA synthesis in the cytoplasm, and fewer incoming viruses from the blood. Nevertheless, cccDNA depletion is expected to require years of NA drug administration (15). Thus, despite the absence of detectable viraemia, the persistent cccDNA minichromosome in the infected liver appears to be the reason that viral clearance does not occur as well as the reason for relapse of viral activity in most chronically infected patients after cessation of antiviral therapy with polymerase inhibitors. Furthermore, if viral suppression is not complete, the selection of resistant variants escaping antiviral therapy may occur (16), at least with the first NAs – molecules such as lamivudine or adefovir. Resistant HBV genomes can be archived in infected hepatocytes when nucleocapsids, produced in the cytoplasm by reverse transcription and containing resistant mutants, are transported back into the nucleus or are permitted to infect new cells and so are added to the cccDNA pool.

During chronic HBV infection, immune-mediated cell injury and compensatory hepatocyte proliferation may favour cccDNA decline, the selection of cccDNA-free cells and the reduction in HBsAg (14). The only available licensed immune modulatory drug for HBV so far, PEG-IFN-α, can induce some inflammation and possibly some cell turnover during therapy. In particular, studies with HBV animal models have shown that antiviral therapy with polymerase inhibitors induced greater cccDNA reductions in animals displaying higher hepatocyte proliferation rates (17). A decrease in cccDNA was also identified in chronically infected woodchuck hepatocytes, when cell turnover was induced *in vitro* by the addition of cellular growth factors and when viral replication was suppressed by adefovir (16, 18). Furthermore, the identification of uninfected cccDNA-negative cell clones containing "traces" of the infection in the form of viral integration indicates that cccDNA clearance can occur without cell destruction (19). Thus, in chronic HBV infection, immune-mediated killing of hepatocytes along with drug induced suppression of viral replication may be instrumental not only in eliminating infected cells but also in inducing hepatocyte proliferation, which in turn, may favour cccDNA and HBsAg loss (13, 20; Fig. 1). On the other hand, studies have shown that very low levels of cccDNA can persist indefinitely, possibly explaining lifelong immune responses to HBV despite clinical resolution of HBV infection (21) and demonstrating that silencing rather than eradication of cccDNA is needed in clinical situations for long-term management of patients. In summary, there is considerable theoretical and experimental evidence suggesting that a combination of NAs with PEG-IFN could result in more extensive suppression of viral replication and antigen production (22), and thus favouring long-term control of CHB (12).

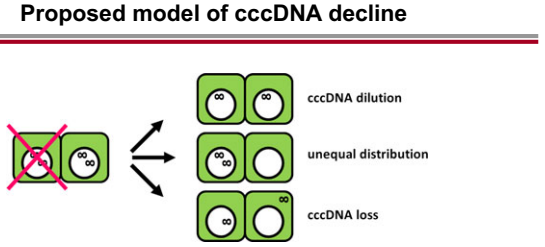

Fig. 1. Cell division in the setting of liver regeneration may induce cccDNA destabilization and formation of cccDNA-free cells, since cccDNA might be diluted, unequally distributed or lost after cell division. The increased cell turnover associated with the necroinflammatory response may be a prerequisite for cccDNA reduction.

Combination of PEG-IFN and NAs in clinical practice

The first pivotal combination therapy studies used lamivudine and PEG-IFN. Although these combination studies failed to demonstrate a long-term clinical benefit after 48 week of treatment and 24 week of follow-up, a more pronounced on-treatment virological response (week 48) was observed with combination therapy than with lamivudine or PEG-IFN alone (23, 24). The increased HBV DNA suppression induced by the combination regimen was associated with a lower incidence of lamivudine resistance. However, with newer, high-potency NAs such as entecavir and tenofovir, concern for HBV associated drug resistance has decreased significantly in the past few years, even when administered alone. The major weakness of the early combination studies was that they focused on a relatively short duration of combination therapy and an immediate stop of both antiviral agents after 1 year of treatment. Thus, whether combination therapy confers an additional benefit compared to monotherapy for treating chronic hepatitis B remained unclear from those studies.

A series of small, uncontrolled studies, with thorough virological analyses showed that the combination of adefovir and PEG-IFN induced a greater reduction in cccDNA in the liver and HBsAg loss in serum of patients with CHB than monotherapy (10, 11, 25). In the first study (8), twenty-six HBsAg-positive CHB patients received combination treatment with adefovir and PEG-IFN for 48 weeks followed immediately by another 96 weeks of adefovir monotherapy (11). Triple liver biopsies before and at the end of combination treatment, as well as at the end of adefovir monotherapy were analysed for intrahepatic HBV DNA. Median intrahepatic cccDNA had decreased by 2.4 $\log_{10}$ after 1 year. Changes in intracellular HBV DNA positively correlated with HBsAg serum reduction and were accompanied by a high number of serological responders. Eight of 15 HBeAg-positive patients lost HBeAg and five developed anti-HBe antibodies during treatment. These eight patients exhibited lower cccDNA levels before and at the end of therapy than patients without HBeAg loss. Four patients even developed anti-HBs antibodies. In particular, the number of HBs-antigen- and HBc-antigen-positive hepatocytes appeared to be significantly lower after 1 year of combination treatment, suggesting that cytolytic mechanisms were involved and/or IFN-mediated suppression of viral antigen production. Reduction in intrahepatic viral load after 48 weeks of combination therapy with PEG-IFN and adefovir was maintained in the following 96 weeks of adefovir monotherapy, but no further significant cccDNA changes occurred between week 48 and week 144, although 2 years of adefovir monotherapy controlled cccDNA levels in most patients. Analysis of intrahepatic HBV DNA species showed that combination therapy with PEG-IFN and adefovir inhibited viral production by 99% and subsequent adefovir monotherapy by only 76%, respectively (Fig. 2), showing that suppression of intrahepatic viral spread was more significant with combination therapy. Of note, two patients developed adefovir resistance during the third year of treatment.

These results were later confirmed (25) by Takkenberg *et al.* in a larger group of patients. This group reported results of combination therapy with PEG-IFN and adefovir for 48 weeks and demonstrated a high rate of HBsAg loss, both in HBeAg-positive (11%) and HBeAg-negative (17%) patients 2 years after treatment ended. Interestingly, the group identified baseline predictors of response, and low baseline HBsAg in HBeAg-negative patients was the only independent significant predictor of HBsAg loss (25). To identify host factors contributing to treatment-induced HBsAg loss, they performed a genome-wide screen of single nucleotide polymorphisms and studied the immunological effect in a follow-up study in the same cohort (26). There were strong associations between SLC16A9 gene variations, carnitine levels and HBsAg loss. Although these are preliminary findings, they could provide interesting areas of research on host immune associated factors involved in treatment-induced HBsAg loss, and if they are confirmed in larger trials, they could play a role in the prediction of treatment outcome by identifying patients with a greater chance of HBsAg loss.

Although combination therapies using PEG-IFN and more potent NAs such as entecavir and tenofovir may be more attractive, the efficacy must be fully evaluated. Several ongoing studies are evaluating combination PEG-IFN and entecavir or tenofovir using simultaneous or add-on approaches. In the largest existing ongoing prospective, controlled, international study (GS-US-174-1049), 740 HBeAg positive and –negative patients received either tenofovir, or PEG-IFN, or the combination of tenofovir plus PEG-IFN with and without tenofovir monotherapy following combination therapy. Patients were stratified by genotype and HBeAg status, and the primary endpoint in this study was the loss of HBsAg. Primary results were presented recently at AASLD 2014 (33; Fig. 3). CHB patients treated with TDF and PEG-IFN combination therapy for 48 weeks

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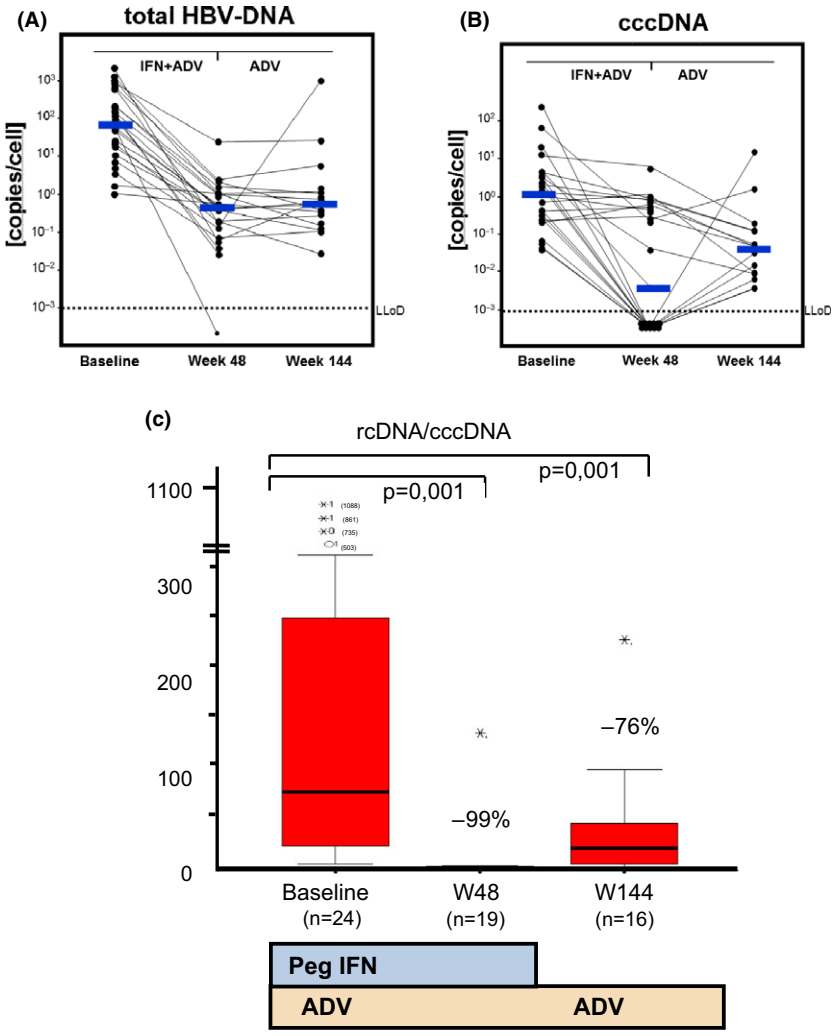

Fig. 2. Reduction in intrahepatic viral load after 48 weeks of PEG-IFN and adefovir was maintained in the following 96 weeks of adefovir monotherapy. No further significant cccDNA changes occurred between week 48 and week 144 (a,b). Analysis of intrahepatic HBV DNA revealed that combination therapy with PEG-IFN and adefovir inhibited viral production by 99% and subsequent adefovir monotherapy by only 76%, respectively (c), showing that suppression of intrahepatic viral spread was more pronounced with combination therapy.

achieved significantly higher rates of HBsAg loss than either therapy given alone (Fig. 3) (P. Marcellin *et al.* personal communication).

In two recently previously published add-on trials, the results were inconclusive in relation to the value of adding PEG-IFN to entecavir (ETV) treatment. In the first multicenter investigator initiated controlled ARES study (27), HBeAg-positive CHB patients with compensated liver disease began ETV-monotherapy and were randomized to receive either PEG-IFNα-2a add-on from week 24–48, or to continue ETV-monotherapy. Response was defined as HBeAg-loss with HBV DNA <200 IU/ml. Responders at week 48 stopped ETV at week 72. Of the 175 patients in the modified intention-to-treat analysis, 85 were allocated to ETV+PEG-IFN add-on therapy and 90 to ETV-monotherapy. At week 96, 26 (31%) patients in the add-on vs. 18 (20%) in the monotherapy arm achieved the response ($P = 0.107$). Twenty (24%) patients in the add-on vs. 10 (11%) in the monotherapy arm achieved HBeAg-seroconversion with HBVDNA <200 IU/ml ($P = 0.029$). Adding-on PEG-IFN to ETV increased the reduction in HBsAg, HBeAg, HBVDNA, and to more sustained responses after ETV-discontinuation [9/14 (64%) vs. 2/8 (25%)], preventing relapse after stNA. One patient who received add-on therapy lost HBsAg. Unfortunately, in this study a PEG-IFN monotherapy arm was missing.

In the second study, 218 treatment-naïve Chinese HBeAg-positive patients were randomized to PEG-IFN for 48 weeks, either as monotherapy, with 24 weeks of ETV added at week 13, or pretreatment with a 24-week course of ETV, with PEG-IFN begun at week 21 (28). The primary endpoint was reduction in quantitative HBeAg from baseline to 24 weeks post-treatment.

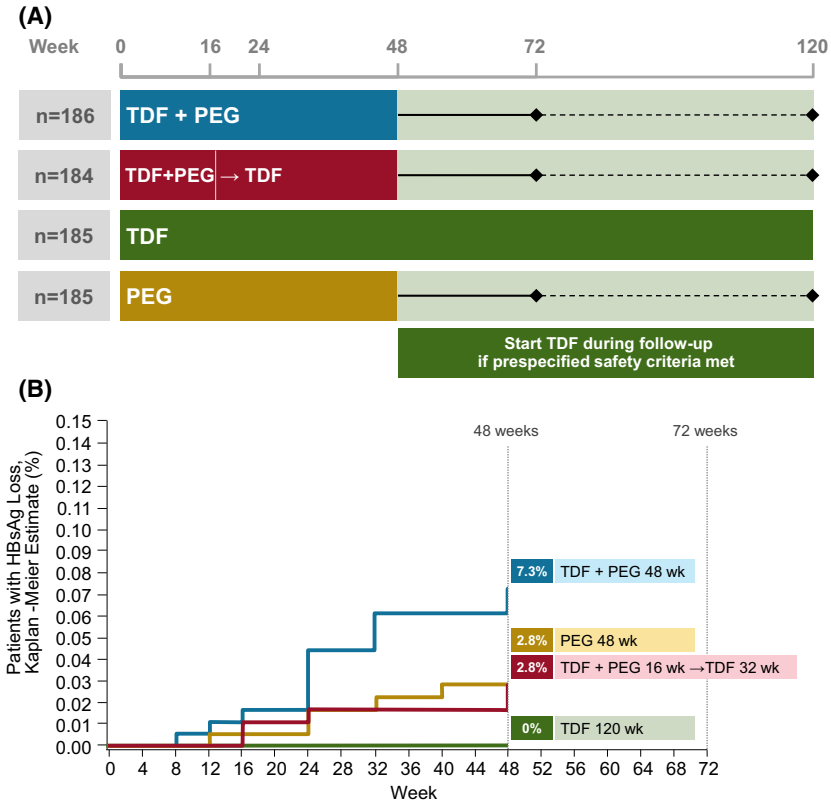

Fig. 3. Study design of study GS-US-174-0149 (a), HBsAg loss in different study arms over time (week 48) (b).

Significant reductions in HBeAg from baseline were achieved in all treatment groups 24 weeks post-treatment. Decreases were comparable across treatment arms. Significantly greater decreases in HBV DNA were achieved with ETV add-on on-treatment, but were not sustained post-treatment. In this study, neither ETV add-on nor ETV pretreatment was shown to be better than 48 weeks of PEG-IFN monotherapy. As a result of the preliminary and conflicting results of combination studies, thus, far combination NAs plus PEG-IFN treatment is not recommended.

Finally, a recent study reported unexpected severe peripheral neuropathy in 7/50 patients who received combination therapy of PEG-IFN and telbivudine, compared to only in 1/54 patients who received telbivudine monotherapy (29). The study was terminated early because of safety concerns, indicating that in depth safety studies must be performed in ongoing combination trials.

Switching from NAs to PEG-IFN may be better than continuing NAs

Recently, switching treatment from entecavir to PEG-IFN after 36 months of continuous entecavir administration with a complete response to entecavir was shown to be better than continuing entecavir treatment by measuring HBeAg seroconversion and HBsAg loss rates in HBeAg positive patients (30). Two hundred CHB patients who had received entecavir for 9–36 months were randomized 1:1 to receive a finite course of PEG-IFN or to continue with entecavir for another 48 weeks. The primary endpoint was HBeAg seroconversion at week 48. Patients who switched to PEG-IFN achieved significantly higher week 48 HBeAg seroconversion rates compared to those who continued entecavir (15% vs. 6%) and only patients who received PEG-IFN achieved HBsAg loss (8.5%). In PEG-IFN treated patients with HBeAg loss and HBsAg <1500 IU/ml at randomization, 33% and 22% achieved HBeAg seroconversion and HBsAg loss respectively. An early on-treatment decline in HBsAg and an HBsAg baseline level of <1500 IU/ml was predictive of response at week 48. The highest rates were observed in patients with week 12 HBsAg levels of <200 IU/ml. This study is interesting for possible response-guided approaches that must be considered in the future and that could help identify patients with the greatest chance of treatment success, i.e. HBsAg loss.

Combination of NAs may have additive effects in selected clinical situations

Combining drugs with the same virological target, such as different NAs, may not have additive therapeutic

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effects. Therefore, EASL CPG (1) is considering combination therapy of potent NAs only as rescue therapy under highly selected circumstances. In rare clinical situations where multiresistant virus strains have been analysed and treated unsuccessfully with several monotherapies, even with IFN, combination rescue therapy might be considered if other issues such as incomplete adherence aspects have been ruled out. For the moment, the number of published studies involving this segment of patients is very limited. The aim of an international European open label, investigator initiated cohort study was to examine the efficacy and safety of a combination of entecavir plus tenofovir in 57 CHB partial responders or multidrug resistant patients (29). Fifty-seven patients (37 HBeAg+), who previously received long-term treatment with a median of three courses of antiviral therapy (range 1–6), about half with advanced liver disease, were included. The median duration of combination therapy was 21 months. HBV DNA levels during combination therapy decreased by a median 3 $\log_{10}$ and 51/57 patients became HBV DNA undetectable. The probability of HBV DNA suppression was not reduced in patients with adefovir or entecavir resistance or in patients with advanced liver disease. Viral suppression resulted in a decline in ALT and five patients lost HBeAg with HBs seroconversion in one patient (31). There was no clinical decompensation in patients with advanced disease, but two patients with cirrhosis and undetectable HBV DNA developed hepatocellular carcinoma. There were no significant safety signals reported in this study. In a follow-up study of a subgroup of patients from this cohort, combination therapy was reduced to monotherapy after successful suppression of viraemia and normalization of transaminases (32). Tenofovir was withdrawn in patients with adefovir resistance, and entecavir was withdrawn in patients with lamivudine resistance. Interestingly, monotherapy with entecavir or tenofovir was efficient and safe in these patients and well tolerated in patients with or without advanced liver disease.

In conclusion, although the efficacy and role of potent and appropriate HBV combination therapies must be further evaluated, this requires large, expensive trials. Thus, the efficacy of potent monotherapies with NAs or PEG-IFN vs. different combinations of PEG-IFN and NAs must be determined from direct clinical experience in the next few years. To date, because of very limited available data, combination therapy is not recommended. EASL considers combination therapy in CHB to be an area requiring further research and supports further assessment of the safety and efficacy of the combination of PEG-IFN with potent NAs such as entecavir or tenofovir to increase anti-HBe or anti-HBs seroconversion rates (1). Furthermore, studies investigating immune responses during antiviral therapy in prospective controlled trials are still scarce. Therefore, besides virological response and the rather simple measurements of HBsAg quantification, in the future the complicated mechanisms of immune responses in treated patients must be further evaluated to identify the optimal HBV therapy of individual patients early on.

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