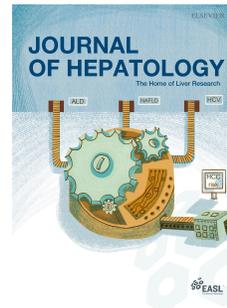


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## **The times they are a-changing – A refined proposal for finite HBV nucleos(t)ide analog therapy**

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**Abstract**

Although discontinuation of nucleos(t)ide analogue (NA) treatment before HBsAg loss is part of all current hepatitis B virus (HBV) treatment guidelines for HBeAg-positive patients who achieved HBeAg seroconversion, a treatment endpoint known to be associated with silencing of HBV transcriptional activity and restoration of HBV-specific immune control, it is still highly controversial whether it is even appropriate to consider NA discontinuation before HBsAg loss in the HBeAg-negative phase. Despite the growing evidence that a relevant, albeit small, proportion of patients with HBeAg-negative disease can be cured by stopping NA treatment, the fear of discontinuation-associated relapse and the uncertainty of how to predict off-therapy response and monitor patients after discontinuation have generated scepticism and subsequently led to low implementation of this concept in the clinic. In this article, we propose a concept in which NA discontinuation-associated relapse is an integral part of the stop-to-cure approach and ultimately the trigger for achieving HBsAg loss. However, the relapse in this sense only becomes functionally effective if HBV-specific immune reinvigoration and silencing of HBV transcriptional activity have been achieved previously under the NA treatment period. The probability with which a functional cure can be achieved but also the severity of post-discontinuation flares depends on the underlying baseline HBV transcriptional activity when NA was started as well as the duration of NA treatment, both factors that we should consider as we move towards individualised cure approaches in the future.

## Treatment concepts of chronic hepatitis B - times they are a changing

Inducing long-term immune control of chronic hepatitis B by a finite treatment approach has been the cornerstone of treatment strategies for decades and was driven by the specific mode of action of interferons which were the only licensed drugs available at that time. At the beginning of the nucleos(t)ide analogue (NA) era, studies also aimed to achieve long-term off-treatment responses through a time-limited administration of these direct antiviral drugs. However, randomized studies proved that NAs were inferior to pegylated interferon alpha in terms of inducing long-term remission, HBeAg seroconversion as well as HBsAg loss (1, 2). It was also shown that virologic relapses, although seen in both, interferon- and NA-treated patients, may differ in terms of the risk of relapse-associated disease decompensation in patients with advanced fibrosis or cirrhosis which was mainly seen in the NA-treated cohort when hepatitis B virus (HBV) replication reappeared to high levels shortly after treatment cessation (1, 2).

The high efficacy and safety of second-generation NAs together with data showing for the first time that the level of HBV replication is the main determinant of disease progression has led to sustained viral load suppression becoming an increasingly desirable endpoint of therapy (3). The prospective tenofovir studies designed to treat patients for 8 years irrespectively of on-treatment serologic response features (i.e. HBeAg loss or HBsAg loss) together with the results of longitudinal entecavir and tenofovir real-world studies have been an important step towards promoting long-term suppression of HBV replication as the main goal of treatment. They have shown that it cannot only halt disease progression but also lead to regression of fibrosis and even cirrhosis in a majority of treated patients (4-7).

Nevertheless, discontinuation of NAs is still part of all current treatment guidelines and is recommended when certain features of response to treatment known to be associated with silencing HBV transcriptional activity and restoration of HBV-specific immune control can be induced (3, 8, 9). The on-treatment induction of HBeAg loss or seroconversion, a serologic hallmark of a silenced disease activity in the natural course of the infection represents such a feature. The main controversy as to when and if NAs can be discontinued at all exists in patients who start antiviral treatment in the HBeAg-negative chronic hepatitis phase, as no serologic markers have yet been established that herald silencing of viral activity has been achieved on-treatment (**Table 1**). However, recent long-term follow-up studies after cessation of NA treatment in HBeAg-negative patients after prolonged on-treatment suppression of HBV replication showed a relatively high proportion of patients remaining in treatment-free remission or even achieving functional cure, i.e. loss of HBsAg, a finding which has challenged the dogma of lifelong treatment necessity in these circumstances (10-14).

Treatment discontinuation-associated relapses in HBV replication are now being discussed as a possible important trigger for the induction of immune-mediated long-term control and functional cure (15). The emergence of new direct antiviral and immune-mediated treatment strategies that also aim to induce a functional cure of HBV infection through a finite duration of treatment have prompted widespread discussion on flare management on the one hand, but also on how to distinguish potential flare-associated HBV responses from the specific modes of action of the studied antiviral regimens. Therefore, there is a heated debate about the extent to which discontinuation of NAs and the associated flare should be part of a new approach to our current treatment management of HBeAg-negative disease that should be applied more widely to increase functional cure rates in this patient population.

### **NA-discontinuation in HBeAg-negative chronic hepatitis B - Where do we stand?**

Despite the 2017 EASL guideline recommendation on the management of chronic HBV infection to consider discontinuation of NA therapy prior to HBsAg loss in selected patients with HBeAg-negative chronic hepatitis B (3) (**Table 1**), the overall use of this strategy in clinical practice is limited. In our personal experience, less than 10% of physicians currently caring for these patients would use this strategy today. There are several possible explanations for this attitude (**Table 2**). Over the past 20 years, guidelines, reviews, original publications, and, not least, presentations at national and international congresses have made the importance of virologic response quite clear. The relevance of undetectable HBV DNA as a surrogate biomarker for a clinically robust endpoint such as HCC prevention has been confirmed by the last 10 years of experience with potent NAs, which showed a significant reduction in HCC incidence rates in patients with long-term maintained virologic response (3, 6, 8, 9). This is one of the strongest arguments for physicians, as well as patients, not to discontinue NA before HBsAg loss. Indeed, a rebound of HBV replication with detectable HBV DNA levels after NA discontinuation is almost universal (10-14). Whether this rebound warrants an increased risk of HCC development in the long-term is still unknown. Prevention of progression to cirrhosis is also closely linked to virologic response but does not require complete suppression of HBV replication with undetectable HBV DNA levels, but rather the reduction of HBV DNA to < 2000 IU/mL with normalization of ALT, as has been demonstrated as a valuable endpoint in numerous interferon therapy trials (3). Another argument often made against stopping NA before HBsAg loss in HBeAg-negative disease is the risk of significant disease relapse and even liver failure, which requires close follow-up and high patient adherence to avoid these adverse consequences. For both patients and physicians, the monitoring associated with the finite treatment approach makes discontinuation of NA more challenging than simply continuing treatment.

There is however some contradiction between the clear general recommendation to discontinue NA therapy after HBeAg seroconversion and the strong caveat to discontinuation of treatment in HBeAg-negative hepatitis B (3, 8). First, the fact that we do not have HBeAg seroconversion as a serologic marker associated with silencing of cccDNA in HBeAg-negative hepatitis does not mean that a similar silencing in HBV transcriptional activity cannot be achieved with antiviral treatment also in a proportion of the HBeAg-negative patients. Second, if not only HBsAg loss but also silencing HBV transcriptional activity and the induction of a true “inactive carrier state” remains an appropriate endpoint in HBeAg-positive hepatitis B, why should this not also be the case for HBeAg-negative disease.

Third, the concern about virologic relapses as the driving argument for the general recommendations not to stop therapy in HBeAg-negative patients is conflicting, because discontinuation of NA is still recommended in HBeAg-positive patients with treatment-induced HBeAg seroconversion, although the risk of relapse in this constellation does not seem to differ from those who started NA treatment in the HBeAg-negative hepatitis phase (16). The opposite is probably true, as recently demonstrated in a prospective study in which pre-treatment HBeAg-positive patients were three times more likely than HBeAg-negative patients to require retreatment for a clinically relevant relapse after stopping NAs (17).

### **Outcome studies - geographical differences matter**

Most of our knowledge on NA discontinuation comes from Asian studies that retrospectively evaluated the outcome of their discontinuation regimens in terms of virologic and biochemical relapse rates in HBeAg-negative patients. It is important to emphasise that this discontinuation approach was mostly driven by local reimbursement policies and not by the attempt to induce a post-discontinuation flare-associated long-term remission. In contrast to prospective European studies that showed that virologic relapses after discontinuation of NAs prior to HBsAg loss are almost universal in HBeAg-negative patients, these retrospective studies showed a rather heterogeneous picture with highly variable biochemical and virologic relapse patterns depending on the relapse definitions, duration of the preceding NA treatment period, as well as specific disease characteristics (18-21). However, prospective Asian studies confirmed virologic relapse rates of over 90% after NA discontinuation, implying that transient mild virologic relapses may have been overlooked in the aforementioned Asian studies due to their retrospective nature (22). In some of these Asian studies, up to 40% of the patients in whom treatment was stopped had cirrhosis at baseline, which was a contraindication to stopping NAs in all Western studies (21). And indeed, liver decompensation and death associated with post-treatment flares were observed so far only in patients with cirrhosis (19, 21). The fact that most Asian studies did not apply

predefined rules for the timing of treatment resumption further complicates comparisons between studies because the timing of retreatment has been shown to have important implications for whether complete post-treatment remission and HBsAg loss can be achieved. Early initiation of NA retreatment could potentially jeopardize the beneficial effect of flare-associated immune activation, as previously shown in interferon-treated patients (21, 23).

Stephanos Hadziyannis' study was the first to open the window to a new finite treatment approach in HBeAg-negative disease that went beyond counting the number of virologic and biochemical flares. After a follow-up period of 6 years following discontinuation of NAs that were administered for at 4-5 years, 39% of patients lost HBsAg without any treatment (10). He hypothesized that part of this success was related to post-treatment flares, which, however, remained untreated and thus may have helped to stimulate immune-mediated mechanisms, leading to long-lasting immune control, much like flares that occur in the natural course of the HBV infection and are known to be followed by long-term remissions and seroconversions.

Fascinated by this concept, prospective studies were initiated aiming to induce a functional cure by a stop to relapse approach with predefined re-treatment rules that allow to tolerate biochemical flares if they do not fulfil criteria being indicative of an incipient liver function impairment. These studies provided strong evidence that discontinuation of NA, compared to continued NA therapy, is overall safe and associated with a more pronounced decline in HBsAg levels and a significantly higher rate of HBsAg loss, which can reach 10-20% during a relatively short follow-up period of 2-3 years (11, 12). In addition, up to 40% enter a true off-treatment carrier stage with suppressed viral activity (10-14). From these studies, it also became evident that HBV infection goes through different phases after discontinuation of NA treatment, the lag phase, the reactivation phase, and the consolidation phase (15). The HBV DNA and ALT flares observed during the reactivation phase are often transient and their further fate can only be assessed during the consolidation phase. Whether there are favourable and unfavourable flares in terms of the induction of long-term remission and HBsAg response and how to distinguish between them is controversial, as is the question of why only a minority of patients achieve a functional cure.

### **Inducing functional cure - The stop-to-relapse hypothesis**

There is no longer any doubt that the recurrence of HBV replication after NA discontinuation represents an important trigger leading to altered HBsAg dynamics and HBsAg loss. But why does this desired HBsAg response occur in only a minority of patients, even though HBV

replication rebound happens universally? Does the outcome depend primarily on the course of the post-treatment discontinuation phase and the flare pattern, or is the fate of what happens after discontinuation already determined during the treatment phase?

From what we know so far, it is not the severe flares with pronounced HBV replication and high ALT levels that are associated with a favorable HBsAg response. Mild HBV DNA rebounds often without marked ALT elevations along with low HBsAg levels are the typical features seen in patients who have lost HBsAg (11, 12, 21, 24, 25).

In our opinion what matters most is what happened during the NA treatment phase: Stopping NA prematurely before HBsAg loss can only be successful in terms of inducing a functional cure if cccDNA transcriptional activity has been silenced to some degree during treatment. The probability with which this goal can be achieved depends on the underlying baseline HBV transcriptional activity when NA was started as well as the duration of NA treatment, i.e. the higher the viral activity in the beginning, the longer the treatment phase must be. However, other characteristics, such as the overall duration of the infection, history of vertical transmission, patients' ethnicity, age, HBV genotypes and perhaps also the NA class may also play a role.

Sulsov and colleagues (26) recently described different patterns of viral control in the HBeAg negative hepatitis phase. Viral replication appeared to be mostly controlled by a combination of cytolytic and non-cytolytic antiviral mechanisms, the latter leading to more efficient viral control in a subset of HBeAg-negative patients characterized by reduced replication efficiency downstream of pgRNA. It is tempting to speculate that this subset of patients with low-replicative HBeAg-negative hepatitis may respond differently to NAs and may not only be less likely to suffer from a severe flare after treatment cessation than their high-replicative HBeAg-negative counterparts but also may have a higher chance of losing HBsAg.

The reinvigoration of a previously dysfunctional innate and adaptive immune response under NA treatment, as evidenced by an increased HBV-specific T cell response, decreased NK cell killing of HBV-specific T cells, and an increase in serum cytokine and chemokine levels could represent a condition sine qua non for a safe and effective NA discontinuation (27-31). Under the condition of both, a silenced HBV transcriptional activity and reinvigoration of innate and adaptive immune responses, innate immune cells in the liver might be activated after NA treatment cessation by a threshold exposure to HBV and express various inflammatory cytokines and chemokines which in turn may lead to long-term immune control of the infection and eventually HBsAg loss (as summarized in 29 and 30) (**Figure**)

### **Definitions of the patient populations benefiting from the finite approach – the role of HBV biomarkers**

Inducing a functional cure is, in our opinion, the main justification for treatment discontinuation in HBeAg-negative disease. Since the overall HBsAg loss rates are however limited, reliable predictors and better definitions of the patient populations that will benefit from this finite treatment approach are needed. This is even more true when the alternative strategy, continuous NA administration, is associated with excellent efficacy and ease of use. Several studies have investigated whether new biomarkers, such as HBV RNA, HBcrAg, and anti-HBc antibody levels, can be used to stratify patients who are more likely to respond after stopping NA or, conversely, those who are more likely to require retreatment (32-37). Although both, serum HBV RNA and HBcrAg levels reflect intrahepatic HBV transcriptional activity to some extent and thus could be valuable tools for predicting outcomes after NA discontinuation, they are not yet ready for "prime time" (38-40). While detectable levels of HBV RNA and HBcrAg at the end of treatment clearly predict an unfavourable outcome, the opposite - non-detectability of these markers - is not highly predictive of HBsAg loss (32, 33, 35-37). Almost all these biomarker studies were retrospective post-hoc observations which is another limitation, as well as the fact that the endpoints for which these markers were assessed differed from study to study. In addition, HBV RNA is not commercially available, and HBcrAg still suffers from limited sensitivity and lack of reimbursement. Currently, quantitative HBsAg levels are the most reliable predictive marker, and a HBsAg decline during treatment and low HBsAg levels at the end of treatment correlate highly with the likelihood of achieving HBsAg loss after discontinuation of NAs (41, 42). The HBsAg cut-off at the end-of-treatment that best predicts functional cure is still under debate and appears to differ between Caucasian and Asian patient populations. However, it has now been convincingly demonstrated that HBsAg levels of < 1000 IU/mL in the Caucasian population and < 100 IU/mL in the Asian population are associated with HBsAg loss rates of more than 20%-30% (13, 21, 29, 31, 42). These functional cure rates appear to justify the effort related to discontinuing NAs, and they are in the range of what we are also targeting as efficacy endpoints for novel antiviral and immunomodulatory agents currently being studied to cure HBV infection (43).

## **Summary and conclusions**

The answer to the question of whether we can go beyond the guidelines in terms of discontinuing NA in HBeAg-negative patients is that it is not a matter of going beyond, but rather of becoming more specific in our recommendations. In early 2017, when the EASL guidelines were written, we underestimated the importance of NA treatment duration in the context of baseline viral activity and overestimated the role of discontinuation-associated relapse as the essential trigger in eliciting the functional cure. Although NA discontinuation-

associated relapse is an integral part of the stop-to-cure approach and ultimately the trigger for achieving HBsAg loss, the relapse becomes functional in this sense only if HBV-specific immune reinvigoration and silencing HBV transcriptional activity has previously been achieved under the NA treatment period. The chances of the immune system being awakened from its slumber depend critically on the baseline transcriptional activity of HBV infection at the start of NA treatment and on the duration for which HBV replication has been completely suppressed by NA treatment. Therefore, we are opposed to specific cut-offs for the duration of NA treatment, as mentioned in the guidelines, to decide on treatment discontinuation, as it may take either shorter or longer to achieve the desired therapeutic effects depending on the baseline HBV transcriptional activity and the individual constitution of the host immune system. Wider use of biomarkers that reflect the transcriptional activity of cccDNA in the HBV DNA-suppressed settings, such as HBV RNA and HBcrAg, but also the ability to monitor innate and adaptive HBV immune responses, would be important to refine the finite approach. Among the routinely available biomarkers, baseline HBV DNA and the dynamics of quantitative HBsAg under treatment help to guide the finite treatment strategy. In the subset of patients who achieve on-treatment HBsAg levels  $< 1000$  IU/mL in Caucasian and  $< 100$  IU/mL in Asian patients treatment discontinuation may result in high functional cure rates of more than 20%-30%, and importantly, the risk of developing severe flares is low. These cure rates, together with the fact that treatment initiation thresholds related to ALT and HBV DNA levels have become more liberal over time, allowing us to start treatment earlier in the disease stage in many patients, are in our opinion a strong argument for recommending a finite NA approach as the therapeutic goal right from the beginning under the above-mentioned circumstances. Early prediction of the long-term outcome of finite treatment approaches not only by end-of-treatment markers but also by early relapse kinetics may further facilitate acceptance of the finite approach. Conversely, novel antiviral and immunomodulatory agents that can help to accelerate the time to achieve silencing of HBV transcriptional activity are particularly needed for patients in whom both baseline and on-treatment characteristics make achievement of a functional cure through a finite NA approach unlikely.

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**Figure legend:**

The chances of functional cure after premature NA discontinuation before HBsAg loss in patients with HBeAg-negative hepatitis B but also the severity of post-discontinuation flares depends on whether silencing of HBV transcriptional activity as well as reinvigoration of the exhausted HBV-specific immune response can be achieved during the NA treatment period. If baseline HBV transcriptional activity is already low, further silencing of its activity by NA-induced viral suppression together with a reinvigoration of HBV-specific immune functions can be achieved after only a limited treatment period. In contrast, a more pronounced baseline HBV transcriptional activity may require longer treatment periods to allow some restoration of exhausted immune functions. It is tempting to speculate that the individual factors of the HBV infection and the host immune system may lead to a constellation where it will never be possible to reach a stage where safe and effective discontinuation of NA is possible. Under the condition of silenced HBV transcriptional activity and reinvigoration of innate and adaptive immune responses, rebound of HBV replication after discontinuation of NA treatment is typically mild and represents the trigger required for long-term immune control of the infection and ultimately HBsAg loss. Severe flares after discontinuation of NA are probably a reflection of high HBV cccDNA transcriptional activity still persisting under NA treatment indicated by high HBsAg, HBcrAg and HBV RNA levels despite undetectable HBV DNA, and are typically associated with a high probability of needing re-treatment and low rates of HBsAg loss.

**Table 1: Current recommendations from international guidelines on early discontinuation of NA before HBsAg loss in patients with HBeAg-negative hepatitis B**

<b>APASL 2016</b>	<b>EASL 2017</b>	<b>AASLD 2018</b>
In patients without liver cirrhosis, the treatment can be withdrawn after treatment for at least 2 years with undetectable HBV DNA documented on three separate occasions, 6 months apart	Discontinuation of NAs in selected non-cirrhotic HBeAg-negative patients who have achieved long-term ( $\geq 3$ years) virological suppression under NAs may be considered if close post-NA monitoring can be guaranteed	The AASLD suggests indefinite antiviral therapy for adults with HBeAg-negative, immune-active CHB unless there is a compelling rationale for treatment discontinuation

According to references 3, 8 and 9; NAs, nucleos(t)ide analogues; CHB, chronic hepatitis B

**Table 2: Pros and Cons of NA discontinuation before HBsAg loss in patients with HBeAg-negative chronic hepatitis B**

<b>PROS</b>	<b>CONS</b>
Higher HBsAg loss rates <sup>a</sup>	Universal HBV DNA rebound
Inactive carrier state in approx. 20-30%	Abnormal ALT levels in many patients
Long-term off therapy in approx. 50% of the patients <sup>b</sup>	ALT flares in some patients
	More intensive monitoring <sup>c</sup>
	Retreatment with NA in 50% of the patients <sup>e</sup>
	Excellent patients' adherence required <sup>f</sup>
	Careful patients' selection <sup>g</sup>
	Assessment of predictors <sup>h</sup>
	Unpredictability of virological and clinical outcomes <sup>l</sup>

a) approximately 5-10% at year 1, higher in selected patients and higher over time; b) estimated at year 1-2; c) at least for the first year off therapy; d) at least for the first year off therapy because of the intense virological and clinical monitoring; e) for retreatment rules, see article; f) compliance to extensive monitoring at least for the first year; g) patients with any evidence of current or previous cirrhosis must continue NA; h) EOT HBsAg levels may help selecting patients with higher likelihood of HBsAg loss off therapy; l) the virological and clinical outcomes cannot be easily predicted.

Figure 1

