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Emilia Hadziyannis & Stephanos Hadziyannis

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REVIEW

Current practice and contrasting views on discontinuation of nucleos(t)ide analog therapy in chronic hepatitis B

Emilia Hadziyannis and Stephanos Hadziyannis

Second Academic Department of Medicine, National and Kapodistrian University of Athens, Hippokration General Hospital, Athens, Greece

ABSTRACT

Introduction: Long-term, even indefinite treatment with nucleos(t)ide analogs (NAs) is the current first-line therapy for patients with chronic hepatitis B (CHB), regardless of its histological stage. Guidelines and recommendations on duration and endpoints of NA therapy in CHB are not identical and change over time.
Areas covered: The authors review NA discontinuation approaches and views with an emphasis on HBeAg-negative patients based on published studies relevant to the topic, stressing on whether or not the optimal endpoint of HBsAg loss is practically achievable.
Expert opinion: Discontinuation of NA therapy in HBeAg-negative noncirrhotic patients has to be considered after long-term effective treatment with controlled liver disease activity, undetectable viremia, and significant decline in serum HBsAg titers. Close post-treatment monitoring is required for early intervention in cases of severe clinical relapse. Immediate retreatment hampers the favorable outcome of HBsAg clearance (functional cure) and should be avoided in transient ALT flares. Predictors of such relapses are still under investigation and include viral and patient factors. For HBeAg-positive noncirrhotic patients, there is wide acceptance of the endpoint of HBeAg seroconversion, after a long consolidation period.

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Chronic Hepatitis B; nucleos(t)ide analog; treatment discontinuation; functional cure; HBV

1. Introduction

Chronic HBV infection has a complex course classified into phases of variable duration, which may be sequential or non-sequential and not every infected patient will undergo through all phases (Figure 1). Antiviral treatment is currently recommended for the phases of active hepatitis either HBeAg-positive or HBeAg-negative and for patients with cirrhosis.

Nucleos(t)ide analogs (NAs) with anti-reverse transcriptase activity, initially applied in the treatment of HIV infection [1], were introduced 20 years ago in the treatment of chronic HBV infection with active liver disease, referred as chronic hepatitis (CHB). They started with Lamivudine [2] and have been followed by compounds with a higher genetic barrier to HBV resistance, namely Adefovir Dipivoxil, Entecavir, Telbivudine, Tenofovir disoproxil and last in 2017 by Tenofovir Alafenamide [3].

Up to 2017, both the European and the American Associations for the Study of the Liver (EASL and AASLD) in their treatment Guidelines were recommending long-term duration of NA therapy for patients with HBeAg-positive and indefinite (lifelong) for those with HBeAg-negative CHB [4,5]. However, different were the views and recommendations of the Asian and Pacific Association for the Study of the Liver (APASL) regarding the duration of NA therapy in CHB. Thus, already from 2005 and 2008, APASL was considering and suggesting discontinuation of NA treatment both for HBeAg-positive and HBeAg-negative patients with CHB, provided that serum HBV DNA would be negative in 3 consecutive assays 6 months apart [6,7]. Basically, this early view of APASL has remained practically unchanged over the years being actually renewed, clarified, and commented critically in the subsequent guideline editions of 2012 and 2015 [8,9]. At the same time, newer worldwide data and evidence on NA therapy in HBeAg-negative CHB modified the earlier views prevailing in Western countries, and new Evidence-Based Treatment Guidelines and recommendations have been published both by EASL and AASLD in 2017 as well as other local organizations (Table 1) [10–16].

Therapies with NAs are aiming at suppression of HBV replication or else at a response reflected by undetectable HBV DNA in the serum or plasma of the patients. This goal is practically achievable in all HBeAg-negative patients by long-term treatment with NAs of high genetic barrier to HBV resistance (Entecavir and Tenofovir), also referred to as first-line NAs. Suppression of HBV replication to serum HBV DNA <2,000 IU/mL and return of serum aminotransferases to normal levels are termed clinical remission [17]. However, in CHB, clinical remission per se, regardless of its duration, does not indicate discontinuation of NA therapy unless combined with other endpoints which differ between HBeAg-positive and HBeAg-negative patients.

The view that dominated in the field of Hepatology was that treatment with NAs should continue up to HBsAg loss and preferably up to the development of anti-HBs. This review article deals with variable and contrasting views and recommendations that are evolving worldwide regarding the discontinuation of long-term NA therapy in patients with CHB.

CONTACT Stephanos Hadziyannis hadziyannis@ath.forthnet.gr Second Academic Department of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Article Highlights

- Discontinuation of NA therapy in HBeAg-positive non-cirrhotic patients is considered in international practice guidelines after HBeAg seroconversion and consolidation treatment
- Discontinuation of NA therapy in HBeAg negative no cirrhotic patients is a safe option that is gaining increasing acceptance but views in certain aspects are still variable and evolving.
- Patients who discontinue treatment should be under close and long-term post-treatment follow-up.
- Relapses, attributed to restoration of HBV immunity, may be beneficial and should not be treated immediately except for severe or persisting cases
- Functional cure is achieved in higher rates in patients who discontinue treatment.
- End of treatment HBsAg level is currently the best predictor of outcome.

2. Discontinuation of NAs in HBeAg-positive CHB

The goals of NA treatment in HBeAg-positive patients are suppression of HBV DNA to undetectable levels by the currently applied real-time PCR assays and clearance of serum HBeAg. The loss of HBsAg or else functional cure of HBV infection is the most desirable endpoint of NA therapy but is not a prerequisite for discontinuation of NAs in HBeAg-positive CHB.

The EASL, AASLD, and WHO guidelines recommend that in patients with HBeAg-positive CHB without evidence of cirrhosis, treatment with NAs can be discontinued after loss of HBeAg from serum and development of antibodies to HBeAg (anti-HBe) confirmed by repeated testing at least 2 months apart [10,12,18]. Moreover, it is also recommended that prerequisites for discontinuation of NAs are persistently normal serum ALT levels and undetectable HBV DNA, after receiving a minimum of 12 months of treatment consolidation. It is required though, that patients who discontinue treatment be followed closely long term for reactivation [19].

The EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection suggests (evidence level II-1, grade of recommendation 1) that the induction of HBeAg loss, with or without anti-HBe seroconversion, is a valuable endpoint which represents often enough a partial immune control [12]. The APASL 2016 guidelines advise as well treatment discontinuation for patients with HBeAg seroconversion, undetectable HBV DNA, and persistently normal ALT levels with 1 to 3 years of consolidation therapy [9]. Treatment

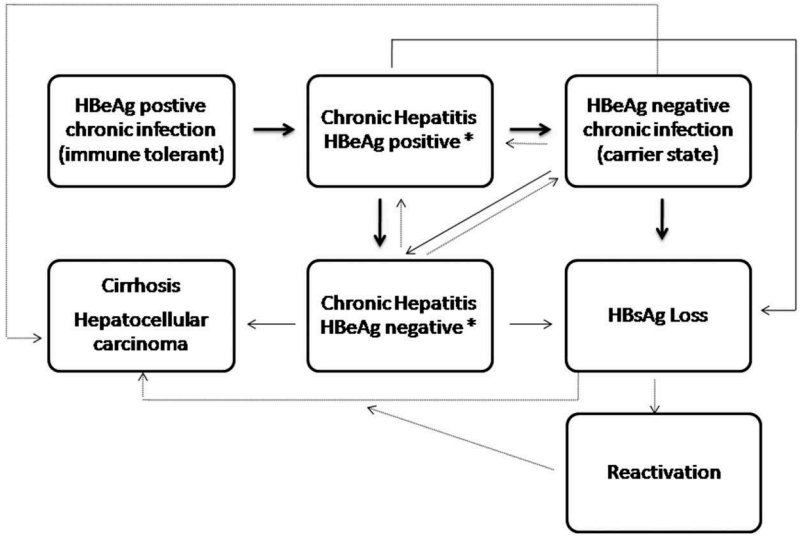

Figure 1. During the complex natural course of chronic hepatitis B virus infection, treatment is recommended in the phases of active disease*.

Table 1. International guidelines on NA treatment discontinuation in noncirrhotic patients.

	HBeAg positive	HBeAg negative
EASL [12]	Treatment can be discontinued in patients with stable HBeAg seroconversion and undetectable HBV DNA after completion of at least 12 months of consolidation therapy	Treatment should be discontinued in patients with confirmed HBsAg loss, with or without anti-HBs seroconversion (safest end point). Treatment discontinuation may be considered in patients with long-term (>3 years) virological suppression under NA(s) if close post-NA monitoring can be guaranteed
AASLD [10]	Treatment discontinuation is suggested in patients who seroconvert to anti-HBe on therapy, after a period of treatment consolidation with persistently normal ALT levels and undetectable serum HBV-DNA levels of at least 12 months	Treatment discontinuation may be considered in patients who have demonstrated loss of HBsAg
APASL [9]	Therapy can be stopped after at least 1 year, but preferably after 3 years of additional therapy after HBeAg seroconversion with undetectable HBV DNA by PCR and persistently normal ALT levels	Treatment can be withdrawn after HBsAg loss following either anti-HBs seroconversion or at least 12 months of a post-HBsAg clearance consolidation period, or after treatment for at least 2 years with undetectable HBV DNA documented on three separate occasions, 6 months apart

discontinuation is not an option for patients with cirrhosis both for HBeAg positive and negative CHB.

There is an overall agreement, in practically all national guidelines, that in HBeAg-positive noncirrhotic patients effective NA therapy, as defined in terms of virological and biochemical response, could be discontinued with the advent of verified HBeAg seroconversion followed with at least 1 year of consolidation therapy.

However, since serum HBsAg usually remains positive and about half of the HBeAg+ patients who discontinue NA treatment under the above criteria will eventually experience viral reactivation [12,20,21], one may choose to continue NA therapy under the treatment guidelines proposed by EASL and AASLD for patients with HBeAg-negative CHB, with the aim of HBsAg loss. HBeAg-positive patients who cleared HBeAg during treatment have been included in studies of long-term NA therapy and have been found to respond better after treatment discontinuation than their *ab initio* HBeAg-negative counterparts, exhibiting higher rates of functional cure and lower relapse rates, especially those of young age and low end of treatment HBsAg levels [22–26]. Although prolongation of NA therapy in HBeAg-positive patients is aiming at functional cure under treatment, this is still a rare event, and HBeAg seronversion is the widely accepted endpoint.

In pretreatment HBe-positive CHB, although there are data of high relapse rates [27], there is not a lot of debate on NA discontinuation, contrary to HBe negative CHB that is discussed next.

3. Discontinuation of NAs in HBeAg-negative CHB

As stated before, up to 2017 the Treatment Guidelines of AASLD and EASL, had been recommending that in patients with HBeAg-negative CHB, effective NA treatment, achieving clinical and virological remission, should continue up to HBsAg loss, preferably up to its seroconversion to anti-HBs [4,5]. However, this outcome, also referred to as functional HBV cure, may not be reached even by lifelong NA-therapy with compounds of high barrier to HBV resistance [28,29]. Thus, indefinite duration of such treatment has been recommended.

On the other hand, sustained clinical and virological remissions had already been reported in 2003 by Fung et al. in some patients with HBeAg-negative CHB after withdrawal of a 2-year therapy with Lamivudine [30]. This antiviral agent was the first NA registered in the treatment of CHB and has a low genetic barrier for HBV resistance. Later on, in a prospective study of HBeAg-negative patients with CHB who had achieved clinical and virological remission under Adefovir therapy of 4 or 5 years' duration, treatment was discontinued and the outcomes over a 6-year period of post-treatment follow-up were published in 2012 [31]. Although practically all patients experienced increases in serum ALT values and HBV viremia, if these relapses were not retreated immediately they were found to be transient, to resolve over time and be followed by HBsAg loss in the majority of the patients. This study was considered pivotal and has been followed by similar prospective studies that evaluated the outcomes of HBeAg-negative CHB after discontinuation of long therapy with the newer NAs with variable results and recommendations that have been discussed in several review articles [20,22,27,32–38].

Thus, current views on discontinuation of long-term treatment with NAs in HBeAg-negative patients with CHB are quite variable. The rational against discontinuation of such treatments in clinical practice is based on the possible risk of severe hepatic flares in patients who relapse, the need for frequent post-treatment monitoring and the lack of solid criteria for retreatment [39,40]. On the other side, discontinuation of NAs, following effective durable viral suppression, has shown promising results regarding long-term remission and increased probability of functional cure [31,33,41–43]. Moreover, lifelong duration of NA treatment poses a significant financial burden on health-care systems and a number of countries do not fully reimburse its cost. Additionally, although few side effects have been reported, the safety of very long-term treatment has to be proved in the future. Furthermore, it is rational to discontinue any therapy if it is of no further benefit to the patient.

Three are the major questions that arise:

3.1. What may happen over time after cessation of NA therapy in HBeAg-negative CHB patients and how frequently and for how long patients should be followed-up and monitored?

From the analysis of relevant data in the world literature on this topic the following four post-treatment outcomes are possible:

(1) Virological remission (VR) with non-detectable serum HBV DNA or detectable at levels lower than 2,000 IU/mL.
(2) Virological relapse, with serum HBV DNA becoming again detectable at levels above 2,000 IU/mL but with normal serum ALT values.
(3) Clinical relapse with serum HBV DNA higher than 2,000 IU/mL and increased ALT values (>2 or 3 fold the upper normal value).
(4) HBsAg loss also referred to as a functional cure of chronic HBV infection.

Patients with HBV DNA higher than 2,000 IU/mL and mild increases of ALT values less than 2 times the upper normal values need to be followed closely since they will frequently evolve either in virological remission and HBsAg loss or in clinical relapse.

Most relevant information is limited to two maximum 3 years of follow-up and refers mainly to the first three outcomes. Data on HBsAg loss are also available in the literature but since in most cases such favorable outcome takes several years post-treatment to be achieved it is underestimated in studies with short follow-up [31,41,44].

On the other hand, although virological and clinical relapses are more frequent in the first post-treatment period, they may also occur long after discontinuation of NA therapy [31,45]. Thus, for a reliable evaluation of post-treatment outcomes, a quite long-term follow-up is required. For the time being, such long-term post-treatment observations have been materialized only in few studies [31,46,47].

Post-treatment outcomes and the possibility of transition from one to the other are depicted in Figure 2. Virological

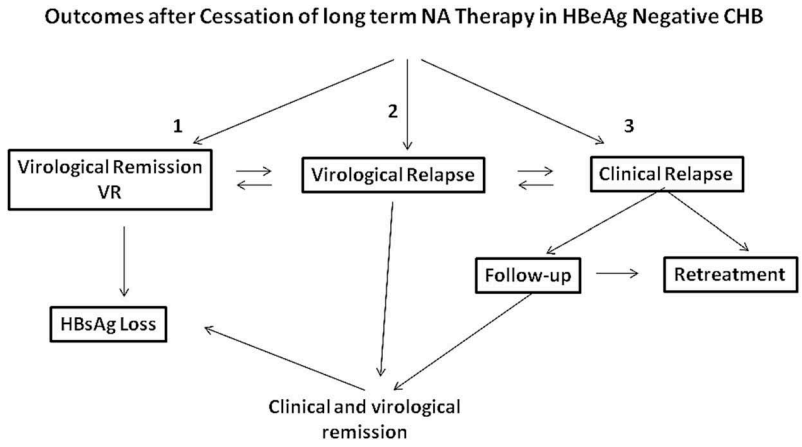

Figure 2. Possible outcomes of HBeAg-negative CHB after cessation of long-term NA therapy.

remission may be followed over time by HBsAg clearance with or without the development of anti-HBs or may evolve into virological relapse which in its turn may either reverse to virological remission or evolve to a clinical relapse of varying severity.

On the basis of hitherto reported data, following cessation of NA therapy, a regular clinical and laboratory evaluation at monthly or even closer at 15-day intervals during the first 3 months is imperative and then every 3–6 months up to HBsAg clearance. The laboratory tests must include HBsAg, HBV DNA, and ALT levels. HBsAg loss may take several years to occur but can be achieved in a significant proportion of patients [48,49]. Seroconversion to anti-HBs follows HBsAg loss, the latter being a robust and durable event [50].

3.2. How safe is to discontinue long-term NA therapy in patients with HBeAg-negative CHB and how to deal with clinical relapses?

In the first systematic review and meta-analysis published in the literature in 2015, it was stressed that clinical relapses occur in <50% of the patients. Off-therapy severe flares are rare and hepatic decompensation has been reported only in one patient with cirrhosis [36]. A subsequent systematic review and additional research articles, further support that with an appropriate stopping rule and a proper off-therapy monitoring plan, cessation of NA therapy is safe at least in non-cirrhotic CHB patients [20,32,33,51].

Close follow up ensures timely detection of relapses. If clinical relapses are immediately retreated, they are followed by prompt resolution of clinical and virological activity but with persistence of HBsAg seropositivity. On the other hand, untreated clinical relapses can resolve spontaneously with HBsAg titers declining over time up to HBsAg loss and even development of anti-HBs [31,33,52,53].

Post-treatment flares in ALT activity, referred to as clinical relapses, actually exhibit typical features of immunologically induced liver necro-inflammation. The sequence of events during clinical relapses is first a resumption of HBV replication with increasing serum HBV DNA levels occur, followed by increases in serum ALT values, serum IP-10 and anti-HBc IgM titers [54]. All recent data support the view that the restoration of the immunological host responsiveness against HBV is required for the achievement of HBsAg loss [55,56]. However, it remains uncertain if this can be accomplished in all CHB patients; even by the addition of therapeutic vaccines [57], and support the view that at the end of long-term NA therapy there is a partial restoration of the T cells with an increase in their functional efficiency that can culminate to functional HBV cure upon its discontinuation [58,59].

On the basis of these observations, we have already proposed that in patients with a major decrease in the number of HBsAg+ hepatocytes and with a major reduction of cccDNA in the liver at end of treatment (EOT) such an immune-mediated anti-HBV effect can be sufficient enough to clear any residual HBV in the liver and terminate to HBsAg clearance [54,60]. This view is further supported by recent immunological observations [61–65].

In the majority of published studies, it is reasonably suggested that retreatment should be considered only for patients experiencing post-treatment clinical relapses that do not tend to resolve spontaneously or for severe flares. A flare is considered severe when ALT is increased >10 fold the upper normal value or >5 fold the upper normal value combined with increased bilirubin >2 mg/dL and/or prolongation of prothrombin time (PT) [49]. It has been suggested that close monitoring of HBsAg titers after stopping NA therapy is important for the differentiation of benign flare that could lead to HBsAg seroclearance, from a flare that would lead to further liver deterioration and has to be treated. Decreasing HBsAg levels after the ALT peak were related with benign flares, and in those patients retreatment could be withheld [53]. Moreover, severe post-treatment relapses may become predictable prior to discontinuation of NA therapy on the basis of serological assays as of pregenomic HBV RNA and of core-related antigen [66]. Such information combined with other variables should be taken into account when considering stopping or continuing NA therapy in individual patients.

3.3. What are the best predictors/determinants of a favorable post-treatment outcome?

The outcomes of CHB after discontinuation of long NA therapy have been linked with the sex and age of patients, modes of

Table 2. Factors evaluated as predictors of sustained response and HBsAg clearance after NA discontinuation.

Parameter	Possible association with response	References
Age	Younger age	[23,25,84,86–89]
Sex	Female	[88,89]
Ethnicity	Caucasian	[89]
Duration of treatment	Long period >3 years	[20]
IL28B polymorphism	No association	[67]
ALT and HBV DNA at baseline	Lower levels	[68,88]
ALT and HBV DNA at the end of therapy	Normal/undetectable	[20. 89]
Serum levels of HBsAg at the end of therapy	<100 IU/mL prediction of functional cure	[31,44,69–74]
Serum levels of HBcrAg	< 3.4 log U/mL	[44,66,80,81,84]
Serum HBV RNA	Undetectable	[66,83]
Liver cccDNA	Low Levels < −0.8 Log copies/genome equivalent	[95,96]
HBsAg liver expression	Pattern and number of positive hepatocytes	[60]
Immunological parameters	HBV specific T cells	[92. 93]

acquisition of HBV infection, HBV genotypes, geographical areas, ethnicity, and other viral-related factors as HBsAg, HBcrAg levels, and HBV RNA in serum. These variables have been evaluated as possible predictors/determinants of short and long post-treatment outcomes and particularly of serum HBsAg loss.

In Table 2, we have summarized several variables that have been evaluated in different studies as predictors of sustained response (or clinical relapse) and of HBsAg clearance.

Low HBsAg titers, in most studies <100 IU/mL; at the end of treatment seem to be the most important independent predictor for subsequent functional cure [31,44,69–74]. Since such low levels of HBsAg are reached only in a small proportion of patients, it is important to recognize other factors linked with the favorable outcome of NA discontinuation [75]. It has been suggested that the titers of anti-HBc at the end of treatment could be used to determine the risk of relapse for patients with HBsAg levels >100 IU/mL [75]. Additionally, serial HBsAg measurements after the end of treatment are important since declining titers are associated with functional cure and increasing titers are predictors of relapse [53,73,76,77].

It is well known that NA therapy can not completely eliminate cccDNA or integrated viral sequences from the nucleus of the infected hepatocytes [78]. HBcrAg and serum HBV RNA have been shown to reflect better than HBsAg levels the transcriptional activity of cccDNA [79]. These markers have been recently associated with the outcome of NA cessation as predictors of severe clinical relapse [44,66,80–84]. Serum HBV RNA is a promising and specific marker mainly for the risk of viral rebound but lacks sensitivity since the majority of HBeAg-negative patients under effective NA treatment become serum HBV RNA negative [85].

Moreover, age seems to be an important factor with significantly lower relapse rates observed in younger <35 years old patients [23,25,84,86–89].

Since immunological responses are important for viral clearance several related biomarkers have been studied. Interferon-inducible protein-10 (IP10) level has been shown to reflect antiviral immunity and has been correlated with HBsAg loss when measured in serum 1 month after treatment discontinuation [44,90]. HBV core and polymerase specific PD-1 + T cells have also been suggested to be a candidate immunological biomarker for the safe discontinuation of treatment [91]. The study for immunological parameters, especially under treatment is ongoing but no clinically applicable predictive immunological biomarkers in serum or peripheral cells have been identified as yet [92,93].

Liver biomarkers, viral and immunological as cccDNA, viral antigens, and IP10, have been evaluated and appear to be more sensitive and specific than serum ones, but since a liver biopsy is needed for their study they not be used regularly as predictors of NA discontinuation [54,60,90,94–96].

A combination of serum biomarkers and other patient factors could probably provide a scoring system that would be a better predictor than individual markers.

These points are under ongoing research and current views continue to be variable and contrasting as discussed in a recent conference organized by EASL and AASL in March 2019 in London and an International Workshop on HBV Cure held in Toronto, Canada in November 2019. Actually, in the first conference therapeutic endpoints, trial designs, appropriate patient populations, and safety issues with regards to novel therapeutic approaches aiming to HBV cure were discussed and in the latter two sessions were dedicated to studies of NA discontinuation, on endpoints and biomarkers, revealing the significance and worldwide interest in this topic. The report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference has been published in the form of guidance for design and endpoints of clinical trials in chronic hepatitis B [97]. In this conference, it was agreed that the primary goal of treatment is sustained HBsAg loss with undetectable HBV DNA after completion of treatment and in the report is stated that more robust data are needed if patients withdrawn from NA therapy are to serve as a reference for assessing efficacy of finite courses of new HBV therapies aiming at functional cure. On this point, large randomized registered clinical studies are undergoing to identify patients who can safely stop NAs with favorable outcomes as are viral remission and functional cure. One example is clinical trial NCT03792919, which will be comparing cessation of long-term NA therapy to ongoing NA treatment, with an estimated enrollment of 2000 participants and completion date June 30, 2024. In this study, the primary outcome is the incidence of HBsAg clearance and secondary outcomes are HBsAg seroconversion, sustained HBV viral remission, liver failure, and the incidence of hepatocellular carcinoma from baseline to the end of the fifth year after cessation of anti-HBV treatment. In another study (NCT04102176) the incidence of HBsAg loss will be evaluated in an estimated population of 150 participants divided into two arms (stopping and continuing treatment). These interventional, randomized, open-label studies are in early stages, currently recruiting patients; thus, results are not anticipated soon.

4. Conclusion

Discontinuation of NA therapy in HBeAg-positive noncirrhotic patients after HBeAg seroconversion and consolidation treatment is considered in all international treatment guidelines. NA treatment cessation in HBeAg-negative noncirrhotic patients is a safe option, provided that close and long-term post-treatment follow-up is feasible. This approach is currently gaining increasing acceptance worldwide. Functional cure is achieved in higher rates in HBeAg-negative patients who discontinue NA treatment. Sensitive and specific predictive biomarkers to guide the decision of NA cessation are under investigation. For the time being, end of treatment HBsAg level is the best predictor of functional cure.

5. Expert Opinion

Discontinuation of NA treatment in noncirrhotic HBeAg-positive patients with CHB after loss of HBeAg followed by 2 years of treatment consolidation is a widely accepted approach. In such patients, who have become HBeAg-negative, whether with or without the development of anti-HBe, continuation of NA therapy up to HBsAg loss (functional HBV cure) is like treating HBeAg-negative CHB but without any evidence that actually liver disease activity is going on.

Contrasting views regarding NA discontinuation pertain to patients with HBeAg-negative chronic HBV infection with active liver disease (CHB), and actually this is the central point on which the present review article has been focused.

Prerequisite for HBV infection to become chronic is that the viral genome of relaxed circular DNA that is present in hepatitis B virions is transformed into circular covalently closed DNA (cccDNA), that persists in the nuclei of infected hepatocytes. This molecule is a stable template for HBV transcription and cannot be eliminated by treatment with NAs regardless of its duration. Long-term NA therapy inhibits the step of reverse transcription of viral RNA to HBV DNA that takes place in the liver and is a prerequisite for HBV replication. Thus, NAs can silence cccDNA but cannot eliminate it from the liver. Even so, by inhibiting the step of reverse transcription of HBV RNA to HBV DNA they suppress viral replication and in the long run clearance of HBsAg had been expected. However, it has turned out that the incidence of this endpoint under NA therapy is very low and may not be achieved even by lifelong duration of such therapy. Nevertheless, mounting evidence from several prospective studies has revealed that HBsAg loss and development of anti-HBs occur much more frequently after discontinuation than during NA therapy. This unexpected finding first reported by our research group has now been widely confirmed worldwide.

HBeAg-negative patients with CHB effectively treated with NAs for five or more years with undetectable serum HBV DNA, upon discontinuation of therapy experience viral reactivation and increase in serum ALT levels. This relapse is attributed to the resumption of HBV replication from viral cccDNA in the liver that triggers immune mechanisms that recognize the viral targets and/or nonspecific immune responses that ultimately destroy infected hepatocytes. These immunological events reduce the pool of cccDNA and also lead to a decrease in HBsAg levels up to HBsAg loss. Hence, flares after NA discontinuation should not be treated immediately, unless they are severe. Since flares could actually be beneficial we believe that in the future, further boosting of the immune system at the time of NA discontinuation could be used for maximizing favorable post-treatment outcomes.

For the time being, cessation of NA treatment in noncirrhotic patients has been shown to be safe, not posing a risk for the patient, provided a close and regular post-treatment monitoring is followed. Moreover, HBsAg loss and subsequent development of anti-HBs, are much more frequent after discontinuation of NAs than during continuous therapy. Thus, if loss of HBsAg with or without seroconversion to anti-HBs is not achieved during a 5-year period of NA therapy we recommend its discontinuation only in noncirrhotic HBeAg-negative patients, provided that HBsAg levels have declined significantly preferably to less than 200 IU/mL and that the patient understands why a strict post-treatment follow-up is necessary and agrees to its implementation. In such patients, there is a chance for functional HBV cure. Since immune parameters play a significant role, we anticipate that appropriate immunological markers will be applied in conjunction with HBsAg levels for the prediction of such favorable outcomes. Moreover, serum HBV RNA, which is currently measured by homemade real-time PCR assays, will be used widely and provide important information on cccDNA transcription levels, and for prediction of severe post-treatment relapse when commercial well standardized and approved assays become available.

An important issue that has been raised is whether NA treatment per se reduces the risk for the development of HCC. It has been well documented that even patients that have achieved functional cure are still at risk for HCC. Residual cccDNA and integrated viral sequences in the genome of hepatocytes are risk factors for hepatocarcinogenesis. In our ongoing studies as well as in the literature there is no evidence of reduced risk for HCC in patients who continue treatment versus those who stop NAs. Therefore, in our opinion discontinuation of NAs does not pose increased risk for HCC but even so the patients should continue post-treatment monitoring for HCC.

Views and recommendations on the duration and end-points of NA therapy in CHB with current first-line compounds will continue to vary geographically depending on the prevailing socioeconomic factors and conditions, at least up to the development of curative therapies. Research for such therapies aiming at virological cure with clearance or silencing of cccDNA from the nuclei of the hepatocytes is ongoing [98,99]. Nevertheless, even if such a virological cure is achieved, integrated viral sequences in the genome of the hepatocytes will remain and could still pose a risk for the development of HBV related hepatocellular carcinoma (HCC) [100]. Until the development of such therapies, discontinuation of NAs in selected patients will be expanding as this approach leads to high rates of the closest to cure outcome of HBsAg loss. Hopefully, sensitive and specific predictive biomarkers will soon be available to guide the decision of NA cessation.

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Declaration of Interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Sande MA, Carpenter CC, Cobbs CG, et al. Antiretroviral therapy for adult HIV-infected patients. Recommendations from a state-of-the-art conference. National institute of allergy and infectious diseases state-of-the-art panel on anti-retroviral therapy for adult HIV-infected patients. Jama. 1993;270(21):2583–2589.
2. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999;341(17):1256–1263.
3. Scott LJ, Chan HLY. Tenofovir Alafenamide: A review in chronic hepatitis B. Drugs. 2017;77(9):1017–1028.
4. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57(1):167–185.
5. Lok AS, McMahon BJ. Chronic hepatitis B: update. Hepatology. 2009;50(3):661–662. 2009.
6. Liaw YF, Leung N, Guan R, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update. Liver Int. 2005;25(3):472–489.
7. Liaw YF, Leung N, Kao JH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2(3):263–283.
8. Liaw YF, Kao JH, Piratvisuth T, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int. 2012;6(3):531–561.
9. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10(1):1–98.
10. Terrault NA, Bzowej NH, Chang KM, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. 2016;63(1):261–283.
11. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67(4):1560–1599.
12. Lampertico P, Agarwal K, Berg T. EASL. Clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67(2):370–398. 2017.
13. Tong MJ, Pan CQ, Han SB, et al. An expert consensus for the management of chronic hepatitis B in Asian Americans. Aliment Pharmacol Ther. 2018;47(8):1181–1200.
14. Arora A, Singh SP, Kumar A, et al. INASL position statements on prevention, diagnosis and management of hepatitis B virus infection in india: the Andaman statements. J Clin Exp Hepatol. 2018;8(1):58–80.
15. Korean Association for the Study of the Liver. KASL clinical practice guidelines for management of chronic hepatitis B. Clin Mol Hepatol. 2019;25(2):93–159.
16. Ghany MG. Current treatment guidelines of chronic hepatitis B: the role of nucleos(t)ide analogues and peginterferon. Best Pract Res Clin Gastroenterol. 2017;31(3):299–309.
17. Suk-Fong Lok A. Hepatitis B treatment: what we know now and what remains to be researched. Hepatol Commun. 2019;3(1):8–19.
18. WHO. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. 2015.
19. Vittal A, Ghany MG. WHO guidelines for prevention, care and treatment of individuals infected with HBV: a US perspective. Clin Liver Dis. 2019;23(3):417–432.
20. Papatheodoridis G, Vlachogiannakos I, Cholongitas E, et al. Discontinuation of oral antivirals in chronic hepatitis B: A systematic review. Hepatology. 2016;63(5):1481–1492.
21. Van Hees S, Chi H, Hansen B, et al. Caucasian ethnicity, but not treatment cessation is associated with HBsAg loss following nucleos(t)ide analogue-induced HBeAg seroconversion. Viruses. 2019;11:8.
22. Buti M, Tsai N, Petersen J, et al. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. Dig Dis Sci. 2015;60(5):1457–1464.
23. Cao J, Chi H, Yu T, et al. Off-treatment hepatitis B virus (HBV) DNA levels and the prediction of relapse after discontinuation of nucleos(t)ide analogue therapy in patients with chronic hepatitis B: a prospective stop study. J Infect Dis. 2017;215(4):581–589.
24. Chen CH, Hsu YC, Lu SN, et al. The incidence and predictors of HBV relapse after cessation of tenofovir therapy in chronic hepatitis B patients. J Viral Hepat. 2018;25(5):590–597.
25. Xu WX, Zhang Q, Zhu X, et al. 48-week outcome after cessation of nucleos(t)ide analogue treatment in chronic hepatitis B patient and the associated factors with relapse. Can J Gastroenterol Hepatol. 2018;1817680:2018.
26. Buti M, Wong DK, Gane E, et al. Safety and efficacy of stopping tenofovir disoproxil fumarate in patients with chronic hepatitis B following at least 8 years of therapy: a prespecified follow-up analysis of two randomised trials. Lancet Gastroenterol Hepatol. 2019;4(4):296–304.
27. Liem KS, Fung S, Wong DK, et al. Limited sustained response after stopping nucleos(t)ide analogues in patients with chronic hepatitis B: results from a randomised controlled trial (Toronto stop study). Gut. 2019;68(12):2206–2213.
28. Chevaliez S, Hezode C, Bahrami S, et al. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol. 2013;58(4):676–683.
29. Striki A, Manolakopoulos S, Deutsch M, et al. Hepatitis B s antigen kinetics during treatment with nucleos(t)ides analogues in patients with hepatitis B e antigen-negative chronic hepatitis B. Liver Int. 2017;37(11):1642–1650.
30. Fung SK, Wong F, Hussain M, et al. Sustained response after a 2-year course of lamivudine treatment of hepatitis B e antigen-negative chronic hepatitis B. J Viral Hepat. 2004;11(5):432–438.
31. Hadziyannis SJ, Sevastianos V, Rapti I, et al. Sustained responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop long-term treatment with adefovir. Gastroenterology. 2012;143(3):629–636.e621.

•• **Pivotal study for discontinuation of NAs, showing high rates of HBsAg loss in long post tratment follow up**

32. Berg T, Simon KG, Mauss S, et al., Long-term response after stopping tenofovir disoproxil fumarate in non-cirrhotic HBeAg-negative patients - FINITE study. J Hepatol. 2017;67(5):918–924.

•• **Recent study confirming the results of NA cessation with NAs with high genetic barrier for resistance.**

33. Papatheodoridis GV, Rigopoulou EI, Papatheodoridi M, et al. DARING-B: discontinuation of effective entecavir or tenofovir disoproxil fumarate long-term therapy before HBsAg loss in non-cirrhotic HBeAg-negative chronic hepatitis B. Antivir Ther. 2018;23(8):677–685.
34. Seto WK, Hui AJ, Wong VW, et al. Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative chronic hepatitis B: a multicentre prospective study. Gut. 2015;64(4):667–672.
35. Wübbolding M, Cornberg M, Höner Zu Siederdissen C. Evidence-based approach to stopping oral antiviral therapy in chronic HBV. Curr Hepatol Rep. 2019;18(4):512–521. 9.
36. Chang ML, Liaw YF, Hadziyannis SJ. Systematic review: cessation of long-term nucleos(t)ide analogue therapy in patients with hepatitis

B e antigen-negative chronic hepatitis B. Aliment Pharmacol Ther. 2015;42(3):243–257.
37. Chen CH, Hu TH, Wang JH, et al. Comparison of HBsAg changes between HBeAg-negative patients who discontinued or maintained entecavir therapy. Hepatol Int. 2019. doi:10.1007/s12072-019-09991-y.
38. Liaw YF. Finite nucleos(t)ide analog therapy in HBeAg-negative chronic hepatitis B: an emerging paradigm shift. Hepatol Int. 2019;13(6):665–673.
39. Marciano S, Gadano A. Why not to stop antiviral treatment in patients with chronic hepatitis B. Liver Int. 2018;38(Suppl 1):97–101.
40. Moreno-Cubero E, Del Arco RTS, Pena-Asensio J, et al. Is it possible to stop nucleos(t)ide analogue treatment in chronic hepatitis B patients? World J Gastroenterol. 1825–1838;24(17):2018.
41. Chen CH, Hung CH, Wang JH, et al. The incidence of hepatitis b surface antigen loss between hepatitis B E antigen-negative noncirrhotic patients who discontinued or continued entecavir therapy. J Infect Dis. 2019;219(10):1624–1633.
42. Muche M, Meyer U, Siegmund B, et al. Sustained off-treatment response after discontinuation of long-term nucleos(t)ide analogue treatment in HBeAg-seronegative hepatitis B: a case series. Top Antivir Med. 2017;25(3):114–117.
43. Dusheiko G, Wang B. Hepatitis B surface antigen loss: too little, too late and the challenge for the future. Gastroenterology. 2019;156(3):548–551.
44. Papatheodoridi M, Hadziyannis E, Berby F, et al. Predictors of hepatitis B surface antigen loss, relapse and retreatment after discontinuation of effective oral antiviral therapy in noncirrhotic HBeAg-negative chronic hepatitis B. J Viral Hepat. 2020;27(2):118–126.
45. Papatheodoridis GV, Manolakopoulos S, Su TH, et al. Significance of definitions of relapse after discontinuation of oral antivirals in HBeAg-negative chronic hepatitis B. Hepatology. 2018;68(2):415–424.
46. Chen DB, Chen YM, Liu J, et al. Durability of efficacy after telbivudine off-treatment in chronic hepatitis B patients. J Clin Virol. 2014;59(1):50–54.
47. Pan HY, Chen L, Yang DH, et al. Ten-year follow-up of hepatitis B relapse after cessation of lamivudine or telbivudine treatment in chronic hepatitis B patients. Clin Microbiol Infect. 2015;21(12):1123.e1121–1129.
48. Hadziyannis S, Liaw YF. Discontinuation of long-term NA therapy in HBeAg-negative chronic hepatitis B. In. Gut. 2015;64(6):1005–1006. England.
49. Papatheodoridi M, Papatheodoridis G. Can we stop nucleoside analogues before HBsAg loss? J Viral Hepat. 2019;26(8):936–941.
- **Excellent recent review with details in definitions of terms concerning NA discontinuation**
50. Alawad AS, Auh S, Suarez D, et al. Durability of spontaneous and treatment-related loss of hepatitis B s antigen. Clin Gastroenterol Hepatol. 2020;18(3):700–709.
51. van Bommel F, Berg T. Stopping long-term treatment with nucleos(t)ide analogues is a favourable option for selected patients with HBeAg-negative chronic hepatitis B. Liver Int. 2018;38(Suppl 1):90–96.
52. Jeng WJ, Chen YC, Chien RN, et al. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. Hepatology. 2018;68(2):425–434.
- **Excellent study in Asian patients, with practical implications on NA discontinuaton**
53. Liaw YF, Jeng WJ, Chang ML. HBsAg kinetics in retreatment decision for off-therapy hepatitis B flare in HBeAg-negative patients. Gastroenterology. 2018;154(8):2280–2281.
54. Hadziyannis SJ. Update on hepatitis B virus infection: focus on treatment. J Clin Transl Hepatol. 2014;2(4):285–291.
55. Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. J Hepatol. 2016;64(1 Suppl):S71–s83.
56. Maini MK, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. J Hepatol. 2016;64(1 Suppl):S60–s70.
57. Ning Q, Wu D, Wang GQ, et al., Roadmap to functional cure of chronic hepatitis B: an expert consensus. J Viral Hepat. 2019;26(10):1146–1155.
- **Comprehensive review of immunological mechanisms leading to HBsAg loss after stopping antiviral therapy.**
58. Ferrari C, Boni C, Rossi M, et al. T cell regulation in HBV-related chronic liver disease. J Hepatol. 2017;66(5):1096–1098.
59. Wang H, Luo H, Wan X, et al. TNF-alpha/IFN-gamma profile of HBV-specific CD4 T cells is associated with liver damage and viral clearance in chronic HBV infection. J Hepatol. 2020;72(1):45–56.
60. Hadziyannis SJ, Vassilopoulos D, Sevastianos V, et al. Can nucleos(t)ide analogue (na) therapy ever be stopped in HBeAg-negative chronic hepatitis B. Curr Hepatol Rep. 2014;13(3):256–263.
61. Rinker F, Zimmer CL, Honer Zu Siederdissen C, et al. Hepatitis B virus-specific T cell responses after stopping nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. J Hepatol. 2018;69(3):584–593.
62. Zimmer CL, Rinker F, Honer Zu Siederdissen C, et al. Increased NK cell function after cessation of long-term nucleos(t)ide analogue treatment in chronic hepatitis B is associated with liver damage and HBsAg loss. J Infect Dis. 2018;217(10):1656–1666.
63. Honer Zu Siederdissen C, Rinker F, Maasoumy B, et al. Viral and host responses after stopping long-term nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. J Infect Dis. 2016;214(10):1492–1497.
64. Jacobi FJ, Wild K, Smits M, et al. OX40 stimulation and PD-L1 blockade synergistically augment HBV-specific CD4 T cells in patients with HBeAg-negative infection. J Hepatol. 2019;70(6):1103–1113.
65. Fanning GC, Zoulim F, Hou J, et al. Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat Rev Drug Discov. 2019;18(11):827–844.
66. Carey I, Gersch J, Wang B, et al. Pre-genomic HBV RNA and HBcrAg predict outcomes in HBeAg negative chronic hepatitis B patients suppressed on nucleos(t)ide analogue therapy. Hepatology. 2019. doi:10.1002/hep.31026.
67. Hadziyannis E, Laras A, Panopoulou E, et al. IL28B polymorphisms in patients with HBeAg negative chronic HBV infection genotype D. Acta Microbiol Hellenica. 2017;62(2):81–89.
68. Jeng WJ, Sheen IS, Chen YC, et al. Off-therapy durability of response to entecavir therapy in hepatitis B e antigen-negative chronic hepatitis B patients. Hepatology. 2013;58(6):1888–1896.
69. Chen CH, Hung CH, Wang JH, et al. Long-term incidence and predictors of hepatitis B surface antigen loss after discontinuing nucleoside analogues in noncirrhotic chronic hepatitis B patients. Clin Microbiol Infect. 2018;24(9):997–1003.
70. Hsu YC, Mo LR, Chang CY, et al. Association between serum level of hepatitis B surface antigen at end of entecavir therapy and risk of relapse in E antigen-negative patients. Clin Gastroenterol Hepatol. 2016;14(10):1490–1498.e1493.
71. Liu J, Li T, Zhang L, et al. The role of hepatitis B surface antigen in nucleos(t)ide analogues cessation among asian patients with chronic hepatitis B: a systematic review. Hepatology. 2019;70(3):1045–1055.
72. Yao CC, Hung CH, Hu TH, et al. Incidence and predictors of HBV relapse after cessation of nucleoside analogues in HBeAg-negative patients with HBsAg</= 200 IU/mL. Sci Rep. 2017;7(1):1839.
73. Jeng W-J, Chang M-L, Liaw Y-F. Off-therapy precipitous HBsAg decline predicts HBsAg loss after finite entecavir therapy in HBeAg-negative patients. J Viral Hepat. 2019;26(8):1019–1026.
74. Chong CH, Lim SG. When can we stop nucleoside analogues in patients with chronic hepatitis B? Liver Int. 2017;37(Suppl 1):52–58.
75. Chi H, Li Z, Hansen BE, et al. Serum level of antibodies against hepatitis b core protein is associated with clinical relapse after discontinuation of nucleos(t)ide analogue therapy. Clin Gastroenterol Hepatol. 2019;17(1):182–191.e181.
76. Liaw YF. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative chronic HBV infection. Nat Rev Gastroenterol Hepatol. 2019;16(10):631–641.

77. Su TH, Yang HC, Tseng TC, et al. Distinct relapse rates and risk predictors after discontinuing tenofovir and entecavir therapy. J Infect Dis. 2018;217(8):1193–1201.
78. Alter HJ, Chisari FV. Is elimination of hepatitis B and C a pipe dream or reality? Gastroenterology. 2019;156(2):294–296.
79. Caviglia GP, Pellicano R, Saracco GM, et al. Hepatitis B core-related antigen: a serum biomarker for intrahepatic covalently-closed-circular DNA. Clin Lab. 2018;64(3):411–412.
80. Mak LY, Wong DK, Cheung KS, et al. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. Aliment Pharmacol Ther. 2018;47 (1):43–54.
81. Caviglia GP, Smedile A. Hepatitis B core-related antigen: a novel biomarker for chronic hepatitis B treatment. Minerva Gastroenterol Dietol. 2017;63(3):169–171.
82. Honer Zu Siederdissen C, Maasoumy B, Cornberg M. New viral biomarkers for Hepatitis B: are we able to change practice? J Viral Hepat. 2018;25(11):1226–1235.
83. Liu YY, Liang XS. Progression and status of antiviral monitoring in patients with chronic hepatitis B: from HBsAg to HBV RNA. World J Hepatol. 2018;10(9):603–611.
84. Jung KS, Park JY, Chon YE, et al. Clinical outcomes and predictors for relapse after cessation of oral antiviral treatment in chronic hepatitis B patients. J Gastroenterol. 2016;51 (8):830–839.
85. Hadziyannis E, Laras A. Viral biomarkers in chronic HBeAg negative HBV infection. Genes (Basel). 2018;9:10.
86. Liu F, Wang L, Li XY, et al. Poor durability of lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients. J Gastroenterol Hepatol. 2011;26(3):456–460.
87. Ha M, Zhang G, Diao S, et al. A prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients with stringent cessation criteria for adefovir. Arch Virol. 2012;157(2):285–290.
88. Chen CH, Lu SN, Hung CH, et al. The role of hepatitis B surface antigen quantification in predicting HBsAg loss and HBV relapse after discontinuation of lamivudine treatment. J Hepatol. 2014;61 (3):515–522.
89. Liu Y, Jia M, Wu S, et al. Predictors of relapse after cessation of nucleos(t)ide analog treatment in HBeAg-negative chronic hepatitis B patients: A meta-analysis. Int J Infect Dis. 2019;86:201–207.
90. Zhao K, Yang T, Sun M, et al. IP-10 expression in patients with chronic HBV infection and its ability to predict the decrease in HBsAg levels after treatment with entecavir. Mol Cells. 2017;40(6):418–425.
91. Rivino L, Le Bert N, Gill US et al. Hepatitis B virus-specific T cells associate with viral control upon nucleos(t)ide-analogue therapy discontinuation. J Clin Invest. 2018;128(2):668–681.
92. Kranidioti H, Manolakopoulos S, Kontos G, et al. Immunological biomarkers as indicators for outcome after discontinuation of nucleos(t)ide analogue therapy in patients with HBeAg-negative chronic hepatitis B. J Viral Hepat. 2019;26(6):697–709.
93. Gill US, Battisti A, Kennedy PTF. Emerging tools in the changing landscape of chronic hepatitis B management. Expert Rev Anti Infect Ther. 2019;17(12):943–955.
94. Lai CL, Wong D, Ip P, et al. Reduction of covalently closed circular DNA with long-term nucleos(t)ide analogue treatment in chronic hepatitis B. J Hepatol. 2017;66(2):275–281.
95. Werle-Lapostolle B, Bowden S, Locarnini S, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126(7):1750–1758.
96. Sung JJ, Wong ML, Bowden S, et al. Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained response to therapy. Gastroenterology. 2005;128(7):1890–1897.
97. Cornberg M, Lok AS, Terrault NA, et al. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference(double dagger). J Hepatol. 2019. doi:10.1002/hep.31030
98. Gane EJ. Future anti-HBV strategies. Liver Int. 2017;37(Suppl 1):40–44.
99. Tang L, Zhao Q, Wu S, et al. The current status and future directions of hepatitis B antiviral drug discovery. Expert Opin Drug Discov. 2017;12(1):5–15.
100. Podlaha O, Wu G, Downie B, et al. Genomic modeling of hepatitis B virus integration frequency in the human genome. PLoS One. 2019;14(7):e0220376.