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Views on stopping nucleos(t)ide analogue therapy in patients with chronic hepatitis B

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Abstract

Long-term treatment with nucleos(t)ide analogs (NAs) is the current first line therapy for patients with chronic hepatitis B (CHB), recommended by most of the current guidelines. NAs prevent disease progression, liver failure, decrease the risk of hepatocellular carcinoma (HCC), and have favorable safety profiles. However, low rates of on-therapy functional cure (hepatitis B surface antigen [HBsAg] loss), which is regarded as the optimal end point, prevent many patients from stopping NA therapy with the need for a lifelong treatment. The higher likelihood of HBsAg loss associated with stopping as compared to continuing NAs has got a lot of attention lately. Recommendations regarding endpoints allowing for safely stopping NA therapy differ between the different international guidelines. Whereas in HBeAg-positive patients, HBeAg seroconversion with at least one year of consolidation therapy is an accepted endpoint of treatment, the recommendations for HBeAg-negative ones differ. Some guidelines propose ≥ 3 years of HBV DNA undetectability to stop NA while others regard HBsAg loss as the only acceptable endpoint to therapy. Stopping NA can lead to substantial rates of virologic relapses and consequent ALT flares in some of them. Moreover, no reliable predictor(s) of post-NA relapses have been identified so far. Quantitative HBsAg is becoming an increasingly promising marker to predict safe NA cessation. On the other hand, investigating the role of the immune system in mediating sustained virologic responses after NA withdrawal is needed to suggest immunological biomarkers to safely stop NA. In this article, we will review relevant literature regarding NA stopping rules and discuss promising viral and immunological biomarkers to predict antiviral responses and thus to help identify patients who are more likely to achieve HBsAg seroclearance.

Highlights

- NA therapy can effectively reduce HBV load but cannot prevent the risk of rebound after treatment interruption due to the presence of cccDNA and integrated HBV sequences in infected hepatocytes.
- NA cessation in HBeAg-positive non-cirrhotic patients is considered after HBeAg seroconversion and consolidation treatment.
- NA cessation in HBeAg-negative patients has become an interesting approach to reach functional cure.
- Better definition of patients with a high chance to achieve HBsAg loss after stopping NAs is needed.
- Retreatment criteria and monitoring frequencies should be standardized.
- End of treatment HBsAg level is a promising predictor of outcome for safe NA discontinuation.
- There is an urgent need to investigate immunological predictors of safe NA cessation, such as HBV-specific CD8+ T cells.

Keywords

Quantitative HBsAg, relapse, immune control, NA discontinuation, HBV infection.

Abbreviations

AASLD, American Association for the Study of Liver Diseases; ADV, adefovir dipivoxil; AHB, Acute hepatitis B; ALT, alanine aminotransferase; anti-HBc, Hepatitis B core antibody; anti-HBe, Hepatitis B e antibody; anti-HBs, hepatitis B surface antigen antibody; APASL, Asian Pacific Association for the Study of the Liver; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CTL, Cytotoxic T cell; CXCL-10, C-X-C motif

chemokine 10; DC, Dendritic cells; DNA, Desoxyribonucleic acid; EASL, European Association for the Study of the Liver; ETV, entecavir; FASLG, Fas Ligand Gene; HBcAg, Hepatitis B core antigen; HBcrAg, Hepatitis B core-related antigen; HBeAg, Hepatitis B e antigen; HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFNγ, interferon gamma; IL, Interleukin; LAM, Lamivudine; NA, nucleos(t)ide analogue; NK cells, Natural Killer cells; PD-1, Programmed cell death protein 1; PEG-IFN, pegylated interferon; snp, single nucleotide polymorphism; TAF, tenofovir alafenamide; T-bet; TBV, Telbivudine; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; TGF-β, Tumor growth factor beta; Tim-3, T-cell immunoglobulin and mucin-domain containing-3; TLR, toll like receptor; TNFα, Tumor necrosis factor alpha; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; WHO, World Health Organization.

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1. Introduction

Hepatitis B virus (HBV) infection remains a major global health concern as over 257 million individuals are estimated to be carriers of hepatitis B 'surface' antigen (HBsAg), the hallmark of chronic HBV infection (CHB)(European Association for the Study of the Liver, 2017; World Health Organization et al., 2017). CHB has potential adverse outcomes, including cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC)(European Association for the Study of the Liver. 2017; Sarin et al., 2016; Terrault et al., 2018). In 2015, hepatitis B resulted in an estimated 887 000 deaths from these liver diseases according to the World Health organization (WHO)(World Health Organization et al., 2017). HBsAg correlates with the presence of covalently closed circular DNA (cccDNA), in Hepatitis B 'e' antigen (HBeAg)-positive CHB patients, and is a key marker of infection(Tout et al., 2020). Its clearance is a reasonable therapeutic end point and is also referred to as a functional cure of CHB(Martinot-Peignoux et al., 2014). Although nucleos(t)ide analogues (NA) and immunomodulators (pegylated interferon (PEG-IFN)) are recommended therapies, most patients take NA due to the easy administration, safety, and efficacy(Cornberg et al., 2020). These therapies reduce viral replication and their aims include improving quality of life and decreasing the risk of liver disease-related complications(Marcellin et al., 2019; Papatheodoridis et al., 2018c) but however they rarely lead to HBsAg seroclearance. Long term NA therapy raises some concerns such as adherence, side effects, and, particularly, costs (Lampertico and Berg, 2018; Papatheodoridis et al., 2016). Additionally, a significant proportion of patients may want to discontinue therapy for various reasons. Thus, defining rules to stop NA therapy is of paramount importance and safety without loss of efficacy must be

considered(Lampertico and Berg, 2018). The concept of NA discontinuation was first mentioned in 2000 APASL guidelines and the first stopping rule for HBeAg-negative CHB was included in 2008 APASL guidelines, driven by cost and drug-resistance issues (Liaw et al., 2008). These recommendations were based on several earlier studies in Asian patients treated with lamivudine (LAM) showing that the sustained response rate 6-12 months after cessation of LAM therapy was around 50% if patients had achieved a maintained virological response before stopping LAM therapy (Chan et al., 2007; Chien and Liaw, 2006; Fung et al., 2004). This NA cessation concept was later initiated in Western countries with the study by Hadziyannis et al. showing a remarkably high HBsAg loss rate when stopping NAs(Hadziyannis et al., 2012).

In this review, we discuss current international guidelines for stopping NA therapy and we evaluate data of HBsAg loss in different categories of CHB patients. Moreover, potential virologic and immunologic predictors of NA cessation may also be considered to precisely determine the outcome of HBV infection, and identify patients who are more likely to achieve HBsAg seroclearance or a sustained response.

2. Goals and outcomes of NA therapy

The goal of therapy for CHB is to improve survival by preventing disease progression and improving quality of life. This is best achieved by long-term NA therapy leading to HBV suppression, associated with a reduction in histological activity, a lower risk of cirrhosis, and reversal of fibrosis(European Association for the Study of the Liver. 2017; Marcellin et al., 2019; Marcellin and Asselah, 2013; Sarin et al., 2016; Terrault et al., 2018). NA mechanism of action consists of viral polymerase inhibition, which

leads to decreased virion assembly, and thus a reduction of the viral replication and load (Asselah et al., 2019). According to the European Association for the Study of the Liver (EASL)(European Association for the Study of the Liver. 2017) and the American Association for the Study of Liver Diseases (AASLD)(Terrault et al., 2018), the currently approved and first-line recommended NA drugs are tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), and entecavir (ETV) due to their high barrier to HBV resistance, their high effectiveness, and their well tolerance. Other NAs such as Lamivudine (LAM), Telbivudine (TBV) and adefovir dipivoxil (ADV) should not be used as first-line treatment options due to their low barrier to HBV resistance causing resistance strains and insufficient virologic response. The main advantage of NA treatment is its predictable high long-term antiviral efficacy leading to undetectable HBV DNA levels in the vast majority of compliant patients as well as its favorable safety profile(European Association for the Study of the Liver. 2017; Lok et al., 2016; Terrault et al., 2018). Over 8 years (Papatheodoridis et al., 2018c) and 10 years(Marcellin et al., 2019), NAs have a favorable safety profile, are well tolerated, and resulted in continued maintenance of virologic suppression.

The ideal endpoint of therapy in HBeAg-positive and HBeAg-negative patients is sustained HBsAg loss with or without seroconversion to anti-HBs, which is usually associated with complete and definitive remission of the activity of CHB (Kim et al., 2014). However, these endpoints are rarely achieved because of the presence of the covalently closed circular DNA (cccDNA) minichromosome, which remains untouched in the nuclei of infected hepatocytes as well as the presence of integrated HBV sequences in hepatocytes genome which can cause reactivation of the disease and relapse of viraemia. Since these goals are rarely reached, surrogate endpoints have been identified to assess efficacy, including improved liver inflammation

(biochemical remission), viral suppression (virologic remission) and HBeAg seroconversion (European Association for the Study of the Liver. 2017). Achievement of these objectives has been shown to stop the inflammation cascade and fibrosis progression (Marcellin et al., 2013). NA treatment rarely achieve a functional cure and necessitate lifelong therapy in addition to other concerns such as adherence (Ford et al., 2018), side effects (Fontana, 2009; Marcellin et al., 2019), and, particularly, costs (Aiello et al., 2016). Therefore, the concept of NA treatment cessation is an interesting strategy to consider and further exploration of the recommended rules of stopping NAs before a functional cure and the benefits gained is needed. It is rational to discontinue any therapy if it is of no further benefit to the patient (Hadziyannis and Hadziyannis, 2020).

3. Aim of NA therapy cessation strategy

Over the last years, there has been a growing body of evidence that assessed the potential benefits, the safety, and the efficacy of stopping long-term NA therapy. One of the first pivotal studies regarding the NA cessation approach was a small, prospective cohort study from Greece that assessed the biochemical and virologic effects of stopping long-term NA therapy in HBeAg-negative CHB patients (Hadziyannis et al., 2012). After being treated for 4–5 years, all 33 patients developed virologic relapse once ADV was stopped, 76% had biochemical relapse, and 45% were retreated. Interestingly, among those with sustained biochemical and virologic remission, 39% reached HBsAg loss during 5.5 years of follow-up (Hadziyannis et al., 2012). This is a remarkable finding compared to the very low HBsAg clearance rate during NA therapy. Quantitative HBsAg (qHBsAg) at the time of stopping therapy was < 1000 IU/mL which suggested that monitoring of HBsAg level can guide the timing of stopping NAs. Since then, a series of studies attempted

to determine the occurrence of HBsAg loss after stopping NA therapy. Chan et al. reported that 20% of patients achieved HBsAg seroclearance after stopping LAM therapy (Chan et al., 2011). The main predictor of HBsAg seroclearance was end of therapy qHBsAg levels < 2 log IU/mL and > 1 log reduction from baseline. A randomized study (the FINITE study)(Berg et al., 2017) compared standard TDF therapy continuation (n=21) against its interruption (n=21) in HBeAg-negative patients that had been under NAs for at least 3,5 years. Thirteen out of 21 patients in the NA stopping group remained off-therapy and 4 of them even achieved HBsAg seroclearance after 3 years of follow-up. Those who stopped therapy had greater reduction in qHBsAg compared to those who continued therapy (0.283 vs 0.088 log reduction respectively)(Berg et al., 2017).

To evaluate stopping NAs, we must consider what are the benefits of their discontinuation. Since long-term NA therapy can already achieve viral suppression and limit the risk of liver-related diseases, the withdrawal of the treatment should aim to have similar or even better outcomes than treatment continuation. HBsAg loss, indicating immune control with sustained virologic remission (Tout et al., 2020), remains the best indicator of long-term favorable outcomes and therefore of NA cessation. Relapse must also be considered, which occurs frequently off-treatment and may lead to clinically significant flares. Determining precise predictors of relapse or sustained remission is crucial to identify which patients should not stop therapy.

3.1 HBV guidelines and recommendations on stopping NA therapy

Three major international scientific guidelines, AASLD, EASL and the Asian Pacific Association for the Study of the Liver (APASL) have proposed stopping rules for

different categories of CHB patients under NA(European Association for the Study of the Liver. 2017; Sarin et al., 2016; Terrault et al., 2018) (Table 1). However, there is no clear consensus regarding when and how to stop the administration of NAs without compromising optimal management in the majority of CHB patients. The ideal endpoint for all subgroups of patients is considered to be the sustained loss of HBsAg, with or without subsequent seroconversion to anti-HBs (European Association for the Study of the Liver. 2017; Sarin et al., 2016; Terrault et al., 2018). Therefore, major guidelines recommend that all CHB patients, including those with compensated cirrhosis, can stop NA therapy if they achieve HBsAg loss that persists for at least 1 year, even if this goal is rarely achieved. Alternatively, surrogate endpoints have been determined to safely discontinue the treatment. In non-cirrhotic HBeAg-positive CHB patients, HBeAg seroconversion is a reliable indication of sustained off-NA response, when it is accompanied by inhibition of HBV replication and persistently normal ALT levels. Thus, all three guidelines recommend that these patients can discontinue therapy if they achieve HBeAg seroconversion with HBV DNA undetectability and normal ALT levels, and after they complete at least 12 months (up to 3 years according to the APASL) of consolidation therapy.

For non-cirrhotic HBeAg-negative CHB patients, APASL guidelines have suggested that NAs can be discontinued after at least 2 years of therapy if serum HBV DNA remains undetectable on 3 separate occasions 6 months apart, which could be mainly driven by cost constraints. Additionally, they also suggest NA withdrawal in the case of HBsAg loss with at least 12 months of a post-HBsAg loss consolidation period even if there was no HBsAg seroconversion (Sarin et al., 2016). Interestingly, the EASL recommendations also suggested that NA discontinuation may be considered in selected non-cirrhotic patients with long-term (≥ 3 years) on-therapy

virologic suppression if close post-treatment monitoring is guaranteed for at least 1 year (European Association for the Study of the Liver. 2017). AASLD guidelines generally do not recommend NA discontinuation for these patients except in the case of HBsAg seroclearance, but however included a recommendation of NA discontinuation as an option if there is a compelling rationale and under the condition of careful monitoring every 3 months for at least 1 year (Terrault et al., 2016). APASL are the only guideline who consider possible stopping of NA therapy, before HBsAg seroclearance, in patients with compensated cirrhosis, in condition of a careful off-therapy monitoring plan, while both EASL and AASLD recommend indefinite NA therapy in cirrhotic CHB patients who remain positive for HBsAg.

3.2 NA cessation in HBeAg-positive CHB patients before HBsAg loss

The main guidelines suggest that pretreatment HBeAg-positive patients, without cirrhosis, can stop NA therapy after reaching HBeAg seroconversion with undetectable HBV DNA and completing 1 to 3 years of consolidation therapy. Several reports have evaluated the durability of HBeAg seroconversion with LAM therapy, resulting in a wide range of durable HBeAg seroconversion rates. Song et al. evaluated patients who underwent HBeAg seroconversion with short duration LAM consolidation therapy (2 to 4 months) and observed a relapse with elevated transaminases and reappearance of HBeAg in 49% of patients at 2 years post-treatment (Song et al., 2000). Another study evaluated the durability of serological responses in 40 HBeAg-positive patients who were treated by LAM (Dienstag et al., 2003). Most HBeAg responses achieved during LAM therapy were durable, and most responders experienced prolonged clinical benefit after HBeAg seroconversion and

subsequent discontinuation of LAM. The relapse rate after HBeAg seroconversion was only 27% with a median period of 3-year follow up (Dienstag et al., 2003). Similarly, a study from Taiwan showed that a duration of LAM consolidation therapy > 48 weeks may be favorable for maintaining durable HBeAg seroconversion (Kuo et al., 2010). Then later, Lee et al. showed that consolidation therapy for more than 1 year after HBeAg seroconversion helped maintain the serological response (Lee et al., 2010). A prospective study of 82 patients demonstrated a cumulating relapse rate of 23.4% at 6 months and 29.4% at 4 years after discontinuation of LAM (Wang et al., 2010), and a second study observed a 3-year cumulative relapse rate of 34.4% in 42 patients (Jin et al., 2012). Recently, a study from Belgium aimed to determine relapse rates and clinical outcomes in a predominantly Caucasian cohort of 98 CHB patients with NA-induced HBeAg seroconversion (Van Hees et al., 2018). Viral relapses were observed in more than half of patients who discontinued treatment with potential fatal outcomes while none of the patients who continued therapy after HBeAg seroconversion relapsed or developed severe hepatic outcomes (Van Hees et al., 2018). Kang et al. assessed the antiviral response in 157 patients who had discontinued LAM for a mean 72 months of follow-up. The cumulative probabilities of virologic relapse at 1, 24, 48, 96, and 120 months were 10.3%, 55.6%, 62.8%, 67.0%, and 67.0%, respectively (Kang et al., 2017). However, most of these studies were long-term retrospective studies that did not correct for selection or measurement bias (Table 2). Therefore, the evidence on NA withdrawal in HBeAg-positive CHB patients is lacking and needs to be based on better designed studies.

Among the prospective studies, Jung et al. evaluated virologic relapse in 45 HBeAg-positive CHB patients who were treated with LAM or ETV. Within 1 year or NA treatment, viral relapse occurred in 57,8 % of the patients (Jung et al., 2016). In

several studies (Chaung et al., 2012; Pan et al., 2013; Reijnders et al., 2010; Sohn et al., 2014) with various NAs, the off-treatment response was not durable despite consolidation therapy after the HBeAg seroconversion. In the largest prospective study with 10-year follow up, 31% of 138 HBeAg-positive CHB patients experienced virologic relapse, almost all of whom (76%) developed HBeAg seroreversion, attesting that this was not durable at the time of stopping therapy (Liu et al., 2018). In a randomized controlled trial from Toronto, 61% of pretreatment HBeAg-positive patients required retreatment compared with 22% of HBeAg-negative patients (Liem et al., 2019).

A systematic review by Papatheodoridis et al., including 25 studies with 1716 patients who discontinued NA and were followed up for > 12 months shows that 51% of HBeAg-positive CHB patients had durable virologic remission at 3 years post-treatment and 76% of them had durable biochemical remission (Papatheodoridis et al., 2016). These rates were higher than in HBeAg-negative CHB patients. Sustained HBeAg seroconversion was found in 95%, 92% and 88% of patients at 6, 12 and 24 months, respectively, following discontinuation of NA therapy (Papatheodoridis et al., 2016). Collectively, these data suggest that the risk of relapse requiring retreatment is relatively high in pretreatment HBeAg-positive patients, even with long consolidation therapy after HBeAg loss. Due to the heterogeneity of the studies and their design, it is, therefore, difficult to confirm the advantage of NA discontinuation in pre-treatment HBeAg-positive CHB patients who undergo HBeAg seroconversion. Another factor leading to clear discrepancies between studies is the variable relapse definition (Papatheodoridis et al., 2018a). Although prolongation of NA therapy in HBeAg-positive patients is aiming at functional cure under treatment, this is still a rare event, and HBeAg seroconversion is the currently accepted endpoint despite

reported data of high relapse rates. Determining the appropriate duration for consolidation therapy is important to achieve sustained remission in those patients. Thus, there is an urgent need to specifically examine the effect of the duration of consolidation therapy on sustained HBeAg seroconversion in future well designed studies.

3.3 NA cessation in HBeAg-negative CHB patients before HBsAg loss

Current interest and debates in stopping NA are largely related to finite NA therapy in HBeAg-negative CHB patients. The guidelines recommend that NA therapy in HBeAg-negative CHB patients, without cirrhosis, can be stopped after ≥3 years of virologic suppression (EASL 2017)(European Association for the Study of the Liver. 2017), after treatment for at least 2 years with undetectable HBV DNA documented on three separate occasions, 6 months apart (APASL 2015)(Sarin et al., 2016), or only after achieving HBsAg loss, except if there is a compelling rationale and under the condition of careful monitoring every 3 months for at least 1 year (AASLD 2018)(Terrault et al., 2018). The previously mentioned study by Hadziyannis et al. was one of the first papers that presented data favoring NA withdrawal in HBeAg-negative CHB patients (Hadziyannis et al., 2012). It showed that stopping adefovir therapy in HBeAg negative patients was associated with a 39% HBsAg seroclearance rate. This high HBsAg seroclearance rate was not observed in subsequent studies probably due to shorter follow-up duration or even the ethnicity of the studied cohort. Additionally, whether the initial increase in HBV DNA, after NA withdrawal, settles down to quiescent disease or severe flares varies widely between different studies and patients and is hard to predict. Some studies defining viral

311 relapse after treatment cessation as HBV DNA>60 IU/mL (Shouval et al., 2009; Sohn
312 et al., 2014), HBV DNA >70 copies/mL(Jung et al., 2011), or HBV DNA >200 IU/mL
313 (Chan et al., 2011), found a very high percentage of HBeAg-negative CHB patients
314 going through viral relapse after NA cessation. Other studies have used levels of
315 HBV DNA >2000 IU/mL to define relapse. A prospective study of 184 Asian patients
316 taking ETV for at least 24 months and using the APASL criteria to guide treatment
317 cessation (treatment for at least 2 years with undetectable HBV DNA on 3 separate
318 occasions, 6 months apart) had a cumulative rate of virologic relapse 74.2% and
319 91.4% at 6 and 12 months post-treatment respectively (Seto et al., 2015). In two
320 studies by Jeng W-J et al. on Asian patients treated with ETV (Jeng et al., 2013) or
321 TDF (Jeng et al., 2016), 46% and 52% relapsed respectively. In another study of 45
322 patients of >3years follow-up, LAM, ETV or ADV therapy, the relapse rate was 49%
323 and 73% at 6 and 12 months (Kim et al., 2013). A recent study on 234 HBeAg-
324 negative CHB patients who received ETV and 119 receiving TDF showed that HBV
325 relapse occurs sooner and is more severe after cessation of TDF than after cessation
326 of ETV (Kuo et al., 2019). These results confirmed earlier reports that showed that
327 discontinuation of TDF rather than ETV was associated with earlier relapse (Höner
328 Zu Siederdissen et al., 2018; Jeng et al., 2016; Su et al., 2018). Off-ETV mostly
329 occurred after 6 months vs within 6 month off-TDF (Jeng et al., 2016). These studies
330 clearly show the difference in relapse timing between ETV and TDF. The reason
331 behind this obvious difference in timing needs to be investigated in future studies,
332 perhaps having to do with pharmacokinetics of different NAs or different interactions
333 with immune responses.

334 In HBeAg-negative CHB patients most of the virologic relapse occurs within the first
335 3-9 months after treatment discontinuation(Fung et al., 2004; He et al., 2013).

Studies with longer follow-up confirm possible sustained off-treatment response and HBsAg loss in HBeAg-negative CHB patients (Table 3). The daring-B study followed 57 patients who discontinued therapy after median virologic remission of 5,3 years and remained under a median of 18 months of follow-up(Papatheodoridis et al., 2018b). For a cutoff of HBV DNA > 2,000 IU/ml, the cumulative virologic relapse rates were 56%, 70% and 72% at 3, 12 and 18 months after therapy. The probability of relapse decreased after month 6.

In a systematic review on NA discontinuation, 2% HBsAg loss was observed (Papatheodoridis et al., 2016). The pooled rates of sustained virologic remission at 12, 24, 36 months in HBeAg-negative patients were 43.7%, 31.3% and 30.1% respectively (Papatheodoridis et al., 2016). In another systematic review of 22 studies including 1732 HBeAg-negative patients, the 1 year off-therapy virologic relapse was < 70%, and clinical relapse (HBV DNA >2000 IU/mL and ALT elevation) occurred in < 50% of patients and <40% required retreatment (M.-L. Chang et al., 2015). Thus, these two recent systematic reviews show that stopping NA is relatively safe with a careful off-therapy monitoring plan. Concerning the safety of NA discontinuation, they suggest that significant complications such as severe flares and decompensation were relatively infrequent and occurred in 0,8% of patients with cirrhosis, while jaundice developed in 2,5%, with 1 death from liver failure (M.-L. Chang et al., 2015; Papatheodoridis et al., 2016). Conversely, a study from Toronto, with majorly Asian patients, found that only 33% of pretreatment HBeAg-negative patients had a sustained off-treatment response, 72 weeks after NA withdrawal (Liem et al., 2019). Although the retreatment criteria in this study was not stringent which allowed to better evaluate the off-NA viral and biochemical kinetics during close-visit monitoring, there were only 27 HBeAg-negative CHB patients which limits the

significance of such results. In another recent study of 106 HBeAg-negative patients who stopped TDF therapy (Buti et al., 2019), almost a third of patients experienced a grade 3 or higher laboratory abnormality (ALT elevations) and serious adverse events occurred in 4% of patients. 35% of patients had both HBV DNA concentrations of less than 2000 IU/mL and ALT concentrations less than the ULN at TFFU week 24(Buti et al., 2019). These notable differences between studies may originate from following different guidelines thereby limiting their scope by design, different definitions of virologic relapse, differences in outcome definitions, or heterogeneity of cohorts. Most of the studies, on which the guidelines are based on, consist of Asian cohorts with genotype B and C, leading to a heterogeneity compared to Caucasian patients with a prominent genotype D. Finally, a recent multicenter study including 276 patients from 16 hospitals in Korea, aimed to determine whether NA discontinuation was as safe as NA continuation after NA-induced HBsAg seroclearance. The authors found that discontinuation of NA was not associated with a higher risk of either HBsAg reversion, serum HBV DNA redetection or HCC development compared with NA continuation (Kim et al., 2020).

3.4 Stopping NA in patients with cirrhosis before HBsAg loss

Patients with cirrhosis are at risk of developing liver decompensation and stopping therapy in this subgroup is controversial due to the risk of liver failure and ascites (Wen-Juei Jeng et al., 2018). While the EASL and the AASLD guidelines(European Association for the Study of the Liver, 2017; Terrault et al., 2018) do not recommend stopping NA therapy, before HBsAg loss, in patients with cirrhosis, under any circumstance, the APASL guidelines(Sarin et al., 2016) may consider it in

385 compensated cirrhosis with a careful monitoring plan. Therefore, data evaluating NA
386 discontinuation, before HBsAg loss, in patients with pretreatment cirrhosis, come
387 mainly from Asian studies (Jeng et al., 2013; Kuo et al., 2010). In a large study from
388 Taiwan, the cumulative probability of liver decompensation was 3% over a > 5 years
389 follow-up and only 1% of patients died (Wen-Juei Jeng et al., 2018). Chen et al.
390 analyzed 586 HBeAg-negative CHB patients with compensated cirrhosis and 205
391 patients, among them, who stopped ETV based on APASL 2008 guidelines (Chen et
392 al., 2015). These patients were matched by propensity scoring to patients who
393 continued treatment and no significant difference was found in liver related
394 complications/deaths between the two groups after a median follow-up of 59 months
395 (Chen et al., 2015). Another study found, similarly, no statistical significance between
396 patients with and without cirrhosis who stopped LAM therapy (Ming-Ling Chang et al.,
397 2015). Jeng et al. assessed 39 cirrhotic patients receiving TDF and found that
398 relapse occurred in 39.5%. Two patients developed hepatic decompensation(Jeng et
399 al., 2016). In the study by Kuo et al., jaundice occurred in 8,5% of patients all of
400 whom had cirrhosis. Of these 7 patients, 6 improved after LAM retreatment and 1
401 patient with decompensated liver disease died 1 week later(Kuo et al., 2010).
402 Furthermore, the HCC risk decreases beyond year 5 of ETV/TDF therapy in
403 Caucasian CHB patients, particularly in those with compensated cirrhosis
404 (Papatheodoridis et al., 2017). The studies discussing the safety of NA
405 discontinuation in cirrhotic CHB patients are not frequent and are usually
406 retrospective, therefore less rigorous than well-executed randomized control trials
407 showing the benefit of continuing therapy in these patients (Liaw et al., 2004).
408 Therefore, there is a need to perform future studies on NA cessation in cirrhotic
409 patients to determine if there is a benefit of stopping the treatment especially since

this is a controversial issue between the different guidelines. Lifelong NA treatment may be an unaffordable burden for healthcare systems in certain countries and thus, the only benefit of stopping therapy in cirrhotic patients could be reducing the costs of treatment.

3.5 Rescue therapy in case of relapse

Cessation of antiviral treatment with NA, before HBsAg loss, may lead to clinical reactivation/relapse, flares, decompensation, overt liver failure and death. Therefore, specific stringent criteria for retreatment are critical for the optimal management of CHB patients who discontinue NA and help preventing significant clinical relapses (Berg et al., 2017; Papatheodoridis et al., 2018a, 2018b). Close post-treatment follow-up with, at least, ALT and HBV DNA determinations is mandatory, and strict and predefined criteria for retreatment are necessary to avoid potential life-long retreatment (Papatheodoridis et al., 2018a). However, the criteria for retreatment have not been standardized resulting in great heterogeneity among different studies (Papatheodoridis et al., 2016, 2018a). For patients with flares, delaying retreatment can be detrimental, while starting retreatment too soon might counteract the potential relapse-associated induction of a functional cure (W.-J. Jeng et al., 2018). Severe hepatic flares require immediate retreatment and it is quite challenging to determine the exact “kind” of flares occurring and base on that observation the decision to retreat patients. Some patients can still be in an intermediate state, neither fulfilling criteria for CHB nor for an inactive carrier state, and therefore require further follow-up for a precise disease activity characterization and to decide whether retreatment should be commenced (Wen-Juei Jeng et al., 2018; Lampertico and Berg, 2018). Therefore, the retreatment decision is based on the severity of HBV flares and the persistence (≥ 3 months) of liver disease activity, taking into consideration,

primordially, the patient's safety (Lampertico and Berg, 2018). Interestingly, HBsAg kinetics during clinical relapse have been proposed to guide retreatment decision; retreatment may be required in those with increasing HBsAg levels, whereas retreatment might be unnecessary in those with decreasing HBsAg levels (Chien and Liaw, 2020; Liaw, 2019; Liaw et al., 2018). This rescue therapy induces virologic and biochemical remission in all non-cirrhotic CHB patients who developed HBV related flares (Papatheodoridis et al., 2016). Concerning patients with persistently mild to moderate liver disease activity, the decision of retreatment relies on persistent elevation of HBV DNA and ALT levels for at least 6 months after stopping therapy(European Association for the Study of the Liver. 2017; Terrault et al., 2018). The DARING-B study shows that retreatment is also required in cases of modest ALT elevations (>3×ULN) and high HBV DNA levels (>100 000 IU/mL) at the same visit (Papatheodoridis et al., 2018b).

Interestingly, the study by Kuo et al., conducted in 342 CHB patients who stopped ETV vs. patients who stopped TDF (Kuo et al., 2019) showed that the rate of off-therapy HBsAg loss was comparable between the two groups after 2 to 3 years of follow-up (Kuo et al., 2019). A large, retrospective cohort study from Taiwan (691 HBeAg-negative CHB patients who stopped treatment according to the APASL stopping rule) revealed a 13% HBsAg loss at 6 years off therapy, much higher than that during therapy(Wen-Juei Jeng et al., 2018). The DARING-B study followed 57 patients who discontinued therapy after median virologic remission of 5,3 years and remained under a median of 18 months of follow-up (Papatheodoridis et al., 2018b). Cumulative rates of HBsAg loss were 5%, 16% and 25% at 6, 12 and 18 months being higher in patients with lower HBsAg levels at treatment discontinuation. Another recent study found a 5% HBsAg loss among 106 HBeAg-negative patients

who stopped TDF therapy (Buti et al., 2019). However, the follow-up duration was 24 weeks and therefore is too short to reflect a sustainable HBsAg loss rate especially among a subgroup of patients achieving a low-replicative state and therefore are potential HBsAg loss candidates if they are followed up for a longer duration after NA cessation(Buti et al., 2019).

As mentioned before in this section, the “retreatment” or “no retreatment” decision in CHB patients with hepatic flares is governed by the outcomes of these, being detrimental or beneficial to the patients. Nevertheless, it should be noted that the “no retreatment” decision is considered to be a strong predictor of off-NA HBsAg loss(Berg et al., 2017; Chen et al., 2019; Liaw, 2020; Liem et al., 2019). Therefore, a proper assessment is crucial to better determine the type of HBV flares, which will help with the retreatment/no retreatment decision.

Figure 1 presents a proposed algorithm for the follow-up of non-cirrhotic CHB patients who stop NA therapy and indications for retreatment.

4. Biomarkers to consider to safely stop NA therapy before HBsAg loss

The evaluation of the probability of post-NA remission has been quite challenging due to the great heterogeneity among the studies which have variations in patient populations, designs, definitions of relapse, post-NA management and duration of follow-up (Papatheodoridis et al., 2016). Thus, the identification of reliable serologic, virologic, or immunologic predictors of post-NA remission or relapse is quite interesting and helps refining current rules of NA cessation. CHB outcomes rely on equilibrium between the virus and the host, therefore different virologic and immunological parameters could be considered as predictors of safe NA

discontinuation. In recent years, numerous studies have investigated several host and viral factors such as patient characteristics including age (Bonino et al., 2007; Ha et al., 2012; Marcellin et al., 2009) and gender (Bonino et al., 2007; Chen et al., 2014), baseline clinical characteristics including HBV DNA (Huang et al., 2003; Jeng et al., 2013), ALT levels (Bonino et al., 2007; Chien and Liaw, 2006; Marcellin et al., 2009), HBsAg (Chen et al., 2018, 2014, 2014; Liang et al., 2011), treatment-related factors including duration of consolidation therapy (Jeng et al., 2013; Jiang et al., 2015; Kuo et al., 2010; Sohn et al., 2014), and virus-related factors, such as genotype (Chien and Liaw, 2006; He et al., 2013) and viral mutations (Liang et al., 2011; Ryu et al., 2003) have also been considered. However, these factors vary between studies because of the various designs, populations, and patient pre-treatment HBeAg status.

In HBeAg-positive CHB patients, the duration of consolidation therapy is the most analyzed predictive factor. Most guidelines consider that prolonged additional administration of NA after HBeAg seroconversion may enhance the durability of sustained response after treatment cessation and most of them consider that a 12-month period offers a more durable sustained response (European Association for the Study of the Liver. 2017; Pan et al., 2013; Ryu et al., 2003; Sarin et al., 2016; Terrault et al., 2018; Wang et al., 2010). A study on LAM cessation showed that consolidation therapy for more than 1 year after HBeAg seroconversion helped maintain the response (Lee et al., 2010). Song et al. showed that high pre-treatment HBV DNA levels were predictive for relapse and that pre-treatment HBV DNA and the duration of consolidation treatment were independent important risk factors for relapse(Song et al., 2000). There have been no meta-analyses examining the effect of the duration of consolidation therapy on sustained HBeAg seroconversion and

there is a need to further explore this aspect. On the other hand, studies showed that the combination of young age and prolonged consolidation treatment gives lower relapse rates (Byun et al., 2005; Chen et al., 2014; Lee et al., 2010; Wang et al., 2010). Chien et al. noticed that patients with genotype C had higher relapse rates than those with genotype B(Chien and Liaw, 2006). Cao et al. showed that the level and persistence of off-treatment elevated HBV DNA levels were useful in the prediction of a subsequent biochemical relapse and may guide off-treatment management (Cao et al., 2017). A study reported a negative correlation between baseline ALT levels and relapse rate (Pan et al., 2013) while another one showed that lower ALT baseline levels correlated with higher rates of HBsAg loss (Chen et al., 2014).

In HBeAg-negative CHB patients, several predictive factors have been associated with a better chance of sustained off-NA remission such as younger age, higher levels of ALT at baseline and at the end of the treatment, lower serum HBV DNA at baseline, low serum HBsAg levels at the end of treatment as well as decreasing HBsAg levels during therapy (Papatheodoridis et al., 2016). Two studies have demonstrated that patients with sustained response are younger than patients with relapse (Ha et al., 2012; Liu et al., 2011). Higher levels of ALT at baseline and at the end of treatment (Hadziyannis et al., 2012) and lower levels of HBV DNA pretreatment (Hadziyannis et al., 2012; Jeng et al., 2013) predicted a sustained response.

In addition to predicting HBsAg loss by its decrease (Martinot-Peignoux et al., 2013), qHBsAg has also been used to predict off-therapy sustained response with NAs, and off-therapy flares (Höner Zu Siederdissen et al., 2016; Martinot-Peignoux et al., 2015, 2014; Qiu et al., 2016). Low levels of serum HBsAg at the end of treatment, or

decreasing levels during therapy or after its cessation were shown to be positive predictors for sustained response (Chan et al., 2011; Hadziyannis et al., 2012). Chen et al. showed that HBsAg cut-off values of 120 and 200 IU/mL predicted a 79% HBsAg loss and 93% post-treatment sustained response respectively (Chen et al., 2014). Conversely, two studies showed no influence of HBsAg kinetics and relapse rates (Jeng et al., 2016; Seto et al., 2015). Several studies identified serum HBsAg levels of <2 log10 IU/mL to be a reliable predictor of a sustained off-treatment response (Chen et al., 2018; Chi et al., 2019; Wen-Juei Jeng et al., 2018; Papatheodoridi and Papatheodoridis, 2020; Su et al., 2018). In a study of 204 HBeAg-negative patients, qHBsAg level ≥1500 IU/mL at discontinuation was the only predictive factor of relapse (Ge et al., 2015). qHBsAg>3 log IU/mL was predictive of a virologic relapse at 1 year in another study (Liang et al., 2011). In the FINITE study, patients who achieved a decrease in HBsAg levels after treatment withdrawal had baseline HBsAg levels<25 000 IU/mL when NA treatment was discontinued (Berg et al., 2017). A recent meta-analysis found that older age, shorter duration of consolidation therapy, lower levels of baseline and EOT HBsAg levels ≥1000 IU/ml were predictive factors of relapse in HBeAg-negative CHB patients who discontinued NA treatment(Y. Liu et al., 2019). Liu et al. showed in a systematic review that an HBsAg<100 IU/mL at EOT is a significant maker for safe NA discontinuation(J. Liu et al., 2019). Two recent studies found that an HBsAg <50 IU/mL(Gao et al., 2019) or HBsAg <40 IU/mL (Tseng et al., 2020) have higher predictive values of better sustained responses in HBeAg-negative CHB patients. More studies outside Asia and therapies using different NAs are needed to define the optimal cut-off levels. Nevertheless, from a practical viewpoint, EOT HBsAg <100 IU/mL, in most studies, seems to be the most important predictor for subsequent functional cure(J. Liu et al.,

559 2019). Additionally, serial HBsAg measurements after EOT are important since
560 decreasing levels are associated with beneficial outcomes and increasing levels are
561 predictors of relapse(Liaw, 2019).

562 Several viral markers have been proposed as potential biomarkers for a safe NA
563 discontinuation. Serum HBV pregenomic RNA (henceforth referred to as HBV RNA)
564 reflects the transcriptional activity of liver cccDNA even during suppression of HBV
565 DNA during NA treatment or very low HBV replication activity (Giersch et al., 2017)
566 and its decline seems to be a good predictor of HBeAg seroconversion (van Bömmel
567 et al., 2015) . Unlike HBsAg, HBV RNA is derived only from cccDNA, and its
568 quantification is not affected by viral antigens or antibody complexes and therefore it
569 may reflect more accurately the transcriptional activity of cccDNA. Levels of serum
570 and intrahepatic HBV RNA have been suggested as a novel marker during NA
571 treatment (Wang et al., 2016; Yu et al., 2018). However, it is commonly undetectable
572 in HBeAg-negative patients (Wang et al., 2016), which makes it interesting only in
573 HBeAg-positive CHB patients. Nevertheless, a recent study suggested that HBV
574 RNA along with another marker can predict outcomes in HBeAg-negative CHB
575 patients suppressed on NA therapy (Carey et al., 2019). In a recent study (Fan et al.,
576 2020) including 127 HBeAg-positive patients who stopped TBV-based therapy after
577 achieving HBeAg seroconversion, HBV RNA was deemed satisfactorily in predicting
578 clinical relapse and HBsAg loss after treatment cessation. Similarly, another study by
579 Seto et al. found serum HBV RNA measurement to be essential for deciding on ETV
580 cessation in CHB patients with low HBsAg levels (Seto et al., 2020).

581 HBV core-related antigen (HBcrAg) includes HBcAg, HBeAg and a pre-core protein
582 (p22cr), and its quantification closely correlates with intrahepatic cccDNA level (Lin
583 and Kao, 2016). It has been also proposed as a novel marker for safe NA

584 discontinuation (Matsumoto et al., 2015, 2007). Low HBcrAg levels were associated
585 with a lower risk of a biochemical relapse following LAM discontinuation (Matsumoto
586 et al., 2015). Furthermore, two studies, including a recent one, suggested that the
587 combination of HBsAg together with HBcrAg can predict safe NA discontinuation
588 (Hsu et al., 2019; Tanaka and Matsumoto, 2014). A retrospective cohort study
589 consisting of 560 patients enrolled in a phase IV, multicenter, randomized, controlled
590 trial treated with NA-based therapy for up to 2 years, reported that baseline anti-
591 HBcAg levels is a strong predictor for HBeAg seroconversion during NA therapy (Fan
592 et al., 2016). Another recent prospective study noticed a trend for an inverse
593 association between anti-HBc levels and clinical relapse in CHB patients off-ETV
594 (Tseng et al., 2018).In summary, some promising virologic markers, such as HBV
595 RNA and HBcrAg, could be useful predictors of response but further cohort
596 validation is still needed.

597

598 ## 5. Immunological response following NA discontinuation

599 The humoral and cellular immune responses play a crucial role in clearing the
600 infection during acute hepatitis B (AHB) (Rehermann and Nascimbeni, 2005).
601 However, this adaptive response is severely impaired in CHB patients due to a high
602 and persistent viral antigens exposure. This immune exhaustion is characterized by
603 an upregulation of regulatory cells, increase of IL-10 and TGF-β levels, high viral
604 antigen levels especially HBsAg, and mitochondrial dysfunction (Mansouri et al.,
605 2018; Ye et al., 2015). This leads to a dysfunction of CD4+ and CD8+ T cells
606 (Fisicaro et al., 2017), decreased numbers and dysfunction of DCs and NKs/NKTs
607 (Fisicaro et al., 2019; Maini and Peppa, 2013; Martinet et al., n.d.; Ouaguia et al.,

2019) up-regulated/enhanced expression of regulatory factors, including the immune checkpoint proteins PD-1, CTLA-4, and Tim-3 (Hoogeveen and Boonstra, 2020); and impaired innate immune response (Faure-Dupuy et al., 2017), especially TLR downregulation and dysfunction (Ma et al., 2018; Tout et al., 2018; Zhang and Lu, 2015).

Multiple studies have shown an increased frequency and improved function of HBV-specific CD4+ and CD8+ T cells in CHB patients after NA therapy and viral load reduction (Boni et al., 2012, 2003). Most outcomes after NA cessation in CHB patients, ranging from functional cure to severe liver inflammation, links intimately to the immune system.

Hence, immunologic biomarkers that discriminate between antiviral responses and pathologic inflammation after stopping therapy could facilitate clinical decisions, limit adverse outcomes, and identify mechanisms leading to functional cure. In this context, we will mainly discuss the immunological activity of T cells and NK cells as well as the cytokinic profile after NA discontinuation.

5.1 T cells

Several studies have shown that, after long-term NA treatment discontinuation, most patients remain with a viral response after a long follow-up (Berg et al., 2017; Hadziyannis et al., 2012) and thus we could speculate that the host's immune system is controlling HBV replication. The reduction in viral load induced by the antiviral therapy would restore the function of HBV-specific CD8+ T cells allowing them to control the virus. Patients with viral control are more likely to have a good immune response against the virus (Maini et al., 2000), in comparison to cases with virologic suppression, that have a dysfunctional response. Therefore, changes in the

phenotype and functionality of HBV-specific CD8+ T cells may account as a valuable predictor of antiviral control and may point us to the patients that can safely stop NA therapy without viral relapse.

Previous studies of NA-treated HBeAg-positive patients show that control of virus replication induced by therapy can lead to a transient restoration of T cell function (Boni et al., 2003; Stoop et al., 2007). To investigate the effect of long-term treatment on T cell responses, Boni et al. compared HBV-specific T cell responses of patients treated with NA with those detected in other conditions of HBV control (Boni et al., 2012). They demonstrated that HBV-specific CD8+ T cell effector abilities were similar between patients after several years of antiviral treatment and acute resolving cases featured by a PD-1+ phenotype. More recently, Rivino et al. studied two cohorts of 29 and 27 HBeAg-negative CHB patients who received NA monotherapy or NA combination. Patients were, then, separated into 2 groups based on the presence or absence of hepatic flares during the 6-month period after the termination of the therapy (Rivino et al., 2018). They demonstrated that the patients who did not relapse after NA cessation featured, during treatment, an increased frequency of functional PD-1+ HBV-specific CD8+ T cells directed against HBcAg and HBV polymerase. However, peripheral T cell expansion or PD-1 expression did not consistently correlate with HBsAg reduction or biochemical reactivation. Rather, CD38 and Ki-67 expression during ALT elevation correlated with HBsAg loss in the 3 patients who achieved functional cure (Rinker et al., 2018). Interestingly, a higher frequency of polymerase-specific CD8+ T cells expressed markers indicating more severe T cell exhaustion and exhibited a reduced expansion capacity than HBcAg-specific CD8+ T cells (Schuch et al., 2019). This indicates that the molecular mechanisms underlying impaired T cell responses differ with respect to the targeted

HBV antigens. Overall, PD-1+ HBV-specific T cells were identified as a potential biomarker for the safe discontinuation of NA therapy for CHB patients (Rivino et al., 2018). In line with these results, it was reported that a high level of anti-HBc at the end of NA treatment was associated with a reduced risk of clinical relapse (Chi et al., 2019) and it would be interesting to determine whether this higher level of anti-HBc is related to the higher HBV-specific T cell activity. As the analysis of PD-1+ HBV-specific T cells is somehow complicated, the development of a practical assay to directly quantify these cells would be of great interest to permit this biomarker to become clinically useful. It would be interesting to know what causes the hepatic flares after the cessation of NA therapy since the magnitude of the HBV-specific T cell responses was low in those patients. Additionally, PD-1 upregulation on HBV-specific CD8+ T cells could either mean an activation before HBV clearance in long-term NA treated CHB patients without HBV flares or exhaustion after persistent and high HBV antigens exposition in untreated CHB patients. Therefore, it is unclear whether anti-PD-1 treatments (Fisicaro et al., 2017, p. 8; Salimzadeh et al., 2018) will enhance HBV clearance in patients with PD-1$^+$ HBV-specific T cells. Further longitudinal investigation of blood and liver samples are needed to determine precise immunological events occurring in patients stopping NA therapy.

5.2 NK cells

The role of NK cells, characterized by the presence of activating and inhibiting receptors, in CHB has thoroughly been debated (Maini and Peppa, 2013). Their presence in the liver several folds higher than the periphery, their contribution to liver damage by killing hepatocytes (Dunn et al., 2007; Maini et al., 2000) as well as their correlation with HBV control makes them interesting cells to study the antiviral response against HBV. On the other hand, multiple studies have shown that NK cells,

682 in CHB, are defective in the production of cytokines like IFN-γ, impairing their
683 noncytolytic antiviral capacity (Oliviero et al., 2009; Peppa et al., 2010). NK cells can
684 also impair antiviral immunity by deleting HBV-specific T cells (Lang et al., 2012;
685 Peppa et al., 2013).

686 A partial restoration of antiviral cytokine production of NK cells was shown, following
687 long-term ETV, compared to naïve CHB patients (Tjwa et al., 2011). To further
688 understand to what extent NA treatment can affect NK cells, Boni et al. studied NK
689 cell phenotype and function in HBeAg-negative patients either untreated or treated
690 with NA. No significant improvement of NK cell function was observed, despite a
691 reduced expression of some activation/proliferation markers, including the death
692 ligand TRAIL indicating a low NK pro-inflammatory phenotype (Boni et al., 2015).
693 This lower inflammatory status of NK cells correlated with a better HBV-specific T cell
694 response (Zimmer et al., 2018). An increase in both degranulation and cytokine
695 production, was found, along with a CD38 upregulation, in correspondence of the
696 ALT flares following NA discontinuation, which was particularly enhanced in the
697 fraction of patients experiencing HBsAg loss (Zimmer et al., 2018). This strongly
698 suggested a role for NK cells in HBsAg seroclearance and in biochemical reactivation
699 following NA discontinuation.
700 These studies not only help to clarify mechanisms of liver inflammation but suggest
701 that NK cells may aid in predicting safe NA discontinuation. Studies in bigger cohorts
702 are needed to confirm this lower inflammatory profile tendency in NK cells in
703 successful NA withdrawal as it can be easily evaluated in laboratory and may reflect
704 an HBV-specific T cell restoration.

705

5.3 Cytokines

An adequate antiviral immune response or, in contrast, liver inflammation, associated with hepatic flares, are strictly affected by a balanced cytokine profile (Rehermann, 2013; Schurich et al., 2013; Xia and Protzer, 2017) which could be useful to determine the fate of HBV infection upon NA discontinuation. Successful viral repression induced by NA therapy leads to antiviral response by promoting proinflammatory cytokines such as IFNγ (Chen et al., 2011) and IL-2(Jiang et al., 2010), and by decreasing regulatory signals such as IL-10 (van der Molen et al., 2006) and TGF-β (Zheng et al., 2012). Serum cytokine studies, in small cohorts, could not predict off-therapy relapse (Höner Zu Siederdissen et al., 2016; Tan et al., 2010), but we could theoretically speculate that the measurement of these cytokines together with HBV-specific T cells or NK cells could help to establish a good cessation point for therapy. Among the cytokines that increased after NA withdrawal included IL-12 (Höner Zu Siederdissen et al., 2016), which is important for the induction and recovery of exhausted adaptive T-cell responses (Schurich et al., 2013). In addition, an increase in sST2 in clinical relapse patients might be associated with an inflammation-related immune response after discontinuation of NA treatment (Xie et al., 2019).

In a recent study aiming to identify an immune signature associated with off-treatment remission to NA therapy, Kranidioti et al. performed microarray analysis of PBMCs from 6 CHB patients who stopped NA therapy (3 with off-treatment remission, 3 with relapse) compared to 5 CHB patients (inactive carriers) as controls (Kranidioti et al., 2019). They found that IFNγ, IL-8, FASLG and CCL4 levels were significantly lower in the patients with off-treatment remission compared to the

patients with relapse. Thus, they identified these 4 genes as potential biomarkers for stopping NA therapy (Kranidioti et al., 2019).

Larger studies are required to determine more adequate cytokine candidates. Additionally, several studies have shown that genetic polymorphisms might influence the immune response in CHB (Galmozzi et al., 2014; Ju et al., 2017). Yu et al. showed an effect of the IL-28B (IFNλ3/4) genotype on the response to LAM at one year(Yu et al., 2013). Among 33 single-nucleotide polymorphisms (SNPs) from 16 immune-related genes, a recent study found that genetic variations in the CXCR5 gene decrease the risk of clinical relapse after NA discontinuation in CHB patients and thus these genotypes could facilitate optimization of NA discontinuation (Wu et al., 2020, p. 5).

An additional important factor is the migration of HBV-specific T cells to the infected liver which is controlled by chemokines and their receptors (Brass and Brenndörfer, 2014; Larrubia et al., 2008). Chemokines drive inflammatory infiltrates, possibly induced by HBV replication (CXCL-8) or IFNγ (CXCL-9/10)(Tan et al., 2010). Studies confirmed the associations between liver disease and chemokine production after stopping NA therapy (Höner Zu Siederdissen et al., 2016; Tan et al., 2010). Previous work reported higher levels of CXCL-10 in patients with HBsAg loss during NA therapy (Jaroszewicz et al., 2011). Another study found that CXCL-10 levels started to significantly increase after the 3rd year of treatment with ETV presenting a promising predictor of HBsAg decline (Papatheodoridis et al., 2014). An efficient viral suppression under NA therapy would restore the migration of functional HBV-specific T cells, helped by a lower inflammatory status of NK cells, to clear infected hepatocytes and thus leading to HBsAg loss (Figure 2). More robust immune biomarkers with simple practical applications, remain important

to optimize NA cessation strategy. Furthermore, future clinical studies on larger scales, should implement the monitoring of such promising immune biomarkers especially HBsAg-specific CD8+ T cells before and during the study to assess the immune environment in CHB patients who have undergone NA discontinuation which will allow a better understanding of the mechanism of off-NA HBsAg loss. Other immune cells, such as B cells, would be interesting to study during NA cessation in order to understand their role in HBV clearance. HBsAg-specific B cells have gained over the last few years a massive interest and constitute a novel attractive target for antiviral strategies toward functional cure of chronic HBV infection (Neumann-Haefelin and Thimme, 2018; Salimzadeh et al., 2018).

6. Future directions and conclusions

Although stopping NA therapy following HBeAg seroconversion is recommended in major guidelines, this approach is associated with relapse in a substantial proportion of patients(Seto et al., 2015). With the relapse rates following HBeAg seroconversion reaching almost 50% of CHB patients, the value of HBeAg seroconversion as a clinical endpoint is a subject of debate and continuing NA therapy in these patients could be more beneficial. Nevertheless, 1 to 3 years of consolidation therapy with normal levels of HBV DNA and ALT are determined to be sufficient to decrease relapse rates.

In HBeAg-negative patients, recent reports confirmed the possibility of sustained off-treatment response and higher HBsAg loss rates compared with the current long-term NA strategy. Thus, this Finite strategy can be considered a rational strategy to

induce a functional cure in carefully selected non-cirrhotic HBeAg-negative patients and with close follow-up monitoring.

It is worthy of mentioning that most of the relevant data so far come from Asia, where the guidelines recommending that NA can be discontinued rather early, are based primarily on reducing costs and not on efficacy. In addition, the systemic evaluation of the results has been quite challenging due to the great heterogeneity of studies. Therefore, prospective well-designed studies with a large number of patients and longer follow-up are needed to confirm the findings and to further refine the criteria for NA withdrawal.

Careful selection of candidates to NA withdrawal requires validated serologic, virologic, or immunologic markers and predictors of relapse or on-treatment responses. HBsAg levels and other promising predictors such as anti-HBc, HBV RNA, and HBcrAg should be prospectively validated in larger cohort studies. NA therapy in patients with liver cirrhosis should be lifelong and should be excluded from this Finite therapy strategy due to the risk of subsequent decompensation or even death.

Moreover, the definition of relapse strongly affects the off-NA rates of relapse and therefore can have a great impact on the probability of retreatment. Retreatment criteria should be standardized. Based on current knowledge, patients with persistent HBV activity and severe flares should be retreated. EASL guidelines specify that the indication for treatment in patients who have discontinued therapy should be in line with the indications for treatment-naïve patients, specifically those with HBV DNA >2,000 IU/ml and ALT >ULN (European Association for the Study of the Liver. 2017). HBsAg kinetics measurements, after EOT, are a very interesting strategy to distinguish beneficial from detrimental flares as early as possible, which may be

extremely challenging to identify(Jeng et al., 2019; Liaw et al., 2018). More frequent HBsAg measurements are required for HBsAg kinetics to be effectively used in retreatment decisions. On the other hand, monitoring frequencies should also be standardized. Monitoring should ideally include measuring ALT and HBV DNA levels monthly for the first 6 months, and thereafter, every 3 months for the first 2 years. Finally, the identification of immunological biomarkers such as HBV-specific T cells, for safe therapy discontinuation, is urgently needed to move forward in the clinical management of CHB patients. A necessary step to translate these findings into the clinic is to develop a reliable, easy to perform, and rapid assay to identify these immune markers in candidates for NA discontinuation. However, the exact mechanism leading to immunological control in these patients is not well understood. The sudden increase in HBV DNA and HBV proteins following NA withdrawal may be an important trigger to re-induce T cell responses against HBV-infected hepatocytes and further studies are needed to clarify these interactions between the virus and the host's immunity.

Figure legends

Fig. 1. Algorithm for the follow-up of non-cirrhotic CHB patients who discontinue NA therapy.

Fig. 2. Immunological changes upon NA-induced viral suppression

High viral DNA and HBV proteins during chronic HBV infection lead to exhausted HBV-specific CD8+ T cells characterized by a dysfunctional phenotype, high and sustained PD-1 expression, and abrogated antiviral responses. NK cells are defective and are characterized by a high inflammatory phenotype that may reflect NK cell-mediated T cell deletion through death receptors like TRAIL. After long-term NA therapy-induced HBV suppression, HBV-specific PD-1+ CD8+ T cells restore their antiviral function through a direct cytolytic activity on HBV-infected hepatocytes and effective antiviral cytokine secretion (IFNγ and IL-2). NK cells have a low inflammatory profile and can restore their antiviral functions. This immune control phase is also characterized by high levels of CXCL-10 which could point to anti-viral control. HBV-specific PD-1+ CD8+ T cells, low inflammatory NK cells and levels of IL-2, IFNγ and CXCL-10 are promising predictors of safe NA therapy discontinuation. HBV: Hepatitis B virus; CXCL10: C-X-C motif chemokine 10 ; IFNγ: interferon gamma; IL-2: interleukin 2; NA: Nucleos(t)ide analogue; NK cell: Natural killer cell; PD-1: Programmed cell death protein 1.

Table 1. Recommendations on stopping NAs by the European, American and Asian Pacific Guidelines for Chronic Hepatitis B, stratified by HBeAg status and cirrhosis

Guideline	EASL (2017)	AASLD (2018)	APASL (2015)
HBeAg positive	-HBsAg seroclearance -HBeAg seroconversion and HBV DNA undetectable with at least 12 months of consolidation therapy	-HBsAg seroclearance -HBeAg seroconversion with at least 12 months of persistently normal ALT levels and undetectable HBV DNA levels (close monitoring every 3 months for at least 1 year)	HBeAg seroconversion with undetectable HBV DNA and persistently normal ALT levels with 1-3 years of consolidation therapy
HBeAg negative	-HBsAg seroclearance -May be considered before HBsAg loss in selected patients with undetectable HBV DNA for at least 3 years if guaranteed close post-treatment monitoring for at least 1 year	HBsAg seroclearance (close monitoring every 3 months for at least 1 year for recurrent viremia, ALT flares, and clinical decompensation). - May be considered if there is a compelling rationale and under careful monitoring every 3 months for at least 1 year.	-HBsAg seroclearance with anti-HBs seroconversion -HBsAg loss with at least 12 months of a post-HBsAg loss consolidation period -After treatment for at least 2 years with undetectable HBV DNA on three separate occasions, 6 months apart
Cirrhosis	Not recommended before HBsAg loss	Not recommended before HBsAg loss	May be considered before HBsAg loss in compensated cirrhosis with careful monitoring plan

AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; EASL, European Association for the Study of the Liver; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen

866 Table 2. Outcomes in HBeAg-positive CHB patients after NA cessation

Reference	Study design	HBeAg (+) patients (n)	Follow-up (years)	NA (mean duration of treatment)	Consolidation treatment (months)	Sero-reversion (%)	Clinical relapse (%)
(Song et al., 2000)	Retro.	34	2	LAM (9 mo)	2,5	38	49,2
(Kuo et al., 2010)	Retro.	124	2	LAM (6 mo)	9	44,3	68,4
(Lee et al., 2010)	Retro.	178	5	LAM (26 mo)	>12	18	30,2
(Wang et al., 2010)	Prosp.	82	4	LAM (24 mo)	16	42,9	29,4
(Van Hees et al., 2018)	Retro.	62	3,8	LAM, TDF, ADV, ETV, LAM+ADV (5,8 years)	8	9	48
(Jung et al., 2016)	Prosp.	45	1	ETV,LAM (8,4 years)	6	n.a.	18
(Reijnders et al., 2010)	Retro.	42	5	LAM, ADV, ETV (26 mo)	>6	44	69
(Liu et al., 2018)	Prosp.	138	10	LAM, ADV, TBV, ETV (27,5 mo)	21	n.a.	31
(Liem et al., 2019)	RCT	18	1,5	ADV, LAM, TBV, TDF(7,4 years)	40	5	6
(Fan et al., 2020)	Prosp.	127	4	TBV(35 mo)	18	17,2	31,5

867 HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; mo, months; n.a., non-applicable;
868 NA, nucleos(t)ide analogue; Prosp., prospective; RCT, randomized controlled trial; Retro,
869 retrospective

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879 Table 3. Outcomes in HBeAg-negative CHB patients after NA cessation

Reference	Study design	HBeAg (-) patients (n)	Follow-up (years)	NA (mean duration of treatment)	HBsAg loss (%)	Clinical relapse (%)	Retreatment (%)
(Seto et al., 2015)	Prosp.	184	1	ETV (36,7 mo)	0	22,8	n.a.
(Kim et al., 2013)	Retro.	45	1	ETV, LAM, ADV (38,4 mo)	n.a.	53,3	n.a.
(Fung et al., 2004)	Retro.	27	1,5	LAM (2 years)	0	30	n.a.
(Wen-Juei Jeng et al., 2018)	Retro.	691	3	ETV, TDF, ADV, LAM, TBV(3 years)	13	61	41
(Papatheodoridis et al., 2018b)	Prosp.	57	1,5	ETV/TDF (≥4 years)	25	33	28
(Liem et al., 2019)	RCT	27	1,5	ADV, LAM, TBV, TDF (7,4 years)	4	19	22
(Berg et al., 2017)	RCT	21	3	TDF(>4 years)	19	19 (Vir*)	38
(Gao et al., 2019)	Retro.	10	1	LAM, ADV, TBV, ETV (42 mo)	20	30	30
(Papatheodoridis et al., 2018a)	Prosp.	130	1,3	ETV, LAM, ADV(5 years)	n.a.	40	33
(Cao et al., 2017)	Prosp.	22	2	ETV, TDF(4 years)	6,1	53	53

880 HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; mo, months; n.a., non-applicable;
881 NA, nucleos(t)ide analogue; Prosp., prospective; RCT, randomized controlled trial; Retro,
882 retrospective; Vir*, virologic relapse.

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References:

Aiello, A., Guarino, M., D'Ausilio, A., Toumi, M., Caporaso, N., Morisco, F., 2016. PMD31 - A Real World Cost Analysis of Serum Hbsag Quantification Test in Patients with Chronic Hepatitis B: The Case of The University of Naples Federico II. Value Health 19, A690. https://doi.org/10.1016/j.jval.2016.09.1972

Asselah, T., Loureiro, D., Boyer, N., Mansouri, A., 2019. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. Lancet Gastroenterol. Hepatol. 4, 883–892. https://doi.org/10.1016/S2468-1253(19)30190-6

Berg, T., Simon, K.-G., Mauss, S., Schott, E., Heyne, R., Klass, D.M., Eisenbach, C., Welzel, T.M., Zachoval, R., Felten, G., Schulze-Zur-Wiesch, J., Cornberg, M., Op den Brouw, M.L., Jump, B., Reiser, H., Gallo, L., Warger, T., Petersen, J., FINITE CHB study investigators [First investigation in stopping TDF treatment after long-term virological suppression in HBeAg-negative chronic hepatitis B], 2017. Long-term response after stopping tenofovir disoproxil fumarate in non-cirrhotic HBeAg-negative patients - FINITE study. J. Hepatol. 67, 918–924. https://doi.org/10.1016/j.jhep.2017.07.012

Boni, C., Laccabue, D., Lampertico, P., Giuberti, T., Viganò, M., Schivazappa, S., Alfieri, A., Pesci, M., Gaeta, G.B., Brancaccio, G., Colombo, M., Missale, G., Ferrari, C., 2012. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. Gastroenterology 143, 963-973.e9. https://doi.org/10.1053/j.gastro.2012.07.014

Boni, C., Lampertico, P., Talamona, L., Giuberti, T., Invernizzi, F., Barili, V., Fisicaro, P., Rossi, M., Cavallo, M.C., Vecchi, A., Pedrazzi, G., Alfieri, A., Colombo, M., Missale, G., Ferrari, C., 2015. Natural killer cell phenotype modulation and natural killer/T-cell interplay in nucleos(t)ide analogue-treated hepatitis e antigen-negative patients with chronic hepatitis B. Hepatol. Baltim. Md 62, 1697–1709. https://doi.org/10.1002/hep.28155

Boni, C., Penna, A., Bertoletti, A., Lamonaca, V., Rapti, I., Missale, G., Pilli, M., Urbani, S., Cavalli, A., Cerioni, S., Panebianco, R., Jenkins, J., Ferrari, C., 2003. Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. J. Hepatol. 39, 595–605. https://doi.org/10.1016/s0168-8278(03)00292-7

Bonino, F., Marcellin, P., Lau, G.K.K., Hadziyannis, S., Jin, R., Piratvisuth, T., Germanidis, G., Yurdaydin, C., Diago, M., Gurel, S., Lai, M.-Y., Brunetto, M.R., Farci, P., Popescu, M., McCloud, P., Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group, 2007. Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B. Gut 56, 699–705. https://doi.org/10.1136/gut.2005.089722

Brass, A., Brenndörfer, E.D., 2014. The Role of Chemokines in Hepatitis C Virus-Mediated Liver Disease. Int. J. Mol. Sci. 15, 4747–4779. https://doi.org/10.3390/ijms15034747

Buti, M., Wong, D.K., Gane, E., Flisiak, R., Manns, M., Kaita, K., Janssen, H.L.A., Op den Brouw, M., Jump, B., Kitrinos, K., Crans, G., Flaherty, J., Gaggar, A., Marcellin, P., 2019. 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. 4, 296–304. https://doi.org/10.1016/S2468-1253(19)30015-9

Byun, K.S., Kwon, O.S., Kim, J.H., Yim, H.J., Chang, Y.J., Kim, J.Y., Yeon, J.E., Park, J.J., Kim, J.S., Bak, Y.T., Lee, C.H., 2005. Factors related to post-treatment relapse in chronic hepatitis B patients who lost HBeAg after lamivudine therapy. J. Gastroenterol. Hepatol. 20, 1838–1842. https://doi.org/10.1111/j.1440-1746.2005.03952.x

Cao, J., Chi, H., Yu, T., Li, Z., Hansen, B.E., Zhang, X., Zhong, C., Sun, J., Hou, J., Janssen, H.L.A., Peng, J., 2017. 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. 215, 581–589. https://doi.org/10.1093/infdis/jix025

Carey, I., Gersch, J., Wang, B., Moigboi, C., Kuhns, M., Cloherty, G., Dusheiko, G., Agarwal, K., 2019. Pre-genomic HBV RNA and HBcrAg predict outcomes in HBeAg negative chronic hepatitis B

patients suppressed on nucleos(t)ide analogue therapy. Hepatol. Baltim. Md. https://doi.org/10.1002/hep.31026
Chan, H.L.-Y., Wang, H., Niu, J., Chim, A.M.-L., Sung, J.J.-Y., 2007. Two-year lamivudine treatment for hepatitis B e antigen-negative chronic hepatitis B: a double-blind, placebo-controlled trial. Antivir. Ther. 12, 345–353.
Chan, H.L.-Y., Wong, G.L.-H., Chim, A.M.-L., Chan, H.-Y., Chu, S.H.-T., Wong, V.W.-S., 2011. Prediction of off-treatment response to lamivudine by serum hepatitis B surface antigen quantification in hepatitis B e antigen-negative patients. Antivir. Ther. 16, 1249–1257. https://doi.org/10.3851/IMP1921
Chang, Ming-Ling, Jeng, W.-J., Liaw, Y.-F., 2015. Clinical events after cessation of lamivudine therapy in patients recovered from hepatitis B flare with hepatic decompensation. Clin. Gastroenterol. Hepatol. Off. Clin. Pract. J. Am. Gastroenterol. Assoc. 13, 979–986. https://doi.org/10.1016/j.cgh.2014.10.023
Chang, M.-L., Liaw, Y.-F., Hadziyannis, S.J., 2015. 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. 42, 243–257. https://doi.org/10.1111/apt.13272
Chaung, K.T., Ha, N.B., Trinh, H.N., Garcia, R.T., Nguyen, H.A., Nguyen, K.K., Garcia, G., Ahmed, A., Keeffe, E.B., Nguyen, M.H., 2012. High frequency of recurrent viremia after hepatitis B e antigen seroconversion and consolidation therapy. J. Clin. Gastroenterol. 46, 865–870. https://doi.org/10.1097/MCG.0b013e31825ceed9
Chen, C.-H., Hsu, Y.-C., Lu, S.-N., Hung, C.-H., Wang, J.-H., Lee, C.-M., Hu, T.-H., 2018. The incidence and predictors of HBV relapse after cessation of tenofovir therapy in chronic hepatitis B patients. J. Viral Hepat. 25, 590–597. https://doi.org/10.1111/jvh.12851
Chen, C.-H., Hung, C.-H., Wang, J.-H., Lu, S.-N., Lai, H.-C., Hu, T.-H., Lin, C.-H., Peng, C.-Y., 2019. 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. 219, 1624–1633. https://doi.org/10.1093/infdis/jiy697
Chen, C.-H., Lu, S.-N., Hung, C.-H., Wang, J.-H., Hu, T.-H., Changchien, C.-S., Lee, C.-M., 2014. The role of hepatitis B surface antigen quantification in predicting HBsAg loss and HBV relapse after discontinuation of lamivudine treatment. J. Hepatol. 61, 515–522. https://doi.org/10.1016/j.jhep.2014.04.029
Chen, Y., Li, X., Ye, B., Yang, X., Wu, W., Chen, B., Pan, X., Cao, H., Li, L., 2011. Effect of telbivudine therapy on the cellular immune response in chronic hepatitis B. Antiviral Res. 91, 23–31. https://doi.org/10.1016/j.antiviral.2011.04.008
Chen, Y.C., Peng, C.Y., Jeng, W.J., Chien, R.N., Liaw, Y.F., 2015. Clinical outcomes after interruption of entecavir therapy in HBeAg-negative chronic hepatitis B patients with compensated cirrhosis. Aliment. Pharmacol. Ther. 42, 1182–1191. https://doi.org/10.1111/apt.13409
Chi, H., Li, Z., Hansen, B.E., Yu, T., Zhang, X., Sun, J., Hou, J., Janssen, H.L.A., Peng, J., 2019. 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. Off. Clin. Pract. J. Am. Gastroenterol. Assoc. 17, 182-191.e1. https://doi.org/10.1016/j.cgh.2018.05.047
Chien, R.-N., Liaw, Y.-F., 2020. Re-treatment for severe hepatitis flare in HBeAg-negative chronic hepatitis B: An appraisal with combined HBsAg/ALT kinetics. J. Viral Hepat. 27, 544–547. https://doi.org/10.1111/jvh.13253
Chien, R.-N., Liaw, Y.-F., 2006. Short-term lamivudine therapy in HBeAg-negative chronic active hepatitis B in Taiwan. Antivir. Ther. 11, 947–952.
Cornberg, M., Lok, A.S.-F., Terrault, N.A., Zoulim, F., 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty, 2020. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference‡. J. Hepatol. 72, 539–557. https://doi.org/10.1016/j.jhep.2019.11.003

Dienstag, J.L., Cianciara, J., Karayalcin, S., Kowdley, K.V., Willems, B., Plisek, S., Woessner, M., Gardner, S., Schiff, E., 2003. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatol. Baltim. Md 37, 748–755. https://doi.org/10.1053/jhep.2003.50117

Dunn, C., Brunetto, M., Reynolds, G., Christophides, T., Kennedy, P.T., Lampertico, P., Das, A., Lopes, A.R., Borrow, P., Williams, K., Humphreys, E., Afford, S., Adams, D.H., Bertoletti, A., Maini, M.K., 2007. Cytokines induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. J. Exp. Med. 204, 667–680. https://doi.org/10.1084/jem.20061287

European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver, 2017. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J. Hepatol. 67, 370–398. https://doi.org/10.1016/j.jhep.2017.03.021

Fan, R., Peng, J., Xie, Q., Tan, D., Xu, M., Niu, J., Wang, H., Ren, H., Chen, X., Wang, M., Sheng, J., Tang, H., Bai, X., Wu, Y., Zhou, B., Sun, J., Hou, J., Chronic Hepatitis B Study Consortium, 2020. Combining HBV RNA and hepatitis B core-related antigen: guidance for safely stopping nucleos(t)ide analogues in HBeAg-positive chronic hepatitis B patients. J. Infect. Dis. https://doi.org/10.1093/infdis/jiaa136

Fan, R., Sun, J., Yuan, Q., Xie, Q., Bai, X., Ning, Q., Cheng, J., Yu, Y., Niu, J., Shi, G., Wang, H., Tan, D., Wan, M., Chen, S., Xu, M., Chen, X., Tang, H., Sheng, J., Lu, F., Jia, J., Zhuang, H., Xia, N., Hou, J., Chronic Hepatitis B Study Consortium, 2016. Baseline quantitative hepatitis B core antibody titre alone strongly predicts HBeAg seroconversion across chronic hepatitis B patients treated with peginterferon or nucleos(t)ide analogues. Gut 65, 313–320. https://doi.org/10.1136/gutjnl-2014-308546

Faure-Dupuy, S., Lucifora, J., Durantel, D., 2017. Interplay between the Hepatitis B Virus and Innate Immunity: From an Understanding to the Development of Therapeutic Concepts. Viruses 9. https://doi.org/10.3390/v9050095

Fisicaro, P., Barili, V., Montanini, B., Acerbi, G., Ferracin, M., Guerrieri, F., Salerno, D., Boni, C., Massari, M., Cavallo, M.C., Grossi, G., Giuberti, T., Lampertico, P., Missale, G., Levrero, M., Ottonello, S., Ferrari, C., 2017. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat. Med. 23, 327–336. https://doi.org/10.1038/nm.4275

Fisicaro, P., Rossi, M., Vecchi, A., Acerbi, G., Barili, V., Laccabue, D., Montali, I., Zecca, A., Penna, A., Missale, G., Ferrari, C., Boni, C., 2019. The Good and the Bad of Natural Killer Cells in Virus Control: Perspective for Anti-HBV Therapy. Int. J. Mol. Sci. 20. https://doi.org/10.3390/ijms20205080

Fontana, R.J., 2009. Side effects of long-term oral antiviral therapy for hepatitis B. Hepatol. Baltim. Md 49, S185-195. https://doi.org/10.1002/hep.22885

Ford, N., Scourse, R., Lemoine, M., Hutin, Y., Bulterys, M., Shubber, Z., Donchuk, D., Wandeler, G., 2018. Adherence to Nucleos(t)ide Analogue Therapies for Chronic Hepatitis B Infection: A Systematic Review and Meta-Analysis. Hepatol. Commun. 1160–1167.

Fung, S.K., Wong, F., Hussain, M., Lok, A.S.F., 2004. Sustained response after a 2-year course of lamivudine treatment of hepatitis B e antigen-negative chronic hepatitis B. J. Viral Hepat. 11, 432–438. https://doi.org/10.1111/j.1365-2893.2004.00556.x

Galmozzi, E., Viganò, M., Lampertico, P., 2014. Systematic review with meta-analysis: do interferon lambda 3 polymorphisms predict the outcome of interferon-therapy in hepatitis B infection? Aliment. Pharmacol. Ther. 39, 569–578. https://doi.org/10.1111/apt.12631

Gao, L., Hu, Y., Shi, X., Li, X., Zhang, D., Ren, H., 2019. 48 weeks outcome after cessation of nucleos(t)ide analogue therapy in chronic hepatitis B patients. Ann. Hepatol. https://doi.org/10.1016/j.aohep.2019.10.006

Ge, G.-H., Ye, Y., Zhou, X.-B., Chen, L., He, C., Wen, D.-F., Tan, Y.-W., 2015. Hepatitis B surface antigen levels of cessation of nucleos(t)ide analogs associated with virological relapse in hepatitis B

surface antigen-negative chronic hepatitis B patients. World J. Gastroenterol. 21, 8653–8659. https://doi.org/10.3748/wjg.v21.i28.8653
Giersch, K., Allweiss, L., Volz, T., Dandri, M., Lütgehetmann, M., 2017. Serum HBV pgRNA as a clinical marker for cccDNA activity. J. Hepatol. 66, 460–462. https://doi.org/10.1016/j.jhep.2016.09.028
Ha, M., Zhang, G., Diao, S., Lin, M., Sun, L., She, H., Kuan, C., Shen, L., Huang, C., Shen, W., Huang, Z., 2012. A prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B patients with stringent cessation criteria for adefovir. Arch. Virol. 157, 285–290. https://doi.org/10.1007/s00705-011-1163-0
Hadziyannis, E., Hadziyannis, S., 2020. Current practice and contrasting views on discontinuation of nucleos(t)ide analog therapy in chronic hepatitis B. Expert Rev. Gastroenterol. Hepatol. 14, 243–251. https://doi.org/10.1080/17474124.2020.1738219
Hadziyannis, S.J., Sevastianos, V., Rapti, I., Vassilopoulos, D., Hadziyannis, E., 2012. Sustained responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop long-term treatment with adefovir. Gastroenterology 143, 629-636.e1. https://doi.org/10.1053/j.gastro.2012.05.039
He, D., Guo, S., Chen, W., Chen, X., Yan, G., Wang, J., Li, M., Zhu, P., Huang, H., Wang, Y., 2013. Long-term outcomes after nucleos(t)ide analogues discontinuation in chronic hepatitis B patients with HBeAg-negative. BMC Infect. Dis. 13, 458. https://doi.org/10.1186/1471-2334-13-458
Höner Zu Siederdissen, C., Hui, A.J., Sukeepaisarnjaroen, W., Tangkijvanich, P., Su, W.W., Nieto, G.E.G., Gineste, P., Nitcheu, J., Crabé, S., Stepien, S., Manns, M.P., Trépo, C., Wedemeyer, H., Cornberg, M., 2018. Contrasting Timing of Virological Relapse After Discontinuation of Tenofovir or Entecavir in Hepatitis B e Antigen-Negative Patients. J. Infect. Dis. 218, 1480–1484. https://doi.org/10.1093/infdis/jiy350
Höner Zu Siederdissen, C., Rinker, F., Maasoumy, B., Wiegand, S.B., Filmann, N., Falk, C.S., Deterding, K., Port, K., Mix, C., Manns, M.P., Herrmann, E., Wedemeyer, H., Kraft, A.R.M., Cornberg, M., 2016. Viral and Host Responses After Stopping Long-term Nucleos(t)ide Analogue Therapy in HBeAg-Negative Chronic Hepatitis B. J. Infect. Dis. 214, 1492–1497. https://doi.org/10.1093/infdis/jiw412
Hoogeveen, R.C., Boonstra, A., 2020. Checkpoint Inhibitors and Therapeutic Vaccines for the Treatment of Chronic HBV Infection. Front. Immunol. 11, 401. https://doi.org/10.3389/fimmu.2020.00401
Hsu, Y.-C., Nguyen, M.H., Mo, L.-R., Wu, M.-S., Yang, T.-H., Chen, C.-C., Tseng, C.-H., Tai, C.-M., Wu, C.-Y., Lin, J.-T., Tanaka, Y., Chang, C.-Y., 2019. Combining hepatitis B core-related and surface antigens at end of nucleos(t)ide analogue treatment to predict off-therapy relapse risk. Aliment. Pharmacol. Ther. 49, 107–115. https://doi.org/10.1111/apt.15058
Huang, Y.-H., Wu, J.-C., Chang, T.-T., Sheen, I.-J., Lee, P.-C., Huo, T.-I., Su, C.-W., Wang, Y.-J., Chang, F.-Y., Lee, S.-D., 2003. Analysis of clinical, biochemical and viral factors associated with early relapse after lamivudine treatment for hepatitis B e antigen-negative chronic hepatitis B patients in Taiwan. J. Viral Hepat. 10, 277–284. https://doi.org/10.1046/j.1365-2893.2003.00428.x
Jaroszewicz, J., Ho, H., Markova, A., Deterding, K., Wursthorn, K., Schulz, S., Bock, C.-T., Tillmann, H.L., Manns, M.P., Wedemeyer, H., Cornberg, M., 2011. Hepatitis B surface antigen (HBsAg) decrease and serum interferon-inducible protein-10 levels as predictive markers for HBsAg loss during treatment with nucleoside/nucleotide analogues. Antivir. Ther. 16, 915–924. https://doi.org/10.3851/IMP1866
Jeng, W.-J., Chang, M.-L., Liaw, Y.-F., 2019. Off-therapy precipitous HBsAg decline predicts HBsAg loss after finite entecavir therapy in HBeAg-negative patients. J. Viral Hepat. 26, 1019–1026. https://doi.org/10.1111/jvh.13114
Jeng, Wen-Juei, Chen, Y.-C., Chien, R.-N., Sheen, I.-S., Liaw, Y.-F., 2018. 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. Hepatol. Baltim. Md 68, 425–434. https://doi.org/10.1002/hep.29640
Jeng, W.-J., Chen, Y.-C., Sheen, I.-S., Lin, C.-L., Hu, T.-H., Chien, R.-N., Liaw, Y.-F., 2016. Clinical Relapse After Cessation of Tenofovir Therapy in Hepatitis B e Antigen-Negative Patients. Clin. Gastroenterol. Hepatol. Off. Clin. Pract. J. Am. Gastroenterol. Assoc. 14, 1813-1820.e1. https://doi.org/10.1016/j.cgh.2016.07.002
Jeng, W.-J., Sheen, I.-S., Chen, Y.-C., Hsu, C.-W., Chien, R.-N., Chu, C.-M., Liaw, Y.-F., 2013. Off-therapy durability of response to entecavir therapy in hepatitis B e antigen-negative chronic hepatitis B patients. Hepatol. Baltim. Md 58, 1888–1896. https://doi.org/10.1002/hep.26549
Jeng, W.-J., Yang, H.-I., Chen, Y.-C., Liu, J., Chien, R.-N., Sheen, I.-S., Chen, C.-J., Liaw, Y.-F., 2018. Increased incidence of HBsAg seroclearance in HBeAg negative chronic hepatitis B patients discontinued Nuc therapy comparing to natural course-a propensity score matched study. J. Hepatol. 68, S88. https://doi.org/10.1016/S0168-8278(18)30394-5
Jiang, J., Huang, Z., He, L., Huang, Y., Su, M., Xie, R., Liang, Y., Fu, W., Huang, X., Guo, W., Zhong, S., Liu, Z., Li, S., Zhu, T., Gao, Z., 2015. Residual amount of HBV DNA in serum is related to relapse in chronic hepatitis B patients after cessation of nucleos(t)ide analogs. J. Clin. Gastroenterol. 49, 323–328. https://doi.org/10.1097/MCG.0000000000000170
Jiang, Y., Ma, Z., Xin, G., Yan, H., Li, W., Xu, H., Hao, C., Niu, J., Zhao, P., 2010. Th1 and Th2 immune response in chronic hepatitis B patients during a long-term treatment with adefovir dipivoxil. Mediators Inflamm. 2010, 143026. https://doi.org/10.1155/2010/143026
Jin, Y.-J., Kim, K.M., Yoo, D., Shim, J.H., Lee, H.C., Chung, Y.-H., Lee, Y.S., Suh, D.J., 2012. Clinical course of chronic hepatitis B patients who were off-treated after lamivudine treatment: analysis of 138 consecutive patients. Virol. J. 9, 239. https://doi.org/10.1186/1743-422X-9-239
Ju, H., Liu, H., Tian, Z.-B., Jiang, Y.-P., Zhang, C.-P., Liu, X.-S., 2017. Association of polymorphisms in key Th-17 immune response genes with HBeAg-positive chronic hepatitis B susceptibility and response to PEG-IFNa-2α. Virology 509, 35–41. https://doi.org/10.1016/j.virol.2017.05.011
Jung, K.S., Park, J.Y., Chon, Y.E., Kim, H.-S., Kang, W., Kim, B.K., Kim, S.U., Kim, D.Y., Han, K.-H., Ahn, S.H., 2016. Clinical outcomes and predictors for relapse after cessation of oral antiviral treatment in chronic hepatitis B patients. J. Gastroenterol. 51, 830–839. https://doi.org/10.1007/s00535-015-1153-1
Jung, Y.K., Yeon, J.E., Lee, K.G., Jung, E.S., Kim, Jeong Han, Kim, Ji Hoon, Seo, Y.S., Yim, H.J., Um, S.H., Ryu, H.S., Byun, K.S., 2011. Virologic response is not durable after adefovir discontinuation in lamivudine-resistant chronic hepatitis B patients. Korean J. Hepatol. 17, 261–267. https://doi.org/10.3350/kjhep.2011.17.4.261
Kang, S.H., Kang, K., Jong Eun, Y., Lee, Y.S., Kim, T.S., Yoo, Y.J., Suh, S.J., Yoon, E.L., Jung, Y.K., Kim, J.H., Seo, Y.S., Yim, H.J., Byun, K.S., 2017. Antiviral response is not sustained after cessation of lamivudine treatment in chronic hepatitis B patients: A 10-year follow-up study. J. Med. Virol. 89, 849–856. https://doi.org/10.1002/jmv.24715
Kim, G.-A., Lim, Y.-S., An, J., Lee, D., Shim, J.H., Kim, K.M., Lee, H.C., Chung, Y.-H., Lee, Y.S., Suh, D.J., 2014. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut 63, 1325–1332. https://doi.org/10.1136/gutjnl-2013-305517
Kim, M.A., Kim, S.U., Sinn, D.H., Jang, J.W., Lim, Y.-S., Ahn, S.H., Shim, J.-J., Seo, Y.S., Baek, Y.H., Kim, S.G., Kim, Y.S., Kim, J.H., Choe, W.H., Yim, H.J., Lee, H.W., Kwon, J.H., Lee, S.W., Jang, J.Y., Kim, H.Y., Park, Y., Kim, G.-A., Yang, H., Lee, H.A., Koh, M., Lee, Y.-S., Kim, M., Chang, Y., Kim, Y.J., Yoon, J.-H., Zoulim, F., Lee, J.-H., 2020. Discontinuation of nucleos(t)ide analogues is not associated with a higher risk of HBsAg seroreversion after antiviral-induced HBsAg seroclearance: a nationwide multicentre study. Gut. https://doi.org/10.1136/gutjnl-2019-320015

Kim, Y.J., Kim, K., Hwang, S.H., Kim, S.S., Lee, D., Cheong, J.Y., Cho, S.W., 2013. Durability after discontinuation of nucleos(t)ide therapy in chronic HBeAg negative hepatitis patients. Clin. Mol. Hepatol. 19, 300–304. https://doi.org/10.3350/cmh.2013.19.3.300

Kranidioti, H., Manolakopoulos, S., Kontos, G., Breen, M.S., Kourikou, A., Deutsch, M., Quesada-Del-Bosque, M.E., Martinez-Nunez, R.T., Naiyer, M.M., Woelk, C.H., Sanchez-Elsner, T., Hadziyannis, E., Papatheodoridis, G., Khakoo, S.I., 2019. 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. 26, 697–709. https://doi.org/10.1111/jvh.13068

Kuo, M.-T., Hu, T.-H., Hung, C.-H., Wang, J.-H., Lu, S.-N., Tsai, K.-L., Chen, C.-H., 2019. Hepatitis B virus relapse rates in chronic hepatitis B patients who discontinue either entecavir or tenofovir. Aliment. Pharmacol. Ther. 49, 218–228. https://doi.org/10.1111/apt.15053

Kuo, Y.-H., Chen, C.-H., Wang, J.-H., Hung, C.-H., Tseng, P.-L., Lu, S.-N., Changchien, C.-S., Lee, C.-M., 2010. Extended lamivudine consolidation therapy in hepatitis B e antigen-positive chronic hepatitis B patients improves sustained hepatitis B e antigen seroconversion. Scand. J. Gastroenterol. 45, 75–81. https://doi.org/10.3109/00365520903394550

Lamivudine Treatment Can Restore T Cell Responsiveness in Chronic Hepatitis B [WWW Document], n.d. URL https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/9727065/?from_term=+Lamivudine+treatment+can+restore+T+cell+responsiveness+in+chronic+hepatitis+B&from_sort=date (accessed 4.25.20).

Lampertico, P., Berg, T., 2018. Less can be more: A finite treatment approach for HBeAg-negative chronic hepatitis B. Hepatol. Baltim. Md 68, 397–400. https://doi.org/10.1002/hep.29821

Lang, P.A., Lang, K.S., Xu, H.C., Grusdat, M., Parish, I.A., Recher, M., Elford, A.R., Dhanji, S., Shaabani, N., Tran, C.W., Dissanayake, D., Rahbar, R., Ghazarian, M., Brüstle, A., Fine, J., Chen, P., Weaver, C.T., Klose, C., Diefenbach, A., Häussinger, D., Carlyle, J.R., Kaech, S.M., Mak, T.W., Ohashi, P.S., 2012. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. Proc. Natl. Acad. Sci. U. S. A. 109, 1210–1215. https://doi.org/10.1073/pnas.1118834109

Larrubia, J.R., Benito-Martínez, S., Calvino, M., Sanz-de-Villalobos, E., Parra-Cid, T., 2008. Role of chemokines and their receptors in viral persistence and liver damage during chronic hepatitis C virus infection. World J. Gastroenterol. 14, 7149–7159. https://doi.org/10.3748/wjg.14.7149

Lee, H.W., Lee, H.J., Hwang, J.S., Sohn, J.H., Jang, J.Y., Han, K.J., Park, J.Y., Kim, D.Y., Ahn, S.H., Paik, Y.H., Lee, C.K., Lee, K.S., Chon, C.Y., Han, K.-H., 2010. Lamivudine maintenance beyond one year after HBeAg seroconversion is a major factor for sustained virologic response in HBeAg-positive chronic hepatitis B. Hepatol. Baltim. Md 51, 415–421. https://doi.org/10.1002/hep.23323

Liang, Y., Jiang, J., Su, M., Liu, Z., Guo, W., Huang, X., Xie, R., Ge, S., Hu, J., Jiang, Z., Zhu, M., Wong, V.W.-S., Chan, H.L.-Y., 2011. Predictors of relapse in chronic hepatitis B after discontinuation of anti-viral therapy. Aliment. Pharmacol. Ther. 34, 344–352. https://doi.org/10.1111/j.1365-2036.2011.04738.x

Liaw, Y.-F., 2020. Hepatitis B flare after cessation of nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B: To retreat or not to retreat. Hepatol. Baltim. Md. https://doi.org/10.1002/hep.31525

Liaw, Y.-F., 2019. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative chronic HBV infection. Nat. Rev. Gastroenterol. Hepatol. 16, 631–641. https://doi.org/10.1038/s41575-019-0197-8

Liaw, Y.-F., Jeng, W.-J., Chang, M.-L., 2018. HBsAg Kinetics in Retreatment Decision for Off-Therapy Hepatitis B Flare in HBeAg-Negative Patients. Gastroenterology 154, 2280–2281. https://doi.org/10.1053/j.gastro.2018.03.066

Liaw, Y.-F., Leung, N., Kao, J.-H., Piratvisuth, T., Gane, E., Han, K.-H., Guan, R., Lau, G.K.K., Locarnini, S., Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study

of the Liver, 2008. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol. Int. 2, 263–283. https://doi.org/10.1007/s12072-008-9080-3

Liaw, Y.-F., Sung, J.J.Y., Chow, W.C., Farrell, G., Lee, C.-Z., Yuen, H., Tanwandee, T., Tao, Q.-M., Shue, K., Keene, O.N., Dixon, J.S., Gray, D.F., Sabbat, J., Cirrhosis Asian Lamivudine Multicentre Study Group, 2004. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N. Engl. J. Med. 351, 1521–1531. https://doi.org/10.1056/NEJMoa033364

Liem, K.S., Fung, S., Wong, D.K., Yim, C., Noureldin, S., Chen, J., Feld, J.J., Hansen, B.E., Janssen, H.L.A., 2019. 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 68, 2206–2213. https://doi.org/10.1136/gutjnl-2019-318981

Lin, C.-L., Kao, J.-H., 2016. New perspectives of biomarkers for the management of chronic hepatitis B. Clin. Mol. Hepatol. 22, 423–431. https://doi.org/10.3350/cmh.2016.0069

Liu, F., Liu, Z.R., Li, T., Liu, Y.D., Zhang, M., Xue, Y., Zhang, L.X., Ye, Q., Fan, X.P., Wang, L., 2018. Varying 10-year off-treatment responses to nucleos(t)ide analogues in patients with chronic hepatitis B according to their pretreatment hepatitis B e antigen status. J. Dig. Dis. 19, 561–571. https://doi.org/10.1111/1751-2980.12654

Liu, F., Wang, L., Li, X.Y., Liu, Y.D., Wang, J.B., Zhang, Z.H., Wang, Y.Z., 2011. 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. 26, 456–460. https://doi.org/10.1111/j.1440-1746.2010.06492.x

Liu, J., Li, T., Zhang, L., Xu, A., 2019. The Role of Hepatitis B Surface Antigen in Nucleos(t)ide Analogues Cessation Among Asian Patients With Chronic Hepatitis B: A Systematic Review. Hepatol. Baltim. Md 70, 1045–1055. https://doi.org/10.1002/hep.30474

Liu, Y., Jia, M., Wu, S., Jiang, W., Feng, Y., 2019. 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. IJID Off. Publ. Int. Soc. Infect. Dis. 86, 201–207. https://doi.org/10.1016/j.ijid.2019.07.036

Lok, A.S.F., McMahon, B.J., Brown, R.S., Wong, J.B., Ahmed, A.T., Farah, W., Almasri, J., Alahdab, F., Benkhadra, K., Mouchli, M.A., Singh, S., Mohamed, E.A., Abu Dabrh, A.M., Prokop, L.J., Wang, Z., Murad, M.H., Mohammed, K., 2016. Antiviral therapy for chronic hepatitis B viral infection in adults: A systematic review and meta-analysis. Hepatol. Baltim. Md 63, 284–306. https://doi.org/10.1002/hep.28280

Ma, Z., Cao, Q., Xiong, Y., Zhang, E., Lu, M., 2018. Interaction between Hepatitis B Virus and Toll-Like Receptors: Current Status and Potential Therapeutic Use for Chronic Hepatitis B. Vaccines 6. https://doi.org/10.3390/vaccines6010006

Maini, M.K., Boni, C., Lee, C.K., Larrubia, J.R., Reignat, S., Ogg, G.S., King, A.S., Herberg, J., Gilson, R., Alisa, A., Williams, R., Vergani, D., Naoumov, N.V., Ferrari, C., Bertoletti, A., 2000. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J. Exp. Med. 191, 1269–1280. https://doi.org/10.1084/jem.191.8.1269

Maini, M.K., Peppa, D., 2013. NK cells: a double-edged sword in chronic hepatitis B virus infection. Front. Immunol. 4, 57. https://doi.org/10.3389/fimmu.2013.00057

Mansouri, A., Gattolliat, C.-H., Asselah, T., 2018. Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases. Gastroenterology 155, 629–647. https://doi.org/10.1053/j.gastro.2018.06.083

Marcellin, P., Asselah, T., 2013. Long-term therapy for chronic hepatitis B: hepatitis B virus DNA suppression leading to cirrhosis reversal. J. Gastroenterol. Hepatol. 28, 912–923. https://doi.org/10.1111/jgh.12213

Marcellin, P., Bonino, F., Lau, G.K.K., Farci, P., Yurdaydin, C., Piratvisuth, T., Jin, R., Gurel, S., Lu, Z.-M., Wu, J., Popescu, M., Hadziyannis, S., Peginterferon alfa-2a in HBeAg-negative Chronic Hepatitis B Study Group, 2009. Sustained response of hepatitis B e antigen-negative patients

3 years after treatment with peginterferon alpha-2a. Gastroenterology 136, 2169-2179.e1–4. https://doi.org/10.1053/j.gastro.2009.03.006
Marcellin, P., Gane, E., Buti, M., Afdhal, N., Sievert, W., Jacobson, I.M., Washington, M.K., Germanidis, G., Flaherty, J.F., Aguilar Schall, R., Bornstein, J.D., Kitrinos, K.M., Subramanian, G.M., McHutchison, J.G., Heathcote, E.J., 2013. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet Lond. Engl. 381, 468–475. https://doi.org/10.1016/S0140-6736(12)61425-1
Marcellin, P., Wong, D.K., Sievert, W., Buggisch, P., Petersen, J., Flisiak, R., Manns, M., Kaita, K., Krastev, Z., Lee, S.S., Cathcart, A.L., Crans, G., Op den Brouw, M., Jump, B., Gaggar, A., Flaherty, J., Buti, M., 2019. Ten-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B virus infection. Liver Int. Off. J. Int. Assoc. Study Liver 39, 1868–1875. https://doi.org/10.1111/liv.14155
Martinet, J., Dufeu-Duchesne, T., Bruder Costa, J., Larrat, S., Marlu, A., Leroy, V., Plumas, J., Aspord, C., n.d. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of natural killer cells in patients with chronic HBV infection. Gastroenterology 143, 1586–1596.e8. https://doi.org/10.1053/j.gastro.2012.08.046
Martinot-Peignoux, M., Asselah, T., Marcellin, P., 2015. HBsAg quantification to optimize treatment monitoring in chronic hepatitis B patients. Liver Int. Off. J. Int. Assoc. Study Liver 35 Suppl 1, 82–90. https://doi.org/10.1111/liv.12735
Martinot-Peignoux, M., Lapalus, M., Asselah, T., Marcellin, P., 2014. HBsAg quantification: useful for monitoring natural history and treatment outcome. Liver Int. Off. J. Int. Assoc. Study Liver 34 Suppl 1, 97–107. https://doi.org/10.1111/liv.12403
Martinot-Peignoux, M., Lapalus, M., Laouénan, C., Lada, O., Netto-Cardoso, A.C.F., Boyer, N., Ripault, M.P., Carvalho-Filho, R., Asselah, T., Marcellin, P., 2013. Prediction of disease reactivation in asymptomatic hepatitis B e antigen-negative chronic hepatitis B patients using baseline serum measurements of HBsAg and HBV-DNA. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 58, 401–407. https://doi.org/10.1016/j.jcv.2013.08.010
Matsumoto, A., Tanaka, E., Minami, M., Okanoue, T., Yatsuhashi, H., Nagaoka, S., Suzuki, F., Kobayashi, M., Chayama, K., Imamura, M., Yotsuyanagi, H., Nakaoka, S., Maki, N., Kawata, S., Kumada, H., Iino, S., Kiyosawa, K., 2007. Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol. Res. Off. J. Jpn. Soc. Hepatol. 37, 661–666. https://doi.org/10.1111/j.1872-034X.2007.00094.x
Matsumoto, A., Yatsuhashi, H., Nagaoka, S., Suzuki, Y., Hosaka, T., Tsuge, M., Chayama, K., Kanda, T., Yokosuka, O., Nishiguchi, S., Saito, M., Miyase, S., Kang, J.-H., Shinkai, N., Tanaka, Y., Umemura, T., Tanaka, E., 2015. Factors associated with the effect of interferon-α sequential therapy in order to discontinue nucleoside/nucleotide analog treatment in patients with chronic hepatitis B. Hepatol. Res. Off. J. Jpn. Soc. Hepatol. 45, 1195–1202. https://doi.org/10.1111/hepr.12488
Neumann-Haefelin, C., Thimme, R., 2018. Entering the spotlight: hepatitis B surface antigen-specific B cells. J. Clin. Invest. 128, 4257–4259. https://doi.org/10.1172/JCI124098
Oliviero, B., Varchetta, S., Paudice, E., Michelone, G., Zaramella, M., Mavilio, D., De Filippi, F., Bruno, S., Mondelli, M.U., 2009. Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. Gastroenterology 137, 1151–1160, 1160.e1–7. https://doi.org/10.1053/j.gastro.2009.05.047
Ouaguia, L., Leroy, V., Dufeu-Duchesne, T., Durantel, D., Decaens, T., Hubert, M., Valladeau-Guilemond, J., Bendriss-Vermare, N., Chaperot, L., Aspord, C., 2019. Circulating and Hepatic BDCA1+, BDCA2+, and BDCA3+ Dendritic Cells Are Differentially Subverted in Patients With Chronic HBV Infection. Front. Immunol. 10, 112. https://doi.org/10.3389/fimmu.2019.00112
Pan, X., Zhang, K., Yang, X., Liang, J., Sun, H., Li, X., Zou, Y., Xu, Qingqiang, An, G., Li, G., Xu, Qihuan, 2013. Relapse rate and associated-factor of recurrence after stopping NUCs therapy with

different prolonged consolidation therapy in HBeAg positive CHB patients. PloS One 8, e68568. https://doi.org/10.1371/journal.pone.0068568
Papatheodoridi, M., Papatheodoridis, G., 2020. Emerging Diagnostic Tools to Decide When to Discontinue Nucleos(t)ide Analogues in Chronic Hepatitis B. Cells 9. https://doi.org/10.3390/cells9020493
Papatheodoridis, G., Goulis, J., Manolakopoulos, S., Margariti, A., Exarchos, X., Kokkonis, G., Hadziyiannis, E., Papaioannou, C., Manesis, E., Pectasides, D., Akriviadis, E., 2014. Changes of HBsAg and interferon-inducible protein 10 serum levels in naive HBeAg-negative chronic hepatitis B patients under 4-year entecavir therapy. J. Hepatol. 60, 62–68. https://doi.org/10.1016/j.jhep.2013.08.023
Papatheodoridis, G., Vlachogiannakos, I., Cholongitas, E., Wursthorn, K., Thomadakis, C., Touloumi, G., Petersen, J., 2016. Discontinuation of oral antivirals in chronic hepatitis B: A systematic review. Hepatol. Baltim. Md 63, 1481–1492. https://doi.org/10.1002/hep.28438
Papatheodoridis, G.V., Idilman, R., Dalekos, G.N., Buti, M., Chi, H., van Boemmel, F., Calleja, J.L., Sypsa, V., Goulis, J., Manolakopoulos, S., Loglio, A., Siakavellas, S., Keskın, O., Gatselis, N., Hansen, B.E., Lehretz, M., de la Revilla, J., Savvidou, S., Kourikou, A., Vlachogiannakos, I., Galanis, K., Yurdaydin, C., Berg, T., Colombo, M., Esteban, R., Janssen, H.L.A., Lampertico, P., 2017. The risk of hepatocellular carcinoma decreases after the first 5 years of entecavir or tenofovir in Caucasians with chronic hepatitis B. Hepatol. Baltim. Md 66, 1444–1453. https://doi.org/10.1002/hep.29320
Papatheodoridis, G.V., Manolakopoulos, S., Su, T.-H., Siakavellas, S., Liu, C.-J., Kourikou, A., Yang, H.-C., Kao, J.-H., 2018a. Significance of definitions of relapse after discontinuation of oral antivirals in HBeAg-negative chronic hepatitis B. Hepatol. Baltim. Md 68, 415–424. https://doi.org/10.1002/hep.29497
Papatheodoridis, G.V., Rigopoulou, E.I., Papatheodoridi, M., Zachou, K., Xourafas, V., Gatselis, N., Hadziyannis, E., Vlachogiannakos, J., Manolakopoulos, S., Dalekos, G.N., 2018b. 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. 23, 677–685. https://doi.org/10.3851/IMP3256
Papatheodoridis, G.V., Sypsa, V., Dalekos, G., Yurdaydin, C., van Boemmel, F., Buti, M., Goulis, J., Calleja, J.L., Chi, H., Manolakopoulos, S., Loglio, A., Siakavellas, S., Gatselis, N., Keskın, O., Lehretz, M., Savvidou, S., de la Revilla, J., Hansen, B.E., Kourikou, A., Vlachogiannakos, I., Galanis, K., Idilman, R., Colombo, M., Esteban, R., Janssen, H.L.A., Berg, T., Lampertico, P., 2018c. Eight-year survival in chronic hepatitis B patients under long-term entecavir or tenofovir therapy is similar to the general population. J. Hepatol. 68, 1129–1136. https://doi.org/10.1016/j.jhep.2018.01.031
Peppa, D., Gill, U.S., Reynolds, G., Easom, N.J.W., Pallett, L.J., Schurich, A., Micco, L., Nebbia, G., Singh, H.D., Adams, D.H., Kennedy, P.T.F., Maini, M.K., 2013. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. J. Exp. Med. 210, 99–114. https://doi.org/10.1084/jem.20121172
Peppa, D., Micco, L., Javaid, A., Kennedy, P.T.F., Schurich, A., Dunn, C., Pallant, C., Ellis, G., Khanna, P., Dusheiko, G., Gilson, R.J., Maini, M.K., 2010. Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathog. 6, e1001227. https://doi.org/10.1371/journal.ppat.1001227
Qiu, Y.-W., Huang, L.-H., Yang, W.-L., Wang, Z., Zhang, B., Li, Y.-G., Su, T.-T., Zhou, H.-Y., Xu, W., Wang, X.-D., Dai, Y.-P., Gan, J.-H., 2016. Hepatitis B surface antigen quantification at hepatitis B e antigen seroconversion predicts virological relapse after the cessation of entecavir treatment in hepatitis B e antigen-positive patients. Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis. 43, 43–48. https://doi.org/10.1016/j.ijid.2015.10.019
Rehermann, B., 2013. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nat. Med. 19, 859–868. https://doi.org/10.1038/nm.3251

Rehermann, B., Nascimbeni, M., 2005. Immunology of hepatitis B virus and hepatitis C virus infection. Nat. Rev. Immunol. 5, 215–229. https://doi.org/10.1038/nri1573

Reijnders, J.G.P., Perquin, M.J., Zhang, N., Hansen, B.E., Janssen, H.L.A., 2010. Nucleos(t)ide analogues only induce temporary hepatitis B e antigen seroconversion in most patients with chronic hepatitis B. Gastroenterology 139, 491–498. https://doi.org/10.1053/j.gastro.2010.03.059

Rinker, F., Zimmer, C.L., Höner Zu Siederdissen, C., Manns, M.P., Kraft, A.R.M., Wedemeyer, H., Björkström, N.K., Cornberg, M., 2018. Hepatitis B virus-specific T cell responses after stopping nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. J. Hepatol. 69, 584–593. https://doi.org/10.1016/j.jhep.2018.05.004

Rivino, L., Le Bert, N., Gill, U.S., Kunasegaran, K., Cheng, Y., Tan, D.Z., Becht, E., Hansi, N.K., Foster, G.R., Su, T.-H., Tseng, T.-C., Lim, S.G., Kao, J.-H., Newell, E.W., Kennedy, P.T., Bertoletti, A., 2018. Hepatitis B virus-specific T cells associate with viral control upon nucleos(t)ide-analogue therapy discontinuation. J. Clin. Invest. 128, 668–681. https://doi.org/10.1172/JCI92812

Ryu, S.H., Chung, Y.-H., Choi, M.H., Kim, J.A., Shin, J.W., Jang, M.K., Park, N.H., Lee, H.C., Lee, Y.S., Suh, D.J., 2003. Long-term additional lamivudine therapy enhances durability of lamivudine-induced HBeAg loss: a prospective study. J. Hepatol. 39, 614–619. https://doi.org/10.1016/s0168-8278(03)00394-5

Salimzadeh, L., Le Bert, N., Dutertre, C.-A., Gill, U.S., Newell, E.W., Frey, C., Hung, M., Novikov, N., Fletcher, S., Kennedy, P.T., Bertoletti, A., 2018. PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. J. Clin. Invest. 128, 4573–4587. https://doi.org/10.1172/JCI121957

Sarin, S.K., Kumar, M., Lau, G.K., Abbas, Z., Chan, H.L.Y., Chen, C.J., Chen, D.S., Chen, H.L., Chen, P.J., Chien, R.N., Dokmeci, A.K., Gane, E., Hou, J.L., Jafri, W., Jia, J., Kim, J.H., Lai, C.L., Lee, H.C., Lim, S.G., Liu, C.J., Locarnini, S., Al Mahtab, M., Mohamed, R., Omata, M., Park, J., Piratvisuth, T., Sharma, B.C., Sollano, J., Wang, F.S., Wei, L., Yuen, M.F., Zheng, S.S., Kao, J.H., 2016. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol. Int. 10, 1–98. https://doi.org/10.1007/s12072-015-9675-4

Schuch, A., Salimi Alizei, E., Heim, K., Wieland, D., Kiraithe, M.M., Kemming, J., Llewellyn-Lacey, S., Sogukpinar, Ö., Ni, Y., Urban, S., Zimmermann, P., Nassal, M., Emmerich, F., Price, D.A., Bengsch, B., Luxenburger, H., Neumann-Haefelin, C., Hofmann, M., Thimme, R., 2019. Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load. Gut 68, 905–915. https://doi.org/10.1136/gutjnl-2018-316641

Schurich, A., Pallett, L.J., Lubowiecki, M., Singh, H.D., Gill, U.S., Kennedy, P.T., Nastouli, E., Tanwar, S., Rosenberg, W., Maini, M.K., 2013. The third signal cytokine IL-12 rescues the anti-viral function of exhausted HBV-specific CD8 T cells. PLoS Pathog. 9, e1003208. https://doi.org/10.1371/journal.ppat.1003208

Seto, W.-K., Hui, A.J., Wong, V.W.-S., Wong, G.L.-H., Liu, K.S.-H., Lai, C.-L., Yuen, M.-F., Chan, H.L.-Y., 2015. Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative chronic hepatitis B: a multicentre prospective study. Gut 64, 667–672. https://doi.org/10.1136/gutjnl-2014-307237

Seto, W.-K., Liu, K.S., Mak, L.-Y., Cloherty, G., Wong, D.K.-H., Gersch, J., Lam, Y.-F., Cheung, K.-S., Chow, N., Ko, K.-L., To, W.-P., Fung, J., Yuen, M.-F., 2020. Role of serum HBV RNA and hepatitis B surface antigen levels in identifying Asian patients with chronic hepatitis B suitable for entecavir cessation. Gut. https://doi.org/10.1136/gutjnl-2020-321116

Shouval, D., Lai, C.-L., Chang, T.-T., Cheinquer, H., Martin, P., Carosi, G., Han, S., Kaymakoglu, S., Tamez, R., Yang, J., Tenney, D., Brett-Smith, H., 2009. Relapse of hepatitis B in HBeAg-negative chronic hepatitis B patients who discontinued successful entecavir treatment: the case for continuous antiviral therapy. J. Hepatol. 50, 289–295. https://doi.org/10.1016/j.jhep.2008.10.017

Sohn, H.R., Min, B.Y., Song, J.C., Seong, M.H., Lee, S.S., Jang, E.S., Shin, C.M., Park, Y.S., Hwang, J.-H., Jeong, S.-H., Kim, N., Lee, D.H., Kim, J.-W., 2014. Off-treatment virologic relapse and outcomes of re-treatment in chronic hepatitis B patients who achieved complete viral suppression with oral nucleos(t)ide analogs. BMC Infect. Dis. 14, 439. https://doi.org/10.1186/1471-2334-14-439

Song, B.C., Suh, D.J., Lee, H.C., Chung, Y.H., Lee, Y.S., 2000. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatol. Baltim. Md 32, 803–806. https://doi.org/10.1053/jhep.2000.16665

Stoop, J.N., van der Molen, R.G., Kuipers, E.J., Kusters, J.G., Janssen, H.L.A., 2007. Inhibition of viral replication reduces regulatory T cells and enhances the antiviral immune response in chronic hepatitis B. Virology 361, 141–148. https://doi.org/10.1016/j.virol.2006.11.018

Su, T.-H., Yang, H.-C., Tseng, T.-C., Liou, J.-M., Liu, C.-H., Chen, C.-L., Chen, P.-J., Chen, D.-S., Liu, C.-J., Kao, J.-H., 2018. Distinct Relapse Rates and Risk Predictors After Discontinuing Tenofovir and Entecavir Therapy. J. Infect. Dis. 217, 1193–1201. https://doi.org/10.1093/infdis/jix690

Tan, A.T., Koh, S., Goh, W., Zhe, H.Y., Gehring, A.J., Lim, S.G., Bertoletti, A., 2010. A longitudinal analysis of innate and adaptive immune profile during hepatic flares in chronic hepatitis B. J. Hepatol. 52, 330–339. https://doi.org/10.1016/j.jhep.2009.12.015

Tanaka, E., Matsumoto, A., 2014. Guidelines for avoiding risks resulting from discontinuation of nucleoside/nucleotide analogs in patients with chronic hepatitis B. Hepatol. Res. Off. J. Jpn. Soc. Hepatol. 44, 1–8. https://doi.org/10.1111/hepr.12108

Terrault, N.A., Bzowej, N.H., Chang, K.-M., Hwang, J.P., Jonas, M.M., Murad, M.H., American Association for the Study of Liver Diseases, 2016. AASLD guidelines for treatment of chronic hepatitis B. Hepatol. Baltim. Md 63, 261–283. https://doi.org/10.1002/hep.28156

Terrault, N.A., Lok, A.S.F., McMahon, B.J., Chang, K.-M., Hwang, J.P., Jonas, M.M., Brown, R.S., Bzowej, N.H., Wong, J.B., 2018. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatol. Baltim. Md 67, 1560–1599. https://doi.org/10.1002/hep.29800

Tjwa, E.T.T.L., van Oord, G.W., Hegmans, J.P., Janssen, H.L.A., Woltman, A.M., 2011. Viral load reduction improves activation and function of natural killer cells in patients with chronic hepatitis B. J. Hepatol. 54, 209–218. https://doi.org/10.1016/j.jhep.2010.07.009

Tout, I., Gomes, M., Ainouze, M., Marotel, M., Pecoul, T., Durantel, D., Vaccarella, S., Dubois, B., Loustaud-Ratti, V., Walzer, T., Alain, S., Chemin, I., Hasan, U., 2018. Hepatitis B Virus Blocks the CRE/CREB Complex and Prevents TLR9 Transcription and Function in Human B Cells. J. Immunol. Baltim. Md 1950 201, 2331–2344. https://doi.org/10.4049/jimmunol.1701726

Tout, I., Loureiro, D., Mansouri, A., Soumelis, V., Boyer, N., Asselah, T., 2020. Hepatitis B Surface Antigen Seroclearance: Immune Mechanisms, Clinical Impact, Importance for Drug Development. J. Hepatol. https://doi.org/10.1016/j.jhep.2020.04.013

Tseng, C.-H., Hsu, Y.-C., Chang, C.-Y., Tseng, T.-C., Wu, M.-S., Lin, J.-T., Kao, J.-H., 2018. Quantification of serum hepatitis B core antibody to predict off-entecavir relapse in patients with chronic hepatitis B. J. Formos. Med. Assoc. Taiwan Yi Zhi 117, 915–921. https://doi.org/10.1016/j.jfma.2017.11.012

Tseng, T.-N., Hu, T.-H., Wang, J.-H., Kuo, Y.-H., Hung, C.-H., Lu, S.-N., Jeng, W.-J., Chen, C.-H., 2020. Incidence and Factors Associated With HBV Relapse After Cessation of Entecavir or Tenofovir in Patients With HBsAg Below 100 IU/mL. Clin. Gastroenterol. Hepatol. Off. Clin. Pract. J. Am. Gastroenterol. Assoc. https://doi.org/10.1016/j.cgh.2020.04.037

van Bömmel, F., Bartens, A., Mysickova, A., Hofmann, J., Krüger, D.H., Berg, T., Edelmann, A., 2015. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatol. Baltim. Md 61, 66–76. https://doi.org/10.1002/hep.27381

van der Molen, R.G., Sprengers, D., Biesta, P.J., Kusters, J.G., Janssen, H.L.A., 2006. Favorable effect of adefovir on the number and functionality of myeloid dendritic cells of patients with chronic HBV. Hepatol. Baltim. Md 44, 907–914. https://doi.org/10.1002/hep.21340

Van Hees, S., Bourgeois, S., Van Vlierberghe, H., Sersté, T., Francque, S., Michielsen, P., Sprengers, D., Reynaert, H., Henrion, J., Negrin Dastis, S., Delwaide, J., Lasser, L., Decaestecker, J., Orlent, H., Janssens, F., Robaeys, G., Colle, I., Stärkel, P., Moreno, C., Nevens, F., Vanwolleghem, T., Belgian NA Stop Study Group, 2018. Stopping nucleos(t)ide analogue treatment in Caucasian hepatitis B patients after HBeAg seroconversion is associated with high relapse rates and fatal outcomes. Aliment. Pharmacol. Ther. 47, 1170–1180. https://doi.org/10.1111/apt.14560

Wang, J., Shen, T., Huang, X., Kumar, G.R., Chen, X., Zeng, Z., Zhang, R., Chen, R., Li, T., Zhang, T., Yuan, Q., Li, P.-C., Huang, Q., Colonno, R., Jia, J., Hou, J., McCrae, M.A., Gao, Z., Ren, H., Xia, N., Zhuang, H., Lu, F., 2016. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. J. Hepatol. 65, 700–710. https://doi.org/10.1016/j.jhep.2016.05.029

Wang, L., Liu, F., Liu, Y.-D., Li, X.-Y., Wang, J.-B., Zhang, Z.-H., Wang, Y.-Z., 2010. Stringent cessation criterion results in better durability of lamivudine treatment: a prospective clinical study in hepatitis B e antigen-positive chronic hepatitis B patients. J. Viral Hepat. 17, 298–304. https://doi.org/10.1111/j.1365-2893.2009.01178.x

World Health Organization, World Health Organization, Global Hepatitis Programme, 2017. Global hepatitis report, 2017.

Wu, Y., Fan, J., Liao, G., Xia, M., Jiang, D., Peng, J., Zhang, X., Liu, H., 2020. Genetic variations in the CXCR5 gene decrease the risk of clinical relapse after discontinuation of nucleos(t)ide analogue therapy in patients with chronic hepatitis B. Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis. 78, 104124. https://doi.org/10.1016/j.meegid.2019.104124

Xia, Y., Protzer, U., 2017. Control of Hepatitis B Virus by Cytokines. Viruses 9. https://doi.org/10.3390/v9010018

Xie, L., Liao, G., Chen, H., Xia, M., Huang, X., Fan, R., Peng, J., Zhang, X., Liu, H., 2019. Elevated expression of serum soluble ST2 in clinical relapse after stopping long-term Nucleos(t)ide analogue therapy for chronic hepatitis B. BMC Infect. Dis. 19, 640. https://doi.org/10.1186/s12879-019-4261-3

Ye, B., Liu, X., Li, X., Kong, H., Tian, L., Chen, Y., 2015. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. Cell Death Dis. 6, e1694. https://doi.org/10.1038/cddis.2015.42

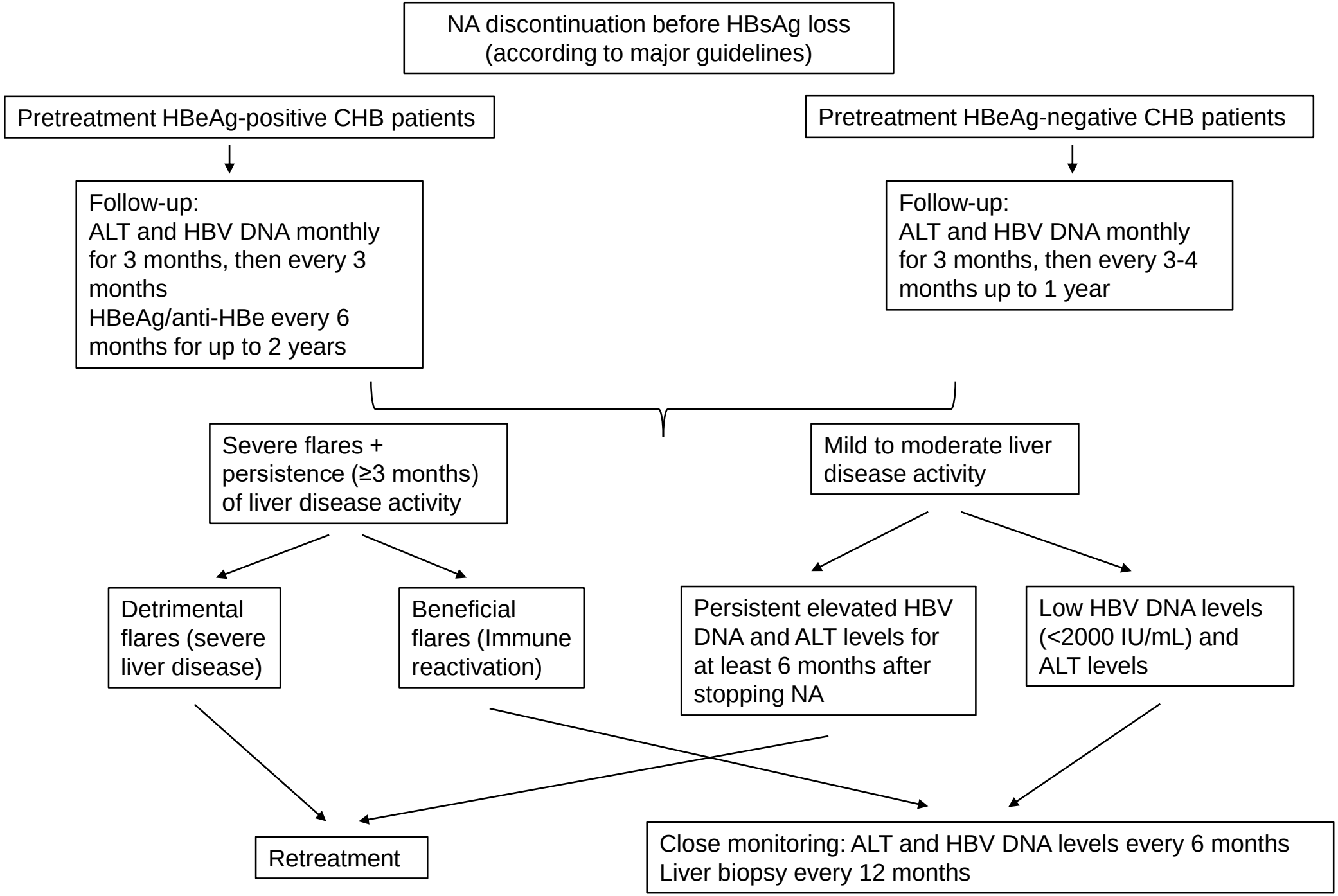
Yu, F., Wang, Y., Yuan, S., Ma, J., Ma, N., Zhang, X., Liu, X., Liu, D., 2013. Association between gene polymorphisms of IL-28 and response to lamivudine in Chinese rural patients with chronic hepatitis B. Scand. J. Gastroenterol. 48, 745–751. https://doi.org/10.3109/00365521.2013.779746

Yu, Y., Wang, J., Li, G., Shen, C., Chen, S., Huang, J., Wang, Xinyu, Zhao, C., Huang, Y., Wang, B., Wang, Xuanyi, Zhang, J., Qiu, C., Zhang, W., 2018. Nucleos(t)ide analogue interruption: Alternative approach to intrahepatic set point for spontaneous control of HBV replication? J. Hepatol. 68, 609–610. https://doi.org/10.1016/j.jhep.2017.09.026

Zhang, E., Lu, M., 2015. Toll-like receptor (TLR)-mediated innate immune responses in the control of hepatitis B virus (HBV) infection. Med. Microbiol. Immunol. (Berl.) 204, 11–20. https://doi.org/10.1007/s00430-014-0370-1

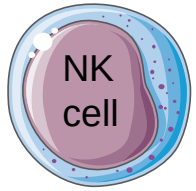
Zheng, Y., Huang, Z., Chen, X., Tian, Y., Tang, J., Zhang, Y., Zhang, X., Zhou, J., Mao, Q., Ni, B., Wang, Q., Wu, Y., 2012. Effects of telbivudine treatment on the circulating $CD4^+$ T-cell subpopulations in chronic hepatitis B patients. Mediators Inflamm. 2012, 789859. https://doi.org/10.1155/2012/789859

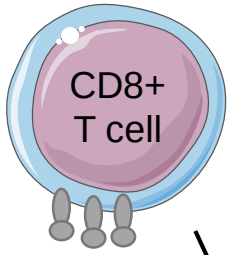
Zimmer, C.L., Rinker, F., Höner Zu Siederdissen, C., Manns, M.P., Wedemeyer, H., Cornberg, M., Björkström, N.K., 2018. Increased NK Cell Function After Cessation of Long-Term Nucleos(t)ide Analogue Treatment in Chronic Hepatitis B Is Associated With Liver Damage and HBsAg Loss. J. Infect. Dis. 217, 1656–1666. https://doi.org/10.1093/infdis/jiy097


NA discontinuation before HBsAg loss (according to major guidelines)
Pretreatment HBeAg-positive CHB patients
Pretreatment HBeAg-negative CHB patients
Follow-up: ALT and HBV DNA monthly for 3 months, then every 3 months HBeAg/anti-HBe every 6 months for up to 2 years
Follow-up: ALT and HBV DNA monthly for 3 months, then every 3-4 months up to 1 year
Severe flares + persistence (≥3 months) of liver disease activity
Mild to moderate liver disease activity
Detrimental flares (severe liver disease)
Beneficial flares (Immune reactivation)
Persistent elevated HBV DNA and ALT levels for at least 6 months after stopping NA
Low HBV DNA levels (<2000 IU/mL) and ALT levels
Retreatment
Close monitoring: ALT and HBV DNA levels every 6 months
Liver biopsy every 12 months

Immune Exhaustion

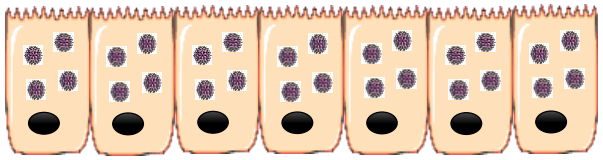
High NK pro-inflammatory phenotype can lead to T cell deletion via death receptors





PD-1

Exhausted PD-1(hi) HBV-specific CD8+ T cells are dysfunctionnal and are unable to mount an effective antivral response



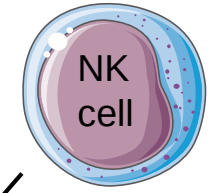
HBV-infected Hepatocytes

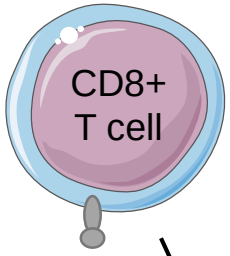
NA therapy

HBV viral suppression

Immune Control

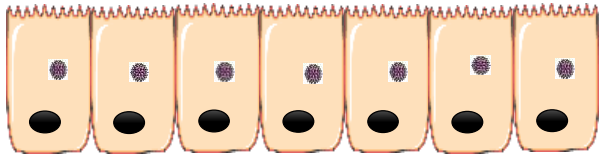
Low NK pro-inflammatory phenotype and restoration of antiviral cytokine production (IFNγ)





PD-1

Effective PD-1+ HBV-specific CD8+ T cells mediate a robust antiviral response (direct cytolytic activity on HBV-infected hepatocytes and a non-cytolytic activity via IFNγ and IL-2 secretion)



HBV-infected Hepatocytes