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

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15 **Abstract**

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

39

## 40 **Highlights**

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

## 55 **Keywords**

56 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; IFN $\gamma$ , 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- $\beta$ , Tumor growth factor beta; Tim-3, T-cell immunoglobulin and mucin-domain containing-3; TLR, toll like receptor; TNF $\alpha$ , Tumor necrosis factor alpha; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; WHO, World Health Organization.

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## 64 **1. Introduction**

65 Hepatitis B virus (HBV) infection remains a major global health concern as over 257  
66 million individuals are estimated to be carriers of hepatitis B 'surface' antigen  
67 (HBsAg), the hallmark of chronic HBV infection (CHB)(European Association for the  
68 Study of the Liver, 2017; World Health Organization et al., 2017). CHB has potential  
69 adverse outcomes, including cirrhosis, hepatic decompensation, and hepatocellular  
70 carcinoma (HCC)(European Association for the Study of the Liver. 2017; Sarin et al.,  
71 2016; Terrault et al., 2018). In 2015, hepatitis B resulted in an estimated 887 000  
72 deaths from these liver diseases according to the World Health organization  
73 (WHO)(World Health Organization et al., 2017). HBsAg correlates with the presence  
74 of covalently closed circular DNA (cccDNA), in Hepatitis B 'e' antigen (HBeAg)-  
75 positive CHB patients, and is a key marker of infection(Tout et al., 2020). Its  
76 clearance is a reasonable therapeutic end point and is also referred to as a functional  
77 cure of CHB(Martinot-Peignoux et al., 2014). Although nucleos(t)ide analogues (NA)  
78 and immunomodulators (pegylated interferon (PEG-IFN)) are recommended  
79 therapies, most patients take NA due to the easy administration, safety, and  
80 efficacy(Cornberg et al., 2020). These therapies reduce viral replication and their  
81 aims include improving quality of life and decreasing the risk of liver disease-related  
82 complications(Marcellin et al., 2019; Papatheodoridis et al., 2018c) but however they  
83 rarely lead to HBsAg seroclearance. Long term NA therapy raises some concerns  
84 such as adherence, side effects, and, particularly, costs (Lampertico and Berg, 2018;  
85 Papatheodoridis et al., 2016). Additionally, a significant proportion of patients may  
86 want to discontinue therapy for various reasons. Thus, defining rules to stop NA  
87 therapy is of paramount importance and safety without loss of efficacy must be

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

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

## 105 **2. Goals and outcomes of NA therapy**

106 The goal of therapy for CHB is to improve survival by preventing disease progression  
107 and improving quality of life. This is best achieved by long-term NA therapy leading to  
108 HBV suppression, associated with a reduction in histological activity, a lower risk of  
109 cirrhosis, and reversal of fibrosis(European Association for the Study of the Liver.  
110 2017; Marcellin et al., 2019; Marcellin and Asselah, 2013; Sarin et al., 2016; Terrault  
111 et al., 2018). NA mechanism of action consists of viral polymerase inhibition, which

112 leads to decreased virion assembly, and thus a reduction of the viral replication and  
113 load (Asselah et al., 2019). According to the European Association for the Study of  
114 the Liver (EASL)(European Association for the Study of the Liver. 2017) and the  
115 American Association for the Study of Liver Diseases (AASLD)(Terrault et al., 2018),  
116 the currently approved and first-line recommended NA drugs are tenofovir disoproxil  
117 fumarate (TDF), tenofovir alafenamide (TAF), and entecavir (ETV) due to their high  
118 barrier to HBV resistance, their high effectiveness, and their well tolerance. Other  
119 NAs such as Lamivudine (LAM), Telbivudine (TBV) and adefovir dipivoxil (ADV)  
120 should not be used as first-line treatment options due to their low barrier to HBV  
121 resistance causing resistance strains and insufficient virologic response. The main  
122 advantage of NA treatment is its predictable high long-term antiviral efficacy leading  
123 to undetectable HBV DNA levels in the vast majority of compliant patients as well as  
124 its favorable safety profile(European Association for the Study of the Liver. 2017; Lok  
125 et al., 2016; Terrault et al., 2018). Over 8 years (Papatheodoridis et al., 2018c) and  
126 10 years(Marcellin et al., 2019), NAs have a favorable safety profile, are well  
127 tolerated, and resulted in continued maintenance of virologic suppression.

128 The ideal endpoint of therapy in HBeAg-positive and HBeAg-negative patients is  
129 sustained HBsAg loss with or without seroconversion to anti-HBs, which is usually  
130 associated with complete and definitive remission of the activity of CHB (Kim et al.,  
131 2014). However, these endpoints are rarely achieved because of the presence of the  
132 covalently closed circular DNA (cccDNA) minichromosome, which remains  
133 untouched in the nuclei of infected hepatocytes as well as the presence of integrated  
134 HBV sequences in hepatocytes genome which can cause reactivation of the disease  
135 and relapse of viraemia. Since these goals are rarely reached, surrogate endpoints  
136 have been identified to assess efficacy, including improved liver inflammation



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

### 148 **3. Aim of NA therapy cessation strategy**

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

162 to determine the occurrence of HBsAg loss after stopping NA therapy. Chan et al.  
163 reported that 20% of patients achieved HBsAg seroclearance after stopping LAM  
164 therapy (Chan et al., 2011). The main predictor of HBsAg seroclearance was end of  
165 therapy qHBsAg levels  $< 2 \log \text{ IU/mL}$  and  $> 1 \log$  reduction from baseline. A  
166 randomized study (the FINITE study)(Berg et al., 2017) compared standard TDF  
167 therapy continuation (n=21) against its interruption (n=21) in HBeAg-negative  
168 patients that had been under NAs for at least 3,5 years. Thirteen out of 21 patients in  
169 the NA stopping group remained off-therapy and 4 of them even achieved HBsAg  
170 seroclearance after 3 years of follow-up. Those who stopped therapy had greater  
171 reduction in qHBsAg compared to those who continued therapy (0.283 vs 0.088 log  
172 reduction respectively)(Berg et al., 2017).  
173 To evaluate stopping NAs, we must consider what are the benefits of their  
174 discontinuation. Since long-term NA therapy can already achieve viral suppression  
175 and limit the risk of liver-related diseases, the withdrawal of the treatment should aim  
176 to have similar or even better outcomes than treatment continuation. HBsAg loss,  
177 indicating immune control with sustained virologic remission (Tout et al., 2020),  
178 remains the best indicator of long-term favorable outcomes and therefore of NA  
179 cessation. Relapse must also be considered, which occurs frequently off-treatment  
180 and may lead to clinically significant flares. Determining precise predictors of relapse  
181 or sustained remission is crucial to identify which patients should not stop therapy.

182

### 183 **3.1 HBV guidelines and recommendations on stopping NA therapy**

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

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

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

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

221

### 222 **3.2 NA cessation in HBeAg-positive CHB patients before HBsAg** 223 **loss**

224 The main guidelines suggest that pretreatment HBeAg-positive patients, without  
225 cirrhosis, can stop NA therapy after reaching HBeAg seroconversion with  
226 undetectable HBV DNA and completing 1 to 3 years of consolidation therapy. Several  
227 reports have evaluated the durability of HBeAg seroconversion with LAM therapy,  
228 resulting in a wide range of durable HBeAg seroconversion rates. Song et al.  
229 evaluated patients who underwent HBeAg seroconversion with short duration LAM  
230 consolidation therapy (2 to 4 months) and observed a relapse with elevated  
231 transaminases and reappearance of HBeAg in 49% of patients at 2 years post-  
232 treatment (Song et al., 2000). Another study evaluated the durability of serological  
233 responses in 40 HBeAg-positive patients who were treated by LAM (Dienstag et al.,  
234 2003). Most HBeAg responses achieved during LAM therapy were durable, and most  
235 responders experienced prolonged clinical benefit after HBeAg seroconversion and

236 subsequent discontinuation of LAM. The relapse rate after HBeAg seroconversion  
237 was only 27% with a median period of 3-year follow up (Dienstag et al., 2003).  
238 Similarly, a study from Taiwan showed that a duration of LAM consolidation therapy >  
239 48 weeks may be favorable for maintaining durable HBeAg seroconversion (Kuo et  
240 al., 2010). Then later, Lee et al. showed that consolidation therapy for more than 1  
241 year after HBeAg seroconversion helped maintain the serological response (Lee et  
242 al., 2010). A prospective study of 82 patients demonstrated a cumulating relapse rate  
243 of 23.4% at 6 months and 29.4% at 4 years after discontinuation of LAM (Wang et al.,  
244 2010), and a second study observed a 3-year cumulative relapse rate of 34.4% in 42  
245 patients (Jin et al., 2012). Recently, a study from Belgium aimed to determine relapse  
246 rates and clinical outcomes in a predominantly Caucasian cohort of 98 CHB patients  
247 with NA-induced HBeAg seroconversion (Van Hees et al., 2018). Viral relapses were  
248 observed in more than half of patients who discontinued treatment with potential fatal  
249 outcomes while none of the patients who continued therapy after HBeAg  
250 seroconversion relapsed or developed severe hepatic outcomes (Van Hees et al.,  
251 2018). Kang et al. assessed the antiviral response in 157 patients who had  
252 discontinued LAM for a mean 72 months of follow-up. The cumulative probabilities of  
253 virologic relapse at 1, 24, 48, 96, and 120 months were 10.3%, 55.6%, 62.8%,  
254 67.0%, and 67.0%, respectively (Kang et al., 2017). However, most of these studies  
255 were long-term retrospective studies that did not correct for selection or  
256 measurement bias (Table 2). Therefore, the evidence on NA withdrawal in HBeAg-  
257 positive CHB patients is lacking and needs to be based on better designed studies.

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

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

270 A systematic review by Papatheodoridis et al., including 25 studies with 1716 patients  
271 who discontinued NA and were followed up for > 12 months shows that 51% of  
272 HBeAg-positive CHB patients had durable virologic remission at 3 years post-  
273 treatment and 76% of them had durable biochemical remission (Papatheodoridis et  
274 al., 2016). These rates were higher than in HBeAg-negative CHB patients. Sustained  
275 HBeAg seroconversion was found in 95%, 92% and 88% of patients at 6, 12 and 24  
276 months, respectively, following discontinuation of NA therapy (Papatheodoridis et al.,  
277 2016). Collectively, these data suggest that the risk of relapse requiring retreatment  
278 is relatively high in pretreatment HBeAg-positive patients, even with long  
279 consolidation therapy after HBeAg loss. Due to the heterogeneity of the studies and  
280 their design, it is, therefore, difficult to confirm the advantage of NA discontinuation in  
281 pre-treatment HBeAg-positive CHB patients who undergo HBeAg seroconversion.  
282 Another factor leading to clear discrepancies between studies is the variable relapse  
283 definition (Papatheodoridis et al., 2018a). Although prolongation of NA therapy in  
284 HBeAg-positive patients is aiming at functional cure under treatment, this is still a  
285 rare event, and HBeAg seroconversion is the currently accepted endpoint despite

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

291

### 292 **3.3 NA cessation in HBeAg-negative CHB patients before HBsAg** 293 **loss**

294 Current interest and debates in stopping NA are largely related to finite NA therapy in  
295 HBeAg-negative CHB patients. The guidelines recommend that NA therapy in  
296 HBeAg-negative CHB patients, without cirrhosis, can be stopped after  $\geq 3$  years of  
297 virologic suppression (EASL 2017)(European Association for the Study of the Liver.  
298 2017), after treatment for at least 2 years with undetectable HBV DNA documented  
299 on three separate occasions, 6 months apart (APASL 2015)(Sarin et al., 2016), or  
300 only after achieving HBsAg loss, except if there is a compelling rationale and under  
301 the condition of careful monitoring every 3 months for at least 1 year (AASLD  
302 2018)(Terrault et al., 2018). The previously mentioned study by Hadziyannis et al.  
303 was one of the first papers that presented data favoring NA withdrawal in HBeAg-  
304 negative CHB patients (Hadziyannis et al., 2012). It showed that stopping adefovir  
305 therapy in HBeAg negative patients was associated with a 39% HBsAg  
306 seroclearance rate. This high HBsAg seroclearance rate was not observed in  
307 subsequent studies probably due to shorter follow-up duration or even the ethnicity of  
308 the studied cohort. Additionally, whether the initial increase in HBV DNA, after NA  
309 withdrawal, settles down to quiescent disease or severe flares varies widely between  
310 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 Siederdisen 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).



336 Studies with longer follow-up confirm possible sustained off-treatment response and  
337 HBsAg loss in HBeAg-negative CHB patients (Table 3). The daring-B study followed  
338 57 patients who discontinued therapy after median virologic remission of 5,3 years  
339 and remained under a median of 18 months of follow-up( Papatheodoridis et al.,  
340 2018b). For a cutoff of HBV DNA > 2,000 IU/ml, the cumulative virologic relapse  
341 rates were 56%, 70% and 72% at 3, 12 and 18 months after therapy. The probability  
342 of relapse decreased after month 6.

343 In a systematic review on NA discontinuation, 2% HBsAg loss was observed  
344 (Papatheodoridis et al., 2016). The pooled rates of sustained virologic remission at  
345 12, 24, 36 months in HBeAg-negative patients were 43.7%, 31.3% and 30.1%  
346 respectively (Papatheodoridis et al., 2016). In another systematic review of 22  
347 studies including 1732 HBeAg-negative patients, the 1 year off-therapy virologic  
348 relapse was < 70%, and clinical relapse (HBV DNA >2000 IU/mL and ALT elevation)  
349 occurred in < 50% of patients and <40% required retreatment (M.-L. Chang et al.,  
350 2015). Thus, these two recent systematic reviews show that stopping NA is relatively  
351 safe with a careful off-therapy monitoring plan. Concerning the safety of NA  
352 discontinuation, they suggest that significant complications such as severe flares and  
353 decompensation were relatively infrequent and occurred in 0,8% of patients with  
354 cirrhosis, while jaundice developed in 2,5%, with 1 death from liver failure (M.-L.  
355 Chang et al., 2015; Papatheodoridis et al., 2016). Conversely, a study from Toronto,  
356 with majorly Asian patients, found that only 33% of pretreatment HBeAg-negative  
357 patients had a sustained off-treatment response, 72 weeks after NA withdrawal (Liem  
358 et al., 2019). Although the retreatment criteria in this study was not stringent which  
359 allowed to better evaluate the off-NA viral and biochemical kinetics during close-visit  
360 monitoring, there were only 27 HBeAg-negative CHB patients which limits the

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

377

### 378 **3.4 Stopping NA in patients with cirrhosis before HBsAg loss**

379 Patients with cirrhosis are at risk of developing liver decompensation and stopping  
380 therapy in this subgroup is controversial due to the risk of liver failure and ascites  
381 (Wen-Juei Jeng et al., 2018). While the EASL and the AASLD guidelines(European  
382 Association for the Study of the Liver, 2017; Terrault et al., 2018) do not recommend  
383 stopping NA therapy, before HBsAg loss, in patients with cirrhosis, under any  
384 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

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

### 414 **3.5 Rescue therapy in case of relapse**

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

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

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

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

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

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

#### 474 **4. Biomarkers to consider to safely stop NA therapy before HBsAg** 475 **loss**

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

484 discontinuation. In recent years, numerous studies have investigated several host  
485 and viral factors such as patient characteristics including age (Bonino et al., 2007; Ha  
486 et al., 2012; Marcellin et al., 2009) and gender (Bonino et al., 2007; Chen et al.,  
487 2014), baseline clinical characteristics including HBV DNA (Huang et al., 2003; Jeng  
488 et al., 2013), ALT levels (Bonino et al., 2007; Chien and Liaw, 2006; Marcellin et al.,  
489 2009), HBsAg (Chen et al., 2018, 2014, 2014; Liang et al., 2011), treatment-related  
490 factors including duration of consolidation therapy (Jeng et al., 2013; Jiang et al.,  
491 2015; Kuo et al., 2010; Sohn et al., 2014), and virus-related factors, such as  
492 genotype (Chien and Liaw, 2006; He et al., 2013) and viral mutations (Liang et al.,  
493 2011; Ryu et al., 2003) have also been considered. However, these factors vary  
494 between studies because of the various designs, populations, and patient pre-  
495 treatment HBeAg status.

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

509 there is a need to further explore this aspect. On the other hand, studies showed  
510 that the combination of young age and prolonged consolidation treatment gives lower  
511 relapse rates (Byun et al., 2005; Chen et al., 2014; Lee et al., 2010; Wang et al.,  
512 2010). Chien et al. noticed that patients with genotype C had higher relapse rates  
513 than those with genotype B (Chien and Liaw, 2006). Cao et al. showed that the level  
514 and persistence of off-treatment elevated HBV DNA levels were useful in the  
515 prediction of a subsequent biochemical relapse and may guide off-treatment  
516 management (Cao et al., 2017). A study reported a negative correlation between  
517 baseline ALT levels and relapse rate (Pan et al., 2013) while another one showed  
518 that lower ALT baseline levels correlated with higher rates of HBsAg loss (Chen et  
519 al., 2014).

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

530 In addition to predicting HBsAg loss by its decrease (Martinot-Peignoux et al., 2013),  
531 qHBsAg has also been used to predict off-therapy sustained response with NAs, and  
532 off-therapy flares (Höner Zu Siederdisen et al., 2016; Martinot-Peignoux et al., 2015,  
533 2014; Qiu et al., 2016). Low levels of serum HBsAg at the end of treatment, or



534 decreasing levels during therapy or after its cessation were shown to be positive  
535 predictors for sustained response (Chan et al., 2011; Hadziyannis et al., 2012). Chen  
536 et al. showed that HBsAg cut-off values of 120 and 200 IU/mL predicted a 79%  
537 HBsAg loss and 93% post-treatment sustained response respectively (Chen et al.,  
538 2014). Conversely, two studies showed no influence of HBsAg kinetics and relapse  
539 rates (Jeng et al., 2016; Seto et al., 2015). Several studies identified serum HBsAg  
540 levels of  $<2 \log_{10}$  IU/mL to be a reliable predictor of a sustained off-treatment  
541 response (Chen et al., 2018; Chi et al., 2019; Wen-Juei Jeng et al., 2018;  
542 Papatheodoridi and Papatheodoridis, 2020; Su et al., 2018). In a study of 204  
543 HBeAg-negative patients, qHBsAg level  $\geq 1500$  IU/mL at discontinuation was the only  
544 predictive factor of relapse (Ge et al., 2015). qHBsAg  $>3 \log$  IU/mL was predictive of a  
545 virologic relapse at 1 year in another study (Liang et al., 2011). In the FINITE study,  
546 patients who achieved a decrease in HBsAg levels after treatment withdrawal had  
547 baseline HBsAg levels  $<25\ 000$  IU/mL when NA treatment was discontinued (Berg et  
548 al., 2017). A recent meta-analysis found that older age, shorter duration of  
549 consolidation therapy, lower levels of baseline and EOT HBsAg levels  $\geq 1000$  IU/ml  
550 were predictive factors of relapse in HBeAg-negative CHB patients who discontinued  
551 NA treatment (Y. Liu et al., 2019). Liu et al. showed in a systematic review that an  
552 HBsAg  $<100$  IU/mL at EOT is a significant marker for safe NA discontinuation (J. Liu et  
553 al., 2019). Two recent studies found that an HBsAg  $<50$  IU/mL (Gao et al., 2019) or  
554 HBsAg  $<40$  IU/mL (Tseng et al., 2020) have higher predictive values of better  
555 sustained responses in HBeAg-negative CHB patients. More studies outside Asia  
556 and therapies using different NAs are needed to define the optimal cut-off levels.  
557 Nevertheless, from a practical viewpoint, EOT HBsAg  $<100$  IU/mL, in most studies,  
558 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- $\beta$  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<sup>+</sup> and CD8<sup>+</sup> T cells  
606 (Fisicaro et al., 2017), decreased numbers and dysfunction of DCs and NKs/NKTs  
607 (Fisicaro et al., 2019; Maini and Peppia, 2013; Martinet et al., n.d.; Ouaguia et al.,

608 2019) up-regulated/enhanced expression of regulatory factors, including the immune  
609 checkpoint proteins PD-1, CTLA-4, and Tim-3 (Hoogeveen and Boonstra, 2020); and  
610 impaired innate immune response (Faure-Dupuy et al., 2017), especially TLR  
611 downregulation and dysfunction (Ma et al., 2018; Tout et al., 2018; Zhang and Lu,  
612 2015).

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

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

## 623 **5.1 T cells**

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

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

635 Previous studies of NA-treated HBeAg-positive patients show that control of virus  
636 replication induced by therapy can lead to a transient restoration of T cell function  
637 (Boni et al., 2003; Stoop et al., 2007). To investigate the effect of long-term treatment  
638 on T cell responses, Boni et al. compared HBV-specific T cell responses of patients  
639 treated with NA with those detected in other conditions of HBV control (Boni et al.,  
640 2012). They demonstrated that HBV-specific CD8<sup>+</sup> T cell effector abilities were  
641 similar between patients after several years of antiviral treatment and acute resolving  
642 cases featured by a PD-1<sup>+</sup> phenotype. More recently, Rivino et al. studied two  
643 cohorts of 29 and 27 HBeAg-negative CHB patients who received NA monotherapy  
644 or NA combination. Patients were, then, separated into 2 groups based on the  
645 presence or absence of hepatic flares during the 6-month period after the termination  
646 of the therapy (Rivino et al., 2018). They demonstrated that the patients who did not  
647 relapse after NA cessation featured, during treatment, an increased frequency of  
648 functional PD-1<sup>+</sup> HBV-specific CD8<sup>+</sup> T cells directed against HBcAg and HBV  
649 polymerase. However, peripheral T cell expansion or PD-1 expression did not  
650 consistently correlate with HBsAg reduction or biochemical reactivation. Rather,  
651 CD38 and Ki-67 expression during ALT elevation correlated with HBsAg loss in the 3  
652 patients who achieved functional cure (Rinker et al., 2018). Interestingly, a higher  
653 frequency of polymerase-specific CD8<sup>+</sup> T cells expressed markers indicating more  
654 severe T cell exhaustion and exhibited a reduced expansion capacity than HBcAg-  
655 specific CD8<sup>+</sup> T cells (Schuch et al., 2019). This indicates that the molecular  
656 mechanisms underlying impaired T cell responses differ with respect to the targeted

657 HBV antigens. Overall, PD-1+ HBV-specific T cells were identified as a potential  
658 biomarker for the safe discontinuation of NA therapy for CHB patients (Rivino et al.,  
659 2018). In line with these results, it was reported that a high level of anti-HBc at the  
660 end of NA treatment was associated with a reduced risk of clinical relapse (Chi et al.,  
661 2019) and it would be interesting to determine whether this higher level of anti-HBc is  
662 related to the higher HBV-specific T cell activity. As the analysis of PD-1+ HBV-  
663 specific T cells is somehow complicated, the development of a practical assay to  
664 directly quantify these cells would be of great interest to permit this biomarker to  
665 become clinically useful. It would be interesting to know what causes the hepatic  
666 flares after the cessation of NA therapy since the magnitude of the HBV-specific T  
667 cell responses was low in those patients. Additionally, PD-1 upregulation on HBV-  
668 specific CD8+ T cells could either mean an activation before HBV clearance in long-  
669 term NA treated CHB patients without HBV flares or exhaustion after persistent and  
670 high HBV antigens exposition in untreated CHB patients. Therefore, it is unclear  
671 whether anti-PD-1 treatments (Fisicaro et al., 2017, p. 8; Salimzadeh et al., 2018) will  
672 enhance HBV clearance in patients with PD-1+ HBV-specific T cells. Further  
673 longitudinal investigation of blood and liver samples are needed to determine precise  
674 immunological events occurring in patients stopping NA therapy.

## 675 **5.2 NK cells**

676 The role of NK cells, characterized by the presence of activating and inhibiting  
677 receptors, in CHB has thoroughly been debated (Maini and Peppia, 2013). Their  
678 presence in the liver several folds higher than the periphery, their contribution to liver  
679 damage by killing hepatocytes (Dunn et al., 2007; Maini et al., 2000) as well as their  
680 correlation with HBV control makes them interesting cells to study the antiviral  
681 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- $\gamma$ , impairing their  
683 noncytolytic antiviral capacity (Oliviero et al., 2009; Peppia et al., 2010). NK cells can  
684 also impair antiviral immunity by deleting HBV-specific T cells (Lang et al., 2012;  
685 Peppia 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

### 706        **5.3    Cytokines**

707    An adequate antiviral immune response or, in contrast, liver inflammation, associated  
708    with hepatic flares, are strictly affected by a balanced cytokine profile (Rehermann,  
709    2013; Schurich et al., 2013; Xia and Protzer, 2017) which could be useful to  
710    determine the fate of HBV infection upon NA discontinuation. Successful viral  
711    repression induced by NA therapy leads to antiviral response by promoting  
712    proinflammatory cytokines such as IFN $\gamma$  (Chen et al., 2011) and IL-2(Jiang et al.,  
713    2010), and by decreasing regulatory signals such as IL-10 (van der Molen et al.,  
714    2006) and TGF- $\beta$  (Zheng et al., 2012). Serum cytokine studies, in small cohorts,  
715    could not predict off-therapy relapse (Höner Zu Siederdisen et al., 2016; Tan et al.,  
716    2010), but we could theoretically speculate that the measurement of these cytokines  
717    together with HBV-specific T cells or NK cells could help to establish a good  
718    cessation point for therapy. Among the cytokines that increased after NA withdrawal  
719    included IL-12 (Höner Zu Siederdisen et al., 2016), which is important for the  
720    induction and recovery of exhausted adaptive T-cell responses (Schurich et al.,  
721    2013). In addition, an increase in sST2 in clinical relapse patients might be  
722    associated with an inflammation-related immune response after discontinuation of NA  
723    treatment                    (Xie                    et                    al.,                    2019).  
724    In a recent study aiming to identify an immune signature associated with off-  
725    treatment remission to NA therapy, Kranidioti et al. performed microarray analysis of  
726    PBMCs from 6 CHB patients who stopped NA therapy (3 with off-treatment  
727    remission, 3 with relapse) compared to 5 CHB patients (inactive carriers) as controls  
728    (Kranidioti et al., 2019). They found that IFN $\gamma$ , IL-8, FASLG and CCL4 levels were  
729    significantly lower in the patients with off-treatment remission compared to the



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

732 Larger studies are required to determine more adequate cytokine candidates.  
733 Additionally, several studies have shown that genetic polymorphisms might influence  
734 the immune response in CHB (Galmozzi et al., 2014; Ju et al., 2017). Yu et al.  
735 showed an effect of the IL-28B (IFN $\lambda$ 3/4) genotype on the response to LAM at one  
736 year (Yu et al., 2013). Among 33 single-nucleotide polymorphisms (SNPs) from 16  
737 immune-related genes, a recent study found that genetic variations in the CXCR5  
738 gene decrease the risk of clinical relapse after NA discontinuation in CHB patients  
739 and thus these genotypes could facilitate optimization of NA discontinuation (Wu et  
740 al., 2020, p. 5).

741 An additional important factor is the migration of HBV-specific T cells to the infected  
742 liver which is controlled by chemokines and their receptors (Brass and Brenndörfer,  
743 2014; Larrubia et al., 2008). Chemokines drive inflammatory infiltrates, possibly  
744 induced by HBV replication (CXCL-8) or IFN $\gamma$  (CXCL-9/10) (Tan et al., 2010). Studies  
745 confirmed the associations between liver disease and chemokine production after  
746 stopping NA therapy (Höner Zu Siederdisen et al., 2016; Tan et al., 2010). Previous  
747 work reported higher levels of CXCL-10 in patients with HBsAg loss during NA  
748 therapy (Jaroszewicz et al., 2011). Another study found that CXCL-10 levels started  
749 to significantly increase after the 3rd year of treatment with ETV presenting a  
750 promising predictor of HBsAg decline (Papatheodoridis et al., 2014).

751 An efficient viral suppression under NA therapy would restore the migration of  
752 functional HBV-specific T cells, helped by a lower inflammatory status of NK cells, to  
753 clear infected hepatocytes and thus leading to HBsAg loss (Figure 2).  
754 More robust immune biomarkers with simple practical applications, remain important

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

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## 766 **6. Future directions and conclusions**

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

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

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

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

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

794 Moreover, the definition of relapse strongly affects the off-NA rates of relapse and  
795 therefore can have a great impact on the probability of retreatment. Retreatment  
796 criteria should be standardized. Based on current knowledge, patients with persistent  
797 HBV activity and severe flares should be retreated. EASL guidelines specify that the  
798 indication for treatment in patients who have discontinued therapy should be in line  
799 with the indications for treatment-naïve patients, specifically those with HBV DNA  
800 >2,000 IU/ml and ALT >ULN (European Association for the Study of the Liver. 2017).  
801 HBsAg kinetics measurements, after EOT, are a very interesting strategy to  
802 distinguish beneficial from detrimental flares as early as possible, which may be

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

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823 **Figure legends**

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

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

829 High viral DNA and HBV proteins during chronic HBV infection lead to exhausted  
830 HBV-specific CD8+ T cells characterized by a dysfunctional phenotype, high and  
831 sustained PD-1 expression, and abrogated antiviral responses. NK cells are defective  
832 and are characterized by a high inflammatory phenotype that may reflect NK cell-  
833 mediated T cell deletion through death receptors like TRAIL. After long-term NA  
834 therapy-induced HBV suppression, HBV-specific PD-1+ CD8+ T cells restore their  
835 antiviral function through a direct cytolytic activity on HBV-infected hepatocytes and  
836 effective antiviral cytokine secretion (IFN $\gamma$  and IL-2). NK cells have a low  
837 inflammatory profile and can restore their antiviral functions. This immune control  
838 phase is also characterized by high levels of CXCL-10 which could point to anti-viral  
839 control. HBV-specific PD-1+ CD8+ T cells, low inflammatory NK cells and levels of IL-  
840 2, IFN $\gamma$  and CXCL-10 are promising predictors of safe NA therapy discontinuation.  
841 HBV: Hepatitis B virus; CXCL10: C-X-C motif chemokine 10 ; IFN $\gamma$ : interferon  
842 gamma; IL-2: interleukin 2; NA: Nucleos(t)ide analogue; NK cell: Natural killer cell;  
843 PD-1: Programmed cell death protein 1.

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

<b>Guideline</b>	<b>EASL (2017)</b>	<b>AASLD (2018)</b>	<b>APASL (2015)</b>
<b>HBeAg positive</b>	-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
<b>HBeAg negative</b>	-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
<b>Cirrhosis</b>	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 ( $\geq 4$ 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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890 **References:**

- 891 Aiello, A., Guarino, M., D'Ausilio, A., Toumi, M., Caporaso, N., Morisco, F., 2016. PMD31 - A Real  
892 World Cost Analysis of Serum Hbsag Quantification Test in Patients with Chronic Hepatitis B:  
893 The Case of The University of Naples Federico II. *Value Health* 19, A690.  
894 <https://doi.org/10.1016/j.jval.2016.09.1972>
- 895 Asselah, T., Loureiro, D., Boyer, N., Mansouri, A., 2019. Targets and future direct-acting antiviral  
896 approaches to achieve hepatitis B virus cure. *Lancet Gastroenterol. Hepatol.* 4, 883–892.  
897 [https://doi.org/10.1016/S2468-1253\(19\)30190-6](https://doi.org/10.1016/S2468-1253(19)30190-6)
- 898 Berg, T., Simon, K.-G., Mauss, S., Schott, E., Heyne, R., Klass, D.M., Eisenbach, C., Welzel, T.M.,  
899 Zachoval, R., Felten, G., Schulze-Zur-Wiesch, J., Cornberg, M., Op den Brouw, M.L., Jump, B.,  
900 Reiser, H., Gallo, L., Warger, T., Petersen, J., FINITE CHB study investigators [First  
901 investigation in stopping TDF treatment after long-term virological suppression in HBeAg-  
902 negative chronic hepatitis B], 2017. Long-term response after stopping tenofovir disoproxil  
903 fumarate in non-cirrhotic HBeAg-negative patients - FINITE study. *J. Hepatol.* 67, 918–924.  
904 <https://doi.org/10.1016/j.jhep.2017.07.012>
- 905 Boni, C., Laccabue, D., Lampertico, P., Giuberti, T., Viganò, M., Schivazappa, S., Alfieri, A., Pesci, M.,  
906 Gaeta, G.B., Brancaccio, G., Colombo, M., Missale, G., Ferrari, C., 2012. Restored function of  
907 HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues.  
908 *Gastroenterology* 143, 963-973.e9. <https://doi.org/10.1053/j.gastro.2012.07.014>
- 909 Boni, C., Lampertico, P., Talamona, L., Giuberti, T., Invernizzi, F., Barili, V., Fisicaro, P., Rossi, M.,  
910 Cavallo, M.C., Vecchi, A., Pedrazzi, G., Alfieri, A., Colombo, M., Missale, G., Ferrari, C., 2015.  
911 Natural killer cell phenotype modulation and natural killer/T-cell interplay in nucleos(t)ide  
912 analogue-treated hepatitis e antigen-negative patients with chronic hepatitis B. *Hepatol.*  
913 *Baltim. Md* 62, 1697–1709. <https://doi.org/10.1002/hep.28155>
- 914 Boni, C., Penna, A., Bertoletti, A., Lamonaca, V., Rapti, I., Missale, G., Pilli, M., Urbani, S., Cavalli, A.,  
915 Cerioni, S., Panebianco, R., Jenkins, J., Ferrari, C., 2003. Transient restoration of anti-viral T  
916 cell responses induced by lamivudine therapy in chronic hepatitis B. *J. Hepatol.* 39, 595–605.  
917 [https://doi.org/10.1016/s0168-8278\(03\)00292-7](https://doi.org/10.1016/s0168-8278(03)00292-7)
- 918 Bonino, F., Marcellin, P., Lau, G.K.K., Hadziyannis, S., Jin, R., Piratvisuth, T., Germanidis, G., Yurdaydin,  
919 C., Diago, M., Gurel, S., Lai, M.-Y., Brunetto, M.R., Farci, P., Popescu, M., McCloud, P.,  
920 Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group, 2007. Predicting  
921 response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative  
922 chronic hepatitis B. *Gut* 56, 699–705. <https://doi.org/10.1136/gut.2005.089722>
- 923 Brass, A., Brenndörfer, E.D., 2014. The Role of Chemokines in Hepatitis C Virus-Mediated Liver  
924 Disease. *Int. J. Mol. Sci.* 15, 4747–4779. <https://doi.org/10.3390/ijms15034747>
- 925 Buti, M., Wong, D.K., Gane, E., Flisiak, R., Manns, M., Kaita, K., Janssen, H.L.A., Op den Brouw, M.,  
926 Jump, B., Kitrinou, K., Crans, G., Flaherty, J., Gaggar, A., Marcellin, P., 2019. Safety and  
927 efficacy of stopping tenofovir disoproxil fumarate in patients with chronic hepatitis B  
928 following at least 8 years of therapy: a prespecified follow-up analysis of two randomised  
929 trials. *Lancet Gastroenterol. Hepatol.* 4, 296–304. [https://doi.org/10.1016/S2468-1253\(19\)30015-9](https://doi.org/10.1016/S2468-1253(19)30015-9)
- 930
- 931 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,  
932 Y.T., Lee, C.H., 2005. Factors related to post-treatment relapse in chronic hepatitis B patients  
933 who lost HBeAg after lamivudine therapy. *J. Gastroenterol. Hepatol.* 20, 1838–1842.  
934 <https://doi.org/10.1111/j.1440-1746.2005.03952.x>
- 935 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.,  
936 2017. Off-Treatment Hepatitis B Virus (HBV) DNA Levels and the Prediction of Relapse After  
937 Discontinuation of Nucleos(t)ide Analogue Therapy in Patients With Chronic Hepatitis B: A  
938 Prospective Stop Study. *J. Infect. Dis.* 215, 581–589. <https://doi.org/10.1093/infdis/jix025>
- 939 Carey, I., Gersch, J., Wang, B., Moigboi, C., Kuhns, M., Cloherty, G., Dusheiko, G., Agarwal, K., 2019.  
940 Pre-genomic HBV RNA and HBcrAg predict outcomes in HBeAg negative chronic hepatitis B

941 patients suppressed on nucleos(t)ide analogue therapy. *Hepatol. Baltim. Md.*  
942 <https://doi.org/10.1002/hep.31026>

943 Chan, H.L.-Y., Wang, H., Niu, J., Chim, A.M.-L., Sung, J.J.-Y., 2007. Two-year lamivudine treatment for  
944 hepatitis B e antigen-negative chronic hepatitis B: a double-blind, placebo-controlled trial.  
945 *Antivir. Ther.* 12, 345–353.

946 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  
947 of off-treatment response to lamivudine by serum hepatitis B surface antigen quantification  
948 in hepatitis B e antigen-negative patients. *Antivir. Ther.* 16, 1249–1257.  
949 <https://doi.org/10.3851/IMP1921>

950 Chang, Ming-Ling, Jeng, W.-J., Liaw, Y.-F., 2015. Clinical events after cessation of lamivudine therapy  
951 in patients recovered from hepatitis B flare with hepatic decompensation. *Clin.*  
952 *Gastroenterol. Hepatol. Off. Clin. Pract. J. Am. Gastroenterol. Assoc.* 13, 979–986.  
953 <https://doi.org/10.1016/j.cgh.2014.10.023>

954 Chang, M.-L., Liaw, Y.-F., Hadziyannis, S.J., 2015. Systematic review: cessation of long-term  
955 nucleos(t)ide analogue therapy in patients with hepatitis B e antigen-negative chronic  
956 hepatitis B. *Aliment. Pharmacol. Ther.* 42, 243–257. <https://doi.org/10.1111/apt.13272>

957 Chaung, K.T., Ha, N.B., Trinh, H.N., Garcia, R.T., Nguyen, H.A., Nguyen, K.K., Garcia, G., Ahmed, A.,  
958 Keeffe, E.B., Nguyen, M.H., 2012. High frequency of recurrent viremia after hepatitis B e  
959 antigen seroconversion and consolidation therapy. *J. Clin. Gastroenterol.* 46, 865–870.  
960 <https://doi.org/10.1097/MCG.0b013e31825ceed9>

961 Chen, C.-H., Hsu, Y.-C., Lu, S.-N., Hung, C.-H., Wang, J.-H., Lee, C.-M., Hu, T.-H., 2018. The incidence  
962 and predictors of HBV relapse after cessation of tenofovir therapy in chronic hepatitis B  
963 patients. *J. Viral Hepat.* 25, 590–597. <https://doi.org/10.1111/jvh.12851>

964 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  
965 Incidence of Hepatitis B Surface Antigen Loss Between Hepatitis B E Antigen-Negative  
966 Noncirrhotic Patients Who Discontinued or Continued Entecavir Therapy. *J. Infect. Dis.* 219,  
967 1624–1633. <https://doi.org/10.1093/infdis/jiy697>

968 Chen, C.-H., Lu, S.-N., Hung, C.-H., Wang, J.-H., Hu, T.-H., Changchien, C.-S., Lee, C.-M., 2014. The role  
969 of hepatitis B surface antigen quantification in predicting HBsAg loss and HBV relapse after  
970 discontinuation of lamivudine treatment. *J. Hepatol.* 61, 515–522.  
971 <https://doi.org/10.1016/j.jhep.2014.04.029>

972 Chen, Y., Li, X., Ye, B., Yang, X., Wu, W., Chen, B., Pan, X., Cao, H., Li, L., 2011. Effect of telbivudine  
973 therapy on the cellular immune response in chronic hepatitis B. *Antiviral Res.* 91, 23–31.  
974 <https://doi.org/10.1016/j.antiviral.2011.04.008>

975 Chen, Y.C., Peng, C.Y., Jeng, W.J., Chien, R.N., Liaw, Y.F., 2015. Clinical outcomes after interruption of  
976 entecavir therapy in HBeAg-negative chronic hepatitis B patients with compensated cirrhosis.  
977 *Aliment. Pharmacol. Ther.* 42, 1182–1191. <https://doi.org/10.1111/apt.13409>

978 Chi, H., Li, Z., Hansen, B.E., Yu, T., Zhang, X., Sun, J., Hou, J., Janssen, H.L.A., Peng, J., 2019. Serum  
979 Level of Antibodies Against Hepatitis B Core Protein Is Associated With Clinical Relapse After  
980 Discontinuation of Nucleos(t)ide Analogue Therapy. *Clin. Gastroenterol. Hepatol. Off. Clin.*  
981 *Pract. J. Am. Gastroenterol. Assoc.* 17, 182-191.e1.  
982 <https://doi.org/10.1016/j.cgh.2018.05.047>

983 Chien, R.-N., Liaw, Y.-F., 2020. Re-treatment for severe hepatitis flare in HBeAg-negative chronic  
984 hepatitis B: An appraisal with combined HBsAg/ALT kinetics. *J. Viral Hepat.* 27, 544–547.  
985 <https://doi.org/10.1111/jvh.13253>

986 Chien, R.-N., Liaw, Y.-F., 2006. Short-term lamivudine therapy in HBeAg-negative chronic active  
987 hepatitis B in Taiwan. *Antivir. Ther.* 11, 947–952.

988 Cornberg, M., Lok, A.S.-F., Terrault, N.A., Zoulim, F., 2019 EASL-AASLD HBV Treatment Endpoints  
989 Conference Faculty, 2020. Guidance for design and endpoints of clinical trials in chronic  
990 hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference†. *J.*  
991 *Hepatol.* 72, 539–557. <https://doi.org/10.1016/j.jhep.2019.11.003>

992 Dienstag, J.L., Cianciara, J., Karayalcin, S., Kowdley, K.V., Willems, B., Plisek, S., Woessner, M.,  
993 Gardner, S., Schiff, E., 2003. Durability of serologic response after lamivudine treatment of  
994 chronic hepatitis B. *Hepatology*. Baltimore, Md 37, 748–755.  
995 <https://doi.org/10.1053/jhep.2003.50117>

996 Dunn, C., Brunetto, M., Reynolds, G., Christophides, T., Kennedy, P.T., Lampertico, P., Das, A., Lopes,  
997 A.R., Borrow, P., Williams, K., Humphreys, E., Afford, S., Adams, D.H., Bertolotti, A., Maini,  
998 M.K., 2007. Cytokines induced during chronic hepatitis B virus infection promote a pathway  
999 for NK cell-mediated liver damage. *J. Exp. Med.* 204, 667–680.  
1000 <https://doi.org/10.1084/jem.20061287>

1001 European Association for the Study of the Liver. Electronic address: [easloffice@easloffice.eu](mailto:easloffice@easloffice.eu),  
1002 European Association for the Study of the Liver, 2017. EASL 2017 Clinical Practice Guidelines  
1003 on the management of hepatitis B virus infection. *J. Hepatology*. 67, 370–398.  
1004 <https://doi.org/10.1016/j.jhep.2017.03.021>

1005 Fan, R., Peng, J., Xie, Q., Tan, D., Xu, M., Niu, J., Wang, H., Ren, H., Chen, X., Wang, M., Sheng, J., Tang,  
1006 H., Bai, X., Wu, Y., Zhou, B., Sun, J., Hou, J., Chronic Hepatitis B Study Consortium, 2020.  
1007 Combining HBV RNA and hepatitis B core-related antigen: guidance for safely stopping  
1008 nucleos(t)ide analogues in HBeAg-positive chronic hepatitis B patients. *J. Infect. Dis.*  
1009 <https://doi.org/10.1093/infdis/jiaa136>

1010 Fan, R., Sun, J., Yuan, Q., Xie, Q., Bai, X., Ning, Q., Cheng, J., Yu, Y., Niu, J., Shi, G., Wang, H., Tan, D.,  
1011 Wan, M., Chen, S., Xu, M., Chen, X., Tang, H., Sheng, J., Lu, F., Jia, J., Zhuang, H., Xia, N., Hou,  
1012 J., Chronic Hepatitis B Study Consortium, 2016. Baseline quantitative hepatitis B core  
1013 antibody titre alone strongly predicts HBeAg seroconversion across chronic hepatitis B  
1014 patients treated with peginterferon or nucleos(t)ide analogues. *Gut* 65, 313–320.  
1015 <https://doi.org/10.1136/gutjnl-2014-308546>

1016 Faure-Dupuy, S., Lucifora, J., Durantel, D., 2017. Interplay between the Hepatitis B Virus and Innate  
1017 Immunity: From an Understanding to the Development of Therapeutic Concepts. *Viruses* 9.  
1018 <https://doi.org/10.3390/v9050095>

1019 Fiscaro, P., Barili, V., Montanini, B., Acerbi, G., Ferracin, M., Guerrieri, F., Salerno, D., Boni, C.,  
1020 Massari, M., Cavallo, M.C., Grossi, G., Giuberti, T., Lampertico, P., Missale, G., Levrero, M.,  
1021 Ottonello, S., Ferrari, C., 2017. Targeting mitochondrial dysfunction can restore antiviral  
1022 activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. *Nat. Med.* 23, 327–336.  
1023 <https://doi.org/10.1038/nm.4275>

1024 Fiscaro, P., Rossi, M., Vecchi, A., Acerbi, G., Barili, V., Laccabue, D., Montali, I., Zecca, A., Penna, A.,  
1025 Missale, G., Ferrari, C., Boni, C., 2019. The Good and the Bad of Natural Killer Cells in Virus  
1026 Control: Perspective for Anti-HBV Therapy. *Int. J. Mol. Sci.* 20.  
1027 <https://doi.org/10.3390/ijms20205080>

1028 Fontana, R.J., 2009. Side effects of long-term oral antiviral therapy for hepatitis B. *Hepatology*. Baltim.  
1029 Md 49, S185-195. <https://doi.org/10.1002/hep.22885>

1030 Ford, N., Scourse, R., Lemoine, M., Hutin, Y., Bulterys, M., Shubber, Z., Donchuk, D., Wandeler, G.,  
1031 2018. Adherence to Nucleos(t)ide Analogue Therapies for Chronic Hepatitis B Infection: A  
1032 Systematic Review and Meta-Analysis. *Hepatology*. Commun. 1160–1167.

1033 Fung, S.K., Wong, F., Hussain, M., Lok, A.S.F., 2004. Sustained response after a 2-year course of  
1034 lamivudine treatment of hepatitis B e antigen-negative chronic hepatitis B. *J. Viral Hepat.* 11,  
1035 432–438. <https://doi.org/10.1111/j.1365-2893.2004.00556.x>

1036 Galmozzi, E., Viganò, M., Lampertico, P., 2014. Systematic review with meta-analysis: do interferon  
1037 lambda 3 polymorphisms predict the outcome of interferon-therapy in hepatitis B infection?  
1038 *Aliment. Pharmacol. Ther.* 39, 569–578. <https://doi.org/10.1111/apt.12631>

1039 Gao, L., Hu, Y., Shi, X., Li, X., Zhang, D., Ren, H., 2019. 48 weeks outcome after cessation of  
1040 nucleos(t)ide analogue therapy in chronic hepatitis B patients. *Ann. Hepatology*.  
1041 <https://doi.org/10.1016/j.aohep.2019.10.006>

1042 Ge, G.-H., Ye, Y., Zhou, X.-B., Chen, L., He, C., Wen, D.-F., Tan, Y.-W., 2015. Hepatitis B surface antigen  
1043 levels of cessation of nucleos(t)ide analogs associated with virological relapse in hepatitis B

1044 surface antigen-negative chronic hepatitis B patients. *World J. Gastroenterol.* 21, 8653–8659.  
1045 <https://doi.org/10.3748/wjg.v21.i28.8653>

1046 Giersch, K., Allweiss, L., Volz, T., Dandri, M., Lütgehetmann, M., 2017. Serum HBV pgRNA as a clinical  
1047 marker for cccDNA activity. *J. Hepatol.* 66, 460–462.  
1048 <https://doi.org/10.1016/j.jhep.2016.09.028>

1049 Ha, M., Zhang, G., Diao, S., Lin, M., Sun, L., She, H., Kuan, C., Shen, L., Huang, C., Shen, W., Huang, Z.,  
1050 2012. A prospective clinical study in hepatitis B e antigen-negative chronic hepatitis B  
1051 patients with stringent cessation criteria for adefovir. *Arch. Virol.* 157, 285–290.  
1052 <https://doi.org/10.1007/s00705-011-1163-0>

1053 Hadziyannis, E., Hadziyannis, S., 2020. Current practice and contrasting views on discontinuation of  
1054 nucleos(t)ide analog therapy in chronic hepatitis B. *Expert Rev. Gastroenterol. Hepatol.* 14,  
1055 243–251. <https://doi.org/10.1080/17474124.2020.1738219>

1056 Hadziyannis, S.J., Sevastianos, V., Rapti, I., Vassilopoulos, D., Hadziyannis, E., 2012. Sustained  
1057 responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop  
1058 long-term treatment with adefovir. *Gastroenterology* 143, 629-636.e1.  
1059 <https://doi.org/10.1053/j.gastro.2012.05.039>

1060 He, D., Guo, S., Chen, W., Chen, X., Yan, G., Wang, J., Li, M., Zhu, P., Huang, H., Wang, Y., 2013. Long-  
1061 term outcomes after nucleos(t)ide analogues discontinuation in chronic hepatitis B patients  
1062 with HBeAg-negative. *BMC Infect. Dis.* 13, 458. <https://doi.org/10.1186/1471-2334-13-458>

1063 Höner Zu Siederdisen, C., Hui, A.J., Sukeepaisarnjaroen, W., Tangkijvanich, P., Su, W.W., Nieto,  
1064 G.E.G., Gineste, P., Nitcheu, J., Crabé, S., Stepien, S., Manns, M.P., Trépo, C., Wedemeyer, H.,  
1065 Cornberg, M., 2018. Contrasting Timing of Virological Relapse After Discontinuation of  
1066 Tenofovir or Entecavir in Hepatitis B e Antigen-Negative Patients. *J. Infect. Dis.* 218, 1480–  
1067 1484. <https://doi.org/10.1093/infdis/jiy350>

1068 Höner Zu Siederdisen, C., Rinker, F., Maasoumy, B., Wiegand, S.B., Filmann, N., Falk, C.S., Deterding,  
1069 K., Port, K., Mix, C., Manns, M.P., Herrmann, E., Wedemeyer, H., Kraft, A.R.M., Cornberg, M.,  
1070 2016. Viral and Host Responses After Stopping Long-term Nucleos(t)ide Analogue Therapy in  
1071 HBeAg-Negative Chronic Hepatitis B. *J. Infect. Dis.* 214, 1492–1497.  
1072 <https://doi.org/10.1093/infdis/jiw412>

1073 Hoogeveen, R.C., Boonstra, A., 2020. Checkpoint Inhibitors and Therapeutic Vaccines for the  
1074 Treatment of Chronic HBV Infection. *Front. Immunol.* 11, 401.  
1075 <https://doi.org/10.3389/fimmu.2020.00401>

1076 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,  
1077 C.-Y., Lin, J.-T., Tanaka, Y., Chang, C.-Y., 2019. Combining hepatitis B core-related and surface  
1078 antigens at end of nucleos(t)ide analogue treatment to predict off-therapy relapse risk.  
1079 *Aliment. Pharmacol. Ther.* 49, 107–115. <https://doi.org/10.1111/apt.15058>

1080 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.-  
1081 Y., Lee, S.-D., 2003. Analysis of clinical, biochemical and viral factors associated with early  
1082 relapse after lamivudine treatment for hepatitis B e antigen-negative chronic hepatitis B  
1083 patients in Taiwan. *J. Viral Hepat.* 10, 277–284. <https://doi.org/10.1046/j.1365-2893.2003.00428.x>

1084

1085 Jaroszewicz, J., Ho, H., Markova, A., Deterding, K., Wursthorn, K., Schulz, S., Bock, C.-T., Tillmann, H.L.,  
1086 Manns, M.P., Wedemeyer, H., Cornberg, M., 2011. Hepatitis B surface antigen (HBsAg)  
1087 decrease and serum interferon-inducible protein-10 levels as predictive markers for HBsAg  
1088 loss during treatment with nucleoside/nucleotide analogues. *Antivir. Ther.* 16, 915–924.  
1089 <https://doi.org/10.3851/IMP1866>

1090 Jeng, W.-J., Chang, M.-L., Liaw, Y.-F., 2019. Off-therapy precipitous HBsAg decline predicts HBsAg loss  
1091 after finite entecavir therapy in HBeAg-negative patients. *J. Viral Hepat.* 26, 1019–1026.  
1092 <https://doi.org/10.1111/jvh.13114>

1093 Jeng, Wen-Juei, Chen, Y.-C., Chien, R.-N., Sheen, I.-S., Liaw, Y.-F., 2018. Incidence and predictors of  
1094 hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in

1095 hepatitis B e antigen-negative chronic hepatitis B. *Hepatology*. Baltimore, Md 68, 425–434.  
1096 <https://doi.org/10.1002/hep.29640>

1097 Jeng, W.-J., Chen, Y.-C., Sheen, I.-S., Lin, C.-L., Hu, T.-H., Chien, R.-N., Liaw, Y.-F., 2016. Clinical Relapse  
1098 After Cessation of Tenofovir Therapy in Hepatitis B e Antigen-Negative Patients. *Clinical  
1099 Gastroenterology and Hepatology*. *Off. Clin. Pract. J. Am. Gastroenterol. Assoc.* 14, 1813-1820.e1.  
1100 <https://doi.org/10.1016/j.cgh.2016.07.002>

1101 Jeng, W.-J., Sheen, I.-S., Chen, Y.-C., Hsu, C.-W., Chien, R.-N., Chu, C.-M., Liaw, Y.-F., 2013. Off-therapy  
1102 durability of response to entecavir therapy in hepatitis B e antigen-negative chronic hepatitis  
1103 B patients. *Hepatology*. Baltimore, Md 58, 1888–1896. <https://doi.org/10.1002/hep.26549>

1104 Jeng, W.-J., Yang, H.-I., Chen, Y.-C., Liu, J., Chien, R.-N., Sheen, I.-S., Chen, C.-J., Liaw, Y.-F., 2018.  
1105 Increased incidence of HBsAg seroclearance in HBeAg negative chronic hepatitis B patients  
1106 discontinued Nuc therapy comparing to natural course-a propensity score matched study. *J.  
1107 Hepatology*. 68, S88. [https://doi.org/10.1016/S0168-8278\(18\)30394-5](https://doi.org/10.1016/S0168-8278(18)30394-5)

1108 Jiang, J., Huang, Z., He, L., Huang, Y., Su, M., Xie, R., Liang, Y., Fu, W., Huang, X., Guo, W., Zhong, S.,  
1109 Liu, Z., Li, S., Zhu, T., Gao, Z., 2015. Residual amount of HBV DNA in serum is related to  
1110 relapse in chronic hepatitis B patients after cessation of nucleos(t)ide analogs. *J. Clin.  
1111 Gastroenterology*. 49, 323–328. <https://doi.org/10.1097/MCG.0000000000000170>

1112 Jiang, Y., Ma, Z., Xin, G., Yan, H., Li, W., Xu, H., Hao, C., Niu, J., Zhao, P., 2010. Th1 and Th2 immune  
1113 response in chronic hepatitis B patients during a long-term treatment with adefovir dipivoxil.  
1114 *Mediators of Inflammation*. 2010, 143026. <https://doi.org/10.1155/2010/143026>

1115 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  
1116 course of chronic hepatitis B patients who were off-treated after lamivudine treatment:  
1117 analysis of 138 consecutive patients. *Virology*. 9, 239. <https://doi.org/10.1186/1743-422X-9-239>

1118

1119 Ju, H., Liu, H., Tian, Z.-B., Jiang, Y.-P., Zhang, C.-P., Liu, X.-S., 2017. Association of polymorphisms in  
1120 key Th-17 immune response genes with HBeAg-positive chronic hepatitis B susceptibility and  
1121 response to PEG-IFN $\alpha$ -2a. *Virology* 509, 35–41. <https://doi.org/10.1016/j.virol.2017.05.011>

1122 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,  
1123 S.H., 2016. Clinical outcomes and predictors for relapse after cessation of oral antiviral  
1124 treatment in chronic hepatitis B patients. *J. Gastroenterology*. 51, 830–839.  
1125 <https://doi.org/10.1007/s00535-015-1153-1>

1126 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.,  
1127 Ryu, H.S., Byun, K.S., 2011. Virologic response is not durable after adefovir discontinuation in  
1128 lamivudine-resistant chronic hepatitis B patients. *Korean J. Hepatology*. 17, 261–267.  
1129 <https://doi.org/10.3350/kjhep.2011.17.4.261>

1130 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,  
1131 J.H., Seo, Y.S., Yim, H.J., Byun, K.S., 2017. Antiviral response is not sustained after cessation of  
1132 lamivudine treatment in chronic hepatitis B patients: A 10-year follow-up study. *J. Med. Virology*.  
1133 89, 849–856. <https://doi.org/10.1002/jmv.24715>

1134 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.,  
1135 2014. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic  
1136 hepatitis B: clinical outcomes and durability. *Gut* 63, 1325–1332.  
1137 <https://doi.org/10.1136/gutjnl-2013-305517>

1138 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,  
1139 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.,  
1140 Kim, H.Y., Park, Y., Kim, G.-A., Yang, H., Lee, H.A., Koh, M., Lee, Y.-S., Kim, M., Chang, Y., Kim,  
1141 Y.J., Yoon, J.-H., Zoulim, F., Lee, J.-H., 2020. Discontinuation of nucleos(t)ide analogues is not  
1142 associated with a higher risk of HBsAg seroreversion after antiviral-induced HBsAg  
1143 seroclearance: a nationwide multicentre study. *Gut*. <https://doi.org/10.1136/gutjnl-2019-320015>

1144

1145 Kim, Y.J., Kim, K., Hwang, S.H., Kim, S.S., Lee, D., Cheong, J.Y., Cho, S.W., 2013. Durability after  
1146 discontinuation of nucleos(t)ide therapy in chronic HBeAg negative hepatitis patients. *Clin.*  
1147 *Mol. Hepatol.* 19, 300–304. <https://doi.org/10.3350/cmh.2013.19.3.300>

1148 Kranidioti, H., Manolakopoulos, S., Kontos, G., Breen, M.S., Kourikou, A., Deutsch, M., Quesada-Del-  
1149 Bosque, M.E., Martinez-Nunez, R.T., Naiyer, M.M., Woelk, C.H., Sanchez-Elsner, T.,  
1150 Hadziyannis, E., Papatheodoridis, G., Khakoo, S.I., 2019. Immunological biomarkers as  
1151 indicators for outcome after discontinuation of nucleos(t)ide analogue therapy in patients  
1152 with HBeAg-negative chronic hepatitis B. *J. Viral Hepat.* 26, 697–709.  
1153 <https://doi.org/10.1111/jvh.13068>

1154 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  
1155 relapse rates in chronic hepatitis B patients who discontinue either entecavir or tenofovir.  
1156 *Aliment. Pharmacol. Ther.* 49, 218–228. <https://doi.org/10.1111/apt.15053>

1157 Kuo, Y.-H., Chen, C.-H., Wang, J.-H., Hung, C.-H., Tseng, P.-L., Lu, S.-N., Changchien, C.-S., Lee, C.-M.,  
1158 2010. Extended lamivudine consolidation therapy in hepatitis B e antigen-positive chronic  
1159 hepatitis B patients improves sustained hepatitis B e antigen seroconversion. *Scand. J.*  
1160 *Gastroenterol.* 45, 75–81. <https://doi.org/10.3109/00365520903394550>

1161 Lamivudine Treatment Can Restore T Cell Responsiveness in Chronic Hepatitis B [WWW Document],  
1162 n.d. URL [https://pubmed-ncbi-nlm-nih-](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)  
1163 [gov.proxy.insermbiblio.inist.fr/9727065/?from\\_term=+Lamivudine+treatment+can+restore+](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)  
1164 [T+cell+responsiveness+in+chronic+hepatitis+B&from\\_sort=date](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).

1165 Lampertico, P., Berg, T., 2018. Less can be more: A finite treatment approach for HBeAg-negative  
1166 chronic hepatitis B. *Hepatol. Baltim. Md* 68, 397–400. <https://doi.org/10.1002/hep.29821>

1167 Lang, P.A., Lang, K.S., Xu, H.C., Grusdat, M., Parish, I.A., Recher, M., Elford, A.R., Dhanji, S., Shaabani,  
1168 N., Tran, C.W., Dissanayake, D., Rahbar, R., Ghazarian, M., Brüstle, A., Fine, J., Chen, P.,  
1169 Weaver, C.T., Klose, C., Diefenbach, A., Häussinger, D., Carlyle, J.R., Kaeck, S.M., Mak, T.W.,  
1170 Ohashi, P.S., 2012. Natural killer cell activation enhances immune pathology and promotes  
1171 chronic infection by limiting CD8+ T-cell immunity. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1210–  
1172 1215. <https://doi.org/10.1073/pnas.1118834109>

1173 Larrubia, J.R., Benito-Martínez, S., Calvino, M., Sanz-de-Villalobos, E., Parra-Cid, T., 2008. Role of  
1174 chemokines and their receptors in viral persistence and liver damage during chronic hepatitis  
1175 C virus infection. *World J. Gastroenterol.* 14, 7149–7159.  
1176 <https://doi.org/10.3748/wjg.14.7149>

1177 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,  
1178 Y.H., Lee, C.K., Lee, K.S., Chon, C.Y., Han, K.-H., 2010. Lamivudine maintenance beyond one  
1179 year after HBeAg seroconversion is a major factor for sustained virologic response in HBeAg-  
1180 positive chronic hepatitis B. *Hepatol. Baltim. Md* 51, 415–421.  
1181 <https://doi.org/10.1002/hep.23323>

1182 Liang, Y., Jiang, J., Su, M., Liu, Z., Guo, W., Huang, X., Xie, R., Ge, S., Hu, J., Jiang, Z., Zhu, M., Wong,  
1183 V.W.-S., Chan, H.L.-Y., 2011. Predictors of relapse in chronic hepatitis B after discontinuation  
1184 of anti-viral therapy. *Aliment. Pharmacol. Ther.* 34, 344–352. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2036.2011.04738.x)  
1185 [2036.2011.04738.x](https://doi.org/10.1111/j.1365-2036.2011.04738.x)

1186 Liaw, Y.-F., 2020. Hepatitis B flare after cessation of nucleos(t)ide analogue therapy in HBeAg-  
1187 negative chronic hepatitis B: To retreat or not to retreat. *Hepatol. Baltim. Md.*  
1188 <https://doi.org/10.1002/hep.31525>

1189 Liaw, Y.-F., 2019. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative  
1190 chronic HBV infection. *Nat. Rev. Gastroenterol. Hepatol.* 16, 631–641.  
1191 <https://doi.org/10.1038/s41575-019-0197-8>

1192 Liaw, Y.-F., Jeng, W.-J., Chang, M.-L., 2018. HBsAg Kinetics in Retreatment Decision for Off-Therapy  
1193 Hepatitis B Flare in HBeAg-Negative Patients. *Gastroenterology* 154, 2280–2281.  
1194 <https://doi.org/10.1053/j.gastro.2018.03.066>

1195 Liaw, Y.-F., Leung, N., Kao, J.-H., Piratvisuth, T., Gane, E., Han, K.-H., Guan, R., Lau, G.K.K., Locarnini,  
1196 S., Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study

1197 of the Liver, 2008. Asian-Pacific consensus statement on the management of chronic  
1198 hepatitis B: a 2008 update. *Hepatol. Int.* 2, 263–283. [https://doi.org/10.1007/s12072-008-](https://doi.org/10.1007/s12072-008-9080-3)  
1199 9080-3

1200 Liaw, Y.-F., Sung, J.J.Y., Chow, W.C., Farrell, G., Lee, C.-Z., Yuen, H., Tanwandee, T., Tao, Q.-M., Shue,  
1201 K., Keene, O.N., Dixon, J.S., Gray, D.F., Sabbat, J., Cirrhosis Asian Lamivudine Multicentre  
1202 Study Group, 2004. Lamivudine for patients with chronic hepatitis B and advanced liver  
1203 disease. *N. Engl. J. Med.* 351, 1521–1531. <https://doi.org/10.1056/NEJMoa033364>

1204 Liem, K.S., Fung, S., Wong, D.K., Yim, C., Noureldin, S., Chen, J., Feld, J.J., Hansen, B.E., Janssen, H.L.A.,  
1205 2019. Limited sustained response after stopping nucleos(t)ide analogues in patients with  
1206 chronic hepatitis B: results from a randomised controlled trial (Toronto STOP study). *Gut* 68,  
1207 2206–2213. <https://doi.org/10.1136/gutjnl-2019-318981>

1208 Lin, C.-L., Kao, J.-H., 2016. New perspectives of biomarkers for the management of chronic hepatitis  
1209 B. *Clin. Mol. Hepatol.* 22, 423–431. <https://doi.org/10.3350/cmh.2016.0069>

1210 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.  
1211 Varying 10-year off-treatment responses to nucleos(t)ide analogues in patients with chronic  
1212 hepatitis B according to their pretreatment hepatitis B e antigen status. *J. Dig. Dis.* 19, 561–  
1213 571. <https://doi.org/10.1111/1751-2980.12654>

1214 Liu, F., Wang, L., Li, X.Y., Liu, Y.D., Wang, J.B., Zhang, Z.H., Wang, Y.Z., 2011. Poor durability of  
1215 lamivudine effectiveness despite stringent cessation criteria: a prospective clinical study in  
1216 hepatitis B e antigen-negative chronic hepatitis B patients. *J. Gastroenterol. Hepatol.* 26,  
1217 456–460. <https://doi.org/10.1111/j.1440-1746.2010.06492.x>

1218 Liu, J., Li, T., Zhang, L., Xu, A., 2019. The Role of Hepatitis B Surface Antigen in Nucleos(t)ide  
1219 Analogues Cessation Among Asian Patients With Chronic Hepatitis B: A Systematic Review.  
1220 *Hepatol. Baltim. Md* 70, 1045–1055. <https://doi.org/10.1002/hep.30474>

1221 Liu, Y., Jia, M., Wu, S., Jiang, W., Feng, Y., 2019. Predictors of relapse after cessation of nucleos(t)ide  
1222 analog treatment in HBeAg-negative chronic hepatitis B patients: A meta-analysis. *Int. J.*  
1223 *Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.* 86, 201–207.  
1224 <https://doi.org/10.1016/j.ijid.2019.07.036>

1225 Lok, A.S.F., McMahon, B.J., Brown, R.S., Wong, J.B., Ahmed, A.T., Farah, W., Almasri, J., Alahdab, F.,  
1226 Benkhadra, K., Mouchli, M.A., Singh, S., Mohamed, E.A., Abu Dabrh, A.M., Prokop, L.J., Wang,  
1227 Z., Murad, M.H., Mohammed, K., 2016. Antiviral therapy for chronic hepatitis B viral infection  
1228 in adults: A systematic review and meta-analysis. *Hepatol. Baltim. Md* 63, 284–306.  
1229 <https://doi.org/10.1002/hep.28280>

1230 Ma, Z., Cao, Q., Xiong, Y., Zhang, E., Lu, M., 2018. Interaction between Hepatitis B Virus and Toll-Like  
1231 Receptors: Current Status and Potential Therapeutic Use for Chronic Hepatitis B. *Vaccines* 6.  
1232 <https://doi.org/10.3390/vaccines6010006>

1233 Maini, M.K., Boni, C., Lee, C.K., Larrubia, J.R., Reignat, S., Ogg, G.S., King, A.S., Herberg, J., Gilson, R.,  
1234 Alisa, A., Williams, R., Vergani, D., Naoumov, N.V., Ferrari, C., Bertolotti, A., 2000. The role of  
1235 virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus  
1236 infection. *J. Exp. Med.* 191, 1269–1280. <https://doi.org/10.1084/jem.191.8.1269>

1237 Maini, M.K., Peppas, D., 2013. NK cells: a double-edged sword in chronic hepatitis B virus infection.  
1238 *Front. Immunol.* 4, 57. <https://doi.org/10.3389/fimmu.2013.00057>

1239 Mansouri, A., Gattolliat, C.-H., Asselah, T., 2018. Mitochondrial Dysfunction and Signaling in Chronic  
1240 Liver Diseases. *Gastroenterology* 155, 629–647.  
1241 <https://doi.org/10.1053/j.gastro.2018.06.083>

1242 Marcellin, P., Asselah, T., 2013. Long-term therapy for chronic hepatitis B: hepatitis B virus DNA  
1243 suppression leading to cirrhosis reversal. *J. Gastroenterol. Hepatol.* 28, 912–923.  
1244 <https://doi.org/10.1111/jgh.12213>

1245 Marcellin, P., Bonino, F., Lau, G.K.K., Farci, P., Yurdaydin, C., Piratvisuth, T., Jin, R., Gurel, S., Lu, Z.-M.,  
1246 Wu, J., Popescu, M., Hadziyannis, S., Peginterferon alfa-2a in HBeAg-negative Chronic  
1247 Hepatitis B Study Group, 2009. Sustained response of hepatitis B e antigen-negative patients

1248 3 years after treatment with peginterferon alpha-2a. *Gastroenterology* 136, 2169-2179.e1-4.  
1249 <https://doi.org/10.1053/j.gastro.2009.03.006>

1250 Marcellin, P., Gane, E., Buti, M., Afdhal, N., Sievert, W., Jacobson, I.M., Washington, M.K.,  
1251 Germanidis, G., Flaherty, J.F., Aguilar Schall, R., Bornstein, J.D., Kitrinis, K.M., Subramanian,  
1252 G.M., McHutchison, J.G., Heathcote, E.J., 2013. Regression of cirrhosis during treatment with  
1253 tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study.  
1254 *Lancet Lond. Engl.* 381, 468-475. [https://doi.org/10.1016/S0140-6736\(12\)61425-1](https://doi.org/10.1016/S0140-6736(12)61425-1)

1255 Marcellin, P., Wong, D.K., Sievert, W., Buggisch, P., Petersen, J., Flisiak, R., Manns, M., Kaita, K.,  
1256 Krastev, Z., Lee, S.S., Cathcart, A.L., Crans, G., Op den Brouw, M., Jump, B., Gaggar, A.,  
1257 Flaherty, J., Buti, M., 2019. Ten-year efficacy and safety of tenofovir disoproxil fumarate  
1258 treatment for chronic hepatitis B virus infection. *Liver Int. Off. J. Int. Assoc. Study Liver* 39,  
1259 1868-1875. <https://doi.org/10.1111/liv.14155>

1260 Martinet, J., Dufeu-Duchesne, T., Bruder Costa, J., Larrat, S., Marlu, A., Leroy, V., Plumas, J., Aspod,  
1261 C., n.d. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of  
1262 natural killer cells in patients with chronic HBV infection. *Gastroenterology* 143, 1586-  
1263 1596.e8. <https://doi.org/10.1053/j.gastro.2012.08.046>

1264 Martinot-Peignoux, M., Asselah, T., Marcellin, P., 2015. HBsAg quantification to optimize treatment  
1265 monitoring in chronic hepatitis B patients. *Liver Int. Off. J. Int. Assoc. Study Liver* 35 Suppl 1,  
1266 82-90. <https://doi.org/10.1111/liv.12735>

1267 Martinot-Peignoux, M., Lapalus, M., Asselah, T., Marcellin, P., 2014. HBsAg quantification: useful for  
1268 monitoring natural history and treatment outcome. *Liver Int. Off. J. Int. Assoc. Study Liver* 34  
1269 Suppl 1, 97-107. <https://doi.org/10.1111/liv.12403>

1270 Martinot-Peignoux, M., Lapalus, M., Laouénan, C., Lada, O., Netto-Cardoso, A.C.F., Boyer, N., Ripault,  
1271 M.P., Carvalho-Filho, R., Asselah, T., Marcellin, P., 2013. Prediction of disease reactivation in  
1272 asymptomatic hepatitis B e antigen-negative chronic hepatitis B patients using baseline  
1273 serum measurements of HBsAg and HBV-DNA. *J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol.*  
1274 58, 401-407. <https://doi.org/10.1016/j.jcv.2013.08.010>

1275 Matsumoto, A., Tanaka, E., Minami, M., Okanoue, T., Yatsushashi, H., Nagaoka, S., Suzuki, F.,  
1276 Kobayashi, M., Chayama, K., Imamura, M., Yotsuyanagi, H., Nakaoka, S., Maki, N., Kawata, S.,  
1277 Kumada, H., Iino, S., Kiyosawa, K., 2007. Low serum level of hepatitis B core-related antigen  
1278 indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. *Hepatol.*  
1279 *Res. Off. J. Jpn. Soc. Hepatol.* 37, 661-666. [https://doi.org/10.1111/j.1872-](https://doi.org/10.1111/j.1872-034X.2007.00094.x)  
1280 [034X.2007.00094.x](https://doi.org/10.1111/j.1872-034X.2007.00094.x)

1281 Matsumoto, A., Yatsushashi, H., Nagaoka, S., Suzuki, Y., Hosaka, T., Tsuge, M., Chayama, K., Kanda, T.,  
1282 Yokosuka, O., Nishiguchi, S., Saito, M., Miyase, S., Kang, J.-H., Shinkai, N., Tanaka, Y.,  
1283 Umemura, T., Tanaka, E., 2015. Factors associated with the effect of interferon- $\alpha$  sequential  
1284 therapy in order to discontinue nucleoside/nucleotide analog treatment in patients with  
1285 chronic hepatitis B. *Hepatol. Res. Off. J. Jpn. Soc. Hepatol.* 45, 1195-1202.  
1286 <https://doi.org/10.1111/hepr.12488>

1287 Neumann-Haefelin, C., Thimme, R., 2018. Entering the spotlight: hepatitis B surface antigen-specific B  
1288 cells. *J. Clin. Invest.* 128, 4257-4259. <https://doi.org/10.1172/JCI124098>

1289 Oliviero, B., Varchetta, S., Paudice, E., Michelone, G., Zaramella, M., Mavilio, D., De Filippi, F., Bruno,  
1290 S., Mondelli, M.U., 2009. Natural killer cell functional dichotomy in chronic hepatitis B and  
1291 chronic hepatitis C virus infections. *Gastroenterology* 137, 1151-1160, 1160.e1-7.  
1292 <https://doi.org/10.1053/j.gastro.2009.05.047>

1293 Ouaguia, L., Leroy, V., Dufeu-Duchesne, T., Durantel, D., Decaens, T., Hubert, M., Valladeau-  
1294 Guilemond, J., Bendriss-Vermare, N., Chaperot, L., Aspod, C., 2019. Circulating and Hepatic  
1295 BDCA1+, BDCA2+, and BDCA3+ Dendritic Cells Are Differentially Subverted in Patients With  
1296 Chronic HBV Infection. *Front. Immunol.* 10, 112. <https://doi.org/10.3389/fimmu.2019.00112>

1297 Pan, X., Zhang, K., Yang, X., Liang, J., Sun, H., Li, X., Zou, Y., Xu, Qingqiang, An, G., Li, G., Xu, Qihuan,  
1298 2013. Relapse rate and associated-factor of recurrence after stopping NUCs therapy with



1299 different prolonged consolidation therapy in HBeAg positive CHB patients. *PloS One* 8,  
1300 e68568. <https://doi.org/10.1371/journal.pone.0068568>

1301 Papatheodoridi, M., Papatheodoridis, G., 2020. Emerging Diagnostic Tools to Decide When to  
1302 Discontinue Nucleos(t)ide Analogues in Chronic Hepatitis B. *Cells* 9.  
1303 <https://doi.org/10.3390/cells9020493>

1304 Papatheodoridis, G., Goulis, J., Manolakopoulos, S., Margariti, A., Exarchos, X., Kokkonis, G.,  
1305 Hadziyiannis, E., Papaioannou, C., Manesis, E., Pectasides, D., Akriviadis, E., 2014. Changes of  
1306 HBsAg and interferon-inducible protein 10 serum levels in naive HBeAg-negative chronic  
1307 hepatitis B patients under 4-year entecavir therapy. *J. Hepatol.* 60, 62–68.  
1308 <https://doi.org/10.1016/j.jhep.2013.08.023>

1309 Papatheodoridis, G., Vlachogiannakos, I., Cholongitas, E., Wursthorn, K., Thomadakis, C., Touloumi,  
1310 G., Petersen, J., 2016. Discontinuation of oral antivirals in chronic hepatitis B: A systematic  
1311 review. *Hepatol. Baltim. Md* 63, 1481–1492. <https://doi.org/10.1002/hep.28438>

1312 Papatheodoridis, G.V., Idilman, R., Dalekos, G.N., Buti, M., Chi, H., van Boemmel, F., Calleja, J.L.,  
1313 Sypsa, V., Goulis, J., Manolakopoulos, S., Loglio, A., Siakavellas, S., Keskin, O., Gatselis, N.,  
1314 Hansen, B.E., Lehretz, M., de la Revilla, J., Savvidou, S., Kourikou, A., Vlachogiannakos, I.,  
1315 Galanis, K., Yurdaydin, C., Berg, T., Colombo, M., Esteban, R., Janssen, H.L.A., Lampertico, P.,  
1316 2017. The risk of hepatocellular carcinoma decreases after the first 5 years of entecavir or  
1317 tenofovir in Caucasians with chronic hepatitis B. *Hepatol. Baltim. Md* 66, 1444–1453.  
1318 <https://doi.org/10.1002/hep.29320>

1319 Papatheodoridis, G.V., Manolakopoulos, S., Su, T.-H., Siakavellas, S., Liu, C.-J., Kourikou, A., Yang, H.-  
1320 C., Kao, J.-H., 2018a. Significance of definitions of relapse after discontinuation of oral  
1321 antivirals in HBeAg-negative chronic hepatitis B. *Hepatol. Baltim. Md* 68, 415–424.  
1322 <https://doi.org/10.1002/hep.29497>

1323 Papatheodoridis, G.V., Rigopoulou, E.I., Papatheodoridi, M., Zachou, K., Xourafas, V., Gatselis, N.,  
1324 Hadziyiannis, E., Vlachogiannakos, J., Manolakopoulos, S., Dalekos, G.N., 2018b. DARING-B:  
1325 discontinuation of effective entecavir or tenofovir disoproxil fumarate long-term therapy  
1326 before HBsAg loss in non-cirrhotic HBeAg-negative chronic hepatitis B. *Antivir. Ther.* 23, 677–  
1327 685. <https://doi.org/10.3851/IMP3256>

1328 Papatheodoridis, G.V., Sypsa, V., Dalekos, G., Yurdaydin, C., van Boemmel, F., Buti, M., Goulis, J.,  
1329 Calleja, J.L., Chi, H., Manolakopoulos, S., Loglio, A., Siakavellas, S., Gatselis, N., Keskin, O.,  
1330 Lehretz, M., Savvidou, S., de la Revilla, J., Hansen, B.E., Kourikou, A., Vlachogiannakos, I.,  
1331 Galanis, K., Idilman, R., Colombo, M., Esteban, R., Janssen, H.L.A., Berg, T., Lampertico, P.,  
1332 2018c. Eight-year survival in chronic hepatitis B patients under long-term entecavir or  
1333 tenofovir therapy is similar to the general population. *J. Hepatol.* 68, 1129–1136.  
1334 <https://doi.org/10.1016/j.jhep.2018.01.031>

1335 Peppas, D., Gill, U.S., Reynolds, G., Easom, N.J.W., Pallett, L.J., Schurich, A., Micco, L., Nebbia, G.,  
1336 Singh, H.D., Adams, D.H., Kennedy, P.T.F., Maini, M.K., 2013. Up-regulation of a death  
1337 receptor renders antiviral T cells susceptible to NK cell-mediated deletion. *J. Exp. Med.* 210,  
1338 99–114. <https://doi.org/10.1084/jem.20121172>

1339 Peppas, D., Micco, L., Javadi, A., Kennedy, P.T.F., Schurich, A., Dunn, C., Pallant, C., Ellis, G., Khanna, P.,  
1340 Dusheiko, G., Gilson, R.J., Maini, M.K., 2010. Blockade of immunosuppressive cytokines  
1341 restores NK cell antiviral function in chronic hepatitis B virus infection. *PLoS Pathog.* 6,  
1342 e1001227. <https://doi.org/10.1371/journal.ppat.1001227>

1343 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,  
1344 X.-D., Dai, Y.-P., Gan, J.-H., 2016. Hepatitis B surface antigen quantification at hepatitis B e  
1345 antigen seroconversion predicts virological relapse after the cessation of entecavir treatment  
1346 in hepatitis B e antigen-positive patients. *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.*  
1347 43, 43–48. <https://doi.org/10.1016/j.ijid.2015.10.019>

1348 Rehmann, B., 2013. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells.  
1349 *Nat. Med.* 19, 859–868. <https://doi.org/10.1038/nm.3251>

1350 Rehermann, B., Nascimbeni, M., 2005. Immunology of hepatitis B virus and hepatitis C virus infection.  
1351 Nat. Rev. Immunol. 5, 215–229. <https://doi.org/10.1038/nri1573>

1352 Reijnders, J.G.P., Perquin, M.J., Zhang, N., Hansen, B.E., Janssen, H.L.A., 2010. Nucleos(t)ide  
1353 analogues only induce temporary hepatitis B e antigen seroconversion in most patients with  
1354 chronic hepatitis B. *Gastroenterology* 139, 491–498.  
1355 <https://doi.org/10.1053/j.gastro.2010.03.059>

1356 Rinker, F., Zimmer, C.L., Höner Zu Siederdisen, C., Manns, M.P., Kraft, A.R.M., Wedemeyer, H.,  
1357 Björkström, N.K., Cornberg, M., 2018. Hepatitis B virus-specific T cell responses after  
1358 stopping nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. *J. Hepatol.*  
1359 69, 584–593. <https://doi.org/10.1016/j.jhep.2018.05.004>

1360 Rivino, L., Le Bert, N., Gill, U.S., Kunasegaran, K., Cheng, Y., Tan, D.Z., Becht, E., Hansi, N.K., Foster,  
1361 G.R., Su, T.-H., Tseng, T.-C., Lim, S.G., Kao, J.-H., Newell, E.W., Kennedy, P.T., Bertoletti, A.,  
1362 2018. Hepatitis B virus-specific T cells associate with viral control upon nucleos(t)ide-  
1363 analogue therapy discontinuation. *J. Clin. Invest.* 128, 668–681.  
1364 <https://doi.org/10.1172/JCI92812>

1365 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.,  
1366 Suh, D.J., 2003. Long-term additional lamivudine therapy enhances durability of lamivudine-  
1367 induced HBeAg loss: a prospective study. *J. Hepatol.* 39, 614–619.  
1368 [https://doi.org/10.1016/s0168-8278\(03\)00394-5](https://doi.org/10.1016/s0168-8278(03)00394-5)

1369 Salimzadeh, L., Le Bert, N., Dutertre, C.-A., Gill, U.S., Newell, E.W., Frey, C., Hung, M., Novikov, N.,  
1370 Fletcher, S., Kennedy, P.T., Bertoletti, A., 2018. PD-1 blockade partially recovers dysfunctional  
1371 virus-specific B cells in chronic hepatitis B infection. *J. Clin. Invest.* 128, 4573–4587.  
1372 <https://doi.org/10.1172/JCI121957>

1373 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.,  
1374 Chien, R.N., Dokmeci, A.K., Gane, E., Hou, J.L., Jafri, W., Jia, J., Kim, J.H., Lai, C.L., Lee, H.C.,  
1375 Lim, S.G., Liu, C.J., Locarnini, S., Al Mahtab, M., Mohamed, R., Omata, M., Park, J., Piratvisuth,  
1376 T., Sharma, B.C., Sollano, J., Wang, F.S., Wei, L., Yuen, M.F., Zheng, S.S., Kao, J.H., 2016. Asian-  
1377 Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol.*  
1378 *Int.* 10, 1–98. <https://doi.org/10.1007/s12072-015-9675-4>

1379 Schuch, A., Salimi Alizej, E., Heim, K., Wieland, D., Kiraithe, M.M., Kemming, J., Llewellyn-Lacey, S.,  
1380 Sogukpinar, Ö., Ni, Y., Urban, S., Zimmermann, P., Nassal, M., Emmerich, F., Price, D.A.,  
1381 Bengsch, B., Luxenburger, H., Neumann-Haefelin, C., Hofmann, M., Thimme, R., 2019.  
1382 Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific  
1383 CD8+ T cells in chronically HBV-infected patients with low viral load. *Gut* 68, 905–915.  
1384 <https://doi.org/10.1136/gutjnl-2018-316641>

1385 Schurich, A., Pallett, L.J., Lubowiecki, M., Singh, H.D., Gill, U.S., Kennedy, P.T., Nastouli, E., Tanwar, S.,  
1386 Rosenberg, W., Maini, M.K., 2013. The third signal cytokine IL-12 rescues the anti-viral  
1387 function of exhausted HBV-specific CD8 T cells. *PLoS Pathog.* 9, e1003208.  
1388 <https://doi.org/10.1371/journal.ppat.1003208>

1389 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.,  
1390 2015. Treatment cessation of entecavir in Asian patients with hepatitis B e antigen negative  
1391 chronic hepatitis B: a multicentre prospective study. *Gut* 64, 667–672.  
1392 <https://doi.org/10.1136/gutjnl-2014-307237>

1393 Seto, W.-K., Liu, K.S., Mak, L.-Y., Cloherty, G., Wong, D.K.-H., Gersch, J., Lam, Y.-F., Cheung, K.-S.,  
1394 Chow, N., Ko, K.-L., To, W.-P., Fung, J., Yuen, M.-F., 2020. Role of serum HBV RNA and  
1395 hepatitis B surface antigen levels in identifying Asian patients with chronic hepatitis B  
1396 suitable for entecavir cessation. *Gut*. <https://doi.org/10.1136/gutjnl-2020-321116>

1397 Shouval, D., Lai, C.-L., Chang, T.-T., Cheinquer, H., Martin, P., Carosi, G., Han, S., Kaymakoglu, S.,  
1398 Tamez, R., Yang, J., Tenney, D., Brett-Smith, H., 2009. Relapse of hepatitis B in HBeAg-  
1399 negative chronic hepatitis B patients who discontinued successful entecavir treatment: the  
1400 case for continuous antiviral therapy. *J. Hepatol.* 50, 289–295.  
1401 <https://doi.org/10.1016/j.jhep.2008.10.017>

1402 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.,  
1403 Jeong, S.-H., Kim, N., Lee, D.H., Kim, J.-W., 2014. Off-treatment virologic relapse and  
1404 outcomes of re-treatment in chronic hepatitis B patients who achieved complete viral  
1405 suppression with oral nucleos(t)ide analogs. *BMC Infect. Dis.* 14, 439.  
1406 <https://doi.org/10.1186/1471-2334-14-439>

1407 Song, B.C., Suh, D.J., Lee, H.C., Chung, Y.H., Lee, Y.S., 2000. Hepatitis B e antigen seroconversion after  
1408 lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. *Hepatology*.  
1409 *Baltim. Md* 32, 803–806. <https://doi.org/10.1053/jhep.2000.16665>

1410 Stoop, J.N., van der Molen, R.G., Kuipers, E.J., Kusters, J.G., Janssen, H.L.A., 2007. Inhibition of viral  
1411 replication reduces regulatory T cells and enhances the antiviral immune response in chronic  
1412 hepatitis B. *Virology* 361, 141–148. <https://doi.org/10.1016/j.virol.2006.11.018>

1413 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.,  
1414 Kao, J.-H., 2018. Distinct Relapse Rates and Risk Predictors After Discontinuing Tenofovir and  
1415 Entecavir Therapy. *J. Infect. Dis.* 217, 1193–1201. <https://doi.org/10.1093/infdis/jix690>

1416 Tan, A.T., Koh, S., Goh, W., Zhe, H.Y., Gehring, A.J., Lim, S.G., Bertolotti, A., 2010. A longitudinal  
1417 analysis of innate and adaptive immune profile during hepatic flares in chronic hepatitis B. *J.*  
1418 *Hepatology*. 52, 330–339. <https://doi.org/10.1016/j.jhep.2009.12.015>

1419 Tanaka, E., Matsumoto, A., 2014. Guidelines for avoiding risks resulting from discontinuation of  
1420 nucleoside/nucleotide analogs in patients with chronic hepatitis B. *Hepatology. Res. Off. J. Jpn.*  
1421 *Soc. Hepatology*. 44, 1–8. <https://doi.org/10.1111/hepr.12108>

1422 Terrault, N.A., Bzowej, N.H., Chang, K.-M., Hwang, J.P., Jonas, M.M., Murad, M.H., American  
1423 Association for the Study of Liver Diseases, 2016. AASLD guidelines for treatment of chronic  
1424 hepatitis B. *Hepatology. Baltim. Md* 63, 261–283. <https://doi.org/10.1002/hep.28156>

1425 Terrault, N.A., Lok, A.S.F., McMahon, B.J., Chang, K.-M., Hwang, J.P., Jonas, M.M., Brown, R.S.,  
1426 Bzowej, N.H., Wong, J.B., 2018. Update on prevention, diagnosis, and treatment of chronic  
1427 hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology. Baltim. Md* 67, 1560–1599.  
1428 <https://doi.org/10.1002/hep.29800>

1429 Tjwa, E.T.T.L., van Oord, G.W., Hegmans, J.P., Janssen, H.L.A., Woltman, A.M., 2011. Viral load  
1430 reduction improves activation and function of natural killer cells in patients with chronic  
1431 hepatitis B. *J. Hepatology*. 54, 209–218. <https://doi.org/10.1016/j.jhep.2010.07.009>

1432 Tout, I., Gomes, M., Ainouze, M., Marotel, M., Pecoul, T., Durantel, D., Vaccarella, S., Dubois, B.,  
1433 Loustaud-Ratti, V., Walzer, T., Alain, S., Chemin, I., Hasan, U., 2018. Hepatitis B Virus Blocks  
1434 the CRE/CREB Complex and Prevents TLR9 Transcription and Function in Human B Cells. *J.*  
1435 *Immunol. Baltim. Md* 1950 201, 2331–2344. <https://doi.org/10.4049/jimmunol.1701726>

1436 Tout, I., Loureiro, D., Mansouri, A., Soumelis, V., Boyer, N., Asselah, T., 2020. Hepatitis B Surface  
1437 Antigen Seroclearance: Immune Mechanisms, Clinical Impact, Importance for Drug  
1438 Development. *J. Hepatology*. <https://doi.org/10.1016/j.jhep.2020.04.013>

1439 Tseng, C.-H., Hsu, Y.-C., Chang, C.-Y., Tseng, T.-C., Wu, M.-S., Lin, J.-T., Kao, J.-H., 2018. Quantification  
1440 of serum hepatitis B core antibody to predict off-entecavir relapse in patients with chronic  
1441 hepatitis B. *J. Formos. Med. Assoc. Taiwan Yi Zhi* 117, 915–921.  
1442 <https://doi.org/10.1016/j.jfma.2017.11.012>

1443 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.  
1444 Incidence and Factors Associated With HBV Relapse After Cessation of Entecavir or Tenofovir  
1445 in Patients With HBsAg Below 100 IU/mL. *Clin. Gastroenterol. Hepatology. Off. Clin. Pract. J. Am.*  
1446 *Gastroenterol. Assoc.* <https://doi.org/10.1016/j.cgh.2020.04.037>

1447 van Bömmel, F., Bartens, A., Mysickova, A., Hofmann, J., Krüger, D.H., Berg, T., Edelmann, A., 2015.  
1448 Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen  
1449 seroconversion during treatment with polymerase inhibitors. *Hepatology. Baltim. Md* 61, 66–76.  
1450 <https://doi.org/10.1002/hep.27381>

1451 van der Molen, R.G., Sprengers, D., Biesta, P.J., Kusters, J.G., Janssen, H.L.A., 2006. Favorable effect of  
1452 adefovir on the number and functionality of myeloid dendritic cells of patients with chronic  
1453 HBV. *Hepatology. Baltim. Md* 44, 907–914. <https://doi.org/10.1002/hep.21340>

1454 Van Hees, S., Bourgeois, S., Van Vlierberghe, H., Sersté, T., Francque, S., Michielsens, P., Sprengers, D.,  
1455 Reynaert, H., Henrion, J., Negrin Dastis, S., Delwaide, J., Lasser, L., Decaestecker, J., Orlent,  
1456 H., Janssens, F., Robaey, G., Colle, I., Stärkel, P., Moreno, C., Nevens, F., Vanwolleghem, T.,  
1457 Belgian NA Stop Study Group, 2018. Stopping nucleos(t)ide analogue treatment in Caucasian  
1458 hepatitis B patients after HBeAg seroconversion is associated with high relapse rates and  
1459 fatal outcomes. *Aliment. Pharmacol. Ther.* 47, 1170–1180.  
1460 <https://doi.org/10.1111/apt.14560>

1461 Wang, J., Shen, T., Huang, X., Kumar, G.R., Chen, X., Zeng, Z., Zhang, R., Chen, R., Li, T., Zhang, T.,  
1462 Yuan, Q., Li, P.-C., Huang, Q., Colonna, R., Jia, J., Hou, J., McCrae, M.A., Gao, Z., Ren, H., Xia,  
1463 N., Zhuang, H., Lu, F., 2016. Serum hepatitis B virus RNA is encapsidated pregenome RNA that  
1464 may be associated with persistence of viral infection and rebound. *J. Hepatol.* 65, 700–710.  
1465 <https://doi.org/10.1016/j.jhep.2016.05.029>

1466 Wang, L., Liu, F., Liu, Y.-D., Li, X.-Y., Wang, J.-B., Zhang, Z.-H., Wang, Y.-Z., 2010. Stringent cessation  
1467 criterion results in better durability of lamivudine treatment: a prospective clinical study in  
1468 hepatitis B e antigen-positive chronic hepatitis B patients. *J. Viral Hepat.* 17, 298–304.  
1469 <https://doi.org/10.1111/j.1365-2893.2009.01178.x>

1470 World Health Organization, World Health Organization, Global Hepatitis Programme, 2017. Global  
1471 hepatitis report, 2017.

1472 Wu, Y., Fan, J., Liao, G., Xia, M., Jiang, D., Peng, J., Zhang, X., Liu, H., 2020. Genetic variations in the  
1473 CXCR5 gene decrease the risk of clinical relapse after discontinuation of nucleos(t)ide  
1474 analogue therapy in patients with chronic hepatitis B. *Infect. Genet. Evol. J. Mol. Epidemiol.*  
1475 *Evol. Genet. Infect. Dis.* 78, 104124. <https://doi.org/10.1016/j.meegid.2019.104124>

1476 Xia, Y., Protzer, U., 2017. Control of Hepatitis B Virus by Cytokines. *Viruses* 9.  
1477 <https://doi.org/10.3390/v9010018>

1478 Xie, L., Liao, G., Chen, H., Xia, M., Huang, X., Fan, R., Peng, J., Zhang, X., Liu, H., 2019. Elevated  
1479 expression of serum soluble ST2 in clinical relapse after stopping long-term Nucleos(t)ide  
1480 analogue therapy for chronic hepatitis B. *BMC Infect. Dis.* 19, 640.  
1481 <https://doi.org/10.1186/s12879-019-4261-3>

1482 Ye, B., Liu, X., Li, X., Kong, H., Tian, L., Chen, Y., 2015. T-cell exhaustion in chronic hepatitis B infection:  
1483 current knowledge and clinical significance. *Cell Death Dis.* 6, e1694.  
1484 <https://doi.org/10.1038/cddis.2015.42>

1485 Yu, F., Wang, Y., Yuan, S., Ma, J., Ma, N., Zhang, X., Liu, X., Liu, D., 2013. Association between gene  
1486 polymorphisms of IL-28 and response to lamivudine in Chinese rural patients with chronic  
1487 hepatitis B. *Scand. J. Gastroenterol.* 48, 745–751.  
1488 <https://doi.org/10.3109/00365521.2013.779746>

1489 Yu, Y., Wang, J., Li, G., Shen, C., Chen, S., Huang, J., Wang, Xinyu, Zhao, C., Huang, Y., Wang, B., Wang,  
1490 Xuanyi, Zhang, J., Qiu, C., Zhang, W., 2018. Nucleos(t)ide analogue interruption: Alternative  
1491 approach to intrahepatic set point for spontaneous control of HBV replication? *J. Hepatol.* 68,  
1492 609–610. <https://doi.org/10.1016/j.jhep.2017.09.026>

1493 Zhang, E., Lu, M., 2015. Toll-like receptor (TLR)-mediated innate immune responses in the control of  
1494 hepatitis B virus (HBV) infection. *Med. Microbiol. Immunol. (Berl.)* 204, 11–20.  
1495 <https://doi.org/10.1007/s00430-014-0370-1>

1496 Zheng, Y., Huang, Z., Chen, X., Tian, Y., Tang, J., Zhang, Y., Zhang, X., Zhou, J., Mao, Q., Ni, B., Wang,  
1497 Q., Wu, Y., 2012. Effects of telbivudine treatment on the circulating CD4<sup>+</sup> T-cell  
1498 subpopulations in chronic hepatitis B patients. *Mediators Inflamm.* 2012, 789859.  
1499 <https://doi.org/10.1155/2012/789859>

1500 Zimmer, C.L., Rinker, F., Höner Zu Siederdisen, C., Manns, M.P., Wedemeyer, H., Cornberg, M.,  
1501 Björkström, N.K., 2018. Increased NK Cell Function After Cessation of Long-Term  
1502 Nucleos(t)ide Analogue Treatment in Chronic Hepatitis B Is Associated With Liver Damage  
1503 and HBsAg Loss. *J. Infect. Dis.* 217, 1656–1666. <https://doi.org/10.1093/infdis/jiy097>

1504

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 ( $\geq 3$  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

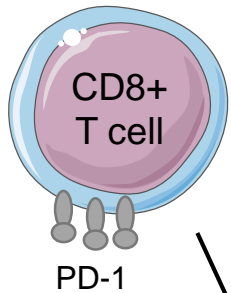
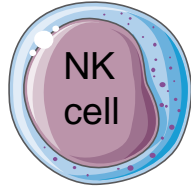
Low HBV DNA levels  
( $< 2000$  IU/mL) and  
ALT levels

Retreatment

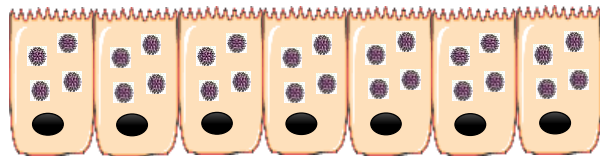
Close monitoring: ALT and HBV DNA levels every 6 months  
Liver biopsy every 12 months

## Immune Exhaustion

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



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



*HBV-infected Hepatocytes*

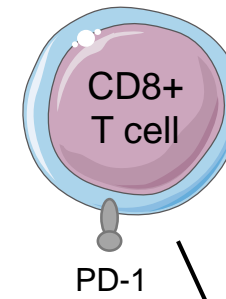
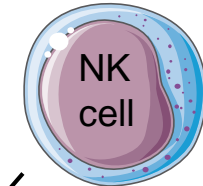
NA therapy



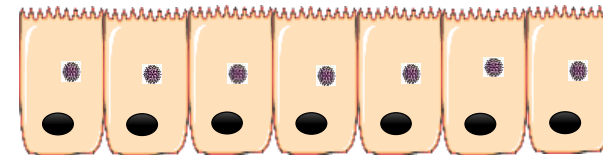
HBV viral suppression

## Immune Control

Low NK pro-inflammatory phenotype and restoration of antiviral cytokine production (IFN $\gamma$ )



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 IFN $\gamma$  and IL-2 secretion)



*HBV-infected Hepatocytes*