Roadmap to Functional Cure of Chronic Hepatitis B: an expert consensus

Running title: Roadmap of CHB Cure

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Abstract

Hepatitis B virus (HBV) infection continues to be a major public health issue worldwide. HBsAg loss is associated with functional remission and improved long-term outcome, and is considered to be a “functional cure” (also referred to as clinical or immunologic cure) for chronic hepatitis B. This ideal goal of therapy can be achieved using optimized combination regimens with direct acting antivirals [e.g. nucleos(t)ide analogues (NAs)] and immunomodulators [e.g. pegylated interferon alpha2a (Peg-IFN)] in selected patients with chronic hepatitis B. Among different combination therapies currently available, those with NA lead-in followed by Peg-IFN in virally suppressed patients has been demonstrated to be effective. This review provides an updated overview of the evidence supporting the use of combination therapies and summarizes expert consensus on the roadmap to attain functional cure for chronic hepatitis B patients.

Key words: Chronic hepatitis B; HBsAg loss; Functional cure; Combination therapy; Consensus

Introduction

Hepatitis B virus (HBV) infection continues to be a major public health burden. Approximately 240 million people worldwide are chronically infected with HBV, contributing to about 30% of cirrhosis and 45% of hepatocellular carcinoma (HCC) cases [1, 2]. The primary goal of hepatitis B treatment is to improve survival by preventing the development of HBV-related cirrhosis, liver failure and HCC [3-6]. HBsAg loss is associated with functional remission and improved long-term outcome[7] and is the ideal goal of therapy (functional cure) recommended by current guidelines[3-6]. However, existing antiviral therapy including immunomodulators [e.g. pegylated interferon alpha2a (Peg-IFN)] or direct acting antivirals (DAA) [e.g. nucleos(t)ide analogues (NAs)] when used alone offers limited capability in delivering functional cure. Curative therapy with the aim of eliminating HBV infection is unlikely to be available in the next decade, thus, there is a pressing need to optimize current therapies. Theoretically, the administration of NA and Peg-IFN with different mechanisms of action in combination against HBV is a promising approach to generate synergistic and complementary effects.
and could be of importance in future therapeutic regimens. Accumulating data have confirmed virological and serological advantages of combination strategy. This review provides an updated analysis of the clinical data supporting the use of combination therapies and summarizes expert consensus on the roadmap to guide hepatologists’ decision-making in attaining functional cure for chronic hepatitis B patients.

**Definition of cure and its key challenges**

**Definition of cure for chronic hepatitis B**

The unprecedented success of curative antiviral therapy for chronic hepatitis C has reignited the search for a cure for hepatitis B [8]. Currently, three categories of cure for chronic hepatitis B have been defined: complete sterilizing cure (also referred to as virological cure), functional cure (also referred to as clinical or immunologic cure), and partial cure [9-11].

Complete sterilizing cure is defined as undetectable serum HBsAg and eradication of HBV DNA from the serum and liver, including intrahepatic covalently-closed-circular DNA (cccDNA) and integrated HBV DNA fragments, with persistent positive serological test results for anti-HBc with or without anti-HBs [9, 10]. Due to the capability of cccDNA to persist and the current lack of therapeutic agents specifically targeting cccDNA, it is almost impossible to reach complete cure.

Functional cure is defined as sustained, undetectable serum HBsAg and HBV DNA with or without seroconversion to anti-HBs, but with the persistence of residual cccDNA, and the absence of spontaneous relapse after the cessation of treatment[12, 13], accompanied by resolution of liver injury and decreased risk of cirrhosis and HCC. This is considered as a successful immunologic control of chronic hepatitis B. Functional cure is a state resembling the spontaneous viral clearance following acute HBV infection, and is the ideal goal of therapy recommended by current guidelines [3-6]. This goal can be reached by using optimized treatment regimens in selected patients with chronic hepatitis B. Partial cure of hepatitis B is characterized by undetectable serum HBV DNA and normal alanine transaminase (ALT) level maintained after treatment cessation, but with detectable HBsAg. Such treatment goal, though achievable, is far from satisfactory in clinical practices [9, 10].
Curing hepatitis B from the virological and immunologic view

Both viral and host factors are involved in HBV chronicity. The HBV genome forms a stable episomal mini-chromosome, the so-called cccDNA, in the nucleus of infected hepatocytes, and HBV DNA sequences are also found to integrate into the host genome, which may render the cellular environment more permissive to virus replication via modulation of HBV transcription [14]. The uniquely intricate genomic organization and replication strategy exploited by HBV enables the maintenance of viral infection [10, 15, 16]. Due to the stable reservoir of cccDNA hepatocytes which have a long half-life, its ability to self-replenish without the need for entry of new virions, and the abundant presence of HBV DNA integration in the host cellular genome, complete cure remains an elusive therapeutic goal [9, 12].

The outcomes of HBV infection rely on a complex interplay between the virus and host immunity. The majority of acutely infected adults can eradicate HBV through broad and vigorous innate and adaptive immune responses against the virus. However, in chronic HBV infection, virus-mediated mechanisms contribute to the limited effectiveness of the antiviral response [15, 17]. HBV is known to have evolved several mechanisms to circumvent the activation of the host antiviral responses [15, 17]. Accumulating data have demonstrated the potential capacity of viral antigens to suppress the innate immune response [18-20] and affect global and HBV-specific B cell functions [21]. Persistent exposure to high antigen concentrations and the tolerizing microenvironment within the liver might contribute to functional exhaustion or deletion of HBV-specific T-cells [22-24].

Although HBsAg can be produced from both episomal cccDNA and the integrated HBV DNA fragments, in which the essential elements of the S gene for HBsAg expression remain largely intact [12, 14], different from complete cure, functional cure is an attainable, realistic goal that can be achieved without absolute eradication of cccDNA and integrated HBV DNA, if the replication-competent HBV is well controlled by the host antiviral immune system. The functional cure for chronic HBV infection typically requires the control of persisting viral genomes and residual infected hepatocytes by the efficient coordination of both innate and adaptive immunities. A strategy with aim of boosting or restoring the defective immune response could help to efficiently control and, in the best case, to eliminate HBV infection [25]. Although novel DAAs or immunomodulators have
been explored and some are under clinical testing, curative therapy capable of eliminating cccDNA is unlikely to be available in the next decade, thus there is a pressing need to optimize current therapies in order to attain functional cure [26].

Current settings and challenges

Currently antiviral regimens include two classes of drugs: immunomodulators, such as Peg-IFN, and antivirals directly acting on viral replication, such as NAs [3-6]. Sustained complete viral suppression, ALT normalization and improvement of liver histology can be achieved by treatment of finite duration with Peg-IFN or by NA maintenance treatment. Antiviral therapy significantly reduces but does not eliminate the risk of HCC development, particularly in cirrhotic patients [27].

Owing to the convenience and their good tolerability, NAs have been widely used to treat chronic hepatitis B. A significant reduction in cccDNA levels was reported after NA treatment[28-30], however, even the preferred NAs, including entecavir (ETV), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF), do not directly target the cccDNA [31] and seldomly achieve sustained immunologic control, making such treatment a lifetime commitment, particularly in HBeAg-negative patients[12, 17]. A life-long pill burden may decrease compliance with NA therapy and lead to concerns over toxicity. There istherefore, an urgent need to come up with a safe, effective alternative to indefinite therapy.

IFN exerts dual effects, through promoting the activity of host immune cells and inducing the production of interferon-stimulated genes (ISGs), which encode directly acting antiviral effector proteins[32]. Furthermore, IFN can also inhibit HBV transcription and reduce the production of viral antigens through enhancing degradation of HBV pre-genomic RNA (pgRNA) and core particles or by modifying epigenetic regulation of cccDNA [33, 34]. The finite duration of IFN treatment can result in marked reduction in serum HBsAg load and durable off-treatment response, however, only in a proportion of patients.
The inability to eliminate the HBV cccDNA and integrated HBV DNA, and to break the immune tolerance against HBV constitute the key challenges to curing chronic HBV infection[13, 35, 36]. NA and Peg-IFN, when used alone, have limited effect on the intranuclear pool of cccDNA, and have partial action on immune response, thus demonstrating limited capability in delivering a functional cure [17]. Peg-IFN promotes the innate immune response, especially the antiviral capacity of CD56bright NK cells, but leads to sustained depletion of CD8+ T cells and does not improve early HBV-specific T cell responses [37], indicating Peg-IFN mediated divergent effects on innate and adaptive immunity [38]. NAs alone do not affect the function of NK cells, however, in patients with elevated ALT, LdT demonstrated a restoration of CD56bright NK cells [39]. Long-term NA therapy with viral suppression showed partial recovery of HBV-specific T cells following expansion [40], revealing that antigen decline may allow reconstitution of functional T-cell responses [26].

NAs and Peg-IFN differentially affect the immune response, and viral suppression mediated by NAs directly enhances the subsequent immunologic response to Peg-IFN [17, 26, 41], providing a mechanistic rationale for combination of both drugs to generate additive or synergistic effects [17, 42]. ADV and Peg-IFN combination therapy could restore HBV-specific T cells in patients achieving HBsAg loss [43]. Successful sequential therapy with ETV and Peg-IFN was associated with early significant restoration of innate and adaptive immune responses [44] and altered expression of ISGs [45]. During sequential treatment, restoration of CD56bright NK cells contributed to HBsAg and cccDNA clearance [46]. Collectively, these data highlight the importance of combination therapy for immune modulation and viral clearance[26].

**Recommendation:**

1. As the ideal goal of anti-HBV therapy, functional cure is defined as sustained, undetectable serum HBsAg and HBV DNA with or without seroconversion to anti-HBs, but with the persistence of residual cccDNA, and the absence of spontaneous relapse after the cessation of treatment.
The major challenges to curing HBV infection include the lack of available drugs to eliminate cccDNA and integrated HBV DNA, and host inability to restore the exhaustion of innate and adaptive immunities against HBV.

The mechanistic rationale for combining direct-acting antivirals (e.g. NAs) and immunomodulators (e.g. Peg-IFN) involves the observations that these two classes of drugs have differential effects on innate and adaptive immunity, and that viral suppression mediated by NAs directly enhances the subsequent immunologic response to Peg-IFN.

Treatment strategies and roadmap

Emerging data confirmed certain clinical benefit of an alternative therapeutic strategy, involving combination of agents with different modes of action (such as NA in combination with Peg-IFN), through harnessing both direct antiviral and immunomodulatory mechanisms [26, 35]. Over the past decade, multiple attempts have been made to optimize the combination strategies, taking into consideration the choice of drugs and timing of their administrations [12, 17]. There are several approaches to administration of combination therapy with NA and Peg-IFN: de novo combination strategy; sequential combination strategy, including switching from NA to Peg-IFN (“switch-to” strategy), or adding Peg-IFN to a stable NA (“add-on” strategy).

De novo combination strategy

Earlier studies have shown that the concomitant administration of Peg-IFN with LAM or ADV provided higher rates of on-treatment virologic response but did not improve rates of post-therapy response or histological outcome, compared with Peg-IFN monotherapy [47-49]. A prematurely terminated study in treatment-naïve HBeAg-positive patients demonstrated that, simultaneous combination therapy with LdT and Peg-IFN led to a higher rate of virologic response and greater decline in HBeAg and HBsAg levels than either therapy alone, however, this combination regimen should be avoided as it carried an increased risk of unexpected severe peripheral neuropathy [50]. A randomized trial in treatment-naive HBeAg-negative patients showed that, combination therapy with
ETV and Peg-IFN contributed to greater on-treatment virologic suppression but failed to improve HBsAg decline and loss (4.8%) over Peg-IFN monotherapy (9.5%) [51]. A recent randomized controlled trial evaluating HBsAg loss in both HBeAg-positive and HBeAg-negative treatment-naive patients showed that, 48 weeks of combination therapy with TDF and Peg-IFN led to a greater on-treatment HBsAg decline at the end of treatment, and a higher rate of HBsAg loss (9.1%) at 24 weeks post-treatment than 16 weeks of combination therapy or either monotherapy alone, particularly in genotype A patients [52]. At week 120, 10.4% of patients treated with combination therapy with TDF and Peg-IFN for 48 weeks lost HBsAg [53]. A recent study in 26 patients with HBV genotype C infection administered both ETV and Peg-IFN simultaneously for 48 weeks showed that, the 5-year cumulative rate of HBsAg loss after the completion of combination therapy was 15% [54].

“Add-on” strategy

This is an alternative strategy, where addition of Peg-IFN to ongoing NA therapy in virally suppressed patients has demonstrated promising results. An observational pilot study in patients on stable oral therapy showed that, the add-on of Peg-IFN induced HBsAg seroconversion in 2 out of 12 patients [55]. A prospective study in HBeAg-negative patients with HBV DNA fully suppressed by long-term NA treatment showed that the addition of Peg-IFN for a maximum of 96 weeks let to HBsAg loss and cessation of NA treatment in 6 of 10 patients [56]. The PEGON study in HBeAg-positive patients treated with ETV or TDF suggested that a 48-week add-on course of Peg-IFN therapy resulted in a numerically higher rate of HBeAg seroconversion than NA monotherapy, though not statistically significant [57]. A retrospective matched-pair study in HBeAg-positive individuals who did not achieve HBeAg seroconversion during prior long-term ETV therapy showed that Peg-IFN add-on led to a higher rate of HBeAg seroconversion (44%) and was more likely to induce HBsAg loss (4%) than ETV monotherapy (6% and 0%). Further analysis revealed that a low baseline level of HBsAg <1000IU/mL and HBsAg decline at week 12 more than 0.5log_{10}IU/mL provided an optimal rate of HBsAg loss [58]. The HERMES study demonstrated that addition of Peg-IFN to ongoing NA therapy significantly decreased HBsAg levels in HBeAg-negative patients with HBV genotype D infection [59]. The PEGAN study in HBeAg-negative patients on
stable NA regimens with virological suppression showed that at week 96, addition of a 48-week course of Peg-IFN did not lead to a significant increase in HBsAg loss (7.8%) than continuous NA monotherapy (3.2%), however, it resulted in a more significant decline in HBsAg titers [60]. The ARES study in HBeAg-positive patients demonstrated that 24 weeks of Peg-IFN added-on to ongoing ETV treatment from week 24 to 48 led to higher proportions of HBeAg loss and seroconversion, and resulted in more decline in HBV DNA and HBsAg levels when compared to ETV monotherapy. Besides, this add-on strategy appeared to prevent relapse after stopping ETV and may thereby facilitate the discontinuation of NAs [61].

“Switch-to” strategy

Several studies suggest that the “switch-to” strategy may induce sustained HBsAg loss and allow patients to discontinue NA treatment. A single-arm pilot study showed that sequential combination therapy with LAM and IFN can induce a sustained virologic response and HBs seroconversion (3/14) in patients not responding to IFN alone [62]. The OSST study in HBeAg-positive patients who maintained virologic suppression and had low HBeAg levels (<100 PEIU/mL) by long-term ETV suggested that switching to a finite 48-week course of Peg-IFN led to significantly higher rates of HBeAg seroconversion and HBsAg loss (8.5%) over continuing ETV monotherapy [63]. One-year follow-up of the patients receiving sequential Peg-IFN therapy showed that rates of HBeAg seroconversion increased from 17.7% at the end of therapy to 38.7% 1-year post-treatment and HBsAg loss was sustained in the majority of patients during off-treatment follow-up [64]. These data were consistent with those reported in several previous or late-breaking studies that switching to Peg-IFN from long-term NA administration accelerates or improves HBsAg decline or loss[65, 66]. The timing of switching may be a pivotal factor determining the treatment outcome. In a randomized trial, 21 weeks of ETV lead-in pretreatment followed by a 48-week course of Peg-IFN did not demonstrate superiority for off-treatment response compared with Peg-IFN monotherapy in treatment-naive HBeAg-positive patients [67]. A prospective study in HBeAg-positive patients who achieved HBeAg seroconversion during prior ETV treatment showed that, 6 of 41 (15%) patients switching to a 48-week course of Peg-IFN had HBsAg loss 24 weeks post-treatment [68]. The NEW
SWITCH study exploring the optimal duration of Peg-IFN treatment with a switch strategy in HBeAg-positive patients who achieved HBeAg loss with prior NA treatment demonstrated that, HBsAg loss rates may be enhanced by extending Peg-IFN treatment from 48 (14.4%) to 96 weeks (20.7%), albeit not being statistically significant [69].

Best approach to combination therapy

A recent meta-analysis of 24 trials involving combination therapy with IFN and NA demonstrated that the “NA-experienced” strategy was more effective than the “De novo” strategy in inducing HBsAg loss for CHB patients at week 48 or 52 (8% versus 11%). Furthermore, the pooled HBsAg loss rate at week 48 or 52 was 14% in patients using the “switch-to” strategy, which was significantly higher than the 8% rate for patients assigned to the “add-on” strategy [70]. A retrospective study in ETV-treated HBeAg-negative patients showed that both “switch-to” Peg-IFN treatment (9%) and “add-on” treatment (15%) led to significantly higher HBsAg loss rates than ETV monotherapy (0%). The response rate (HBsAg decline >1 log IU/mL) in the switch-to, add-on, and ETV monotherapy arms was 60%, 40%, and 2%, respectively at week 48 [71]. A non-randomized study in HBeAg-negative NA-treated patients with undetectable viremia showed that, HBsAg reduction > 1 log IU/mL was observed in 8 of 10 patients switching to Peg-IFN therapy and in 2 of 11 patients using add-on Peg-IFN therapy, suggesting that switch-to strategy might be superior to add-on strategy in reducing HBsAg titers. It is hypothesized that NA cessation in switch-to strategy may activate the host immune response which could help bolster the effects of Peg-IFN [72]. In an ongoing randomised controlled study (SWAP), patients treated with long-term NA treatment were assigned to add-on or switch-to Peg-IFN for 48 weeks, or to continue NA as controls. Preliminary analysis showed that overall HBsAg loss was higher in both add-on (9.0%) and switch-to (8.9%) than in controls (0%), whereas the switch arm had significantly higher virological relapse (30.2%) than controls (3.3%) and the add-on arm (2.0%), respectively. It is noteworthy that this study enrolled a subgroup of patients with compensated cirrhosis who were at high risk for relapse following cessation of NA therapy [73].

Though it is hard to draw firm conclusions from these studies regarding which combination strategy will be most beneficial, since Peg-IFN induced innate immune activation directly benefits from the
suppression of HBV replication, NA treatment usually requires several months to completely suppress HBV viremia and years to decrease HBsAg levels, eventually allowing partial restoration of HBV-specific T cell responses. It is conceivable that choice of drugs (potent NA with Peg-IFN), the time schedule of therapy (NA lead-in followed by Peg-IFN) as well as selection of patients (with stably suppressed viremia and low viral antigenemia) might be the critical factors in determining efficacy.

Roadmap towards functional cure by combination of DAA and immunomodulators

Baseline and early on-treatment quantitative HBsAg levels may predict which patients have the highest likelihood of achieving HBsAg loss with Peg-IFN treatment and identify the subset of patients who may benefit from extended treatment[74-76]. Both the OSST study and NEW SWITCH studies demonstrated that the combination of HBeAg loss and HBsAg < 1500 IU/ml at the time of switching was associated with high probability of HBsAg loss (22.2%-26.5%) at week 48 following sequential Peg-IFN treatment, whereas HBsAg level ≥ 1500 IU/ml was associated with a low rate of HBsAg loss (1.6%-3.8%). Furthermore, patients with HBsAg levels of <200 IU/mL at week 12 or 24 had the greatest chance of HBsAg loss at week 48 (48.9%-77.8%). In contrast, individuals with HBsAg levels of ≥ 1500 IU/mL at week 12 or ≥ 200 IU/mL at week 24 had a minimal chance of achieving HBsAg loss (0%-1.7%), therefore, stopping Peg-IFN therapy may be considered [63, 69]. Consistent with these data, a recent study investigating “switching-to” Peg-IFN as a strategy to stop NA also showed that patients with baseline HBsAg <1500 IU/mL have 20% chance of developing HBsAg loss, whereas baseline HBsAg <500 IU/mL is the best predictor for HBsAg loss (50%) [68]. We thereby propose a roadmap integrating strategies of baseline- and response-guided therapy based upon HBsAg kinetics for sequential combination therapy with DAA (e.g. ETV) and immunomodulators (e.g. Peg-IFN) (Figure 1). According to this roadmap, the optimized patients with low baseline level of HBsAg and HBeAg loss are more likely to achieve clinical cure by sequential Peg-IFN therapy, which is supported by several recent studies. The Endeavor study in patients who achieved HBeAg loss during long-term ETV treatment showed that switching to combination therapy with IFN plus recombinant human IL-2 and therapeutic vaccine lead to higher rates of HBsAg loss (9.38%) than
switching to IFN alone or continuing ETV monotherapy, particularly in those with low initial HBsAg levels <1500IU/mL (27.3%) [77]. Currently several studies in the optimized patients with potentially higher chance of functional cure confirm the beneficial effect of the baseline-guided strategy. A randomized controlled trial showed that in virally suppressed patients undergoing NA treatment with low HBsAg (< 2,000 IU/ml), switching to a 60-week course of Peg-IFN from a stable NA regimen promotes HBsAg loss (32.6%) and HBsAg seroconversion (27.9%) at the end of treatment [78]. A randomized controlled trial (Anchor) in patients with low HBsAg levels (<3000IU/mL) showed that sequential combination therapy with Peg-IFN with or without GM-CSF led to significantly higher rates of HBsAg loss (21.1%-27.78%) and HBsAg seroconversion (19.44%-21.21%) than ETV alone (0%) [79]. The I CURE study explored the efficacy of sequential Peg-IFN treatment in NA experienced patients with low level HBsAg (<1000IU/mL) and negative HBeAg. In this study, 66.67% patients achieved clinical cure. After treatment cessation, 80% of patients maintained complete response at week 24 of follow-up [80]. In the PYRAMID study aiming to verify the response-guided treatment strategy, HBeAg-positive patients who achieved viral suppression with HBsAg<5000IU/ml and HBeAg<100PEIU/ml by NA, received 24 weeks of add-on Peg-IFN therapy, then patients with HBsAg<200IU/ml at week 24 continued combination treatment for a further 24 weeks; those with HBsAg ≥200IU/mL were randomized to combination treatment or NAs alone for 24 weeks. For patients with HBsAg<200IU/ml at week 24, 56.5% achieved HBsAg loss at week 72, whereas if HBsAg level was ≥ 200IU/ml at week 24, only 4.5% of the patients continuing combination treatment and none of the patients stopping Peg-IFN at week 24 achieved HBsAg loss [81]. These data confirm that the response-guided treatment strategy with HBsAg <200 IU/ml at week 24 could help identify which patients would benefit from continued combination treatment.
Recommendation:

4 The choice of drugs (potent DAAs e.g. ETV with immunomodulators e.g. Peg-IFN), the time schedule of therapy (lead-in DAAs e.g. NA followed by immunomodulators e.g. Peg-IFN) as well as selection of patients (with stably suppressed viremia and low viral antigenemia) might be the critical factors in determining efficacy of combination therapy.

5 Sequential combination therapy using ETV first followed by Peg-IFN in a “switch-to” or “add-on” way shows promising results in patients who achieved HBV DNA suppression by NAs, particularly in those with HBeAg loss and low HBsAg titers.

6 The roadmap integrating strategies of baseline- and response-guided therapy based upon HBsAg kinetics can guide treatment decision on sequential combination therapy with DAA (e.g. ETV) and immunomodulators (e.g. Peg-IFN).

7 Low baseline (<1500IU/mL) together with HBeAg loss under DAAs such as ETV and early on-treatment quantitative HBsAg levels (<200IU/mL) may predict which patients have the highest likelihood of achieving HBsAg loss with sequential combined immunomodulators (e.g. Peg-IFN) treatment.

8 Individuals with HBsAg levels of ≥1500 IU/mL at week 12 or ≥200 IU/mL at week 24 had a minimal chance of achieving HBsAg loss, therefore, stopping Peg-IFN therapy or alternative treatment may be considered.

Long-term follow-up and monitoring

In the long journey of treatment to functional cure, regular follow-up and close monitoring should be carried out. The need for liver biopsy limits the use of the method for quantifying intrahepatic cccDNA and intrahepatic total HBV DNA levels in clinical practice. Non-invasive surrogate markers of treatment response are thus required.
Predictor and monitoring

The serum HBsAg level has been associated with the active transcription of cccDNA or translation of messenger RNA. Baseline and early on-treatment quantitative HBsAg levels are a well-established biomarker to predict HBsAg loss after HBeAg seroconversion [82, 83]. Even for the inactive HBsAg carriers with initial HBsAg titers <1000IU/mL, 44.7% of these receiving a 96-week course of Peg-IFN alone or in combination with ADV achieved HBsAg loss [84]. The approach of response-guided therapy including HBsAg <1500IU/ml or HBV DNA <10^5 copies/mL at week 24 would help identify patients who will benefit from continued Peg-IFN treatment [85]. In addition, quantitation of HBsAg at the end-of-therapy may predict the risk of relapse after functional cure was achieved [86]. A large cohort-based study showed that patients with persistently normal ALT after HBeAg seroclearance had a high rate of HBsAg seroclearance (70.3%) at 20 years after HBeAg seroclearance [87].

The baseline anti-HBc level may be a novel biomarker for predicting treatment response during antiviral therapy with NA or Peg-IFN in HBeAg positive patients [88-91]. Quantification of anti-HBc levels significantly predict serum undetectable HBV DNA and HBsAg seroclearance in HBeAg-negative, treatment-naive patients [92]. Levels of hepatitis B core-related antigen (HBcrAg), composed of HBV core antigen (HBcAg), HBeAg, and a 22-kDa precore protein [93], shows a good correlation with intrahepatic cccDNA level. When combined with the HBsAg level, HBcrAg level may help predict the risk of relapse after treatment cessation [94]. Serum HBV RNA is encapsidated pregenomic RNA and can be used as a potential biomarker reflecting the active transcription of intrahepatic cccDNA [95]. Serum HBV RNA may serve as an early predictor of HBeAg seroconversion during antiviral therapy [96] and a potential predictor of the viral rebound and HBsAg reversion post treatment withdrawal [95, 97].

Before initiating Peg-IFN based combination therapy, measurement of key baseline parameters is required, such as the quantitative HBsAg, levels of HBV DNA, HBeAg and biochemical markers, liver ultrasound and transient elastography, thyroid function, mental condition, autoimmune antibodies, fundus examination and routine diagnostic tests to rule out other diseases including diabetes, hypertension or lung disease, which is significant for determining the appropriate therapy.
and predicting treatment response. Monitoring the above-mentioned parameters shall be carried out periodically for assessment of adverse events and effectiveness during treatment, which is essential for evaluating the severity of adverse events and whether there is response to therapy.

**Long term follow-up**

In patients who achieve HBsAg loss or seroconversion, periodical follow-up visits should be done once in 3 months at the beginning, and the time interval of the follow-up visits could be gradually extended for patients without HBsAg reversion beyond 6 months. If there is any recurrence detected during the follow-up, retreatment is still effective.

HBsAg and HBV DNA reversion could still occur in patients with HBsAg loss due to the reduction in immune control of HBV infection. Patients with HBsAg loss should be evaluated for levels of quantitative HBsAg, HBV DNA and ALT annually.

Many studies showed that although HBsAg loss can significantly reduce the risk for HCC and hepatic events, HCC can still develop in a proportion of CHB patient after HBsAg loss, even in those without cirrhosis. Closer attention should be paid to the patients with established cirrhosis, older than 50 years at the time of HBsAg loss, male gender, and HCC family history [98].

Patients with advanced fibrosis or cirrhosis who achieve HBsAg loss should remain under surveillance for HCC every 6 months by AFP, ultrasound, even CT or MRI, and for esophageal varices by endoscopy if varices were present at pre-treatment endoscopy. Patients with pre-existing cofactors for liver disease, such as alcohol consumption, obesity and/or type 2 diabetes, NASH or NAFLD, may determine that additional assessments are necessary.

A recent study showed that the risk of hepatic events and liver-related mortality was similar in patients with complete viral suppression and HBsAg loss. This may suggest that hepatic necroinflammation and injury is the main driver of hepatic events. The presence of cirrhosis remains a major risk factor for hepatic events and liver-related death in patients[99]. Therefore, HBV DNA and ALT levels surveillance after HBsAg loss should continue.
HBsAg-negative, anti-HBe-positive patients receiving immunosuppressive agents, anti-tumor chemotherapy are at risk of HBV reactivation [4, 100]. Levels of ALT, HBV-DNA and HBsAg should be assessed before initiating such treatments and be closely monitored during and post treatments.

**Recommendation:**

9 Before initiating immunomodulator (e.g. Peg-IFN) combination therapy, measurement of key baseline parameters including HBsAg titers is required to guide the treatment decision and predict efficacy and safety. Monitoring shall be carried out periodically for assessment of adverse events and effectiveness during treatment.

10 Monitoring for HBV reactivation, HCC and hepatic events is necessary, despite HBsAg loss achievement.

**Future prospective**

The limitations of available treatment options for hepatitis B and unique virological and immunologic mechanism involved in HBV chronicity make complete cure of HBV infection an elusive therapeutic goal. Complete HBV control depends not only on sustained viral suppression, but also on the induction of an effective antiviral immune response. Efforts to develop DAAs targeting various steps of the HBV life cycle, including HBV entry (NTCP inhibitor), HBV cccDNA production and processing (cccDNA inhibitors), viral replication and viral protein expression (capsid assembly modifiers, RNA interference, Nucleic Acid Polymers, etc.) and immunomodulator interventions that promote cytokine-mediated innate immunity and restore adaptive immunity [25] are under way and some have entered preclinical and/or early clinical evaluation. With new therapeutic approaches expected to be available in the near future, combination of DAAs with immunomodulators may synergistically restore the host immune responses and eliminate cccDNA, ultimately leading to a complete cure of HBV infection.
Future research may focus on the following unresolved issues and unmet needs:

1. More studies are warranted to confirm the predictive value of the immunologic and virological biomarkers for functional cure and treatment failure.

2. Future confirmatory studies are also needed to verify the proposed roadmap for sequential combination therapy with DAAs (e.g. NAs) and immunomodulators (e.g. Peg-IFN).

3. There is a need to evaluate the long-term durability of HBsAg loss after completion of a finite treatment and to identify markers that predict durable off-treatment response.

4. Long-term follow-up studies are also needed to assess the risk and prediction of HCC and hepatic events after functional cure is achieved by combination therapy.

5. The challenges in curing chronic hepatitis B are still very daunting, whilst development of new DAAs with novel targets in the HBV life cycle or immunomodulators to restore immune responses against HBV is urgently needed.

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Figure legends:

Figure 1. Roadmap for sequential combination therapy with direct-acting antivirals (DAAs) [e.g. nucleos(t)ide analogues (NAs)] and immunomodulators (e.g. pegylated interferon).

Abbreviation: HBsAg, HBV surface antigen; HBeAg, HBV e antigen; BGT, Baseline guided therapy; RGT, Response guided therapy; DAA, direct-acting antiviral.