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VIEWPOINTS

Chronic Hepatitis B Finite Treatment: Similar and Different Concerns With New Drug Classes

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Chronic hepatitis B, a major cause of liver disease and cancer, affects >250 million people worldwide. Currently there is no cure, only suppressive therapies. Efforts to develop finite curative hepatitis B virus (HBV) therapies are underway, consisting of combinations of multiple novel agents with or without nucleos(t)ide reverse-transcriptase inhibitors. The HBV Forum convened a webinar in July 2021, along with subsequent working group discussions to address how and when to stop finite therapy for demonstration of sustained off-treatment efficacy and safety responses. Participants included leading experts in academia, clinical practice, pharmaceutical companies, patient representatives, and regulatory agencies. This Viewpoints article outlines areas of consensus within our multistakeholder group for stopping finite therapies in chronic hepatitis B investigational studies, including trial design, patient selection, outcomes, biomarkers, predefined stopping criteria, predefined retreatment criteria, duration of investigational therapies, and follow-up after stopping therapy. Future research of unmet needs are discussed.

Keywords. chronic hepatitis B; stopping finite therapy; new combination therapy; functional cure; HBV biomarkers.

New approaches to treating chronic hepatitis B (CHB) infection aim for a functional cure, defined as sustained hepatitis B virus (HBV) DNA suppression and loss of hepatitis B surface antigen (HBsAg) levels with or without the detection of antibody to HBsAg after cessation of all treatment (ie, finite therapy) [1]. Functional cure is rarely achieved after long-term therapy with nucleos(t)ide analogue reverse-transcriptase inhibitors (NrtIs) but occurs in up to 10% of patients after pegylated interferon therapy [2]. Partial cure, a more modest goal, is defined as off-treatment sustained HBV DNA suppression (less than the lower limit of quantitation) with HBsAg levels <100 IU/mL [1, 3]. Evaluation of therapies for both end points requires finite therapy, a major change from how NrtIs were approved, based on on-treatment HBV DNA suppression and alanine aminotransferase (ALT) normalization.

There is a dearth of data on stopping new investigational therapies to guide clinical development. Stopping NrtI treatment has provided valuable insights, but it is unclear whether these apply to curative strategies in development. The HBV Forum at the Forum for Collaborative Research aims to advance the regulatory science for novel HBV therapeutic interventions and associated morbid conditions in real time by providing an independent and neutral environment for ongoing multistakeholder dialogue. This Viewpoints article discusses areas where patients, academics, pharmaceutical companies, and regulatory agencies, participating in an HBV Forum working group, reached consensus on an approach to stopping all HBV treatment in clinical trials with novel HBV agents, including combination regimens of direct-acting antivirals (DAAs) with or without immunomodulatory agents. This includes establishing criteria to ensure safe and timely retreatment in the event of HBV reactivation after stopping treatment and whether these criteria can/should be standardized for all drug classes in development.

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DATA FROM STUDIES EVALUATING STOPPING NrtI

There are few prospective randomized studies of NrtI discontinuation, but observational clinical studies may inform a framework for investigational therapies by setting criteria for finite investigational therapies and identifying subgroups of patients more likely to achieve functional cure. NrtI discontinuation is associated with long-term benefits in a proportion of patients, with loss of HBsAg varying from 2% and 20% and with lower rates observed in Asian compared with European and North American studies [4–6]. The goal for new therapies is to improve these results. Table 1 highlights some patient characteristics affecting HBsAg loss during/after NrtI therapy. Importantly, stopping NrtI before HBsAg loss is not without safety risk. In a Taiwan-based study of >10 000 patients from the National Lab Database, 6.6% of patients had severe flares, the majority within 2 years, with a 0.79% rate of death or liver transplantation [13]. These adverse events occurred mostly, but not entirely, in patients with cirrhosis, supporting the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) recommendations against NrtI discontinuation in patients with cirrhosis [2, 14].

ROLE OF QUANTITATIVE HBsAg ASSESSMENT IN STOPPING NrtI THERAPY

HBsAg levels at the end of treatment (EOT) is the best-studied predictor of posttreatment response after stopping NrtIs, with varying results across studies [5, 8]. The lower the EOT HBsAg levels, the lower the risk of virologic (usually defined by HBV DNA >2000 IU/mL) and biochemical (usually defined by elevated ALT levels >2 times the upper limit of normal) relapse. In a systematic review of 1716 patients [15], EOT HBsAg levels of <100 and ≥100 IU/mL, were associated with sustained HBsAg loss (>12 months off therapy) of 21%–59% versus 3%–7.4%, respectively, and with virologic relapse rates of 9%–20% versus 31%–87% [15]. In the RETRACT-B study, HBsAg loss after 4 years off therapy was 43%, 7.4%, and 1.1% in those with EOT HBsAg <100, 100–1000, and >1000 IU/mL, respectively [8]. Wu et al [16] reported 5-year follow-up in 2451 patients who had received pegylated interferon: the cumulative rates of HBsAg loss were 30%, 10%, and 0% for patients with EOT HBsAg levels of <10, 10–100, and >100 IU/mL respectively. The negative predictive value of EOT HBsAg >10 IU/mL was 97.9% [16].

Using EOT value requires only a single-time measurement, easily done in clinical practice. Working group members agreed that low HBsAg levels at EOT best "predict" off-treatment response, but consensus was not reached concerning the optimal EOT HBsAg cutoff level associated with an acceptable low rate of disease relapse and satisfactory rate of HBsAg loss. While an HBsAg EOT value of <100 IU/mL was supported by most, <1000 IU/mL and <10 IU/mL were also advised. Clearly, more patients would be eligible for stopping therapy using a cutoff of <100 rather than <10 IU/mL; however, this would likely result in more patients experiencing virologic and biochemical relapse.

OTHER BIOMARKERS TO INFORM STOPPING NOVEL HBV THERAPIES

Lower hepatitis B core-related antigen (HBcrAg) levels are associated with lower virologic relapses and higher HBsAg loss after stopping NrtI therapy [11, 17]. Among patients with EOT HBsAg levels <100 IU/mL, the 3-year virologic relapse rates in patients with pretreatment HBcrAg levels <4.7 or ≥4.7 $\log_{10}$ U/mL were 20% and 60% ($P = .003$), respectively [12]. Among patients with EOT HBsAg ≥100 IU/mL, clinical relapse rates in those with EOT HBcrAg <4.7 or ≥4.7 $\log_{10}$ U/mL were 29% and 78%, respectively ($P < .001$) [17]. Those with EOT HBsAg >100 IU/mL and detectable HBcrAg (>2 $\log_{10}$ U/mL) had negligible HBsAg loss after stopping therapy [5]. These studies support using EOT HBsAg combined with HBcrAg to improve the prediction of sustained HBsAg loss off treatment. In Japan, HBsAg and HBcrAg are used to guide stopping NrtI treatment [18].

EOT HBV RNA is independently associated with off-treatment virologic relapse [9, 19]. In patients who were hepatitis B e antigen (HBeAg) positive when starting NrtIs, an EOT HBsAg level <100 IU/mL along with undetectable HBV RNA was associated with only 5% virologic relapse at 24 months after stopping NrtIs [20]. In patients who were HBeAg negative when starting NrtIs, the 48-week virologic relapse rate was 9% if the EOT HBsAg level was <10 IU/mL along with HBV RNA less than the lower limit of quantitation [9]. Combining EOT HBcrAg <4 $\log_{10}$ U/mL with undetectable HBV RNA resulted in a HBsAg loss of 16% and no biochemical relapse 4 years after stopping therapy [19]. EOT undetectable HBV RNA in combination with low HBsAg levels was associated with lower virologic and biochemical relapse rates, which may improve patient selection for stopping therapy. Unfortunately, no approved commercial HBV RNA assay and international standard are available. Use of assays with different sensitivities complicates cross-study comparisons.

The role of antibody levels to HB core antigen levels is less clear, and results are variable. Quantitative assays for this antibody are not readily available. In summary, there are consistent data indicating that EOT HBsAg <100 IU/mL, HBcrAg <4 $\log_{10}$ U/mL, and HBV RNA negativity (target not detected) may improve the identification of patients more likely to benefit from stopping treatment. Currently HBV RNA and HBcrAg lack sensitivity, but if these markers remain positive at EOT the likelihood for HBsAg loss is low.

Table 1. Patient Characteristics Affecting Hepatitis B Surface Antigen Loss During/After Nucleos(t)ide Analogue Reverse-Transcriptase Inhibitor Therapy

Characteristic of Patients With CHB	Specifics	Relevance to New Therapies
Sex	Female patients achieve HBsAg seroclearance at an older age [7]	This suggests that female patients may be less immunologically primed for HBsAg loss at a younger age
Race and ethnicity	Asians require lower threshold of quantitative HBsAg at EOT to clear HBsAg off NrtI treatment [8]	The race association is likely multifactorial, but this argues for stratification to ensure that race is not a confounder
Age	Older age is associated with higher rates of spontaneous HBsAg loss [7], and younger patients are more likely to have sustained off-treatment responses after NrtI discontinuation [7]	Balanced age distribution may be important to eliminate potential confounding effects of age on HBsAg loss
NrtI suppressed	Undetectable HBV RNA is associated with lower rates of relapse after NrtI discontinuation [5, 9]; detectable HBV RNA has low sensitivity but high specificity for predicting the need for retreatment after NrtI discontinuation [5, 9]	HBV RNA may be a better marker for silencing cccDNA transcriptional activity
NrtI type	Differential timing and rates of early virologic/biochemical relapse after TDF vs ETV discontinuation [8]	Given the focus on NrtI-suppressed patients, consider stratification on NrtI type to avoid potential confounding
HBeAg status	HBeAg positivity at the start of NrtI treatment is associated with higher rates of HBsAg loss after NrtI discontinuation than initially HBeAg-negative CHB [10]	Targeting patients on NrtI therapy who were initially HBeAg positive is expected to yield higher rates of HBsAg loss; this is an important variable for stratification
HBV genotype	High rates of HBsAg loss with genotype A [5], especially A2 [10;] among Asians, genotype C is higher than genotype B [5]	Most relevant to studies conducted outside Asia
EOT HBsAg	EOT HBsAg levels <100 IU/mL in Asian patients and <1000 IU/mL in white patients are associated with higher rates of HBsAg loss [4, 8]	Aim for threshold that will capture maximum success and reduce relapse–<100 IU/mL at EOT
HBcrAg	Lower levels of HBcrAg EOT are associated with lower rates of ALT flares and higher rates of HBsAg loss [5, 11, 12]	As a marker of HBV transcriptional activity, HBcrAg may be useful in guiding treatment discontinuation

Abbreviations: ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; EOT, end of treatment; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide analogue reverse-transcriptase inhibitor; TDF, tenofovir disoproxil fumarate.

IMMUNE MONITORING TO INFORM STOPPING NOVEL HBV THERAPIES

Immune biomarkers should complement viral biomarkers by measuring immune responses that can help predict when and/or in whom to stop treatment [21], but their ability to "predict" off-treatment responses remains speculative. Robust immune correlates of functional cure are lacking. EOT functional HBV-specific T cells have been shown to correlate with maintenance of viral suppression after treatment withdrawal [22] which may be relevant to DAAs. Data on bepirovirsen, an antisense oligonucleotide therapy, showed that participants with decreased quantitative HBsAg also experienced ALT flares, accompanied by increases in soluble proteins suggestive of innate and adaptive immune activation [23].

Regimens including immunomodulatory therapies are even more likely to require immune biomarkers to predict their success and to guide the termination of finite therapies, as exemplified by a single low dose of programmed cell death protein 1 blockade (anti-PD1) trial, where the best responder showed an expansion of HBV-specific T cells temporally correlating with loss of HBsAg [24]. However, increases in HBV-specific T-cell responses have been noted without virologic responses in several trials of therapeutic vaccines and Toll-like receptor agonists [21, 25], perhaps because T-cell boosting was too low level to exert an efficient in vivo effect.

Current immunologic research studies use laborious assays on separated peripheral blood mononuclear cells to detect low-frequency HBV-specific T- and B-cell populations and require specialty laboratories. These assays could be modified to make them suitable for a whole-blood screening assay, like Quantiferon assay. These would not only be easier to scale up for use in nonspecialist settings but would also detect direct ex vivo responses that should be more reflective of antiviral potential. Ultimately, a serum screening assay that could detect a predictive combination of soluble immune mediators, such as cytokines with or without metabolites, would be most practical. Validating such an assay will require assessment of different patient populations receiving different DAAs and/or immunomodulatory agents.

The potential utility of such serum screening, combined with a machine-learning approach, has been used to predict virologic relapse after NrtI discontinuation [23, 26]. Broad unbiased analyses of liver and peripheral cellular and serum compartments in groups who do or do not control HBV after withdrawal of novel drugs will aid the identification of robust immune biomarkers. Liver fine-needle aspirates will facilitate longitudinal monitoring of compartmentalized viral and immune biomarkers, while liver biopsies are needed for histologic assessment or information on immune cell topology [27].

PATIENT PARTICIPATION AND SELECTION

Patient advocates for CHB are increasingly vocal and eager to provide input on acceptability of new drugs and trial design. This has been highly successful in the AIDS Clinical Trial Group with long-standing strong community advisory boards with community advocates on every study team from the time of study inception. Treatment guidelines and health insurance coverage around the world differ in how long patients receive long-term NrtI. While health professionals believe long-term NrtIs are safe and lead to better outcomes, patients are not uniformly interested or willing to take long-term or indefinite therapy, as long-term monitoring can impose great financial and logistical burdens [28]. Living with HBV affects many realms of life and stigma, and discrimination continue to occur around the world with major barriers to diagnosis and ongoing care [28].

A 281-patient survey, presented at the 2022 AASLD-EASL HBV/HDV Treatment Endpoints Conference in June 2022 (Jacki Chen, personal communication), indicated that the majority (75.1%) were willing to take novel treatments for ≥1 year to achieve a cure; 42.5% were willing to take an injectable for ≥1 year, and 37.4% for ≤6 months. US minorities report less interest in entering studies compared with those of other ethnicities or from other countries [29]. More studies are needed to understand the patient perspective.

Finite therapy leading to HBsAg loss with subsequent decreased risk of end-stage liver disease and/or hepatocellular carcinoma is of great interest, and an acceptable treatment duration requires patient acceptance. Careful selection of patients for enrollment in clinical trials for new CHB therapies is critical for the safety of trial participants and demonstration of efficacy. Table 1 highlights some important patient characteristics that may affect responses to new therapies. Initially, clinical trials should exclude patients with advanced fibrosis and cirrhosis for safety reasons.

All participants should undergo abdominal imaging as well as noninvasive evaluation of fibrosis stage before entry into studies. As safety data are accumulated and characterized, specific populations (eg, patients with cirrhosis or decompensated cirrhosis or children) can be incorporated into the development plans [1]. Human immunodeficiency virus (HIV)/HBV coinfection presents an unique issue in the assessment of finite therapy and functional cure as anti-HBV–containing antiretroviral therapy should not be stopped [2]. This is because of the risk of reactivation of CHB via covalently closed circular DNA (cccDNA), as noted with anti-CD20 therapy or after switching to non–HBV-containing antiretroviral therapy [2, 30]. However, individuals with HIV/HBV coinfection should be included in studies of new HBV therapies to assess HBsAg loss.

FRAMEWORK OF FINITE DURATION THERAPY WITH NOVEL AGENTS

On-treatment responses may or may not predict off-treatment response and functional cure. Both responses likely vary by pretreatment disease characteristics and the mechanism of action (MOA) of the intervention. Whether DAAs and immune modulatory agents in development will have similar prognostic indicators to NrtI and interferons is not yet known (see Table 1). Potential ways to improve the feasibility of stopping therapy include use of HBsAg-lowering agents, such as small interfering or silencing RNAs. However, these achieve only low rates of HBsAg loss, and HBsAg levels generally rebound after therapy is stopped [31].

Results of a phase 2b trial of bepirovirsen, an antisense oligonucleotide, showed approximately 10% HBsAg loss after 24 weeks off treatment, with or without NrtI [32]. Participants with lower pretreatment HBsAg levels had higher HBsAg loss. These studies are encouraging, showing that driving down HBsAg levels promotes HBsAg clearance. Alternatively, the use of a combination of additional biomarkers (discussed above) could be explored to select which patients with EOT HBsAg <100 IU/mL could stop therapy, or all investigational therapies could be stopped while continuing NrtI until a predetermined end point (eg, sustained DNA suppression/HBsAg loss), which may reduce the risk of severe flares, decompensation, and death.

Future finite and curative regimens will likely consist of combinations of multiple novel agents with or without NrtI. Virus-targeting drugs in development include ≥3 MOAs: inhibition of viral entry or replication or reduction in antigen burden. It is expected that none of those mechanisms alone will lead to high HBsAg loss rates unless they can restore HBV-specific immunity. Immune-targeting drugs in development for treatment of CHB include Toll-like receptor agonists, therapeutic vaccines, monoclonal antibodies against HBsAg, and checkpoint inhibitors. However, immune modulation alone will likely also be insufficient to lead to HBsAg loss without viral suppression in most patients, unless HBsAg levels are very low, as has been seen with anti-PD1 [24] and anti–PDL-1 [33]. Stopping criteria for regimens without immune modulation may differ from those with immune modulation. Loss of HBsAg may occur at different times during or after therapy using different investigational agents.

Pretreatment characteristics and potential biomarkers that may predict response must be systematically collected and analyzed as part of clinical trials. Six months after discontinuation of all therapies is a pragmatic choice for establishing "sustained" HBsAg loss. However, there may be more seroreversions to HBsAg positivity beyond 6 months, and HBsAg loss may increase over time, highlighting the need for longer-term follow-up studies of new drugs.

Table 2. Criteria for Restarting Nucleos(t)ide Analogue Reverse-Transcriptase Inhibitor

Study	INR Bilirubin Decompensation	Maximum ALT	ALT Criteria for Restarting Nrtl[a]			HBV DNA, IU/mL	HBeAg Seroreversion
			ALT	HBV DNA, IU/mL	Time Frame		
Studies on stopping Nrtls							
Berg et al [37]	Yes	>10× ULN[b,c]	>2–5× ULN	>20 000	>12 wk	ND	ND
	…	…	>5–10× ULN	…	4 wk	…	…
Papatheodoridis et al [38]	Yes	>10× ULN	>3× ULN	>100 000	…	>20 000 [b]	ND
	…	…	>5× ULN[d]	…		…	…
			>ULN	>2000	for 3 visits		
Liem et al [39]	ND	>15× ULN	200–600 U/L	…	For 6–8 wk	ND	ND
	…	…	>5× ULN	…	Twice in 4 wk	…	…
	…	…	>10 ULN	…	Twice in 4 wk	…	…
	…	…	>10× ULN	…	…	…	…
Terrault et al [10]	Yes	…	ALT ≥300 (M); ≥ 200 (F)	…	Thrice in 4 wk	≥10 000	Yes
	…	…	ALT ≥150 (M); ≥ 100 (F)	…	Thrice in 12 wk	…	…
Pharmaceutical companies with new HBV drugs							
Assembly Biosciences	Yes	>10× ULN	≥2–5× ULN	>2000	For 12 wk	ND	ND
	…	…	≥5–10× ULN	>2000	For 4 wk	…	…
Gilead Sciences	Yes	>10× ULN	>ULN	>20 000 (HBeAg pos)[b]	For 8 wk	ND	ND
	…	…	>ULN	>2000 (HBeAg neg)[b]	For 8 wk	ND	ND
Janssen	Yes	NI	>5× ULN	>2000[b,c]	…	>20 000[b,c]	Yes
	…	…	…	…	…	>100 000[e]	…
Vir Biotechnology	Yes	>10× ULN	>10× ULN	2000[b]	…	>20 000[b,c]	Yes
	…	…	2–5× ULN	2000[b]	For 12 wk	>100 000[e]	…
	…	…	5–10× ULN	2000[b]	For 4 wk	…	…

Abbreviations: ALT, alanine aminotransferase; F, female patients; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HBsAg seroreversion, positive serum HBsAg after a negative HBsAg result; INR, international normalized ratio; M, male patients; ND, no data included; neg, negative; NI, data not included in article or criteria; Nrtls, nucleos(t)ide analogue reverse-transcriptase inhibitors; pos, positive; ULN, upper limit of normal.

[a]These columns represent criteria for restarting Nrtl if ALT (measured as times ULN or in units per milliliter) reaches a certain level with/without a predetermined HBV DNA level for the specific period of time.

[b]Consider restarting Nrtl.

[c]Confirmed.

[d]Plus bilirubin >2 mg/dL

[e]Restart Nrtl immediately.

Table 3. Consensus Among Stakeholders for Finite Therapy of Chronic Hepatitis B Investigational Studies

Area of Interest	Consensus
Design of studies	Designing new finite duration therapeutic regimens to achieve functional cure is complex owing to the differing MOAs and heterogeneity based on patient characteristics; early patient input will enhance acceptability of new drugs and trial design; combining new regimens from different industry partners is encouraged.
Patient selection	Initial studies of finite and curative investigational therapies should focus on enrollment of patients without cirrhosis and with minimal fibrosis for the safety of trial participants, especially when finite treatments are assessed; special populations should be added after safety and efficacy is established.
Outcomes	Functional cure is defined as HBsAg loss, with or without detection of antibody to HBsAg (anti-HBs >10 IU/mL), and HBV DNA below the LLOQ sustained for ≥24 wk off all treatment; partial cure is defined as HBsAg positivity, and HBV DNA below the LLOQ sustained for ≥24 wk off all treatment and should be included as a secondary end point.
Biomarkers	New treatment regimens should achieve on-treatment suppressed HBV DNA and RNA and significant reductions in HBsAg (ideally HBsAg negativity) to increase the chances of achieving functional cure and to minimize risks of virologic and clinical relapse; HBsAg level at EOT is the most promising biomarker associated with lower chance of disease relapse and higher likelihood of HBsAg loss after stopping therapy; EOT HBsAg <100 IU/mL, HBcrAg <4 $\log_{10}$ U/mL, and HBV RNA negativity may improve accuracy in identifying patients who could benefit from stopping treatment.
Predefined stopping criteria	Predefined stopping criteria should include low or negative HBsAg, negative HBV DNA, and normal or slightly elevated ALT.
Predefined retreatment criteria	The threshold for retreating study participant needs to be carefully predefined in the protocol based on latest data to allow adequate time to see an off-treatment response while ensuring patient safety; off-treatment monitoring must be frequent (every 2–4 wk), with rapid turnaround of liver and virologic (HBV DNA, quantitative HBsAg) tests.
Duration of investigational therapies	The duration and complexity of any treatment regimens should be acceptable to the patient population (duration ideally ≤48 wk).
Follow-up after stopping therapy	Patients should be followed up for at least 48 wks; long-term follow-up studies are recommended to assess durability or response, additional HBsAg loss, and late relapse.

Abbreviations: ALT, alanine aminotransferase; anti-HBs, antibody to HBsAg; EOT, end of treatment; HBsAg, hepatitis B surface antigen; LLOQ, lower limit of quantitation; MOAs, mechanisms of action.

FRAMEWORK FOR RESTARTING SUPPRESSIVE THERAPY/NrtI

Close monitoring off treatment will identify patients who would benefit from restarting suppressive therapy. In an NrtI-stopping study, postponing retreatment as long as possible led to 39% HBsAg seroclearance [34]. Jeng et al[4] found that patients with biochemical relapse who were not retreated had a 7.34 times higher incidence of HBsAg loss than those who received retreatment (6-year incidence, 19% vs 1%, respectively), highlighting the difficulty of determining whether or not therapy should be restarted.

Whatever the potential benefits of HBsAg loss, these need to be balanced against the potential harm to patients. Thus, patients need very close monitoring and rapid turnaround of liver and viral markers. Severe adverse outcomes after NrtI withdrawal have been reported, mainly, but not exclusively, in patients with preexisting liver cirrhosis [13]. Virologic relapse after NrtI discontinuation is associated with the risk of ALT flare. The notion that ALT flares are required for HBsAg loss was challenged in an HBV Research Network study, finding that HBsAg declines were more likely in patients without ALT flares [35]. Flares may not occur when investigational agents with other MOAs are stopped.

Table 4. Future Needs in Investigational Trials of Chronic Hepatitis B

Area of Interest	Specific Need
Definitions	Uniform definitions of cure (functional or partial) and inactive state, as well as biochemical and virologic relapse, should be used across trials.
Predictors	Predictors of success should be systematically evaluated in all trials, including those with NrtI discontinuation and should include quantitative HBsAg and other markers, such as HBV RNA and HBcrAg.
Stopping criteria	Stopping criteria for low level of HBsAg need validation in clinical trials; different criteria may need to be developed for regimens depending on whether different MOAs (viral inhibitors and/or immune modulators) are incorporated in a treatment regimen.
Source of HBsAg	There is a major need for assays able to differentiate between iDNA and cccDNA in serum as the source of HBsAg [40].
Immunology	No immune biomarkers predict functional cure currently, and they should be tailored to reflect the MOAs of different agents and identify immune system targets (prioritizing the analysis of HBsAg-specific T and B cells for drugs targeting HBsAg and ensuring analysis of liver immunity if using a liver-targeted agent, such as LNA oligonucleotide targeting PD-L1); new methods are needed for measuring restoration of HBV-specific immune control.
Trial samples	All trials should include banked serum/plasma samples, and PBMCs, minimally at pretreatment and EOT to screen and evaluate potential immune and virolologic markers; pathogenesis-focused trials should also include fine-needle aspiration and liver biopsy.
Drug resistance	Assessment for resistance against all drugs in the regimen will be an important consideration for participants with lack of on-treatment response and/or relapse after stopping a finite treatment regimen.

Abbreviations: cccDNA, covalently closed circular DNA; EOT, end of treatment; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; iDNA, integrated DNA; LNA, locked nucleic acid; MOAs, mechanisms of action; NrtI, nucleos(t)ide reverse-transcriptase inhibitor; PBMCs, peripheral blood mononuclear cells; PD-L1, programmed death ligand 1.

In the REEF-2 European study of virologically suppressed HBeAg-negative patients with noncirrhotic CHB, 48-week treatment with either (1) the small interfering RNA JNJ-3989, the capsid assembly modulator JNJ-6379, and an NrtI or (2) a placebo and an NrtI did not lead to functional cure 48 weeks after stopping all treatment [36]. During 48-week follow-up, 27.3% versus 7.1% restarted NrtI in the control versus active arms, respectively [36]. More ALT flares (>3 times the upper limit of normal) were observed in the NrtI control arm (39% vs 5%) than in the active arm.

Various virologic and biochemical criteria have been applied to initiate retreatment in studies of NrtI discontinuation and after stopping new investigational drugs, as shown in Table 2. The monitoring and retreatment criteria proposed in various studies strive to account for rapid virologic relapse and risk of severe flare, which might not be observed with new MOAs. Additional biomarkers (HBcrAg, HBV RNA) have been evaluated for their role in predicting response to NrtI discontinuation (see above) and their specific role with new therapies needs to be further evaluated.

CONSENSUS AND FUTURE NEEDS

There were many areas of overall consensus within our group, as outlined in Table 3. While the most promising biomarker is EOT HBsAg level, there was no stakeholder agreement of a safe HBsAg threshold at which to stop all therapy, with a majority supporting <100 IU/mL. All agreed that predefined stopping criteria should include negative HBsAg and negative HBV DNA results and normal or slightly elevated ALT levels, but no consensus was reached on stopping criteria in patients with detectable HBsAg. While common, aligned stopping criteria across studies are desirable, the final criteria need to consider the type of regimen (viral targeting vs immune modulators).

Many areas need additional research (Table 4). Some of these should be built into future clinical trials, including uniform definitions of cure (functional or partial) and inactive state as well as biochemical and virologic relapse. Predictors of success as seen with NrtI discontinuation studies should be systematically evaluated in addition to other viral markers. Assays are needed that differentiate the source of HBsAg between integrated DNA and cccDNA in serum (presently requiring liver tissue [40]). Some patients with partial cures may have no active cccDNA if HBsAg is wholly derived from integrated DNA. All trials should include banked samples, minimally at pretreatment and EOT to allow for studies of immunologic and virologic predictors of success and relapse. As more data from investigational studies become available, stakeholders should reconvene to refine these issues and expand successful therapies to more patients.

Notes

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