New paradigms in hepatitis B management: only diamonds are forever

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Abstract

Introduction: The hepatitis B virus (HBV) causes chronic hepatitis B (CHB) in ~350 million people worldwide who have an increased risk of end-stage liver disease and/or hepatocellular carcinoma.

Sources of data: Several peer-reviewed papers featuring new approaches to anti-HBV management. Additionally, we also reviewed recent abstract presentations at international congresses.

Areas of agreement: There has been great progress in CHB therapy with the development of standard and pegylated interferon (i.e. PEG-IFN) as well as nucleos/tide analogs (NAs). IFN has both antiviral and immunomodulatory effects and through immune-mediated destruction of infected hepatocytes offers the possibility of finite therapy. However, this ‘killing for a cure’ antiviral strategy may not be tolerated in many, especially in cirrhotic patients. NAs inhibit viral reverse transcriptase, have few side effects and prevent liver disease progression, but cannot offer a cure as they have little effect on the resilient HBV covalently closed circular DNA (cccDNA) intermediate. Moreover, NAs such as tenofovir and entecavir offer a high genetic barrier to resistance, but are expensive and not readily available in many global regions.

Growing points: Despite significant treatment advances, there is increased recognition of the need for improved anti-HBV treatments, and new virologic tests for monitoring treatment response.

Areas of controversy: The role of quantitative hepatitis B surface antigen, intrahepatic cccDNA levels and viral genotype in selecting treatment candidates and refining NA stopping rules.
Areas timely for developing new research: Potential new therapies include viral entry inhibitors, RNA interference technologies (i.e. RNAi) and small molecules that modulate cccDNA transcription, as well as novel immunomodulatory therapies to boost HBV-specific T cell responses. The ultimate goal of new tests and anti-HBV therapies is to reduce the burden and expense of life-long CHB treatment, as ‘only diamonds are forever’.

Key words: chronic hepatitis B, cure, quantitative HBV surface antigen, HBV cccDNA, immune therapy, nucleos/tide analog

Introduction

James Bond: “You expect me to talk?”
Auric Goldfinger: “No Mr. Bond, I expect you to die!”
(from Goldfinger, Eon Productions, 1964)

Approximately, 350 million people worldwide have chronic hepatitis B (CHB) and are at risk for end-stage liver disease, cirrhosis and hepatocellular carcinoma (HCC).1,2

The HBV is an ancient human pathogen, as evidenced by its discovery in the mummified remains of a child from the 16th century AD Korean dynasty; the viral sequence origin was calculated to date from 3000 to 100 000 years ago.3 In the nearly half-century, since its discovery, despite our best efforts, the virus, like Ian Fleming’s legendary secret agent, simply will not die.

HBV is a small DNA virus that infects the cell by interacting with its recently identified sodium taurocholate cotransporting polypeptide surface receptor (NTCP).4 Following viral entry, the virus genome forms a highly stable intranuclear minichromosome known as covalently closed circular DNA (HBV cccDNA), which is the transcriptional template for all viral proteins (i.e. HBV surface or envelope (S), polymerase or reverse transcriptase (P/RT), precore (i.e. HBeAg in serum), core or nucleocapsid (C) and X protein.5 The persistence of HBV cccDNA is believed to be the central mechanism for CHB virus infection. The HBV is a non-cytopathic virus, and viral clearance as well as liver injury is mediated by HBV-specific cluster differentiation (CD8+) cytotoxic T cells.6 The detection of HBV surface antigen (HBsAg) in serum for >6 months confirms CHB.

The loss of serum HBsAg is considered a clinical cure, although HBV replication may continue at low levels this indicates robust immune control of viral infection. However, HBsAg loss is rarely achieved either from spontaneous immune-mediated clearance or by current therapy due in part to the persistence of HBV cccDNA.

The recommended first-line CHB drugs include potent nucleos/tide analogs (NAs), such as tenofovir disoproxil fumarate (TDF) or entecavir (ETV).7–9 In HBV endemic regions, older generation NA such as adefovir, telbivudine and lamivudine are still used due to lower cost. The latter drugs have a higher risk of drug resistance and treatment failure with long-term therapy.10 Current guidelines advise that NA can be stopped if certain clinical end points are achieved, i.e. HBV e Ag (HBeAg)/antibody to HBeAg (anti-HBe) seroconversion. However, NA cessation often leads to HBV rebound,11,12 and despite viral suppression, patients remain at risk for HCC development, especially if they are cirrhotic.13,14

There is a need for accurate biomarkers that can predict serum HBsAg loss and candidates who can safely discontinue NA. Studies suggest that the quantification of HBsAg and HBV cccDNA levels may predict durable off-treatment NA response, defined as persistently normal ALT and HBV DNA <2000 IU/ml (10 000 copies/ml).15–17 HBV cccDNA persistence plays a key role in viral reactivation after treatment withdrawal. In the current review, we provide an overview of the role of recent virological biomarkers in CHB management from the perspective of refining NA stopping rules and predicting HBsAg loss. Finally, we summarize new drugs in development that offer the ultimate hope in reducing...
the need for life-long NA therapy. Although NA may be effective, they are limited by cost, the potential for side effects such as renal and metabolic bone disease, and antiviral drug resistance and treatment failure can occur in those that are not adherent to therapy, especially if receiving the older generation NA.\textsuperscript{18}

Summary of current anti-HBV treatment guidelines

HBV infection is a dynamic disease modulated by the host immune response. The natural course has been divided into four phases that are defined as: inactive CHB, HBV e antigen positive (HBeAg+) immune tolerant, HBeAg (+) immune active and HBeAg negative (−) CHB.\textsuperscript{19} Recent data indicate however, that these arbitrary clinical classifications are not entirely accurate as immune cells from supposedly immune tolerant individuals exhibit significant HBV-specific immune responses.\textsuperscript{20} Regardless, it is recommended that patients with protracted immune-active flares and reactivation of HBeAg (−) hepatitis may need therapy as treatment reduces the risk of HBV-related cirrhosis, liver decompensation and HCC development.\textsuperscript{7,8} Approved first-line anti-HBV therapy includes interferon (i.e. standard or Peg-IFN-alpha) and NA (i.e. TDF and ETV). Although Peg-IFN is for a finite 48 weeks, it has many side effects, requires subcutaneous administration and is only effective in ~20–30% of CHB carriers. However, due to the immunomodulatory effects of IFN, there is a greater chance of off-treatment HBsAg clearance. Oral NAs are safer with few adverse effects and can even be used in cirrhotic patients, but require prolonged therapy. The predicted time to HBsAg loss in NA-treated patients is >30 years, thus lifetime therapy is required in the vast majority of HBV-infected patients.\textsuperscript{21–23} In one recent report of HBeAg positive patients on long-term NA therapy who achieve HBeAg seroconversion and received prolonged consolidation therapy (i.e. continued NA treatment for 3 years), the observed rate of HBsAg loss was reported at ~9\%.\textsuperscript{24} Similarly, the long-term registration trials with tenofovir showed a HBsAg loss of only 4\%.\textsuperscript{25} Although, expert guidelines recommend that HBeAg (+) patients may stop NA after a 12-month consolidation therapy after achieving HBeAg seroconversion, many relapse with treatment cessation.\textsuperscript{11,26} In HBeAg (−) CHB, the Asian-Pacific guidelines suggest that NA may be stopped at 24 months after a patient achieves undetectable HBV DNA, but this is not recommended by other expert societies unless HBsAg loss occurs.\textsuperscript{7,8,27} In clinical trials with ETV or TDF, HBsAg loss occurred in only 5–8% of HBeAg (+) patients after 3 years and was not observed in HBeAg (−) CHB. Recent reviews of NA vs. IFN on-treatment responses have been published.\textsuperscript{28–33}

Potential new markers to guide current antiviral therapy

The clinical utility of quantitative HBsAg in assessing response to NA therapy

HBsAg clearance is considered the closest event to a cure for CHB, reflecting host immunological control of infection. Patients who achieve HBsAg loss have a more favorable prognosis, lower risk of liver fibrosis and HCC development, and in some have cirrhosis regression. HBsAg is produced via different pathways in the HBV lifecycle; (i) it forms the envelope protein for the mature HBV virion, (ii) it is secreted as noninfectious subviral HBsAg spheres or filamentous forms and (iii) it is produced from HBV DNA integrated into the host genome.\textsuperscript{34,35} Thus, serum quantitative HBsAg (qHBsAg) reflects transcription from both active HBV cccDNA molecules and integrated viral sequences. Studies have shown that serum HBsAg titers are a surrogate marker of infected cells and correlate with intrahepatic HBV DNA and cccDNA activity.\textsuperscript{36–41} Large Asian cohort studies have established that low qHBsAg titers (<1000 IU/l) along with HBV DNA <2000/ml reliably identifies minimal risk inactive CHB carriers, and is significantly associated with spontaneous HBsAg loss.\textsuperscript{37,42–45} It has been proposed that HBsAg quantification can be used as a marker of intrahepatic HBV cccDNA levels in patients on long-term NA therapy. A more rapid HBsAg decline in the first year after starting NA, or reduction rate of >0.166 log IU/ml/year may predict HBsAg seroclearance.\textsuperscript{46–48} Since NAs do not directly target HBV cccDNA, this may be related to partial
restoration of immune control against HBV in the first year with initiation of NA therapy. In Asian patients, studies suggest that qHBsAg <100 IU/ml predicts lower risk of relapse with NA cessation. In European patients, with HBV genotype D infection, qHBsAg titers <1000 IU/ml could predict maintained remission and a subsequent HBsAg loss. In most studies of HBeAg (+) patients, qHBsAg positively correlated to HBV DNA and HBV cccDNA levels in the liver; however, there appears to be a disconnection between qHBsAg and viral replication in HBeAg (−) CHB. This is possibly due to more viral integration events as well as the presence of more virus-associated HBsAg in highly viremic HBeAg positive patients. Although differences in inclusion criteria limited comparison across studies, HBeAg (+) patients on NA treatment show greater qHBsAg decline compared with HBeAg (−) CHB, especially if higher baseline ALT levels. The on-treatment predictive value of qHBsAg needs to be studied in sufficiently large numbers of both HBeAg (−) and HBeAg (+) CHB at defined time points, and using the same definition of response. Studies in Western countries are needed to confirm the results in Asian regions, since there are genotype-specific differences in HBsAg kinetics in response to NA therapy. HBsAg quantification may be a promising useful tool to predict CHB patients who can discontinue NA with a low risk of relapse. Further long-term prospective evaluation is needed regarding the impact of baseline HBsAg levels and the kinetics of qHBsAg decline in identifying patients who will have a durable off-treatment NA response.

An accurate stopping rule for NA-treated patients is highly desirable. Quantitative HBsAg reflects the transcriptional activity of HBV cccDNA rather than the absolute number of HBV cccDNA copies. Previous studies have been small, retrospective analyses, qHBsAg were assessed at only a few time points and most were in Asian or European populations with limited HBV genotype distributions. Standardized qHBsAg assays have only recently become commercially available, and they cannot distinguish between the different forms of HBsAg produced, either virion associated, subviral forms or produced from integrated sequences. The HBV S or envelope open reading frame completely overlaps the P gene. Long-term NA therapy, especially those with a lower genetic barrier to resistance can lead to mutations in the HBV P and may result in critical changes in the S proteins. It is unclear whether these mutations affect HBsAg titers in clinically relevant terms. They have been associated with hepatocyte HBsAg retention and potential oncogenic (i.e. HCC) risk. This information is needed to identify relevant qHBsAg clinical cut-offs in all genotypes, and how best to apply them in clinical practice.

The role of intrahepatic HBV cccDNA quantification in assessing response to NA therapy

An essential step in the HBV lifecycle is the formation of the HBV cccDNA in the nucleus, which serves as a template for progeny virus, and recycles from the cytoplasm to the nucleus to renew the cccDNA reservoir. The challenge of antiviral therapy is to clear the HBV cccDNA pool, which is maintained at ∼5–50 copies per infected cell. Current drugs fail to eradicate the cccDNA reservoir, although they do reduce the cccDNA pool due to lower intracellular recycling of rcDNA back to the nucleus. Many studies suggest that HBV cccDNA quantification is a potential tool in evaluating anti-HBV therapeutic efficacy and in estimating treatment endpoints. However, the study of cccDNA has been limited by the requirement for liver biopsies, which are difficult to collect (especially from NA-treated patients without active disease), sampling error, and the lack of sensitive and specific quantitative assays. NA has been reported to reduce intrahepatic as well as serum cccDNA, presumably released from hepatocytes following cell death. Wong et al. analyzed paired liver biopsies from 117 patients before and after 1 year of therapy with both high and less potent NA, there was a 4–7 log decline in serum and intrahepatic HBV DNA, but an overall small reduction of qHBsAg and cccDNA. Only 5/117 patients had undetectable HBV cccDNA after 1 year of NA. It is unknown whether long-term NAs have a greater effect on HBV cccDNA decline. This is clinically relevant since the failure to eliminate the HBV cccDNA as well as loss of host immune control results in viral rebound after NA.
cessation. Moreover, the cccDNA can inherit and ‘amplify’ drug resistant mutations leading to virological breakthrough in those treated with NA that have a lower genetic barrier to resistance. Understanding the dynamics of cccDNA persistence despite long-term NA is critical in identifying patients who can safely stop long-term NA.

The role of HBV genotypes in predicting IFN and NA treatment response

There are 10 HBV genotypes (HBV A–J) with ~4–8% nucleotide sequence divergence. HBV genotypes have a distinct geographical distribution with differences in disease profile. Significant genotype-specific differences in the response to IFN therapy occur, as well as differences in qHBsAg decline. Other studies report genotype-specific differences in NA response, resistance to lamivudine or adefovir and durability of HBeAg seroconversion. This has less clinical relevance with the newer potent NA, however alternative therapy endpoints such as HBsAg loss and HCC potential may be identified. In summary, clinically relevant features of HBV genotypes include: the rate and durability of HBeAg loss/seroconversion (A and D > B and C), spontaneous HBeAg loss (B > C) and cirrhosis (C), HCC risk (C in Asians, F in Alaska Natives) and response to antivirals (A and B > C and D). The role of genotypes in CHB management has been extensively reviewed.

Novel HBV therapies under preclinical and clinical development

The primary objectives of treatment are the long-term control of HBV replication and to induce remission of disease (Table 1). This has been achieved in the vast majority of patients treated with potent NA (i.e. ETV and TDF), and with Peg-IFN-alpha treatment of patients with the best cellular immunity. Recent newer anti-HBV drugs development has focused on improvement of current NA and IFN-based therapies as well as other novel HBV lifecycle targets (Fig. 1). A comprehensive overview of strategies to eradicate HBV has been recently reviewed.

New modified NA, besifovir and tenofovir alafenamide

All NAs are competitive inhibitors of the natural endogenous intracellular nucleotide. Their incorporation in nascent viral DNA results in premature

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**Table 1** Summary of novel anti-HBV therapies in clinical development (see Fig. 1)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Preclinical/clinical</th>
<th>Manufacturer</th>
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<tbody>
<tr>
<td>– Tenofovir</td>
<td>Modified NA</td>
<td>1. TAF: Phase 2</td>
<td>1. TAF: Gilead Sciences</td>
</tr>
<tr>
<td>– Besifovir</td>
<td>2. Besifovir: similar to TDF, rapid conversion to active metabolite in liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peg-IFN-lambda</td>
<td>Type 3 IFN (IL-29) Immunomodulatory more limited receptor distribution</td>
<td>Phase 2: no difference to Peg-IFN-alpha</td>
<td>Bristol Myers Squibb (drug development suspended)</td>
</tr>
<tr>
<td>GS9620</td>
<td>TLR-7 agonist innate immunity presystemic immune response</td>
<td>Phase 2</td>
<td>Gilead Sciences</td>
</tr>
<tr>
<td>GS-4774</td>
<td>Recombinant T cell vaccine with all HBV antigens</td>
<td>Phase 2</td>
<td>Gilead Sciences</td>
</tr>
<tr>
<td>Myrcludex®</td>
<td>Entry inhibitor</td>
<td>Phase 2a</td>
<td>Hepatera with Myr-GmbH</td>
</tr>
<tr>
<td>NVR3-778</td>
<td>Capsid (core) inhibitor</td>
<td>Phase 1a</td>
<td>Novira therapeutics</td>
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<tr>
<td>– ARC520</td>
<td>RNAi-targeted delivery of HBV-specific interfering RNA, inhibit HBsAg</td>
<td>1. ARC 50: Phase 2a</td>
<td>1. ARC 50: arrowhead research</td>
</tr>
<tr>
<td>– TKM-HBV</td>
<td></td>
<td>2. TK-HBV: Phases 1 and 2a in 2015</td>
<td>2. TK-HBV: Tekmira</td>
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chain termination by preventing the incorporation of the next nucleotide in the viral DNA strand, which is therefore very effective in suppressing HBV replication. Besifovir (LB80380) is an acyclic nucleotide phosphonate that is structurally similar to adefovir and tenofovir and rapidly converted into active metabolites in liver and intestine. Preliminary studies showed that the drug is effective in HBV DNA suppression for both treatment-naïve and lamivudine-resistant CHB patients. In a Phase 2b multicenter randomized trial of besifovir versus ETV for 48 weeks in treatment-naïve CHB Asian patients, 90 and 150 mg daily of besifovir were non-inferior to ETV 0.5 mg daily. The only significant side effect of besifovir was L-carnitine depletion, requiring carnitine supplementation. It is unknown whether this is related to mitochondrial toxicity and carnitine depletion, as is observed with anti-HIV antiviral therapy.

Tenofovir alafenamide (TAF) is a next generation pro-drug of tenofovir. Phase 2 data of TAF compared with TDF for treatment of HIV (without HBV co-infection) have recently been published. Treatment-naïve HIV-positive patients given the single tablet regimen that contained either TAF or TDF achieved a high rate of virological response (i.e. suppression in HIV RNA). Compared with those receiving TDF, HIV-positive patients on TAF experienced significantly smaller changes in estimated creatinine clearance, renal tubular proteinuria and bone mineral density. In HIV, TAF-treated patients showed greater viral suppression and higher intracellular active drug levels in peripheral blood mononuclear cells compared with TDF, at approximately 1/10th the dose.

Phase 3 studies of TAF vs TDF for treatment of HBV monoinfection are underway; preliminary data suggest that TAF may have enhanced hepatic uptake and hepatocyte activation. Given that HBV infects and replicates within immune cells (i.e. PBMC), this may translate into greater antiviral efficacy, although this issue remains a controversial and requires further investigation.

Pegylated interferon lambda

IFN-l is a recombinant IL-29 protein that is a member of the Type III IFN family, IL-29 signals through a receptor complex specific to the lambda family. Unlike IFN-alpha, IFN-l has a more limited extrahepatic receptor distribution that reduces risk for systemic extrahepatic adverse events. Phase-2 studies have been published in abstract form for evaluation of pegylated interferon lambda (Peg-IFN-l) compared with standard Peg-IFN-alpha in CHB patients. On-treatment, lambda IFN showed greater early impact on HBV DNA and qHBsAg, and comparable serologic/virologic responses at treatment end. Post-dosing, however, Peg-IFN-alpha
HBeAg seroconversion was higher, with key secondary results mostly favoring alpha. As a result, the manufacturer has halted further clinical development of Peg-IFN-lambda for treatment of CHB.

Boosting innate immunity toll-like receptor agonists

Toll-like receptor (TLR) agonists aim to stimulate the innate immune system cytokine cascade induced following acute HBV infection. The induction of robust antiviral cytokine responses (i.e. tumor necrosis factor-a, IFN-alpha, IFN-gamma and interleukin-1beta) may ultimately induce a long-lasting HBV-specific adaptive immune response. In preclinical studies with an oral TLR-7 agonist (GS9620), a rapid and sustained reduction in viral load and surface antigen levels was observed in animal models, and at GS-9620 doses ≥2 mg was associated with induction of chemokines/cytokines and IFN-stimulated genes in healthy volunteers. Preliminary data of phase 1 clinical trials of GS-9620 in both treatment naive and virologically suppressed CHB patients showed that induction in expression of interferon-stimulated gene (ISG15 and CCL8) was observed in the majority of patients, without significant systemic adverse events. Phase 2 studies with GS-9620 are currently ongoing.

Boosting HBV-specific adaptive immunity, therapeutic HBV vaccines, programmed cell death-1 ligand blockade on CD8+ T cells and adoptive T cell receptor transfer

Therapeutic vaccines also aim to elicit the patient’s immune system in treatment of CHB. The clinical failure of early vaccine therapies was attributed to the single targeting of the envelope (surface) antigen, produced in high quantity in CHB patients. Thus, HBV therapeutic vaccines targeting different HBV proteins with greater ability to stimulate HBV-specific T-cell immune responses have been developed. A therapeutic vaccine comprising particulate HBsAg and HBcAg and a saponin-based adjuvant was able to restore potent multifunctional HBV-specific CD8 +T-cell responses in HBV transgenic mice without causing liver disease. In a phase 1/2, multicenter trial, immunization with an HBV envelope-expressing DNA vaccine did not decrease the risk of relapse in NA-treated patients or the rate of virological breakthrough and did not restore the anti-HBV immune response despite effective viral suppression by analogs. A phase 3 clinical trial of a therapeutic vaccine based on immunogenic complexes composed of HBsAg and anti-human HBsAg antibody has shown some level of efficacy in vaccine therapy. However, a virological response (HBeAg seroconversion) was not only observed in 20% of individuals treated with the vaccine but also in the control group treated with alum adjuvant alone. The immunological mechanisms causing this clinical response induced by vaccination were not directly investigated in this trial. A possible explanation could be provided by the ability of alum to mediate activation of inflammatory monocyte-derived dendritic cells.

HBV persistence is associated with functional exhaustion of HBV-specific CD8+ T cells that express the inhibitory receptor programed cell death-1 (PD-1). Improvement in HBV-specific T cell function was noted in vitro by inhibiting the PD-1/PD-ligand 1 (PD-L1) on CD8 +T cells. In a recent study by Liu et al., in the woodchuck model of HBV, blockade of PD-1/PD-L1 pathway, in combination with ETV treatment and DNA vaccination potently enhanced the function of virus-specific T cells. The authors concluded that these study results may have an impact on future immunotherapeutic clinical trials in CHB patients.

Engineering HBV-specific T cells through transfer of HBV-specific T cell receptor (TCR) has also been recently proposed as a therapeutic strategy to enhance T-cell immunity in many CHB patients. In one recent interesting case report of a transplant patient with metastatic HBV-related HCC, adoptive transfer of gene-modified T cells survived, expanded and mediated a reduction in HBsAg levels without liver inflammation or other toxicity.

Finally, Gilead Sciences is also developing a yeast-based immunotherapeutic vaccine engineered to HBV-specific antigens (GS-4774). Phase 1 studies with GS4774 showed that the drug was safe, well-tolerated and induced HBV-specific immune responses at all doses evaluated. Phase 2 studies with GS-4774 are ongoing.
HBV entry inhibitors, Myrcludex-B

The HBV entry inhibitor in most advanced clinical development is Myrcludex-B®.93 The drug consists of acylated peptides derived from the large HBV envelope protein and targets the essential bile acid receptor for HBV (NTCP) to block HBV entry in hepatocytes.94 These peptides were shown to block virus entry both in vitro and in vivo in mice repopulated with primary human or Tupaia hepatocytes. Recent results reported from a small Phase 2a study showed a dose-dependent effect on HBV DNA with a >1 log decline at week 12 in 75% (6/8) patients receiving 10-mg of Myrcludex-B, which was maintained through week 24.95 These data suggest that inhibition of HBV entry constitutes a therapeutic approach to prevent primary HBV infection, such as after liver transplantation, and it may also control virus infection in chronically infected patients.96 The authors concluded that inhibition of entry and intrahepatic spread of HBV may play an essential role in future curative regimes.

HBV core (capsid) inhibitors, NVR 3-778

Several preclinical studies have evaluated the role of novel HBV agents targeting the HBV core (capsid) assembly which represents a critical step in the HBV lifecycle.97–99 NVR3-778 is the first in-class HBV core inhibitor to advance to clinical testing. In Phase 1a trial of healthy volunteers, oral dosing was well-tolerated at all dose levels (50–800 mg), and PK studies suggest that a once daily dose of ≥200 mg may be sufficient for prolonged HBV inhibition.100 NVR3-778 has now advanced to Phase 1b testing.

RNA interference technologies

This therapeutic approach aims to modulate the expression of viral proteins, such as HbsAg and HBeAg, which are believed to play a role in induction of T-cell exhaustion. This could potentially be achieved by using RNA interference-based therapeutics that target expression of specific viral RNAs.101 In a mouse model of HBV infection, it was recently shown that targeted delivery of HBV-specific small interfering RNA in hepatocytes lead to suppression of viral RNA, DNA and protein.102 Phase 1b and Phase 2 clinical trial studies are underway or planned for 2015 using RNAi technologies. Recent preliminary data with ARC-520, a novel small interfering RNA-containing, liver-targeted therapeutic that is designed to reduce all HBV transcripts was reported. The study assessed HbsAg reduction with ARC-520 vs. placebo in HBeAg-negative CHB patients receiving long-term ETV. In all cohorts to date, the percent HbsAg reduction was statistically significant vs. placebo. This is the first report, albeit preliminary, in CHB patients that a reduction in HBsAg can be mediated through RNA interference.103

HBV cccDNA inhibition/degradation

Degradation of cccDNA may be achieved by utilizing enzymes termed transcription activator-like effector nucleases (TALENs) that can cleave sequence-specific DNA targets. HBV cccDNA-specific TALENs were shown to significantly reduce cccDNA levels without apparent cytotoxic effect when introduced in HCC cell lines. In vivo, in mice hydrodynamically injected with monomeric full-length HBV DNA, introduction of TALENs resulted in a significant reduction of serum HbsAg, HBeAg and liver pregenomic RNA and enhanced the antiviral effects induced by IFN-a when used in combination therapy.104

Lucifora et al. recently published data showing that both IFN-alpha and lymphotoxin-b receptor (LTbR) activation in HBV-infected cells are capable of mediating degradation of cccDNA in infected hepatocytes without hepatotoxicity.105 LTbR signaling in HBV-infected cells lines, primary hepatocytes and human liver needle biopsies was shown to induce the upregulation of cytidine deaminases of the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide like) family of proteins that can specifically target the cccDNA for deamination and degradation. It is speculated that in terms of resolution of infection, moderate cccDNA deamination occurred even without IFN-alpha, providing a possible explanation for the low frequency of spontaneous clearance seen in chronically infected patients (2% of patients per year).106 As noted by Chisari et al.,107 LTbR agonists have been shown to trigger apoptosis, hepatocellular proliferation,
inflammation and HCC, raising significant safety considerations regarding regulatory approval of LTβR activation. Human studies are needed, regarding the use of agents directly targeting HBV cccDNA. However, if proven safe, and if these results can be independently confirmed, would represent a critical first step toward development of a virologic cure for CHB.

Summary

Although current highly potent anti-HBV NA such as TDF and ETV is life prolonging and can prevent disease complications, they are not curative. Many patients have virological relapse after NA cessation and HBsAg loss is rarely achieved. Moreover, CHB patients, even despite NA, remain at continued risk for HCC development. Novel drugs are needed to destroy the HBV cccDNA ‘master template’, and/or harness the ability of the host immune system against the virus.

While elimination of cccDNA from infected cells represents a desirable goal, it may not be necessary for the ‘functional or clinical’ cure of CHB, which may be as defined as robust immune control of the HBV despite low-level viral persistence. In fact, individuals with resolved acute HBV infection do not achieve complete viral eradication but are able to control the virus indefinitely without any signs of liver damage. A thought-provoking concept is to view CHB as an inflammatory rather than a viral infection, and thus not fixate on eradicating (killing) the virus, but rather aim to reduce intrahepatic inflammation. We believe that a true ‘cure’ (i.e. complete eradication of the HBV) for CHB will require combination therapy including suppression of viral replication, specific targeting of HBV cccDNA, as well as immunotherapies that improve T cell function allowing sustained off-treatment immunological control of viral infection.

Conflict of interest statement

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