Will we need novel combinations to cure HBV infection?

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Abstract
Chronic hepatitis B is a numerically important cause of cirrhosis and hepatocellular carcinoma. Nucleoside analogue therapy may modify the risk. However, maintenance suppressive therapy is required, as a functional cure (generally defined as loss of HBsAg off treatment) is an uncommon outcome of antiviral treatment. Currently numerous investigational agents being developed to either interfere with specific steps in HBV replication or as host cellular targeting agents, that inhibit viral replication, and deplete or inactivate cccDNA, or as immune modulators. Synergistic mechanisms will be needed to incorporate a decrease in HBV transcription, impairment of transcription from HBV genomes, loss of cccDNA or altered epigenetic regulation of cccDNA transcription, and immune modulation or immunologically stimulated hepatocyte cell turnover. Nucleoside analogue suppressed patients are being included in many current trials. Trials are progressing to combination therapy as additive or synergistic effects are sought. These trials will provide important insights into the biology of HBV and perturbations of the immune response, required to effect HBsAg loss at different stages of the disease. The prospect of cures of hepatitis B would ensure that a wide range of patients could be deemed candidates for treatment with new compounds if these were highly effective, finite and safe. Withdrawal of therapy in short-term trials is challenging because short-term therapies may risk severe hepatitis flares, and hepatic decompensation. The limited clinical trial data to date suggest that combination therapy is inevitable.

Keywords
antiviral therapy, Hepatitis B, nucleoside analogues, treatment of hepatitis B, viral eradication
Chronic hepatitis B is a numerically important cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide. HBV can cause HCC in the absence of cirrhosis, but most cases worldwide occur in patients with cirrhosis (70%-90%). Indications for antiviral therapy using nucleoside analogues or pegylated interferon (PEG IFN) have been expanding to include earlier treatment to prevent progression to cirrhosis. The prospect of cures of hepatitis B would ensure that a wide range of patients could be deemed candidates for treatment with new compounds if these were highly effective, finite and safe.

Nucleoside analogue suppression of HBV replication modifies the risk of HCC amongst patients with chronic hepatitis B. Nucleoside analogue therapy is associated with a considerable reduction in risk but not elimination of HCC in patients with chronic hepatitis B. In many cases long-term treatment is required, although treatment may be discontinued in a proportion. Finite, circumscribed courses of treatment with nucleoside analogues and functional cures (generally defined as loss of HBsAg off treatment) are an uncommon conclusion of antiviral treatment. Current HBV therapies suppress viral replication but do not generally cure the disease, as they do not eliminate cccDNA, or integrated viral genomes. Thus, most of patients with chronic hepatitis B require maintenance suppressive therapy. A complete sterilizing cure with viral eradication and elimination of all HBV gene products from the host is improbable at present.

2 | HBsAg CLEARANCE

Spontaneous clearance of HBsAg occurs infrequently at rates of 1% per year in treatment naïve adults with chronic hepatitis B. Typically, HBsAg loss has been observed in those with inactive disease and lower degrees of replication encountered in HBeAg-negative infection, and low quantitative HBsAg concentrations. However, HBsAg loss can occur after intense exacerbations of chronic disease in patients with high levels of HBV replication, (or during and after cessation of nucleoside analogue and PEG IFN therapy). Lower concentrations of HBsAg (<1000 IU/mL or lower, and HBV DNA concentrations < 2000 IU/mL) favour HBsAg loss. HBsAg loss in patients treated with nucleoside analogues is similarly unusual (<1%), but can modify the aftermath of disease. The rates are ostensibly higher in patients treated with PEG IFN or combinations of nucleoside analogues and PEG IFN. Baseline and on-treatment HBsAg levels that go some way to predicting loss of HBsAg and determine interferon therapy failure have been identified. Incident cirrhosis after HBsAg seroclearance is rare, if it has not developed beforehand. The incidence of HCC is reduced but not prevented if HBsAg loss is achieved. A large registry of treated patients in Hong Kong indicated that achieving HBsAg seroclearance reduced the risk of HCC to patients, compared to achieving complete viral suppression with prolonged nucleoside analogue treatment; the complications of cirrhosis were not however altered. Moreover, only 2.1% of patients lost HBsAg in this cohort during follow-up. The potential advantage of HBsAg loss may be lost, however, if it first occurs in patients > 50 years old with pre-existing cirrhosis.

3 | FUNCTIONAL CURES: ACHIEVING THE GOAL OF HBsAg LOSS WITH IMPROVED TREATMENTS

A functional cure is defined as sustained loss of HBsAg, with or without acquisition of anti-HBs and undetectable HBV DNA 6 months after completing treatment. This definition recognizes that HBV genomes are not cleared from the liver. Although a functional cure cannot be considered a true cure, because of persistent integrated viral genomes, HBsAg loss is a recognizable endpoint for ongoing clinical trials of new HBV treatments. HBsAg loss, and thus an improved clinical outcome could be obtained in a higher proportion of patients than is now possible with nucleoside analogues or PEG IFN. A reduction in the HBsAg antigen load could improve immunomodulatory strategies. Measurements of HBsAg are assays are standardized (if the sensitivity of detection and lower limits of quantification are defined). Once HBsAg loss is achieved, the rationale is that there will be no further need for therapy. Based on the observed effects of a reduction in HBV replication, it is hoped that a functional cure, achieved at a relatively young age, will prevent progression to cirrhosis and HCC. However, follow-up of patients with past chronic hepatitis B, especially those with cirrhosis, will be needed to confirm a long-term benefit.

4 | VIROLOGY OF HEPATITIS B AND PROSPECTS FOR A CURE

Hepatitis B virus (HBV) is a member of the hepadnaviridae virus family. The virion comprises an enveloped, 42 nm diameter DNA virus. The complete virion encloses a partially double stranded relaxed circular DNA (rcDNA) genome of 3200 base pairs.
life cycle of HBV involves several steps: viral entry, uncoating, nuclear import, transcription, nucleocapsid assembly reverse transcription and viral secretion from host hepatocytes. HBV enters hepatocytes through its receptor, sodium taurocholate cotransporting polypeptide (NTCP). After uncoating the nucleocapsid, relaxed circular DNA (rcDNA) is released, and imported into the nucleus. Replication commences by repair and conversion of the rcDNA to covalently closed circular DNA, the circular DNA minichromosome in the nucleus. (cccDNA). The stable episomal cccDNA acts as the template for transcription of HBV mRNAs for subsequent translation to the viral proteins. cccDNA is thought to be synthesized from rcDNA, derived from incoming virions, and replenished from intracellular nucleocapsids which shuttle double-stranded DNA genomes to the nucleus. Four distinct viral transcripts, the pre-genomicRNA (pgRNA), preS1, preS2 and HBx RNAs are synthesized and subsequently translated into seven viral proteins. Core protein (HBcAg) and the HBV polymerase are transcribed from pgRNA, while secretory e antigen (HBeAg) is transcribed from precore RNA. Two types of virions are secreted from the hepatocyte: a population of complete DNA containing virions containing mature nucleocapsids with the partially double-stranded, rcDNA genome and a larger population containing an empty capsid with no DNA or containing RNA.

HBV DNA genomic fragments integrate into the genome of hepatocytes, but it is not thought that integration is mandatory for replication of HBV. In addition to infectious virions, HBV replication results in the excess production and release of subviral empty envelope particles, devoid of viral capsid or genome. The high HBsAg antigen load found in persistent infection, particularly in HBeAg-positive patients may cause profound antigen-specific immune dysfunction and exhaustion.

HBsAg can be transcribed from ccc/DNA but in HBeAg negative patients the probable predominant source of HBsAg is from RNAs transcribed from integrant HBV DNA. This source of HBsAg is relatively inaccessible without cell loss. A better understanding of differences in the composition and source of subviral particles of HBsAg derived from covalently closed circular DNA vs integrated HBV DNA will assist therapeutic strategies.

5 | STRATEGIES TO PROMOTE HBsAg LOSS

HBsAg loss may be promoted by cessation of nucleoside analogues but the results vary and most patients relapse. The strategy cannot be used in patients with severe liver disease because of the risk of decompensation if the disease is exacerbated. Nucleoside analogues can be discontinued after HBeAg seroconversion, in patients with undetectable HBV DNA and consolidation with 12 months or more of additional treatment. The guidelines in HBeAg-negative patients differ. EASL guidelines indicate that nucleoside analogue treatment can be stopped in certain patients (without cirrhosis) who have had undetectable HBV DNA in blood for >3 years. AASLD guidelines advise indefinite treatment unless HBsAg is undetectable. Relapse is common and reports of HBsAg loss vary considerably. Numerous studies have summarized the variability in response in heterogenous groups of patients. Rates of HBsAg loss also vary considerably in prospective studies. Combinations of PEG IFN and nucleoside analogues result in higher rates of HBsAg loss than nucleoside analogues therapy alone. IFN alfa decreases cccDNA transcription via epigenetic modification in experimental systems.22

Several steps in the replication of HBV are potential drug targets. Numerous agents are under development as immune modulators, to interfere with specific steps in HBV replication or as host targeting agents that inhibit viral replication by modifying host cellular function, or as immune modulators. Combination strategies will likely invoke deepening the inhibition of HBV replication, lowering viral antigen concentrations (particularly HBsAg) and enhancing the immune response. The investigational treatment landscape is discussed elsewhere in this issue. These potential treatments include targeted HBV entry inhibitors, inhibitors of cccDNA formation, inducers of cccDNA cleavage or transcription inhibition and epigenetic modifiers, core and capsid inhibitors, or perturbations of capsid morphogenesis, RNA interference therapies, HBsAg interaction and assembly or release inhibitors, and multiple immunomodulatory agents including toll like receptors agonists, immune checkpoint inhibitors, therapeutic vaccines, immunological engineering of T cells and cytokines including pathogen receptor agonists. HBX protein, a regulatory protein, transcribed from HBx RNA enhances cccDNA transcription and is an attractive viral target to silence cccDNA transcription.

6 | CURRENT STUDIES OF COMBINATION ANTIVIRAL THERAPY

While HBsAg loss remains an attractive goal, the target patient populations for antiviral and immunomodulatory trials and the appropriate sequence of treatment in patients at different stages of the disease are unknown. The unique virology of HBV poses difficulties for cure: HBV genomic fragments integrate into the genome of the hepatocyte. HBsAg can be transcribed from both cccDNA and integrated viral genomes. The latter source of HBsAg is relatively inaccessible without cell loss of DNA editing. HBsAg antigen load may causes profound antigen specific immune dysfunction and exhaustion. Nonetheless, several steps in the replication cycle are druggable targets and numerous investigational agents that interfere with specific steps in HBV replication or host cellular targeting are being tested in clinical trials. The evidence is still being gathered, however both HBeAg-positive and HBeAg-negative patients are being included in current trials. Empirical trial design and adaptive methodology are being applied to clarify appropriate combinations until we acquire insights into the mechanisms that determine response. Synergistic mechanisms will probably be needed to achieve a decrease in HBV transcription, impairment of transcription from HBV genomes, loss of cccDNA or altered epigenetic regulation of
common adverse effect.

Gastrointestinal symptoms were the most frequent study-related adverse event. Across all dose levels, 12/30 (40%) patients 24 weeks after 48 weeks of treatment. HBsAg clearance of new therapies: several cytokines including IFNalpha and lymphokines beta receptor agonists lead to upregulation of APOBEC3A and capsid shuttling.

Clinical trials are evaluating combination therapy. Sequential combinatorial therapy ideally should result in a rapid and precipitous decrease in HBsAg to precede immunomodulatory therapy. The effect of profound reductions in HBsAg upon restoration of immune reactivity, perhaps coupled with immunomodulatory therapy will be of interest, but restoration of immune reactivity has not yet been proven. Nonetheless, GalNac small interfering RNA knockdown of HBsAg in transgenic mice has provided a sophisticated proof of concept.

New capsid assembly modulators (CpAMs) (reviewed elsewhere in this edition) definitively deepen inhibition of HBV replication by disrupting early and late stages of HBV replication. Class I CpAMs induce capsid formation of disrupted morphology. The classification and terminology of CpAMs is currently being reviewed: broadly one class of CpAMs allow the assembly of morphologically normal capsids which are devoid of pgRNA, i.e. empty capsids. A second class results in the formation of aberrant capsids. CpAMs inhibit encapsidation of pgRNA and may inhibit both cccDNA formation or replenishment via inhibition of the capsid uncoating step after viral entry and capsid shuttling. These agents result in a decrease in HBV DNA and HBV RNA. Current trials are examining the efficacy of dual therapy with CpAMs plus a nucleoside analogue: The combination of ABI-HO731 (300 mg) plus entecavir for 24 weeks deepened HBV DNA and HBV RNA suppression in both naïve and nucleoside analogue treated patients. Encouragingly, ARC-520 given as a single dose in combination with entecavir resulted in decreases in HBV DNA in HBeAg-positive and HBeAg-negative patients and a decrease in HBsAg concentrations in HBeAg-negative patients. The initially observed lack of effect in HBeAg-negative patients, (because of truncated viral sequences derived from integrated HBV genomes) has been countered by re-designed siRNAs that target all HBV transcripts. GalNac delivery improves uptake.

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integrated HBV DNA, may prove promising. JNJ73763989 (previously ARO-HBV) given subcutaneously to both HBsAg-positive and negative patients, and to nucleoside analogue experienced or naïve patients has been assessed in a recent study.\(^{49}\) Patients received three subcutaneous doses of 25, 50, 100, 200, 300 or 400 mg JNJ73763989 every 4 weeks on days 1, 27 and 57; treated patients continued nucleoside analogue therapy beyond the end of the JNJ73763989 dosing. At day 113 the mean HBsAg \(\log_{10}\) reduction from day one ranged from 1 to 1.75. In interim data the proportion of patients who achieved a greater than 1 \(\log_{10}\) reduction in HBsAg from day one at nadir ranged from 4/8 of those given 25 mg to 8/8 of those receiving higher doses. Thus 97% of patients (31/32) achieved a greater than 1 \(\log_{10}\) reduction in HBsAg. Evaluation is ongoing. In an exploratory triple combination trial, RNAi JNJ73763989, was given by subcutaneous injection together with a CpAM (JNJ-56136379, at a dose of 250 mg orally daily) for 12 weeks to HBsAg positive and negative patients on entecavir or tenofovir. The combination resulted in a mean 1.7 log change in HBsAg from day 1.\(^{49}\)

Nucleic acid polymers, which block assembly and release of subviral particles, followed by pegylated interferon or with tenofovir, led to reductions in HBsAg (and anti-HBs development). The exact mode of action is not clear but accelerated intracellular degradation of HBsAg is a putative possibility.\(^{50-54}\) New classes of phosphoramide nucleic acid polymers (STOPs) such as ALG-10,093 are a class of potent oligonucleotides that appear to reduce HBsAg secretion possibly by affecting protein trafficking perhaps resulting in putative degradation of HBsAg protein.\(^{55}\)

Mechanisms to correct undetectable or weak HBV specific CD8 + T cell response and T cell exhaustion- probably as a result of chronic antigenic stimulation and loss of T cell effector function- are being sought. Immunotherapeutic strategies, (including interferon) either used alone, de novo or in combination with antiviral agents currently include TLR-7 and TLR-8 agonists, therapeutic vaccines, checkpoint modulators, a RIG-I agonist and anti-HBV antibodies. However serious adverse events have recently been reported in patients receiving inarigivir.\(^{56}\) In a woodchuck model blocking PD-L1 together with HBV therapeutic vaccination reduced HBV DNA concentrations.\(^{57,58}\) Unfortunately it has proven more difficult to validate the efficacy of immunomodulator therapies in human studies.\(^{59-65}\) A recent combination of a TLR7 agonist RO7011785 in 3 cohorts of chronic hepatitis B patients, including virologically suppressed patients has been primarily designed to show the safety of the combination. The combination shows evidence of immune activation but the short 6 week dosing period to date does not allow an effect on HBsAg pharmacodynamics as yet. Longer duration studies are planned.\(^{66}\) GS –9688, an oral selective small molecule agonist of TL8R8 has been evaluated in a phase 2 study of 48 HBeAg-positive or negative patients suppressed on nucleoside analogue therapy. 3.0 mg, 1.5 mg or GS-9688 placebo were given once a week for 24 weeks in combination with nucleoside analogues. One HBeAg negative patient in the study (1.5 mg group) achieved the primary endpoint of \(\geq 1\) log10 IU/ml decline in HBsAg concentrations at week 24 and two other patients in the study (HBeAg-negative and positive) achieved HBsAg loss at week 24.\(^{67}\)

### 7 | EXPERIMENTAL COMBINATIONS

The combination of a liver targeted HBV locked nucleic acid antisense oligonucleotide with RO7020531, a TLR7-agonist in the AAV HBV mouse model in mice treated for 8 weeks showed that the combination reduces HBsAg and HBV DNA concentrations compared to monotherapy. The combination also delayed rebound for several weeks after the end of treatment.\(^{68}\) Treatment of HBV infected HepG2 2-NTCP cells with HBV specific siRNA inhibited HBV replication and suppressed HBV antigen production. The reduced antigen production initially suppresses CDb T cell recognition, but CDb T cell recognition shows evidence of subsequent recovery after siRNA treatment.\(^{69}\) A hypothesis has been proposed that a liver targeted PD-L1 locked nucleic acid antisense oligonucleotide can result in effective suppression of PDL1 expression in the liver with the potential to overcome the immune tolerance observed in hepatitis B virus infection.\(^{70}\)

Experimental combination therapy with a CaPM (Bay41-4109 alone or combination with IFN alpha activates an innate immune response in HBV infected HepG2-hNTCP cells, primary human hepatocytes and human liver chimeric TK-NOG mice.\(^{71}\)

**New putative serum biomarkers** of infection include HBV core related antigen (HBcrAg), theoretically comprising hepatitis B core antigen, HBeAg and the 22 kDa precore protein encoded by the precore-core gene.\(^{72}\) HBV RNA is detectable in serum. Therefore, HBcrAg or HBV RNA may be useful viral markers that are independent of HBV DNA for monitoring the antiviral effect of nucleoside analogues. These biomarkers can remain detectable in serum in patients with undetectable HBV DNA; HBcrAg and HBV RNA may correlate with cccDNA and provide an important clinical surrogate and marker of transcriptional activity of cccDNA; reductions in pgRNA result in a reduction in subsequent viral reverse transcription.

### 8 | CONCLUSIONS

New compounds are currently limited to clinical trials and proof of mechanism and safety studies. Strategies with a combination of agents and additive or synergistic effects being sought. These agents will provide important insights into the biology of HBV and perturbations of the immune response, required to effect HBsAg loss at different stages of the disease. New combination therapies for HBV will require individualization but broad eligibility is sought. The safety of new therapies will be paramount given the safety of currently approved nucleos(t)ide analogues. Patients with cirrhosis are being excluded from early phase trials. Harm vs benefit in young adults will require careful consideration. Withdrawal of therapy in short-term trials is challenging because short-term therapies may have the risk of severe hepatitis flares, hepatic decompensation or
death. Therapies in development that rely on altering CD8 T cell recognition will require a deeper understanding of the effects of new inhibitory compounds and interactions between hepatocyte antigen expression, inflammatory cytokines and adaptive immune responses. Combination treatments appear to be an unavoidable strategy for improving functional cure. The cost of combination curative treatments may become problematic in low- and middle-income countries, given the low cost of generic nucleoside analogues and the minimal monitoring required.

CONFLICT OF INTEREST
None declared.

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