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Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure

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Around 257 million people worldwide have chronic hepatitis B virus (HBV) infection, which leads to almost 1 million deaths per year from complications, mainly decompensated cirrhosis and hepatocellular carcinoma. The development of effective treatments for hepatitis C virus has led to hope for a cure for HBV. Current treatments for HBV infection include pegylated interferon-alfa, which is associated with modest efficacy and poor tolerability, or nucleoside analogues, which require lifelong administration and rarely achieve hepatitis B surface antigen (HBsAg) loss. Understanding the HBV lifecycle is essential to develop new approaches, since each step is a potential target for drug development. New direct-acting antivirals for HBV in development include entry inhibitors, capsid assembly modulators, and drugs targeting cccDNA or HBV RNA, and HBsAg secretion inhibitors. In this Review, we discuss potential targets and direct-acting antiviral approaches in development.

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Introduction

About a third of people worldwide have been exposed to hepatitis B virus (HBV),[1] and around 257 million people are living with HBV infection (defined as being hepatitis B surface antigen [HBsAg]-positive). In 2015, HBV infection resulted in 887 000 deaths, mostly from complications including cirrhosis and hepatocellular carcinoma.[2,3] Although a prophylactic vaccine is available, vaccination campaigns are not well-implemented and new infections still occur.

Several guidelines have been published for management of patients with HBV infection.[4–6] Current treatments for HBV include pegylated interferon-alfa or antiviral nucleoside analogues. Peginterferon-alfa 2a has the advantage of inducing sustained response after a defined course of treatment, but rates of response are low and treatment has a poor safety profile. The nucleoside analogues tenofovir disoproxil fumarate (TDF), tenofovir alafenamide, and entecavir are currently the most potent drugs to suppress HBV. These drugs are also associated with little drug resistance, are easy to take orally, have few side-effects, and require little monitoring. However, they require lifelong administration because they do not eliminate the viral genome that persists in infected hepatocytes.[7]

In addition, access to diagnosis and treatment of HBV is limited in many resource-constrained settings. In 2015, 22 million (9%) of the 257 million people living with chronic HBV infection knew their diagnosis.[2] Of the 22 million people diagnosed, only 8% (1·7 million people) were receiving treatment. Many people are diagnosed only when they already have advanced liver disease.

Therefore, there is an urgent need to develop new limited-course therapies that can cure HBV infection. There has been fantastic enthusiasm following the development of effective treatments for hepatitis C virus, leading to increasing hope for a cure for HBV.[8]

HBV virology and potential targets for new direct-acting antivirals

HBV is a small non-cytopathic enveloped DNA virus of the Hepadnaviridae family.[9] HBV infects hepatocytes, where it establishes its replication cycle and persists in the nucleus. The HBV virion is a lipid-based spherical structure measuring approximately 42–47 nm in diameter, on which three different HBsAg are exposed: the small (S), medium (M), and large (L) viral envelope proteins (figure 1A).[9] The large envelope protein contains the receptor binding domain that is involved in viral entry.[10] At infection, this receptor binding domain interacts with human sodium taurocholate co-transporting polypeptide receptor (hNTCP or SLC10A1), which is expressed on hepatocytes.[11] This interaction allows viral entry into hepatocytes by endocytosis, and the release of HBV nucleocapsid into cytoplasm.[12] Targeting HBV entry by preventing the interaction with NTCP receptor or via another step is an attractive therapeutic strategy to prevent infection.

The nucleocapsid, formed by dimers of HBV core proteins (HBc), contains a partly double-stranded DNA genome in relaxed circular conformation (rcDNA).[13] The circular conformation is the result of short cohesive overlapping sequences at the 5′ ends of two viral DNA strands (figure 1B). This rcDNA is about 3·2 kb in length and is covalently linked to HBV polymerase at the 5′ of the minus DNA strand. At the 5′ end of the plus DNA strand there is a RNA primer derived from pre-genomic RNA (pgRNA), important for reverse transcription. In the cytoplasm of infected cells, the nucleocapsid is transported to the nuclear membrane by microtubule-mediated transport and the rcDNA is released into the nucleus.[14] The HBV lifecycle is represented in figure 2A.

rcDNA can integrate into the host genome and lead to cancer development via genomic alterations.[15] This integration does not play a direct role in the HBV replication cycle, but can serve as a template for HBsAg secretion.[16] rcDNA is converted by the host DNA repair machinery into episomal DNA called covalently closed circular DNA (cccDNA).[17] cccDNA binds to host histones and forms a stable mini-chromosome that acts as a transcriptional template for all viral transcripts.[18]

The HBV genome contains four overlapping open reading frames that produce five viral transcripts using

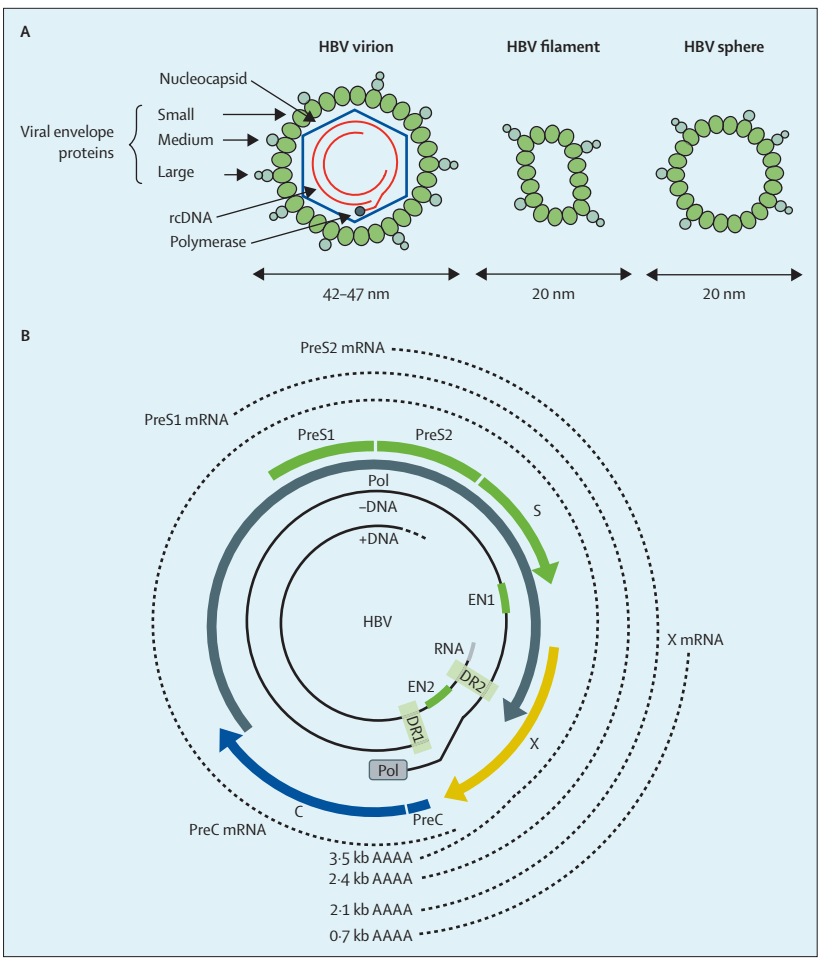

Figure 1: **HBV virion structure (A) and genome organisation (B)**
HBV=hepatitis B virus. rcDNA=relaxed circular DNA. PreC=pre-capsid. PreS1=pre-surface 1. PreS2=pre-surface 2. Pol=HBV polymerase. EN1=enhancer element 1. EN2=enhancer element 2. DR1=direct repeat 1. DR2=direct repeat 2. X=HBV regulatory x protein. C=capsid:HBc. S=surface. ProC=precore.

the human RNA polymerase II (preC mRNA, pgRNAs, PreS1 mRNA, PreS2 mRNA, and X mRNA), which are translated due to alternative splicing of the pgRNAs into seven viral proteins using the cell's machinery (figure 1B).

After the translation of viral proteins, HBV polymerase interacts with the epsilon structure of 5′ pgRNA and, at the same time, core proteins interact with pgRNA, which triggers packaging to new viral capsids. Some capsid assembly inhibitors are in development. The encapsidated pgRNA is retro-transcribed into rcDNA and serves as a template for the minus DNA strand synthesised by viral polymerase (the plus DNA strand is synthesised later). The nucleocapsid can return to the nucleus and be recycled to amplify the cccDNA pool and contribute to cccDNA persistence. Alternatively, nucleocapsids join the endoplasmic reticulum where HBsAg accumulates, and are excreted out of the cells by the secretion pathway. The excess production of HBsAg results in secretion of subviral non-infectious particles that plays an important role in immune evasion. Understanding the HBV cycle is of great importance, since each step is a potential target for drug development (figure 2B).

Candidates for future treatments: which patients should be treated?

With current therapies, all patients with HBeAg positive or negative chronic hepatitis B, defined by HBV DNA more than 2000 IU/mL, alanine aminotransferase (ALT) above the upper limit of normal, or at least moderate liver necroinflammation or fibrosis, are candidates for treatment. Also, patients with compensated or decompensated cirrhosis, and those with a family history of hepatocellular carcinoma, are also candidates for therapy. Patients with HBeAg-negative chronic HBV infection, previously termed inactive carriers, are characterised by the presence of serum antibodies to HBeAg (anti-HBe), undetectable or low (<2000 IU/mL) HBV DNA and normal ALT. These patients have good prognosis and are not candidates for treatment. However, they need lifelong follow-up, as there is a risk of reactivation, and they are at risk of developing extrahepatic manifestations. Concerns regarding HBV DNA integration have been raised, and relatively abundant integrant-derived viral RNAs have been reported in tissue harvested from chronic HBV carriers.[19] In the first step of drug development, these patients might not be candidates for therapy. However, an HBV treatment with high efficacy and favourable safety could be a treatment for all HBV-infected individuals.

Patients with extrahepatic HBV-related manifestations are also candidates for therapy. Several extrahepatic complications are associated with chronic HBV infection, including polyarteritis nodosa and vasculitis; glomerulonephritis; and cutaneous, neurological, and haematological complications. These extrahepatic manifestations can be associated with substantial morbidity and mortality. Awareness and recognition of these manifestations is important to allow early diagnosis and treatment, which can be highly successful. Patients with HBV infection might have comorbidities (eg, alcohol use, non-alcoholic steatohepatitis, diabetes) that can contribute to liver disease progression, which must also be considered and managed.

Endpoints for clinical trials

The primary treatment goals for patients with HBV infection are to increase survival and to prevent progression of the disease, which can manifest as cirrhosis, liver failure, or hepatocellular carcinoma (figure 3). Treatment goals can be achieved by sustained suppression of HBV replication associated with normalisation of serum ALT concentration, thereby reducing the histological activity of chronic hepatitis B and reducing the risk of fibrosis progression. Blocking HBV replication is crucial but must be maintained after treatment if optimal outcomes are to be achieved. Virological response during nucleotide

analogue therapy is defined as undetectable HBV DNA by a sensitive PCR assay with a limit of detection of 10 IU/mL.[4–6] With pegylated interferon-alfa, virological response is defined as serum HBV DNA less than 2000 IU/mL, usually assessed 6 months after the start of treatment, and again at the end of therapy. Sustained off-therapy virological response is defined as serum HBV DNA less than 2000 IU/mL for at least 12 months after the end of therapy.

Ideally, the aim of treatment would be to obtain a serological response with HBsAg loss and HBsAg seroconversion—ie, a functional cure—which is associated with an excellent prognosis. The optimal endpoint of therapy is HBsAg loss, and treatment can be stopped in patients without cirrhosis if this endpoint is achieved.

In immunocompetent adults, the usual outcome of HBV infection is recovery from an episode of acute self-limited HBV infection, which represents functional cure, rather than complete or virological cure, and lifelong protective immunity. When chronic infection is established, HBsAg seroconversion (loss of HBsAg and development of anti-HBs) is rarely observed, possibly due to induction of tolerance and impaired immune response. The annual incidence of HBsAg seroconversion is 1–2% worldwide.[20]

HBsAg seroclearance is one of the most important endpoints of chronic HBV infection since it is associated with a reduced risk of hepatocellular carcinoma compared with patients who are HBsAg-persistent carriers. A systematic review and meta-analysis[21] provided precise estimates of HBsAg seroclearance rates between subgroups and populations. The authors found a low rate of HBsAg seroclearance in untreated and treated patients (pooled annual rate approximately 1%). In a population-based prospective study,[22] eight (1·2%) of 652 participants with HBsAg seroclearance developed hepatocellular carcinoma over a 9-year surveillance programme. The cumulative hepatocellular carcinoma incidence rate was significantly lower in patients with HBsAg seroclearance than that in HBsAg-persistent carriers but was slightly higher than that in HBsAg-negative participants. Moreover, the risk of hepatocellular carcinoma and hepatic events were compared in nucleos(t)ide analogue-treated patients with and without HBsAg seroclearance.[23] Among 20 263 nucleos(t)ide analogue-treated patients with chronic hepatitis B, patients with nucleos(t)ide analogue-induced HBsAg seroclearance on top of complete viral suppression had a lower risk of hepatocellular carcinoma (but not hepatic events) than those only achieving complete viral suppression with long-term nucleos(t)ide analogue treatment. Understanding how these patients recovered and how HBsAg seroclearance was achieved will be important to develop new treatment strategies.

Future treatments should allow the potential for immune-mediated control of HBV infection, with higher rates of HBe seroconversion and the possibility of off-treatment viral suppression, with loss of HBsAg. HBsAg loss (with or without seroconversion) is considered the ultimate goal of therapy.

Elimination of cccDNA would be an indicator of completely cured HBV infection. However, liver biopsy is

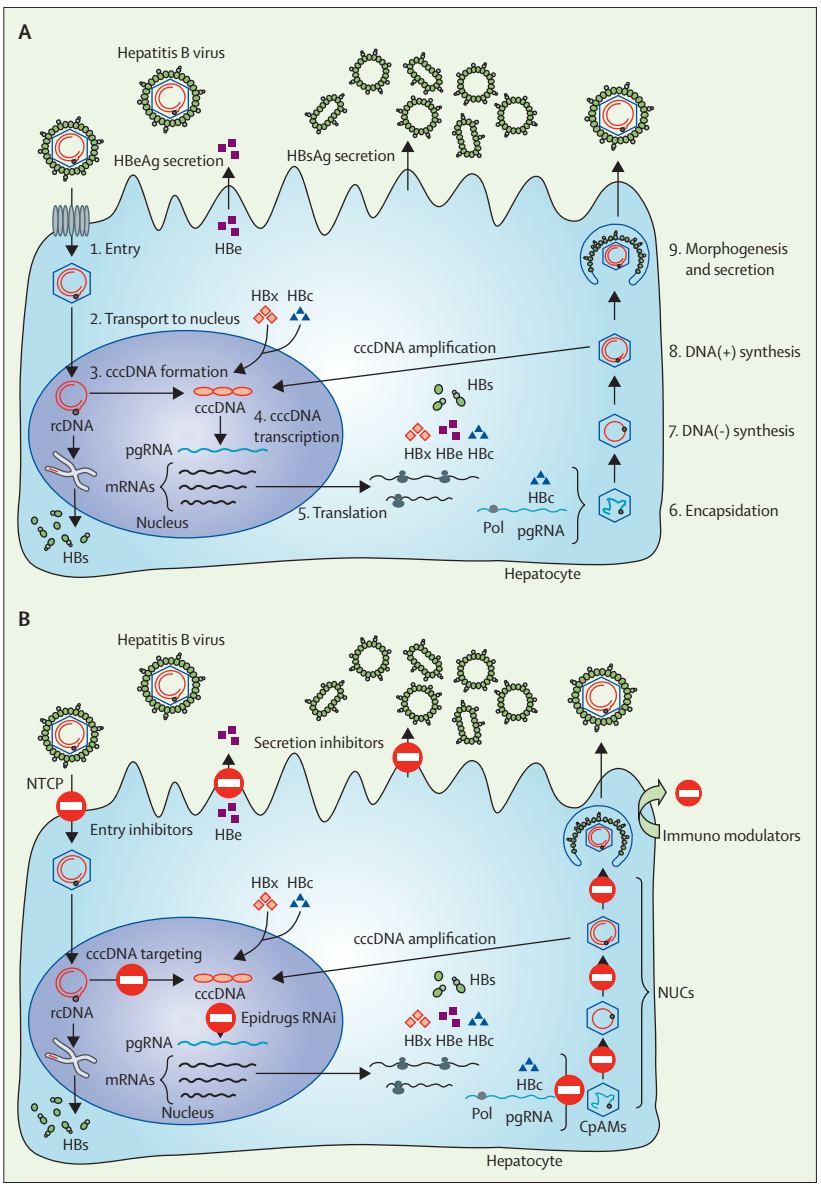

Figure 2: **Lifecycle of HBV and therapeutic targets (antivirals in development)**
(A) Lifecycle of HBV: (1) HBV infects hepatocytes after binding to the NTCP receptor. (2) HBV nucleocapsid is transported to nucleus by cellular motors and rcDNA is delivered into the nucleus. rcDNA can be integrated into the human genome. (3) rcDNA is converted into cccDNA by host factors and forms a stable mini-chromosome. (4) cccDNA serves as a template for all viral RNAs (5), which are translated into HBV proteins. (6) pgRNA, linked to HBV polymerase, is packaged into capsid particles. pgRNA serves as a template for rcDNA synthesis, first the minus DNA (7), then the plus DNA (8). The nucleocapsid can be recycled into the nucleus and amplifies cccDNA. (9) Morphogenesis and secretion of HBV virions and non-infectious subviral particles follow the cellular assembly and the secretion pathway. (B) Schematic representation of therapeutic strategies against HBV. All replication steps can be potentially targeted to inhibit HBV replication. HBV=Hepatitis B virus. NTCP=sodium-taurocholate cotransporting polypeptide receptor. HBeAg=hepatitis B e antigen. HBsAg=hepatitis B surface antigen. cccDNA=covalently closed circular DNA. RNAi=RNA interference. rcDNA=relaxed circular DNA. pgRNA=pregenomic DNA. HBs=HBV surface antigen. HBe=HBV e antigen. Pol=HBV polymerase. NUCs=nucleos(t)ide analogue. CpAMs=capsid assembly modulators.

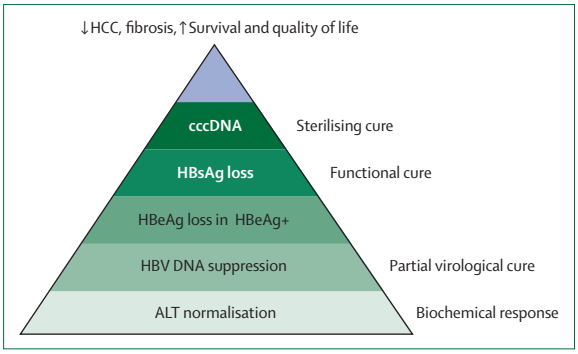

Figure 3: **Treatment goals for patients with HBV infection**
HCC=hepatocellular carcinoma. cccDNA=covalently closed circular DNA. HBeAg=hepatitis B e antigen. ALT=alanine aminotransferase.

	Stage of development	Compound	Company	ClinicalTrials.gov
Entry inhibitors (HDV-HBV coinfection)	Phase 2	Bulevirtide (previously Myrcludex)	Myr GmbH	NCT02888106
Capsid assembly modulators	Phase 2	JNJ-56136379	Janssen	NCT03361956
	Phase 2	GLS4	HEC Pharma	NCT03638076
	Phase 1b/2a	ABI-H0731	Assembly	NCT03576066
	Phase 1	RO7049389	Roche	NCT02952924
	Phase 1	AB-506	Arbutus	NCT02631096
siRNA	Phase 2	ARB-1467	Arbutus	NCT02631096
	Phase 1/2a	ARO-HBV	Arrowhead	NCT03365947
	Phase 1	RO7062931	Roche	NCT03038113
	Phase 1	VIR-2218	Alnylam and Vir	NCT03672188
Antisense oligonucleotide	Phase 2	GSK3389404	Ionis Pharmaceuticals with GlaxoSmithKline	NCT03020745
	Phase 2	GSK3228836		NCT02981602
HBsAg secretion inhibitors	Phase 2	REP 2139-Mg+REP 2165-Mg	Replicor	NCT02565719
	Phase 2	REP 2165-Ca	Replicor	NCT02876419

siRNA=small interfering RNA. HBsAg=hepatitis B s antigen. HBV=hepatitis B virus. HDV=hepatitis D virus.

Table: **Direct-acting antiviral approaches in development**

needed to measure cccDNA, which confers mortality and morbidity risks. Furthermore, there is no standard method to measure cccDNA.[24] Since quantifying cccDNA requires an invasive procedure, serum biomarkers reflecting intrahepatic cccDNA activity are needed. Data suggest[25] that circulating HBV RNA could serve as a surrogate marker for cccDNA. Even though methods of serum HBV RNA detection vary from study to study, and sensitivity must be improved, it has shown promising preliminary results.

Hepatitis B core-related antigen (HBcrAg) is a biomarker consisting of several antigens expressed from the precore or core gene: HBcAg, HBeAg, and prec22 precursor protein.[26] Serum HBcrAg could partly reflect intrahepatic DNA and cccDNA in hepatocytes, in particular in HBeAg-positive patients.[27] HBcrAg has also been used to monitor treatment and predict therapeutic efficacy.[28] Some data[29] suggest an association between serum HBcrAg concentrations and intrahepatic cccDNA, its transcription, and necroinflammation and fibrosis.

Serum HBsAg concentration has been shown to reflect active intrahepatic cccDNA and to have additional value as a marker of on-treatment efficacy, although the correlation is not strong.[30] There is an association between serum HBsAg concentration and the intrahepatic concentration of cccDNA, with the highest concentrations occurring in patients with HBeAg-positive hepatitis B and the lowest in patients with resolved hepatitis.[31–33]

The combined use of HBsAg and HBV RNA quantification with HBV DNA measurements could help to predict treatment outcome and to monitor the effects of therapy. Monitoring serum HBsAg concentrations in patients with HBV infection during treatment could provide substantial complementary information to HBV DNA measurements, although this is complicated because HBsAg could also be produced by integrated HBV DNA.[16]

Direct-acting antiviral approaches in development

Several direct-acting antiviral approaches are in development. Direct-acting antiviral approaches with new mechanisms of action might have the capacity to decrease cccDNA and HBsAg, leading to the possibility of immune restoration. Therefore, some compounds could have a dual role: direct-acting antiviral approaches and host immune restoration. DAAs under development are shown in the table.

Entry inhibitors

Bulevirtide (previously Myrcludex [MYR, Burgwedel]) is a synthetic lipopeptide that is derived from pre-S1 domain of HBV envelope protein.[34] Since bulevirtide contains the NTCP-binding pre-S1 domain of HBV envelope protein, it competitively binds to NTCP, the major functional receptor for HBV entry into hepatocytes, thereby inhibiting attachment of HBV to NTCP. Bulevirtide monotherapy for 24 weeks induced serum hepatitis D virus (HDV) RNA decline in an ongoing phase 2 trial (MYR202, NCT03546621) of patients with HBV and HDV coinfection, without affecting HBsAg.[35] In this study, 60 HBeAg-negative patients with chronic co-infection with HBV and HDV were randomly assigned to four groups. Patients received 180 µg pegylated interferon-alfa once per week, subcutaneous 2 mg bulevirtide once per day plus pegylated interferon-alfa, 5 mg bulevirtide once per day plus pegylated interferon-alfa, or 2 mg bulevirtide once per day alone for 48 weeks. Bulevirtide was well tolerated with mild to moderate drug-related adverse events mainly caused by increases in total bile acids. There was no pruritus. Most reported adverse events were deemed to be related to pegylated interferon-alfa. No serious adverse event was reported during the treatment period. At week 48, serum HDV RNA declined in all bulevirtide groups. At week 48, HDV RNA was undetectable in two (13%) of 15 patients

receiving pegylated interferon-alfa alone, 10 (67%) of 15 patients receiving 2 mg bulevirtide plus pegylated interferon-alfa, eight (57%) of 14 patients receiving 5 mg bulevirtide plus pegylated interferon-alfa, and two (14%) of 14 patients receiving bulevirtide alone. ALT normalisation at week 48 was pronounced in patients receiving bulevirtide alone (10 [71%] of 14) compared with four (29%) of 14 in patients receiving pegylated interferon-alfa alone, four (27%) of 15 in patients receiving 2 mg bulevirtide plus pegylated interferon-alfa, and six (40%) of 15 in patients receiving 5 mg bulevirtide plus pegylated interferon-alfa. Remarkably, HBsAg declined by more than 1 $\log_{10}$ IU/mL in seven (47%) of 15 patients receiving 2 mg bulevirtide plus pegylated interferon-alfa and in three (21%) of 14 patients receiving 5 mg bulevirtide plus pegylated interferon-alfa. No HBsAg change was observed under monotherapy. Eight paired biopsies available from patients receiving bulevirtide alone showed intrahepatic HDV RNA decline of median 1·80 $\log_{10}$ IU/mL and reduction in necro-inflammation (six [75%] of eight) and in liver fibrosis (four [50%] of eight) at week 48. A median 2·4 $\log_{10}$ IU/mL HDV RNA reduction was observed in the combination groups (n=5). All groups showed strong reduction of Hepatitis Delta Antigen (HDAg)-positive cells.[35]

The authors concluded that administration of bulevirtide for 48 weeks alone and in combination with pegylated interferon-alfa was safe. Combination therapy showed a strong synergism on HDV RNA decline and induced profound HBsAg declines in many patients. A lower dose of bulevirtide might have higher efficacy when combined with pegylated interferon because of a synergistic effect with interferon, with higher doses of interferon stimulating gene induction than with higher bulevirtide doses.

This study[35] provides the first evidence that entry inhibition by bulevirtide in combination with pegylated interferon-alfa has curative potential for chronic HDV and HBV infection. Use of this class of inhibitors might protect hepatocytes from new HBV infection.

Capsid assembly modulators

The HBV core protein is involved in several steps of the HBV lifecycle and is an attractive target for direct-acting antiviral approaches; a number of capsid assembly modulators are in development (table). JNJ-56136379 (Janssen, Ringsakiddy, Ireland) binds to the HBV core protein and disrupts early and late-stage processes in the HBV lifecycle.[36] JNJ-56136379 has a dual mechanism of action: the primary mechanism, which acts at the late stage of the HBV lifecycle, involves interference with capsid assembly kinetics, prevention of (pg)RNA encapsidation, and blockade of HBV DNA production. In primary human hepatocytes, JNJ-56136379 acts via its primary mechanism of action at low concentrations. The secondary mechanism of action, which functions at the early stage of the HBV lifecycle, involves inhibition of the formation of cccDNA. This secondary mechanism of JNJ-56136379 is believed to require higher concentrations of drug and an increased duration of treatment to affect HBsAg production.

JNJ-56136379 HPB1001 (NCT02662712) is an ongoing phase 1b global, randomised, double-blind, placebo-controlled study to evaluate the safety, tolerability, pharmacokinetics and efficacy of JNJ-56136379 in treatment-naive patients with chronic hepatitis B.[36] In part two of the study, four different oral doses of JNJ-56136379 or placebo were evaluated in treatment-naive patients with chronic hepatitis B without cirrhosis. At each dose, 12 patients were treated for 4 weeks and followed up for 8 weeks. All four doses of JNJ-56136379 were efficacious, with HBV DNA decreases after 4 weeks of treatment. After discontinuation of treatment, patients returned to baseline HBV DNA. No relevant changes in HBsAg or HBeAg were observed with any of the doses studied. Baseline HBV RNA generally decreased, although baseline HBV RNA was low (3·37–5·59 $\log_{10}$ IU/mL). All four doses tested were safe and well tolerated. This treatment showed potent antiviral activity by reducing HBV DNA and HBV RNA, and displayed dose-proportional pharmacokinetics. A phase 2a study (JADE; NCT03361956) is ongoing in treatment-naive and virologically suppressed patients with chronic hepatitis B, both HBeAg-positive and HBeAg-negative, at 75 mg and 250 mg per day doses, both alone and in combination with nucleos(t)ide analogues.

NVR 3-778 (Janssen, Ringsakiddy, Ireland) is an HBV capsid assembly modulator that can inhibit HBV replication. A phase 1 study (NCT02401737) was done in 73 HBeAg-positive patients with chronic HBV infection without cirrhosis.[37] Patients were randomly assigned to receive oral NVR 3-778 at increasing doses or placebo for 4 weeks. Additional groups received combination treatment with pegylated interferon and NVR 3-778 (600 mg twice daily) or pegylated interferon with placebo. Reductions in serum HBV DNA and HBV RNA were observed in patients receiving NVR 3-778 1200 mg/day or more. The largest mean reduction in HBV DNA was observed in the group given NVR 3-778 plus pegylated interferon (1·97 $\log_{10}$ IU/mL), compared with the groups given NVR 3-778 (1·43 $\log_{10}$ IU/mL) or pegylated interferon alone (1·06 $\log_{10}$ IU/mL). The mean reduction in HBV RNA was also greatest in the group given NVR 3-778 plus pegylated interferon (2·09 $\log_{10}$ copies per mL), compared with the groups given NVR 3-778 (1·42 $\log_{10}$ copies per mL) or pegylated interferon alone (0·89 $\log_{10}$ copies per mL). No significant mean reduction in HBsAg was reported during the 4-week treatment period. There were no discontinuations and no pattern of dose-related adverse effects with NVR 3-778.

Final results of a phase 1b 28-day study of ABI-H0731 (Assemblybio, San Francisco, CA, USA), exploring the

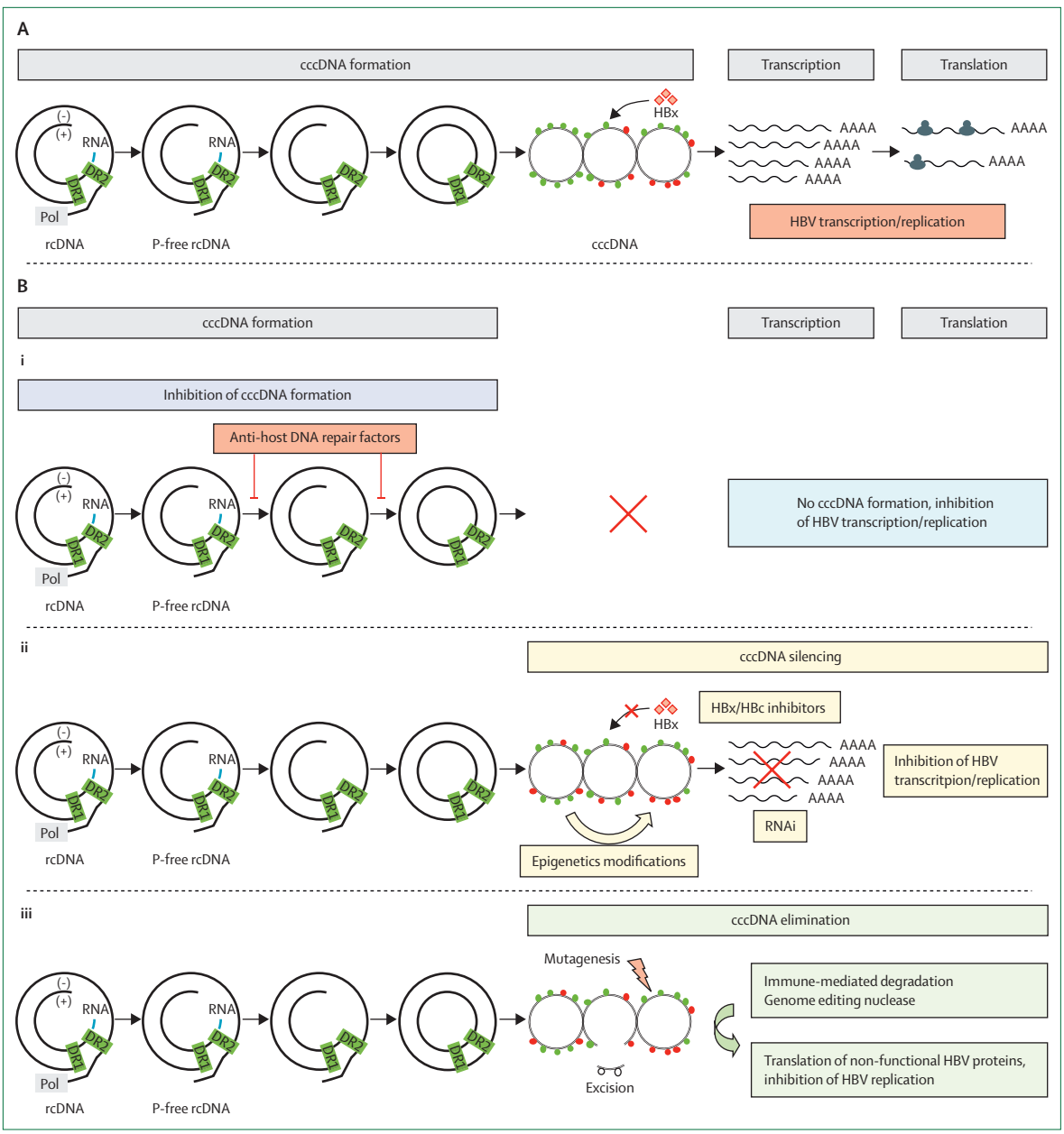

Figure 4: **Formation and targeting of cccDNA**
rcDNA is converted into cccDNA by the host DNA repair machinery. cccDNA can be targeted by (i) inhibition of formation, (ii) silencing, or (iii) elimination. HBV=hepatitis B virus. HBc=HBV core protein. HBx=HBV x protein. cccDNA=covalently closed circular DNA. rcDNA=relaxed circular DNA. Pol=HBV polymerase. DR1=direct repeat 1. DR2=direct repeat 2. RNAi=RNA interference.

safety, pharmacokinetics and pharmacodynamics of the drug in patients with chronic hepatitis B, have been reported.[38] ABI-H0731 is a potent and selective core inhibitor (also known as a core protein allosteric modifier [CpAM]) being developed to improve cure rates for chronic hepatitis B infection. 38 non-cirrhotic patients with chronic hepatitis B were randomly assigned (10:2) to receive either ABI-H0731 (100, 200, 300, or 400 mg) or placebo, and treated once daily for up to 28 days in a double-blind, placebo-controlled study. Overall, ABI-H0731 was safe and well-tolerated. There were no serious adverse events, nor clinically significant drug-related, dose-dependent, or treatment emergent laboratory findings. One patient at 400 mg developed a grade 3 rash after 10 days of dosing that resolved following drug discontinuation. All other drug related treatment-emergent adverse events were considered to be mild (grade 1), and there were no other treatment discontinuations or dose modifications. A patient with suboptimal DNA response had a known resistance

mutation at baseline. Excluding this patient, median maximum $\log_{10}$ IU/mL HBV DNA declines per cohort ranged from 1·5 (range 0·7–3·6) at 100 mg per day to 2·7 (0·8–4·0) at 300 mg per day, with a median 1·4 $\log_{10}$ IU/mL decline in the 100 mg cohort and 2·2 $\log_{10}$ IU/mL decline in the 300 mg cohort in HBeAg-positive patients, and a median 3·0 $\log_{10}$ IU/mL decline in the 100 mg cohort and 2·5 $\log_{10}$ IU/mL decline in the 300 mg cohort in HBeAg-negative patients. Similar maximum declines (approximately 4 $\log_{10}$ IU/mL) were seen in both the 300 mg and 400 mg groups. RNA declines in HBeAg-positive patients paralleled the observed DNA declines ($p<0·001$). The 300 mg per day dose of ABI-H0731 is being evaluated in phase 2 studies in combination with oral nucleos(t)ides (NCT03576066).

Capsid assembly modulators are specific to HBV and prevent the formation of the viral capsid in the cytoplasm. In-vitro resistant variants to the capsid have been described, and in theory some patients could develop resistance. Whether long-term capsid accumulation in the cytoplasm of hepatocytes might trigger the unfolded protein response and autophagy, with potential toxicity, is unclear.

Inhibitors of cccDNA

Current treatments for HBV do not cure the infection because they do not eliminate the cccDNA that persists in the nucleus of infected cells (figure 4A). New therapeutic strategies focus on inhibiting its formation or removing HBV cccDNA through silencing or elimination from infected cells (figure 4B). A number of drugs that target cccDNA are in development (table).

After the cytoplasmic release of the HBV capsid containing HBV rcDNA, rcDNA is delivered to the nucleus of infected cells and repaired into a cccDNA stable mini-chromosome. All steps leading to this conversion are potential targets for new drugs. One such target is tyrosyl-DNA-phosphodiesterase 2, a DNA repair enzyme that is implicated in p-free-rcDNA formation through removing a viral polymerase from rcDNA.[39] 5′-flap structure is removed by flap-endonuclease 1, which represents another target.[40] The synthesis of the positive strand of the rcDNA is completed by DNA polymerase κ, and DNA ligases 1 and 3 are involved in cccDNA formation.[41,42] To treat HBV infection, DNA ligase inhibitors that were developed as anti-cancer drugs could be used. For instance, Cai and colleagues[43] have identified two structurally related disubstituted sulfonamide compounds that block cccDNA formation.

In the nucleus of infected cells, cccDNA is linked by host histones and viral proteins to form a functional mini-chromosome, making it subject to cellular epigenetic regulations.[44] Regulation of cccDNA transcription by epigenetic modification could represent a new class of antivirals (epidrugs) for treatment of chronic hepatitis B.[45] Some cellular actors and viral proteins promote cccDNA transcription, while others inhibit it. HBx can act directly and indirectly on cccDNA epigenetic dynamics and promotes cccDNA transcriptional activity, making HBV x protein (HBx) a favoured target.[46] HBc could also alter the epigenetics of cccDNA.[18] Using inhibitors to block HBx and its cellular partners in combination with HBc inhibitors could lead to silencing of cccDNA transcriptional activity and blocking of HBV replication. These therapies based on RNA interference can target HBV mRNAs with high specificity. All HBV transcripts are overlapping, which means that a single RNA interfering dose could silence several HBV transcripts. This could result in the inhibition of viral replication and in the restoration of HBV-specific T-cell activity by reducing viral surface and precore protein secretion.[47] HBsAg concentration was significantly reduced using small interfering RNA (siRNA) that targets HBV cccDNA in the serum of patients and HBeAg-positive chimpanzees.[16]

Partial cccDNA degradation can be obtained by immune-mediated degradation through actors such as interferon alfa, lymphotoxin-β receptor agonist, tumour necrosis factor-alfa, and interferon gamma, by upregulating cellular effectors such as apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3A/3B (also known as APOBEC3A/3B cytidine deaminase), which will deaminate the cccDNA in the nucleus.[48] Several trials of siRNA and antisense oligonucleotides are in early development and data are not yet available (NCT03772249; NCT02631096, NCT03365947, NCT03038113, NCT03672188; table).

Progress in the field of genetic editing has allowed the development of new approaches using genetic modification, such as nucleases for targeting and eradicating nuclear HBV cccDNA. These genetically modified nucleases include zinc finger nucleases, transcription activator-like effector nucleases, and programmable RNA-guided DNA endonuclease-like, clustered regularly interspaced palindromic repeats (CRISPR) with CRISPR-associated protein (CRISPR/Cas9 systems).[49–51] These agents can specifically target cccDNA to induce mutations, damage DNA, and generate insertions and deletions within cccDNA. In-vitro data show[52] that using a CRISPR/Cas9 system that targets conserved regions within HBV cccDNA suppresses viral gene expression, reduces HBV replication, and reduces the nuclear cccDNA pool. Remaining challenges include how to deliver this gene-editing into the nuclei of infected hepatocytes, how to target viral genome DNA rather than host genomic or mitochondrial DNA, and how to counteract the DNA damage response mediated by *TP53* pathway during the gene-editing process.[53]

Finally, an RNA interference approach might lead to undesirable off-target effects in human host cells, which might represent a real obstacle. Cross-reactivity with human mRNA is a risk and novel strategies should be defined to minimise this risk. Morever, gene-editing approaches might be complicated by off-target side-effects.[54]

Search strategy and selection criteria

We searched PubMed, Ovid MEDLINE, the Cochrane Central Register of Controlled Trials, and Web of Science for articles published in English from March 1, 2012, to March 1, 2019, and also relevant articles outside this period, using the terms HBV, chronic hepatitis B, treatment, and direct-acting antivirals. Conference proceedings, abstract books, and references from relevant studies were also examined.

HBsAg secretion inhibitors

In addition to infectious virion secretion (Dane's Particle), the excess production of HBsAg results in secretion of subviral non-infectious particles that play an important role in immune evasion by inducing immune tolerance and exhaustion.[55]

In agreement with this concept, nucleic acid polymers such as REP 2139 and REP 2165 are new antiviral drugs that block the assembly of subviral particles, preventing the release of HBsAg and allowing its clearance and restoration of functional control of infection when combined with various immunotherapies.

In a clinical trial,[56] safety and efficacy of REP 2139 and pegylated interferon alfa-2a in 12 patients with chronic HBV and hepatitis D virus (HDV) co-infection (NCT02233075).

Nine patients had suppressed HBV DNA (<10 IU/mL) at the end of treatment, which was maintained by seven patients and newly established in an eighth patient at 1 year follow-up. 11 patients became HDV RNA-negative during treatment, with nine remaining HDV RNA-negative at the end of treatment; seven of these patients were still HDV RNA-negative at 1 year follow-up. At 1 year follow-up, normalisation of serum aminotransferases occurred in nine of 12 patients. Based on these findings, phase 2 studies are ongoing (table).

Recently, Usman and colleagues characterised the dynamic changes of HBV quasispecies within the major hydrophilic region (MHR) of the pre-S/S open reading frame including the a determinant in responders and nonresponders to REP 2139-Ca.[57] The MHR mutations were more frequently observed in responders than in nonresponders. No mutations were observed in a determinant of major quasispecies population which may interfere with the detection of HBsAg by diagnostic assays. No specific mutations were found within the MHR which could explain patients' poor HBsAg response during REP 2139-Ca therapy.

Finally, nucleic acid polymers have shown promising results in term of efficacy and safety in a small number of patients. However, further data are required to confirm these preliminary results and novel clinical trials have been registered (table).

Nucleic acid polymers are new antiviral drugs that block the assembly of subviral particles, thus preventing the release of HBsAg and allowing its clearance and restoration of functional control of infection when combined with various immunotherapies (table).[55]

Conclusion

There is an urgent need to develop new therapies that can be given for a limited course and that could cure HBV infection. A cure for HBV could improve survival (by reducing the risk of reactivation and of hepatocellular carcinoma) and improve quality of life. HBV cure will also have the advantage of curing HDV infection. There might also be secondary benefits, such as increasing screening and linkage to care, which might reduce discrimination.

Understanding the HBV lifecycle is of great importance, since each step is a potential target for drug development. It is also important to clarify the clinical relevance of integrated HBV DNA. Development of novel HBV therapies could be aided further by the availability of improved cellular and animal infection models. Surrogate markers associated with increased survival will also help to develop a cure. Since quantifying cccDNA requires an invasive procedure, serum biomarkers reflecting intrahepatic cccDNA activity are needed. Circulating HBV RNA could serve as such a surrogate marker. HBsAg seroclearance is one of the most important endpoints of chronic HBV infection and is associated with a reduced risk of hepatocellular carcinoma. The combined use of HBsAg and HBV RNA quantification and HBcrAg measurements could help predict treatment outcome and to monitor therapy. Future studies of HBV biomarkers are needed to analyse their specificity and sensitivity.

Investigation of the risk–benefit ratio for new drugs is essential, especially given the excellent safety of approved nucleos(t)ide analogues. Comparison of different drugs with different modes of action is difficult at present, since data are currently limited. Several compounds are in the early stages of development, with many of the trials ongoing and without complete data available. Moreover, drugs in development have been studied for short durations (usually 4 weeks at first); efficacy and safety need long-term evaluation.

In early development studies, some endpoints might, in the future, be adapted to the mechanism of action of the compound tested. It will be important to understand how HBV-infected patients recover and how HBsAg seroclearance is achieved to develop new treatment strategies. In the nearer term, patients with active chronic hepatitis and those already receiving therapy could be candidates for new drugs. If these drugs are shown to provide a high probability of cure with favourable safety, all patients with HBV infection may become candidates for therapy.

Several direct-acting antiviral approaches are in development. Direct-acting antiviral approaches with new mechanisms of action could have the capacity to decrease cccDNA and HBsAg, leading to the possibility

of immune restoration. Therefore, some compounds could have a dual role: acting directly on the virus and also via host immune restoration. Although this Review does not deal with immunotherapy, combinations of antiviral therapy (targeting several steps in the HBV lifecycle to suppress viral replication and viral antigen production) and immune modulatory therapy (to restore immune response to HBV) are probably needed to achieve the goal of HBV cure. But which specific combination of agents will be needed is at present uncertain.

Promising new treatment options in development include entry inhibitors, capsid assembly modulators, and drugs targeting cccDNA or HBV RNA. All pathways and combinations should be investigated to help achieve functional cure. We believe that a functional cure after a finite course of novel antiviral and immunomodulatory therapies, achieved in a much higher proportion of patients than is possible with existing treatments, is an attainable goal.

Contributors

TA designed, prepared, and supervised the manuscript. All the authors contributed to the drafting of the review, the critical revision of the manuscript, and the final approval of the version to be published.

Declaration of interests

TA has acted as a speaker and investigator for Janssen, Gilead, Roche, and Merck. NB has acted a speaker and investigator for Janssen, Gilead, Roche and Merck. DL and AM declare no competing interests.

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