New therapeutic agents for chronic hepatitis B

Mayur Brahmania, Jordan Feld, Ambreen Arif, Harry L A Janssen

The treatment goal for chronic hepatitis B is true eradication of the hepatitis B virus, but this is rarely achieved with first-line treatment regimens because of an inability to disrupt covalently closed circular DNA and an inadequate host immune response. Therefore, new antiviral agents are needed to target various stages of the hepatitis B virus lifecycle and modulation of the immune system. This Review provides a summary of available regimens with their strengths and limitations, and highlights future therapeutic strategies to target the virus and host immune response. These new agents can hopefully lead to a finite duration of treatment, and provide a functional and durable cure for chronic hepatitis B infection.

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Toronto Centre for Liver Diseases, Toronto Western Hospital, University Health Network, Toronto, ON, Canada (M Brahmania MD, J Feld MD, A Arif MD, Prof H L A Janssen MD); **and Department of Gastroenterology and Hepatology, Erasmus Medical Center University Hospital, Rotterdam, Netherlands** (Prof H L A Janssen)

Correspondence to: Prof Harry L A Janssen, Toronto Center for Liver Diseases, Toronto Western Hospital, University Health Network, Toronto, ON M5T 2S8, Canada **harry.janssen@uhn.ca**

Introduction

Chronic hepatitis B affects about 250 million people worldwide and can lead to liver cirrhosis, liver failure, hepatocellular carcinoma, and death.[1–3] Worldwide, 30% of cirrhosis and roughly 53% of hepatocellular carcinoma is attributed to chronic hepatitis B.[1] The ideal goal of antiviral treatment is the complete eradication of the hepatitis B virus (HBV) from infected individuals, but this is rarely achieved because covalently closed circular DNA (cccDNA) remains in hepatocytes, and immune control over HBV is difficult to induce. HBsAg loss or HBeAg seroconversion with viral suppression is deemed a successful response to therapy because of improved survival, reduction in hepatocellular carcinoma, and prevention of disease progression.[4–6] First-line antiviral treatment options include nucleos(t)ide analogues such as entecavir or tenofovir, and pegylated interferon (peginterferon), which is mainly an immune modulator. Unfortunately, HBsAg loss is rarely achieved with these treatments and lifelong treatment is often needed, particularly with nucleos(t)ide analogues.[7–10] Although an interim milestone to control HBV infection has been achieved through successful vaccination programmes beginning in the early 1980s,[11] the treatment of chronic hepatitis B will remain a challenge because of the millions of chronically infected individuals and new incident infections resulting from incomplete vaccine coverage rates.[12] More than 30 agents are in development, including novel strategies that target the virus and the host (table 1). Although some agents might not reach clinical use, others could become part of our treatment arsenal in the not too distant future. This Review highlights treatment approaches and agents in phase 1, 2, and 3 clinical trials (and selected preclinical studies) to show how various steps in the viral lifecycle and stimulation of the host immune response might be used in the future to find a functional and durable cure for chronic hepatitis B.

The hepatitis B virus replication cycle

HBV is a circular DNA virus with a partly double-stranded genome that belongs to the Hepadnaviridae family. It is a small, spherical structure containing a nucleocapsid composed of self-assembling core proteins and an outer lipid envelope made up of viral surface proteins—ie, HBsAg. The envelope is associated with binding and entry into hepatocytes, and the nucleocapsid encloses viral DNA and a DNA polymerase with reverse transcriptase activity.[13] The replication cycle of HBV is a complex process that begins with attachment of the virus to the cell-associated heparan sulfate proteoglycans, followed by the virus irreversibly binding to the hepatocyte-specific cellular sodium-taurocholate co-transporting polypeptide receptor (NTCP).[14,15] After binding, HBV enters hepatocytes through either endocytosis or fusion with the viral envelope at the plasma membrane.[16] As viral nucleocapsids are released into the cytoplasm, relaxed circular DNA (rcDNA), with covalently linked polymerase, enters the cell nucleus where the rcDNA genome is converted to the highly stable cccDNA. cccDNA is a template for transcription of viral mRNAs that code for three surface proteins, the HBV X protein, and pregenomic RNA, which is translated to produce the core and polymerase proteins and serves as the RNA template for reverse transcription of nascent viral genomes.[17,18] The HBV core proteins are translated from the pregenomic RNA and self-assemble into nucleocapsids, which encapsidate pregenomic RNA and HBV polymerase, allowing genome replication to proceed. HBV polymerase is the only enzymatically active protein coded by the virus, and has RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase H activity. Inside nucleocapsids, HBV polymerase uses pregenomic RNA as a template to synthesise the minus-strand viral DNA via its RNA-dependent DNA polymerase activity, which is then used as the template for plus strand DNA synthesis.[19] RNase H activity is needed to degrade the pregenomic RNA template during minus-strand DNA synthesis, which is initiated by a protein priming mechanism in which HBV polymerase itself serves a protein primer and a catalyst.[20] The process eventually leads to nucleocapsid maturation with formation of rcDNA, which can either be enveloped by the surface proteins and secreted out of the cell as a virion particle, or delivered into the nucleus to amplify the existing cccDNA pool (figure).[21]

Antiviral treatments for chronic hepatitis B

International liver organisations (American Association for the Study of Liver Diseases, European Association for the Study of the Liver, and Asian Pacific Association

	Mechanism of action	Example drug	Phase of clinical trial
Virus targeting			
Entry inhibitor	Targets NTCP receptor to inhibit virus entry	Myrcludex B	2a
cccDNA degrader, silencer, or eliminator	Upregulation of APOBEC3A and 3B proteins causing cccDNA degradation; direct destruction of cccDNA; inhibiting rcDNA conversion to cccDNA; targeting the epigenetic control of cccDNA function	Lymphotoxin-β receptor Interferon alfa BSBI-25 Zinc finger nucleases Di-substituted sulfonamides	1 Preclinical Preclinical Preclinical Preclinical
RNA interference or gene silencer	RNA molecules inhibiting gene expression and release of new virions	ARC-520 ISIS-HBVRX ALN-HBV TKM-HBV NUC B 1000 DNA-directed RNA interference ALN-PDL	2 2 Preclinical Preclinical Preclinical Preclinical Preclinical
Assembly effector	Inhibits HBV replication by causing destabilisation of viral nucleocapsid	HAPs (Bay 41–4109 and GLS4) Phenylpropenamide (AT-61 and 130) Isothiafludine NVR 3–778 GLS4	1 1 Preclinical 2 2
HBsAg release inhibitor	Inhibits the release of HBsAg and HBsAg SVPs to boost restoration of host immune response	REP 2139-Ca Rep 2165 Rep 2055 Nitazoxanide Triazolo-pyrimidine inhibitors	2 Preclinical Preclinical Preclinical Preclinical
New nucleos(t)ide analogues	DNA polymerase inhibitor	GS-7340 (tenofovir alafenamide) AGX-1009 LB80380 (besifovir) MIV-2 (lagociclovir valactate) CMX 157	3 1 3 1 2
Cyclophilin inhibitor	Blocks HBV protein interaction with host cell cyclophilin and contributes to immune stimulation	CPI-431–32 OCB-030 Alisporivir	Preclinical Preclinical Preclinical
Immune system			
Immunomodulators	Boost host immune responses and have antiviral effect	Peginterferon lambda Cytokines (interleukins 7, 12, 18, 21) GS-9620 (TLR-7 agonist) CYT-003 (TLR-9 agonist) PD-1 blockade Stimulators of interferon genes agonist SB 9200 HBV	2b 1 2 Preclinical Preclinical Preclinical 2
Therapeutic vaccines	Induce and stimulate CD4 and CD8 T-cell response	GS-4774 (tarmogen) HBsAg-antiHBs immunoglobulin HB110 ABX203 HBsAg/HBcAg combination (NASVAC) DNA vaccines T-cell peptide epitome vaccine DV-601 TG-1050 (transgene) GI-13020 NOV-205	2 3 1 2 3 Preclinical 1b 1 1 1 1

NTCP=sodium-taurocholate co-transporting polypeptide. APOBEC=apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like. cccDNA=covalently closed circular DNA. HBV=hepatitis B virus. HAPs=heteroaryldihydropyrimidines. SVP=subviral particles.

Table 1: **Future treatments for chronic hepatitis B**

for the Study of the Liver) recommend three first-line agents for the treatment of patients with treatment-naive chronic hepatitis B, including nucleos(t)ide analogues and immunomodulators (tables 2 and 3).[22–24] Nucleos(t)ide analogues (entecavir and tenofovir) are able to suppress viral replication by inhibiting the reverse transcriptase activity of HBV polymerase, thus preventing viral replication. They have become the mainstay of chronic hepatitis B treatment, as they are potent antivirals with few side-effects and a high barrier to resistance. Both entecavir and tenofovir have shown efficacy in treating patients with chronic hepatitis B with HBeAg seroconversion rates about 30% and HBsAg loss rates between 4·4% and 13% after 5–8 years of follow-up.[9,10] Interferon alfa and long-acting peginterferon are cytokines with immunomodulatory

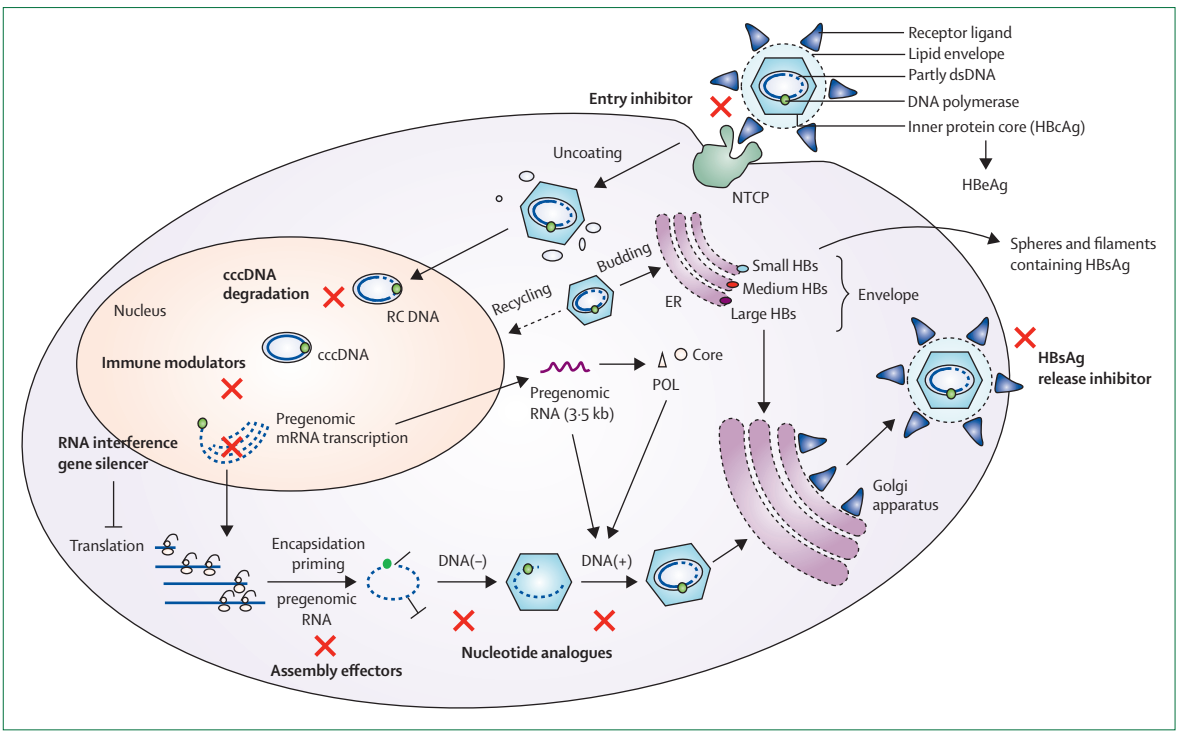

Figure: **The HBV lifecycle and potential therapeutic targets**
After HBV virions attach to the NTCP receptor, they are uncoated and transported to the nucleus, in which cccDNA serves as a template for viral transcription of pregenomic RNA, which then directs the synthesis of viral DNA and mRNA encoding all viral proteins and securing HBV persistence. Genomic replication of HBV happens via virally encoded polymerase and a reverse transcriptase. Encoded polymerase uses pregenomic RNA as a template to synthesise the minus-strand viral DNA via its RNA-dependent DNA polymerisation activity, which is then used by the encoded polymerase as the template for the plus strand DNA synthesis. The process eventually leads to nucleocapsid maturation as rcDNA is formed which can be either enveloped or secreted out of the cell as a virion particle or be delivered into the nucleus to amplify the cccDNA pool. Potential targets of the HBV lifecycle (indicated by red crosses) include entry inhibitors, cccDNA degradation, immune modulation, RNA interference, assembly effectors, HBV DNA polymerase inhibitors, and HBsAg release inhibitors. HBV=hepatitis B virus. NTCP=sodium-taurocholate co-transporting polypeptide. cccDNA=covalently closed circular DNA. rcDNA=relaxed circular DNA. ER=endoplasmic reticulum. HBs=envelope protein.

and antiviral effects, with potential advantages that include finite therapy, the absence of resistance mutations, and durable HBsAg seroconversion rates that are noted in about 10% of patients.[7,8,25]

Limitations of current therapy

True cure of HBV will need to either eliminate cccDNA or prevent transcriptional activity of cccDNA, but this is rarely achieved even with the use of both nucleos(t)ide analogues and interferon. Nucleos(t)ide analogues interfere late in the viral lifecycle, preventing DNA replication, but they do not affect the transcriptional activity of cccDNA or viral protein production, and very rarely induce immune control. HBsAg loss is therefore rare, and lifelong treatment is often needed to prevent viral rebound after treatment cessation,[26,27] which might be in part due to the DNA-based genome of HBV, and a replication cycle that relies on a reverse transcriptase, which is error prone. HBV can process mutations at a high frequency because of viral replication, which results in a diverse population of viral variants in infected individuals, although the variants could have diminished replication competence and might not survive. Alternatively, nucleos(t)ide analogues might select for drug resistance mutations, although so far this has not been seen with tenofovir and in less than 2% of nucleoside-naive patients given entecavir, which is much lower than the resistance rates with nucleosides such as lamivudine and telbivudine.[10,28,29] Peginterferon mediates repression of cccDNA activity and has immune modulatory properties, which can result in viral clearance, but response was only recorded in a few individuals, and treatment uptake is limited by systemic side-effects, the need for subcutaneous injections every week, and safety concerns in patients with cirrhosis.[30,31] As the understanding of HBV has improved, it has become clear that both viral factors (eg, core or precore mutants, replication errors) and determinants of the host immune response (eg, HLA haplotype, T-cell exhaustion) play a part in viral persistence. Novel strategies are therefore needed to create a shift in the treatment of chronic hepatitis B.

Combination therapy

Over the past decade there has been substantial interest in combining nucleos(t)ide analogues and peginterferon therapy with the hope that these agents might act synergistically. Suppressive therapy with potent nucleos(t)ide analogues might reduce virally mediated

	AASLD (USA)[21]	EASL (Europe)[22]	APASL (Asia)[23]
Treatment definitely recommended	HBV DNA >20 000 ALT >2 times ULN	HBV DNA >20 000 ALT >ULN Liver biopsy showing moderate to severe inflammation or fibrosis	HBV DNA >20 000 ALT >2 times ULN
Treatment should be considered	HBV DNA 2000–20 000 ALT 1–2 times ULN Older than 40 years, or liver biopsy showing moderate/severe inflammation/fibrosis	HBV DNA >20 000 ALT <ULN, or liver biopsy showing moderate to severe inflammation or fibrosis	HBV DNA >20 000 ALT <ULN, and liver biopsy showing moderate to severe inflammation or fibrosis
Observe	HBV DNA <20 000 ALT <ULN	HBV DNA <2000 ALT <ULN	HBV DNA <2000 ALT <ULN
Preferred first-line treatment	Peginterferon Entecavir Tenofovir	Peginterferon Entecavir Tenofovir	Peginterferon Entecavir Tenofovir

HBV DNA in IU/mL. AASLD=American Association for the Study of Liver Disease. EASL=European Association for the Study of Liver. APASL=Asian Pacific Association for the Study of the Liver. HBV=hepatitis B virus. ALT=alanine transaminase. ULN=Upper Limit of Normal.

Table 2: **Treatment recommendations from international organisations for HBeAg-positive patients**

	AASLD (USA)[21]	EASL (Europe)[22]	APASL (Asia)[23]
Treatment definitely recommended	HBV DNA >20 000 ALT >2 times ULN	HBV DNA >2000 ALT >ULN Liver biopsy showing moderate to severe inflammation or fibrosis	HBV DNA >2000 ALT >2 times ULN
Treatment should be considered	HBV DNA 2000–20 000 ALT 1–2 times ULN Older than 40 years, or liver biopsy showing moderate to severe inflammation or fibrosis	HBV DNA >2000 ALT <ULN, or liver biopsy showing moderate to severe inflammation or fibrosis	HBV DNA 2000–20 000 ALT 1–2 times ULN Older than 40 years, or liver biopsy showing moderate to severe inflammation or fibrosis
Observe	HBV DNA <20 000 ALT <ULN	HBV DNA <2000 ALT <ULN	HBV DNA <2000 ALT <ULN
Preferred first-line treatment	Pegylated interferon Entecavir Tenofovir	Pegylated interferon Entecavir Tenofovir	Pegylated interferon Entecavir Tenofovir

HBV DNA in IU/mL. AASLD=American Association for the Study of Liver Disease. EASL=European Association for the Study of Liver. APASL=Asian Pacific Association for the Study of the Liver. HBV=hepatitis B virus. ALT=alanine transaminase. ULN=upper limit of normal.

Table 3: **Treatment recommendations from international organisations for HBeAg-negative patients**

T-cell exhaustion, to allow HBV-specific T cells to be more responsive upon peginterferon treatment. Combination therapy might reduce the risk of breakthrough resistance to long-term nucleos(t)ide analogues. Initial trials combining lamivudine with peginterferon were disappointing, with response rates similar to those noted for peginterferon alone. Treatment strategies with the more potent nucleos(t)ide analogues entecavir and tenofovir, either as sequential (nucleos[t]ide analogue followed by peginterferon, or peginterferon followed by nucleos[t]ide analogue), or concomitantly, showed more promising results, with improvement in HBsAg decline and increasing rates of sustained HBeAg and HBsAg seroconversion following therapy. Definitive trials are needed, because phase 2 studies so far have been underpowered or lacked key control arms, making it difficult to draw strong conclusions.[32–36] In view of the poor cure rates with monotherapy with either peginterferon or nucleos(t)ide analogues, combination of immunomodulatory therapies or potent nucleos(t)ide analogue therapy with one of the virus-targeting systems (antigen inhibitors) and cccDNA inhibitors could ultimately prove to be the best option to cure persistent infection.

Virus targeting

Entry inhibitors

NTCP has been identified as a functional receptor for HBV, because it interacts with a key region of the HBV envelope to allow entry of both HBV and hepatitis D virus, which also uses the HBV surface proteins.[15,37] An ideal mechanism to eliminate new HBV infection is to silence NTCP. Myrcludex B, a synthetic lipopeptide that targets NTCP, has been shown to efficiently prevent viral spread from infected human hepatocytes ex vivo and in vivo.[38–42] In a phase 2a clinical trial[43] assessing safety and tolerability of myrcludex B, 40 HBeAg-negative patients with chronic hepatitis B (HBV DNA >2000 IU/mL, median HBV DNA 4·7 $\log_{10}$ IU/mL, without cirrhosis) were treated for 24 weeks with doses

ranging between 0·5 and 10 mg once a day. In the 10 mg group, HBV DNA concentrations decreased by >1 $\log_{10}$ IU/mL at week 12 in six (75%) of eight patients, but a lower response rate (7 [17%] of 40 patients) was noted in the lower dose cohorts. Furthermore, alanine transaminase (ALT) normalisation was reported in 22 (55%) of 40 patients, but there was no noteworthy change in HBsAg concentrations, which is often used as a surrogate marker of active cccDNA transcription.[43–45] Because the activity of myrcludex B includes inactivation of the NTCP receptor, trouble with bile salt homoeostasis, hyperbilirubinaemia, and the metabolism of some drugs might be an issue, particularly if long-term therapy is needed.[46] Although myrcludex can prevent the infection of new hepatocytes, it might be less effective in pre-existing infection. However, the ability to target NTCP might lead to future strategies using combination therapy with nucleos(t)ide analogues, peginterferon, or agents discussed in this Review, to increase clearance of previously infected hepatocytes. Myrcludex B might be attractive in the liver transplant setting, in which an entry inhibitor could prevent reinfection of the new liver.

cccDNA degradation, silencing, and elimination

After attachment to the NTCP receptor, HBV undergoes endocytosis, uncoating, and delivery of nucleocapsids to the nucleus where rcDNA is converted to cccDNA. Nucleos(t)ide analogue-based treatments can block the replication and formation of new cccDNA; however, they have negligible effect on existing cccDNA, which is key to viral persistence and reactivation after cessation of therapy.[26,27] Strategies that use zinc finger nucleases and disubstituted sulfonamide compounds can inhibit cccDNA transcription by direct destruction of cccDNA, inhibiting rcDNA conversion to cccDNA, and by targeting the epigenetic control of cccDNA function. However, these agents are in the early phases of development and have only been studied in cell culture and primary duck hepatocytes.[47–49] Another promising new approach is the activation of apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) proteins, which interact with the HBV core and translocate to the nucleus where they deaminate and subsequently degrade cccDNA. Results of well designed studies showed that activation of the lymphotoxin-β receptor (LTβR) upregulates APOBEC3B, and interferon alfa upregulates APOBEC3A. In-vivo and in-vitro stimulation of LTβR led to a reduction in HBV DNA and cccDNA concentrations with persistent effects after cessation of therapy and no hepatotoxic effects. However, long-term human data are still needed for LTβR agonists, as activation lasting longer than 1 year has been associated with development of hepatocellular carcinoma.[50] Nevertheless, nucleos(t)ide analogue use followed by LTβR activation for a short period could be a novel strategy to eliminate cccDNA reservoirs.

An alternative strategy to target cccDNA is the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) system, which uses a target RNA with sequence specificity for conserved regions of DNA to guide the Cas9 nuclease to cleave the DNA at that site. Several groups have shown that conserved regions of cccDNA can be targeted with the CRISPR system and by combining several target RNAs, potent suppression and even complete elimination of cccDNA from infected cells and transgenic mice was achieved.[51–53] The CRISPR strategy holds great promise for human gene therapy broadly but could be particularly useful for targeting a stable viral genome like that of HBV.

RNA interference and gene silencing

Small interfering RNAs (RNAi) bind and inactivate host or viral mRNA, preventing protein translation. Through target of RNA replicative intermediates, HBV is potentially susceptible to RNAi approaches. Developments in RNAi technology have overcome the challenge of delivery, which was the major barrier to clinical use. Stable RNAi-targeting HBV mRNA intermediates were shown to reduce expression of viral proteins (HBsAg and HBeAg) in mouse and chimpanzee models.[54–56] A phase 2a randomised controlled trial[57] studied 18 Asian HBeAg-negative patients who were taking entecavir and received a single intravenous dose of ARC-520 (1 mg, 2 mg, or 3 mg), a stable pooled siRNA mixture to target all of the HBV RNA replicative intermediates, and showed a 22% mean reduction of HBsAg concentrations from baseline in the 2 mg/kg group, without any treatment-related side-effects after 85 days of follow-up. Studies will assess if preliminary declines in HBsAg concentrations provide any clinically relevant outcomes and if higher doses can be used.

Gene silencing can be achieved by introducing a short antisense oligonucleotide complementary to an RNA target. Billioud and colleagues[58] showed a dose-dependent (≥11 mg/kg per week) reduction of HBsAg concentrations of ≥2 logs after 4 weeks of treatment in vivo; however, combination treatment with entecavir was not substantially better than entecavir alone and HBsAg concentrations returned to baseline after 4 weeks from initial injection. ARC-520 and antisense oligonucleotide therapies have shown some early positive results, but further improvement of stable drug delivery methods and larger trials in heterogeneous populations with durable endpoints are needed to support the results seen thus far. Restoration of HBV-specific immune function, which is directly impaired by HBeAg and HBsAg, could be an added benefit of using RNAi to reduce concentrations of these viral proteins. If immune responses are improved with RNA silencing, there might be substantial additional therapeutic potential to this approach.

Assembly inhibitors

Hepatitis B reverse transcriptase is an important enzyme for viral replication and is the target of nucleos(t)ide analogue therapy. Targeting a single step in the viral lifecycle, particularly late in replication, has not been adequate to lead to viral clearance and resistance was an issue with early agents. Targeting of various steps in the viral assembly pathways could have additional benefits. Assembly inhibitors disrupt the HBV lifecycle by destabilising the nucleocapsid and by blocking RNA packaging to produce empty capsids without genetic information.[59] Preclinical studies[60–65] have shown that both the heteroaryldihydropyrimidine (ie, Bay 41-4109 and GLS4) and phenylpropenamide (AT-61 and AT-130) families of compounds inhibit HBV replication by misdirecting assembly causing destabilisation of capsids to produce incompetent viral particles for reverse transcription. Another compound, NVR 3-778, likewise acting as a direct HBV core and capsid protein inhibitor, progressed to phase 2 trials after showing that 40, predominantly white, men who received a single oral dose of 50 mg, 150 mg, 400 mg, or 800 mg reported no treatment or dose-related adverse events.[66] Moreover, the pharmacokinetic profile of NVR 3-778 showed that doses of 200 mg or more achieved sufficient plasma concentrations to inhibit HBV DNA for more than 24 h in cell cultures.[66] Phase 1b assessment of dose-related safety, pharmacokinetics, antiviral efficacy, and potency of combination therapy with peginterferon are in progress.

HBsAg release inhibitor

HBV persistence is at least partly mediated by suppression of host immune responses by viral proteins. HBsAg was seen to suppress cytokine production and induce T-cell tolerance and exhaustion.[67] Hence, control of HBsAg secretion was proposed as a strategy to restore HBV-specific T-cell-mediated immune control. Nitazoxanide, an antimicrobial with active metabolite tizoxanide, showed the ability to reduce HBsAg, HBeAg, HBcAg concentrations, and might act in synergy with lamivudine or adefovir in vitro, although, clinical trials have not progressed after small initial studies.[68,69] Similarly, a group of triazolo pyrimidine inhibitors have garnered interest as a result of their abilities both to inhibit HBsAg, and to be active against HBV variants that are resistant to nucleos(t)ide analogues. Although the exact mechanism of action is still under investigation, the inhibitors were seen to have no acute or long-term toxic effects in vivo, and displayed desirable pharmacokinetic profiles.[70]

HBsAg might have direct immunomodulatory effects on T cells, but HBV could use HBsAg subviral particles to blunt the host immune system and mediate immunological tolerance. Subviral particles are particles with HBV proteins on their surface that do not contain nucleocapsids or viral nucleic acids, which makes subviral particles non-infectious. The reason for their overproduction and their contribution to HBV pathogenesis is still unclear, but they are thought to mediate tolerance by serving as a decoy by adsorbing neutralising antibodies to delay the clearance of infection.[71–74] REP 2139-Ca is in phase 2 trials (study number not available) and acts as a nucleic acid-based polymer to inhibit the release of subviral particles from infected hepatocytes, which might lead to restoration of the immune response. In a pilot study,[75] 12 patients positive for HBeAg and without cirrhosis were given REP 2139-Ca followed by peginterferon or thymosin alpha. Patients had reductions in concentration of HBV DNA and HBsAg, with four patients reporting HBsAg loss. In view of these favourable results, further studies are planned.

New nucleos(t)ide analogues

Nucleos(t)ide analogues are able to suppress an essential step in viral replication by inhibiting reverse transcriptase and have been approved for use since 2008. Formerly known as GS 7340, tenofovir alafenamide is another prodrug of tenofovir designed to resist rapid metabolism in the plasma (common with the first generation prodrug tenofovir) thereby efficiently delivering active drug (tenofovir diphospate) to infected hepatocytes. Tenofovir alafenamide is converted to tenofovir (ie, a nucleoside monophosphate analogue) by cathepsin A and carboxylesterase-1 (Ces1), which is highly expressed on hepatocytes, and is subsequently converted to tenofovir diphospate by adenylate kinase and nucleotide diphosphate kinase. Tenofovir diphospate is taken up into hepatocytes where it is phosphorylated to the triphosphate, which inhibits the activity of HBV reverse transcriptase by competing with natural substrates and causing DNA termination after being incorporated into viral DNA.[76] In an open label phase 1b study[77] participants were randomly allocated to receive tenofovir alafenamide at doses of 8 mg, 25 mg, 40 mg, or 120 mg, or tenofovir 300 mg, once a day for 28 days. The mean HBV DNA decline at week 4 was 2·7 logs and was equivalent to tenofovir with no noteworthy adverse events such as nephrotoxic effects or bone mineral loss. Beause tenofovir alafenamide has similar antiretroviral efficacy to tenofovir, the smaller doses (ten times lower dose), and paucity of systemic effects will probably cause it to replace tenofovir as the primary drug choice to treat chronic hepatitis B. Phase 3 trials have been completed with results expected by late 2015. Besifovir, an acyclic nucleotide phosphonate, is a nucleos(t)ide analogue that is structurally similar to tenofovir. Besifovir has a rapid intracellular phosphorylation and is effective in inhibiting HBV reverse transcriptase.[78] A phase 2b randomised trial[79] including both HBeAg-positive and HBeAg-negative Asian patients at doses of 90 mg and 150 mg per day combined with entecavir 0·5 mg per day for 48 weeks showed identical reduction in HBV DNA, ALT concentrations and seroconversion rates as patients

given entecavir alone. No resistant mutations or renal toxicity were noted in this study; however, L-carnitine depletion was recorded in the besofovir group, which required oral L-carnitine supplementation. Although besofovir shows favourable characteristics in terms of viral suppression, questions around long-term safety might limit its use.[79] Other compounds in phase 2 clinical trials[80–82] include CMX 157, AGX-1009, and lagociclovir valactate (MIV-210), which have shown promising phase 1 results (reduction in HBV DNA and cccDNA concentrations). Despite these new agents, any nucleos(t)ide analogue-based monotherapy will probably provide negligible HBsAg loss and cccDNA degradation, therefore strategies combining or sequentially adding other agents will probably be the mainstay of chronic hepatitis B therapy in the future.

Cyclophilin inhibitors

Cyclophilins are a group of proteins that are implicated in immunosuppression, although the exact mechanism of action is not wholly understood. Preclinical studies[83,84] with alisporivir in hepatitis C infection showed that cyclophilin inhibitors can block replication and contribute to immune system stimulation, to achieve higher sustained virological response (80%) than peginterferon and ribavirin for 24 weeks (58%). An in-vitro trial[85] of alisporivir in HBV showed dose-dependent reduction in HBV DNA and HBsAg concentrations after 72 h of treatment. The mechanism of action is thought to be related to interference at several sites of the HBV lifecycle. Further studies in human beings are needed to establish whether cyclophilin's mechanism of action will be enough to be given as a single agent or with combination therapy and to define a safety profile.

Immune targeting

Immunomodulators change the host response to HBV, with an aim to improve immune control or lead to viral eradication. Immune modulators considered for HBV therapy include interleukins, chemokines, and cytokines such as peginterferon alfa.

Interferon lambda

There are three classes in the interferon family. Interferon alfa and beta are type I interferons, and signal through a receptor that is present on every cell in the body. By contrast, interferon gamma is a type II interferon that binds to a receptor seen only on specialised immune cells. Members of the interferon lambda family, or type III interferons, bind to a receptor seen mainly on epithelial cells. Through engagement of its receptor, interferon lambda activates the Janus kinase-transducer and activator of transcription (JAK-STAT) pathway leading to induction of a similar range of genes as seen with type I interferons. These genes, collectively known as interferon-stimulated genes (ISGs), have antiviral, antiproliferative, and generally immunomodulatory properties. Although ISG induction is probably needed for viral clearance, the specific functions of the various ISGs are not well characterised and could cause the side-effects associated with peginterferon alfa treatment. Because of the modest distribution of the type III interferon-receptor, interferon lambda was proposed as an alternative interferon that would stimulate the same antiviral ISGs in hepatocytes but with a better side-effect profile, because of reduced ISG expression in non-hepatic tissues. A pegylated version of interferon lambda was assessed in the phase 2b LIRA-B study,[86] in which HBeAg-positive (HBV DNA >10^5 IU/mL, ALT >1 times upper limit of normal [ULN] 47 U/L) interferon-naive patients were randomly assigned (1:1) to 180 µg subcutaneous peginterferon lambda or 180 µg subcutaneous peginterferon alfa per week for 48 weeks (both subcutaneously). Peginterferon lambda showed greater HBV DNA–ALT normalisation and HBsAg decline from baseline to the end of treatment; however, 24 weeks after the end of treatment peginterferon alfa showed higher rates of HBeAg seroconversion (25%) than peginterferon lambda (11%) and virological suppression with fewer ALT flares than peginterferon alfa.[86,87] The findings suggest that the systemic effects of interferon could be important for off-treatment sustainability of responses. This might suggest that HBV clearance is more associated with a potent adaptive immune response, and thus T-cell activation by the interferon is crucial for clearance as shown by the flare in aminotransferases often seen with HBV clearance during interferon therapy. By contrast, hepatitis C is probably cleared by interferon-based therapy through the direct intracellular effect of induced ISGs with T cells having a less important role. There is probably little use for peginterferon lambda as a sole therapeutic option for chronic hepatitis B.

Other cytokine-based therapy

The innate immune response to HBV might have a role in elimination of chronic hepatitis B infection. Cytokines such as interleukin 7, interleukin 12, interleukin 18, and interleukin 1 play a part in B-cell and T-cell development and maintenance. These cytokines have a role in stimulating natural killer T cells to restore exhausted HBV-specific CD8 T cells, thus promoting inhibition of HBV replication. These molecules have shown promise in laboratory trials and are being assessed in phase 1 trials.[88–92]

PD-1 inhibitors

Persistence of HBV might be related to a blunted immune response to the virus, which is a combination of T-cell downregulation, dysfunction, and exhaustion. Programmed death-1 (PD-1) and its ligand programmed death ligand-1/2 (PD-L1/2) are expressed on T cells and play a part in T-cell regulation.[93–99] In-vitro and in-vivo studies with PD-1 and PD-L1/2 antagonists either alone, or combined with nucleos(t)ide analogues or vaccine

treatment have led to increased HBV-specific T-cell responses. Studies in woodchuck models[100,101] have shown similar results in the ability to boost T-cell responses, leading to inhibition of viral replication with elimination of cccDNA in a few woodchucks. Although trials in chronic hepatitis B patients are scarce, PD-1 antagonists in cancer patients have shown promising results with tumour regression in some patients with various tumour types. However, concerns have been raised about PD-1 antagonism because of toxic effects seen in oncology trials, with serious grade 3 or 4 adverse events reported in 9% of patients.[102] Thus the use of PD-1 antagonists might be restricted to a relatively healthy chronic hepatitis B population in view of the side-effect profile; however, second generation PD-1 inhibitors might be more selective and hold greater promise.

Toll-like receptor agonists

Toll-like receptors (TLRs) are important pathogen recognition receptors that stimulate innate and adaptive immune responses upon exposure to specific ligands. HBV downregulates TLRs in an attempt to evade innate immune responses. TLR agonists have been proposed as novel therapies that might induce endogenous interferon production and other innate responses, leading to induction of ISGs and other signalling cascades that inhibit HBV replication.[103] The most promising data so far have focused on stimulating TLR7, which is usually activated by RNA viruses but can be stimulated by several small molecules. After successfully showing that the oral TLR7 agonist GS 9620 could induce long-term suppression of HBV DNA in chimpanzees and stimulate production of interferon alfa, GS 9620 was tested in human trials with 75 healthy people.[104,105] Oral doses (single dose of 0·3 mg to 12 mg) of GS 9620 were well absorbed and well tolerated in doses up to 12 mg. The main adverse events were influenza-like symptoms similar to those seen with exogenous peginterferon alfa therapy but these were only seen in patients who received doses of 8 mg or 12 mg. Notably, serum interferon alfa was only detected at these higher doses, whereas ISG activation was seen at doses as low as 2 mg. The major advantages of TLR-7 therapy include having an oral formulation and the ability to use lower dosing regimens because activation happens in the gastrointestinal tract, enabling rapid uptake by liver through the portal circulation. Ultimately, it remains to be seen if clinically significant changes in HBsAg or HBV DNA concentrations can be achieved at doses that do not produce interferon-like side-effects.

Therapeutic vaccination

Activation of the adaptive immune system to upregulate CD4 and CD8 T-cell responses to neutralise HBV is another potential mechanism to eliminate chronic hepatitis B. Previous vaccines, used alone or in conjunction with nucleos(t)ide analogues, have shown safety but induced weak responses with low rates of HBeAg and HBsAg seroconversion. The main challenge with therapeutic vaccination is the inability to break tolerance, thus new vaccine strategies targeting different HBV proteins or adenoviral vaccine vectors (TG-1050) are being studied.[106–114] GS-4774 (tarmogen) is a recombinant heat-killed whole yeast vaccine, expressing several viral antigens including HBV X, HBV S, and core antigens, which can induce both CD4 and CD8 T-cell responses. In a single-centre safety trial, immune responses were assessed by use of enzyme-linked immunospot (ELISpot) and lymphocyte proliferation assays (LPA) in 49 healthy individuals without previous HBV vaccination receiving 10, 40, or 80 yeast units (1 YU=1 × 10^7 yeast cells) of GS-4774 either per week or per month for a total of 57 days. Irrespective of protocols, GS-4774 was well tolerated with no adverse events. Most patients showed a T-cell response when assessed by at least one of the assays at the end of the 85-day follow-up.[115,116] Phase 2 trials are enrolling to assess GS-4774 alone or in combination with tenofovir in chronic hepatitis B patients (NCT019433799). The first nasal vaccine, nasvac, is based on a combination of surface and core antigens of HBV, which synergistically induces priming of T cells (via activation of B cells) enabling them to act as antigen presenting cells.[117] A phase 1 double-blinded, placebo-controlled randomised clinical trial[118] in 19 healthy men (with no serological markers of HBV) substantiated the safety and tolerability of the agent. Sneezing was the most common adverse event (34·1%), and was self-limited. Phase 2 trials have been completed with results eagerly expected.

The antigen-antibody (HBsAg-HBIG) immunogenic complex with alum (YIC) is another vaccine approach that has been assessed. In a trial[119,120] of 450 patients with chronic hepatitis B, 12 doses of either YIC or alum (placebo) alone were given for 24 weeks with no significant differences reported in HBV DNA suppression, HBeAg seroconversion rates, or normalisation of liver function tests between the two groups. The absence of effect might be caused by immune exhaustion in the host.

Although therapeutic vaccines have long been an attractive approach for chronic hepatitis B, challenges with this strategy remain. Overcoming immune exhaustion seems to be the biggest hurdle, but other issues such as differences between genotypes and the best possible route will need to be established. Combining vaccines with other therapies might be an alternative. Viral suppression with potent nucleos(t)ide analogue therapy might reduce T-cell exhaustion; possibly enhancing the chances that subsequent therapeutic vaccination would be effective.

Conclusion

Control of HBV has greatly evolved over the past 20 years, with the development of an effective treatment algorithm and the introduction of widespread vaccination

Search strategy and selection criteria

References for the Review were identified through searches on PubMed for articles published from January, 1990, to May, 2015. The search terms used were "chronic hepatitis B", "new therapeutic agents", "assembly effectors", "RNA interference", "cccDNA degradation", "entry inhibitors", "HBsAg inhibitor, "hepatitis B virus", "vaccines", "immunomodulators". Relevant articles published between 1990 and 2015 were identified through searches in the authors' personal files and in Google Scholar. Articles resulting from these searches and relevant references cited in those articles were reviewed. Additionally, the Hepatitis B Foundation, updated June 22, 2015, was monitored for new therapeutics. The status of agents was verified by cross-referencing ClinicalTrials.gov. The review largely concentrates on phase 1, 2, or 3 trials; however, selected preclinical trials are included in view of the plausible possibility that they could cure HBV, and to provide a comprehensive review. Only articles published in English were included.

programmes. Unfortunately, available antiviral agents do not constitute a functional cure for chronic hepatitis B, and are limited by side-effects. However, with the emergence of activity in developing novel agents targeting different stages in the HBV lifecycle and host immune response, the current theory might still hold promise for cure of HBV. The discovery of the NTCP receptor to prevent HBV entry into hepatocytes along with potent nucleos(t)ides can prevent new cccDNA formation, which might be combined with antigen or cccDNA inhibitors to disturb existing HBV particles. This in itself might mount an immune response, leading to functional cure. If needed, direct immune-modulating agents can be added to this framework if their safety profile will allow widespread use. Nevertheless, although most agents are in early phase development, collectively they hold great promise for the future and hopefully help us achieve a cure in patients living with chronic hepatitis B.

Contributors

MB, AA, JF, and HLAJ were responsible for the design, integrity and the accuracy of the article, and for critical revision for important intellectual content. MB and AA were responsible for drafting of the manuscript.

Declaration of interests

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