Drugs in Development for Hepatitis B Targeting cccDNA

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ABSTRACT

To completely eliminate HBV infection, the total eradication of covalently closed circular DNA (cccDNA), the mini-chromosome that provides the template for transcription of all viral mRNAs, would be required. Nuclear cccDNA accumulates in hepatocyte nuclei where it persists as a stable epigenome. Because the HBV genome is integrated into the host cell with this persistence of cccDNA a complete cure in which all cccDNA and integrated virus is eliminated is not something we currently have but is considered the ultimate aim of future therapies. The dozens of experimental CHB treatments currently being developed fall into two main categories: direct-acting antivirals that impede viral replication at a specific point and host-targeting agents that modify host cell function in a way that inhibits viral replication, including both immune modulators and agents that target other host functions. Each of these can target cccDNA directly or indirectly. Discussed here are agents currently in the clinical stage of development for CHB treatment that target cccDNA directly and those that may affect cccDNA indirectly.

Keywords: hepatitis B virus, antivirals, immunomodulators, cccDNA
Introduction

Worldwide, approximately 292 million people are chronically infected with hepatitis B virus (HBV),\(^1\) approximately 75% of whom reside in Asia and 12% in Africa.\(^2\) Although the prevalence of chronic hepatitis B (CHB) is much lower in Western countries, it is estimated that even in the United States as many as 2.2 million people may be chronically infected.\(^3\) In Cuba, there are insufficient epidemiologic data to accurately estimate national CHB prevalence but since the inclusion of hepatitis B vaccine in the national immunization program in 1992 there has been a decline in the annual reported cases.\(^4\) CHB is one of the leading causes of liver disease, cirrhosis, and hepatocellular carcinoma (HCC) worldwide.\(^1\) In Cuba, liver cirrhosis was the ninth leading cause of death in 2018.\(^5\) Thus, there is considerable interest in developing effective CHB therapies.

The only currently approved therapies are long-term treatment with nucleos(t)ide analogues (NUCs) and a finite course of pegylated interferon-alpha (PEG-IFN-α).\(^6\) Although these therapies have substantial benefits, decreasing the risks of HCC and liver decompensation and increasing survival,\(^6-8\) the elimination of hepatitis B surface antigen (HBsAg) does not usually occur. HBsAg loss is seen in only 3-8% of either HBeAg-negative or HBeAg-positive CHB patients at 48-52 weeks of PEG-IFN-α therapy.\(^6,9,10\) HBsAg loss is in the same range for patients receiving NUC therapy, only occurring in 0% to 11.8% of HBeAg-positive patients and 0.3% to 5% of HBeAg-negative patients even after multiple years of therapy.\(^11-14\) Thus, new therapies that could substantially increase HBsAg loss and potentially allow therapy to be discontinued are of great interest.

To completely eliminate HBV infection, the total eradication of covalently closed circular DNA (cccDNA), the mini-chromosome that provides the template for transcription of all viral mRNAs,\(^15,16\) would be required.\(^17\) Nuclear cccDNA accumulates in hepatocyte nuclei where it persists as a stable epigenome.\(^16,18,19\) Because the HBV genome is integrated into the host cell with this persistence of cccDNA a complete cure in which all cccDNA and integrated virus is eliminated is not something we currently have but is considered the ultimate aim of future therapies.\(^20\)
Drugs in Development

The dozens of experimental CHB treatments currently being developed fall into two main categories: (1) direct-acting antivirals (DAAs) that impede viral replication at a specific point and (2) host-targeting agents that modify host cell function in a way that inhibits viral replication, including both immune modulators and agents that target other host functions. Each of these can target cccDNA directly or indirectly. Discussed here are agents currently in the clinical stage of development for CHB treatment that target cccDNA directly and those that may affect cccDNA indirectly. A broader summary of agents currently under development for the treatment of CHB are summarized in the Table.

Direct-acting Antivirals

Antisense Oligonucleotides

Multiple antisense oligonucleotides (ASOs) are currently in development. It is thought that they may yield a decrease in cccDNA by blocking core production and capsid formation. In a duck model, use of polyethyleneimine-based ASOs has been shown to result in significant reductions in viremia, intrahepatic HBV DNA, HBV RNA, and surface and core proteins. This may represent an effect on cccDNA in the nucleus. It is also known that ASOs can downregulate asialoglycoprotein receptor 1 (ASGPR1) which HBV upregulates, thus blocking HBV replication by inhibiting hepatic endocytosis of HBV.

cccDNA Formation and Transcription Inhibitors

Repressing cccDNA can occur by blocking its formation, expression, or stability. Cell studies have shown that there might be agents that could decrease or eliminate cccDNA. Two disubstituted sulfonamides (DSS), CCC-0975 and CCC-0346, have been shown to inhibit the formation of cccDNA from rcDNA by suppressing rcDNA deproteinization; CCC-0975 was shown to reduce cccDNA biosynthesis. In another approach, cell studies have shown that activation of the lymphotoxin β receptor (LTβR) with a super-agonistic tetravalent bispecific antibody (BS1) and a
bivalent anti-LTβR monoclonal antibody (CBE11) results in non-hepatotoxic degradation of cccDNA.\(^{24}\) Inhibition of cccDNA formation has also been achieved by targeting the HBV viral genome with endonucleases including meganucleases, transcription activator like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and the CRISPR (clustered, regularly interspaced, short palindromic repeat)/Cas 9 genome editing tool.\(^{25}\)

**Core/Capsid Inhibitors**

Several agents have been developed that counter HBV DNA replication by disturbing capsids or countering core particle assembly.\(^{26}\) The result may be decreased cccDNA levels. Included in this class are the heteroaryldihydropyrimidines (HAPs) Bay 41-4109, HAP-1, GLS-4, HAP-18 and NVR-010-001-E2.\(^{27,28}\)

**HBsAg Release Inhibitors**

In another approach, nucleic acid polymers (NAPs) have been studied because of their ability to inhibit protein interactions involved in viral replication. Their possible effect on cccDNA is hypothetical via reductions in HBcrAg and HBV RNA levels in the blood. In one small study of 12 patients treated with NAP HBsAg release inhibitor REP 2139-Ca followed, in responders, by peginterferon alpha-2a and/or thymosin alpha-1, there was a substantial decline in HBV DNA and HBsAg levels and HBV DNA the majority continued HBV DNA declines off treatment before subsequent rebound.\(^{67}\) This agent is currently in phase 1 and 2 clinical trials in combination with Peg-IFN and TDF.

**Reverse Transcriptase Inhibitors**

Because nucleos(t)ide analogue reverse transcriptase (RT) inhibitors (NUCs) do not directly suppress cccDNA viral transcription or translation they are not considered major inhibitors of cccDNA, although a 1-2 log reduction in cccDNA has been seen in some studies in liver tissue where cccDNA levels could be measured. Clevudine, a NUC approved for HBV treatment in South Korea and the Philippines,\(^{29}\) is no longer used as a solo agent because of adverse effects (myopathy) and drug resistance but it is used in lower doses in combination with adefovir.\(^{30-32}\)
a new form of clevudine that it is thought may affect cccDNA levels via a new mechanism is entering phase I trials. Although Lai and colleagues have reported that prolonged NUC treatment (median 126 months), markedly reduced cccDNA, it was also shown that, although reduced, serum HBsAg levels remained detectable in 42 of 43 patients.\textsuperscript{33} Long-term treatment with NUCs can be associated with drug toxicity and drug resistance.\textsuperscript{34,35} Therefore, there is a need to identify compounds that can lead to eradication of the virus.

**RNA Interference Therapies**

RNA interference (RNAi) therapies directly target hepatitis B virus mRNA transcripts and have been shown to do so with high specificity. They reduce HBsAg and HBcAg production through the use of small, non-coding RNAs that regulate the expression of genetic information.\textsuperscript{36} This may restore host immunity\textsuperscript{37} and decrease cccDNA replenishment.

**Host-Targeting Agents**

Included in this category are a broad variety of agents that modify various aspects of host cell function in ways that inhibit viral replication. These agents can be broadly subdivided into immune modulators and agents that target other host functions.\textsuperscript{21} Such host-targeting agents may be able to boost both innate and adaptive immunity, thus helping to clear HBV-infected hepatocytes.

**Engineered T cells**

Engineering T cells is designed to boost the attraction of T-cell receptors for specific antigens. Preclinical studies have been promising. In HBV transgenic mice, CD8(+) T cells engineered to express HBV-specific chimeric antigen receptors (CARs) were shown to recognize multiple HBV subtypes and to be able to engraft and expand, localizing to the liver and quickly decreasing HBV replication.\textsuperscript{38} In an important recent study by Protzer and colleagues, in HBV-infected hepatoma cells, co-culture with T cells engineered to express high-affinity T-cell receptors specific for HBV core or envelope proteins led to undetectable levels of cccDNA and viral antigens.\textsuperscript{39} In HBV-infected humanized mice, adoptive transfer of T-cell receptor-grafted T cells led to clearance of
HBV-infected hepatocytes and major decreases in HBV viral load and cccDNA. A clinical trial of therapy with engineered T cells in patients with HBV-associated HCC is now planned. Although promising, the very high cost of this individualized therapy and the serious adverse effects seen in other diseases where it has been studied will no doubt limit its use, especially in the developing world.

**Entry Inhibitors**

Entry inhibitors could block HBV entry into hepatocytes before cccDNA is even formed. They work by either blocking HBV binding to the cell receptor(s) or HBV attachment to hepatocytes. Myrcludex B is a synthetic N-myristoylated peptide that competitively attaches to the sodium taurocholate cotransporting polypeptide (NTCP) receptor, thus preventing HBV from entering hepatocytes. In one study it was shown that myrcludex B prevented intrahepatic virus spreading in humanized mice and hindered amplification of intrahepatic cccDNA by blocking the conversion of rcDNA to cccDNA.

**Immunomodulatory Agents**

Much work has been done on approaches to altering the immune response to HBV in order to restore effective antiviral immune responses and many immunomodulatory agents are currently being assessed, including therapeutic vaccines, engineered T cells, toll-like receptor agonists, immune checkpoint inhibitors, and others.

**Toll-like Receptor Agonists**

It is known that HBV reduces the toll-like receptor (TLR) antiviral activity of liver cells. These receptors are key players in immune responses because they sense pathogens and boost inflammatory cytokine release and the adaptive immune responses that follow. TLR7 stimulation mediates an endogenous type I interferon response which is key for developing an effective immunity against HBV. In preclinical studies, activation of intrahepatic innate immune responses with TLRs 3/7/8/9 or STING agonists has been shown to suppress HBV. Multiple doses of the TLR7 agonist GS-9620 given to chimpanzees were shown to result in significant reductions in
viral load, HBsAg, and HBeAg but, after phase 1 trials did not show reductions in HBsAg levels or HBV DNA with monotherapy, its usefulness in combination with TDF is now being studied in phase 2 trials.

Other Immune Modulators and Associated Therapies

Current studies are assessing the immune restoration and vaccine adjuvant effects of certain cytokines and cytokine receptor agonists which it is thought might eliminate cccDNA. SB 9200 is a novel therapy that may not only have direct antiviral properties but also have the ability to prompt endogenous IFN-mediated immune responses in HBV-infected cells. The latter is accomplished by activation of retinoic acid-inducible gene 1 (RIG-I) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2). In woodchucks, daily dosing of SB 9200 for 12 weeks resulted in substantial reductions in viral load and in surface antigen, the latter thought to result from suppression of cccDNA synthesis or transcription.

Because the effectiveness of some immune modulators may be reduced by high antigen levels the CRISPR (clustered, regularly interspaced, short palindromic repeat)/Cas9 genome editing tool that has been called a type of molecular scissors has been studied as an approach to removing HBV cccDNA, both in cell studies and using a mouse model. Although it is hoped that this technique could ultimately be used to directly remove cccDNA, thus improving the effectiveness of certain immune modulators, recent studies showing that it may damage DNA located far from the target DNA has led researchers to urge additional cautionary measures as these therapies are developed.

Conclusion

Although it is clear that current HBV therapies reduce progression to chronic liver disease and its sequelae, many different treatments with multiple targets, including both virologic approaches and host immune approaches, are being studied to achieve the ultimate goal: elimination of cccDNA. Almost all of these approaches are in pre-clinical or phase 1 or 2 trials. Until a true sterilizing cure can be achieved in which all cccDNA and integrated virus are removed, the approaches that work the best with CHB may be a combination of effective antiviral therapies and host-targeting
agents. Research advances have shown that such a combined approach may lead to what has been termed a functional cure for CHB in the not distant future.

**References**


