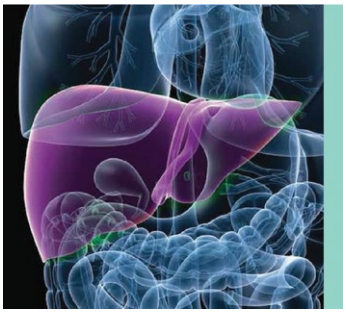

Disease Pathways and Mechanisms of Potential Drug Targets

Marc G. Ghany, M.D., M.HSc., and Timothy M. Block, Ph.D.*

Hepatitis B virus (HBV) is a small, partially double-stranded DNA virus that can cause acute and chronic hepatitis. Chronic HBV infection is a major cause of cirrhosis and hepatocellular carcinoma.[1] Despite the availability of a protective vaccine for more than 30 years, it is estimated there are 257 million persons worldwide with chronic HBV infection and 887,000 deaths annually from complications of cirrhosis and hepatocellular carcinoma.[2] Chronic infection is due to the persistence of the covalently closed circular DNA (cccDNA) as an episomal mini-chromosome in the hepatocyte nucleus and to the ability of HBV to evade the immune system.

Current therapy consists of two classes of antiviral agents, nucleos(t)ide analogues and interferon.[3] Nucleos(t)ide analogues are small-molecule, competitive inhibitors of the viral reverse transcriptase. They block formation of the nascent HBV DNA, thereby inhibiting HBV replication.[4] The exact mode of action of interferon is not known, but it is generally assumed to function by induction of dozens of different host proteins with direct antiviral activities and others that stimulate the host adaptive immunological response. There is also recent evidence that interferons cause repression of transcription of the HBV cccDNA[5] and may even induce cytidine deaminases

Abbreviations: anti-HBs, hepatitis B surface antibody; APOBEC3A, apolipoprotein B mRNA editing enzyme catalytic subunit 3A; CAS9, CRISPR-associated protein 9; cccDNA, covalently closed circular DNA; CRISPR, clustered regularly interspaced short palindromic repeats; DAA, direct-acting antiviral agent; DHQ, dihydroxyquinoline; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HSP, heat shock protein; IDAA, indirect-acting antiviral agent; NTCP, sodium taurocholate cotransporting polypeptide; PD-1, programmed death 1; PD-L1, programmed death ligand 1; RIG-I, retinoic acid–inducible gene I; RNAi, RNA interference; siRNA, small interfering RNA.

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[*]This study was supported by Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Received 30 May 2018; Accepted 20 June 2018

Potential conflict of interest: M.G.G. is an employee of the U.S. Government and has no financial conflicts of interest to disclose.

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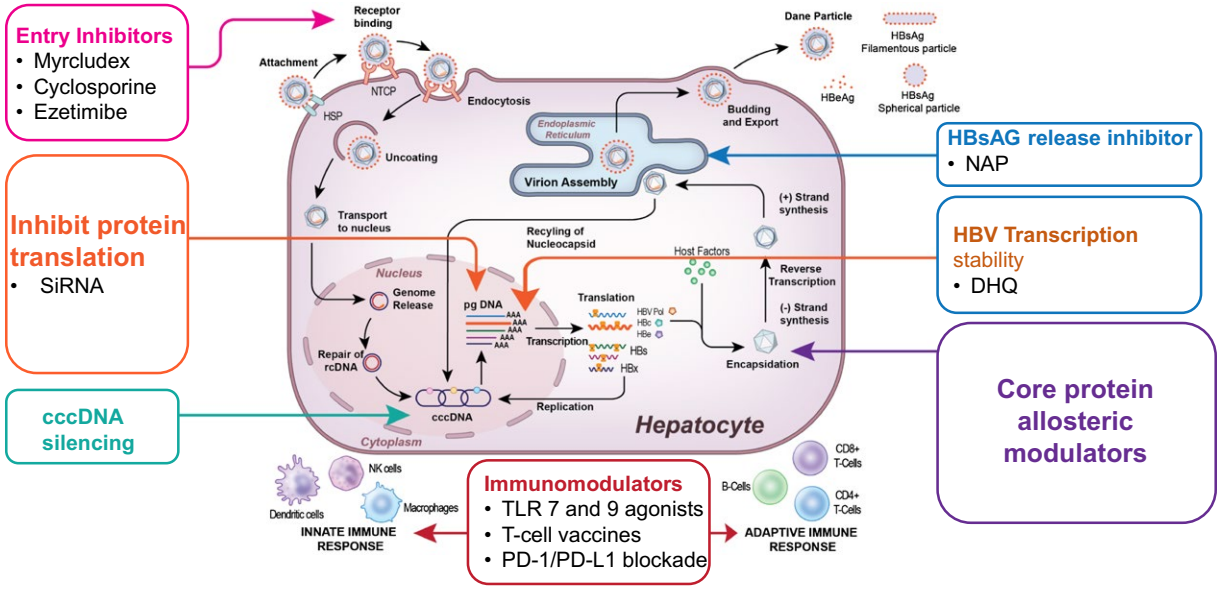

FIG 1 Novel approaches for HBV cure. A better understanding of HBV biology has identified a number of new targets for drug development, and at least seven new classes of compounds are being pursued as therapy for HBV. These include entry inhibitors such as Myrcludex B and monoclonal antibodies. Targeting the viral mRNA using nucleic acid approaches (e.g. siRNA) are promising but delivery to the hepatocyte nucleus remains a challenge. Eliminating or silencing cccDNA is the holy grail of HBV therapy. The availability of gene-editing technology such as CRISPR/CAS9 now makes this a feasible approach, but the clinical applicability is limited by how to efficiently deliver this to all HBV-infected cells. Core protein allosteric modulators are an attractive approach because of the importance of the core particle for the viral life cycle, and several compounds are in early-stage development. The viral polymerase still remains a target for drug development, and agents that target the RNaseH function of the polymerase are being pursued. Releasing inhibitors that block HBsAg or virion secretion are being developed and appear promising, but there is the concern of the effect of retention of viral proteins or complete virions within the hepatocyte. Finally, antiviral approaches alone may not be enough, and strategies to boost the innate or adaptive immune responses using novel immunomodulatory approaches also are being developed including TRL agonists, checkpoint inhibitors, T cell reprogramming, and T cell vaccines. Abbreviations: HSP, heat shock protein; NAP, Nucleic acid polymer; NK, natural killer; PD-1, programmed death 1; PD-L1, programmed death ligand 1.

(apolipoprotein B mRNA editing enzyme catalytic subunit 3A [APOBEC3A] and APOBEC3B) that cause cccDNA degradation.[6-8] Although current antiviral agents are effective at inhibiting viral replication and reducing complications of chronic HBV infection, they are not curative and in the case of nucleos(t)ide analogues must be administered long term, if not indefinitely, because of persistence of the cccDNA.[3]

The development of new treatments for HBV has been hampered by the small and compact nature of the HBV genome with relatively few druggable viral targets. However, a better understanding of the viral life cycle together with advancements in *in vitro* culture systems and animal models have led to the identification of a number of novel therapeutic approaches against HBV, as illustrated in Fig. 1. There are now more than 30 investigational agents in the pipeline of development, targeting specific viral gene products (direct-acting antiviral agents [DAAs]) and host targets (indirect-acting antiviral agents [IDAAs]).[9] The focus of current drug development is on achieving functional cure, defined as sustained loss of hepatitis B surface antigen (HBsAg) with or without concomitant development of hepatitis B surface antibody (anti-HBs), without the need for ongoing therapy.[10] This brief review will highlight key developments in antiviral and immunomodulatory therapy and the rationale for choosing these approaches.

ENTRY INHIBITORS

HBV entry into hepatocytes requires coordinated binding to heparin sulfate proteoglycans, a low-affinity receptor, to mediate hepatocyte attachment, followed by a high-affinity interaction with the sodium taurocholate cotransporting polypeptide (NTCP) for viral internalization.[11,12] The NTCP receptor confers the species specificity to HBV.[13,14] Understanding this process has led to the development of several classes of specific and nonspecific inhibitors of viral entry (see Table 1).[15-18] Entry inhibitors may be useful therapeutically by preventing *de novo* infection of uninfected hepatocytes.

TABLE 1. VIRAL AND HOST TARGETS, MECHANISM OF ACTION, AND NOVEL COMPOUNDS IN DEVELOPMENT FOR CHRONIC HEPATITIS B

Target	Mechanism of Action	Class	Compounds in Development
Viral entry	Antibodies targeting pre-S1 or small surface protein	Monoclonal antibodies	GC1102
	Attachment inhibitors that prevent viral interaction with entry receptors	Heparin	
		Poly-L-lysine	
	Reversibly/irreversibly block the NTCP receptor	Conjugated bile salts	
		Synthetic *N*-acylated pre-S1	Myrcludex B
		Cyclosporine	
cccDNA	Inactivate cccDNA	Zinc finger nucleases	
		Transcription activator-like effector nuclease	
		CRISPR/cas9 system	EBT106 HBV CRISPR-CAS9 lipid nanoparticle
	Degrade cccDNA	Interferon α,γ	
		Tumor necrosis factor-α	
		Lymphotoxin- receptor agonists	
	Functionally silence cccDNA	Epigenomic modifiers	
Viral transcripts	Degrade mRNA	siRNA	ARB-1467 ARB-1740 ALN-HBV Hepbarna (BB-HB-331) Lunar-HBV
	Bind viral mRNA to prevent viral protein production	Antisense oligonucleotides	IONIS-HBVRx (GSK3228836) IONIS-HBVLRx (GSK33389404)
	Cause degradation of HBV RNA in the nucleus	DHQ	AB452 RG7834
	Downregulate viral mRNA	Farnesoid X receptor α agonist	EYP001
Core assembly modulators	Inhibit encapsidation of pregenomic RNA or nucleocapsid assembly	Heteroaryldihydropyrimidines (HAPS)	Morphothiadin (GLS4)
		Phenylpropenamide	AT-61; AT-130
		Pyridazinone derivatives	3711
		Sulfamoylbenzamide	AB-423 JNJ56136379 NVR 3-778
		Isothiafludine	NZ-4
		2-Amino-n-(2,6-dichloropyridin-3-yl) acetamide derivatives	BCM-599
		5,5′-bis[8-(phenylamino)-1- naphthalenesulfonate]	Bis-ANS
HBsAg release inhibitors	Synthetic oligonucleotides that bind HBsAg	Nucleic acid polymers	Rep 2139 Rep 2165
Boost innate immunity	Agonists of sensing arm of innate immune system	Toll-like receptor-7 agonist	RO6864018 (RG7795, ANA773
		Toll-like receptor-8 agonist	GS-9688
		RIG-I and Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) agonist	Inarigivir (SB9200)
		STING agonists	
		TCR-like antibodies	
Boost humoral immunity		Anti-HBs	

TABLE 1. CONTINUED

Target	Mechanism of Action	Class	Compounds in Development
Boost adaptive immunity	Checkpoint inhibitors	Anti-CTLA-4	
		Anti-PD-1	Nivolumab
	Engineering new HBV-specific T cells	TCR gene transfer	LTCR-H2-1
		Chimeric antigen receptor-T cells (CAR-T)	
Therapeutic vaccines	Induction of HBV-specific B and T cells	T cell vaccines	HepTcell
		DNA vaccines	HB-110 INO-1800
		Viral vectors expressing HBV proteins	TG1050 TomegaVax HBV

TARGETING THE cccDNA

cccDNA serves as the template for viral transcription and plays a key role in the viral life cycle. Its persistence in the nucleus of infected hepatocytes is a major reason why cure of HBV is currently not possible. Therefore, strategies to eliminate cccDNA are the holy grail of HBV therapy. However, our understanding of the molecular mechanisms that regulate control of cccDNA activity in hepatocytes is limited. A number of approaches are currently being investigated to eradicate cccDNA, including inactivation through the generation of sequence-specific endonucleases with zinc finger nucleases or to cleave cccDNA by using transcription activator-like effector nucleases or clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CAS9) system.[19-21] Additional approaches being pursued are to use agents to upregulate APOBEC3A/B deaminases (see Table 1) that noncytolytically degrade cccDNA and to functionally silence cccDNA through modifiers of epigenetic regulation.[17] The clinical applicability of all of these approaches is currently limited

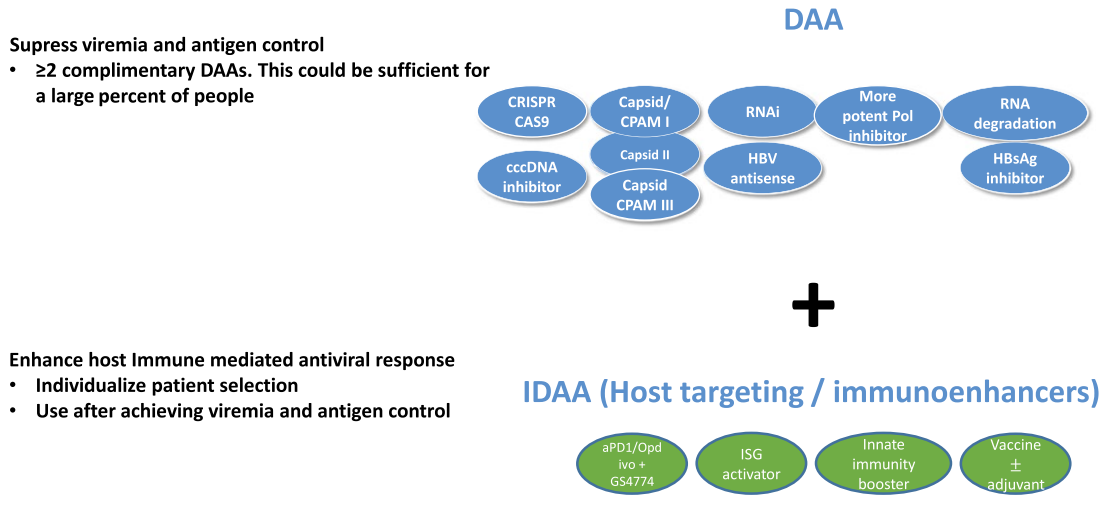

FIG 2 Possible combination therapy strategies to treat chronic HBV. As new therapies to manage HBV become available, it seems likely that combination therapy will be used. Each oval represents a distinct drug in development that is considered either direct acting (blue) or indirect acting (green), based on its mechanism of action. The IDAAs include those that target the host immune systems. One approach will be to first use DAAs to achieve suppression of viremia and antigenemia followed by IDAAs to clear remaining virus and effect functional cure. Abbreviation: CPAM, Core protein allosteric modulators; RNAi, RNA interference.

by efficient and targeted delivery of these molecules to all HBV-infected cells and concerns for off-target effects.

TARGETING VIRAL TRANSCRIPTS

Four viral genes are transcribed from the cccDNA template by host RNA polymerase II in the cell nucleus. These mRNA transcripts (core, polymerase, surface, and X genes) encode all the viral proteins. Technological advancements now permit gene silencing through the use of small interfering RNAs (siRNAs) that target viral transcripts and induce their degradation by the RNA-induced silencing complex/argonaute 2 and by the use of antisense oligonucleotides that block protein expression through steric hindrance and/or RNA degradation by ribonuclease H cleavage (Table 1).[22] Recently, small dihydroxyquinoline (DHQ) molecules have been identified that appear to selectively cause degradation of HBV transcripts, accomplishing much of what siRNA achieves.[23,24] These represent an entirely new approach to HBV antivirals and are in the development pipeline, having been shown to be effective *in vitro* and *in vivo*. The precise molecular target of these drugs is not clear but requires that the targeted RNA contain *cis*-acting RNA sequences and may involve host degradation pathways. Cellular toxicity and how to efficiently deliver these compounds remain a challenge.

TARGETING NUCLEOCAPSID ASSEMBLY

The HBV core protein has many important roles in the HBV life cycle including assembly of viral nucleocapsids. It is the site of HBV DNA replication, stability of cccDNA through modification of chromatin, regulation of viral transcription, and modulation of the host innate immune response. Thus, development of modulators of nucleocapsid assembly have long been an attractive therapeutic target. Unfortunately, the first-generation compounds were limited by hepatotoxicity.[25] There are now many newer core protein allosteric modulators that have been shown to inhibit encapsidation of pregenomic RNA or nucleocapsid assembly, or both (Table 1).[26-31] Potential advantages of these compounds include high selectivity and broad spectrum of activity against all HBV genotypes.

HBsAg RELEASE INHIBITORS

Clearance of HBsAg is one of the goals of a functional cure. HBsAg is made in vast excess of what is needed for enveloping viral nucleocapsids to complete virion assembly, and it has been speculated that HBsAg may contribute to the immune exhaustion observed in patients with chronic HBV infection. Thus, there have been efforts to block release of HBsAg from hepatocytes. A number of compounds have shown promise (see Table 1).[32-34] However, there are concerns about the long-term consequences of HBsAg retention in the hepatocyte because this has been associated with the development of hepatocellular carcinoma in animal models.[35]

IMMUNOLOGICAL APPROACHES

Chronic hepatitis B is characterized by a weak innate immune response, a defective humoral response with absence of neutralizing antibodies, and an exhausted adaptive response.[36] Therefore, the other major effort in HBV therapeutics is to develop strategies to reinvigorate or boost the immune response. Broadly, strategies to induce an innate immune response include use of agonists of the sensing arm of the innate immune response (e.g. toll-like receptor or retinoic acid–inducible gene I (RIG-I) agonists) or to augment the response by selectively targeting HBV-infected hepatocytes using T cell receptor-like antibodies conjugated with inhibitory cytokines.[37] Approaches to enhance the adaptive immune response include strategies to boost the limited HBV-specific T cells already present by using checkpoint inhibitors,[38,39] or to genetically engineer new HBV-specific T cells via T cell receptor gene transfer or chimeric antigen receptor T (CAR-T) cells that can be adoptively transferred to patients.[37,40] There has also been renewed interest in developing therapeutic vaccines that can break T cell tolerance to HBV proteins and stimulate HBV-specific T cell responses in patients with chronic infection.[37] Protein, DNA, and T cell vaccines have been tested, but studies of therapeutic vaccination have been unsuccessful probably because they targeted only HBsAg. Newer approaches using vaccines incorporating multiple HBV proteins with or without adjuvants are in development.[37] Major limitations of these immune-based approaches are

the potential to induce severe hepatitis flares, autoimmunity, and in the case of adoptive T cell therapy, issues with scalability.

COMBINATION THERAPY

It is very likely that combinations of antiviral therapy targeting multiple steps in the viral life cycle or combination antiviral/immunomodulatory approaches will be needed to achieve the goal of functional cure. Which specific agents will be required is currently not known, but with the development of new therapeutic agents, a combination of a DAA and host-acting, perhaps immunorestorative agents, as shown in Fig. 2, will be possible. Although effective suppressive therapy exists for HBV, the development of multiple novel approaches brings achieving the goal of a functional cure into the realm of possibility.

CORRESPONDENCE

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