HHS Public Access

Author manuscript

Adv Exp Med Biol. Author manuscript; available in PMC 2022 April 07.

Published in final edited form as:

Adv Exp Med Biol. 2021 ; 1322: 115–138. doi:10.1007/978-981-16-0267-2_5.

Moving Fast Toward Hepatitis B Virus Elimination

Leda Bassit[#],

Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Children's Healthcare of Atlanta, Atlanta, GA, USA

Suzane Kioko Ono[#],

Department of Gastroenterology, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil

Raymond F. Schinazi

Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Children's Healthcare of Atlanta, Atlanta, GA, USA

[#] These authors contributed equally to this work.

Abstract

Currently, there are two safe and effective therapeutic strategies for chronic hepatitis B treatment, namely, nucleoside analogs and interferon alpha (pegylated or non-pegylated). These treatments can control viral replication and improve survival; however, they do not eliminate the virus and therefore require long-term continued therapy. In addition, there are significant concerns about virus rebound on discontinuation of therapy and the development of fibrosis and hepatocellular carcinoma despite therapy. Therefore, the search for new, more effective, and safer antiviral agents that can cure hepatitis B virus (HBV) continues. Anti-HBV drug discovery and development is fundamentally impacted by our current understanding of HBV replication, disease physiopathology, and persistence of HBV covalently closed circular DNA (cccDNA). Several HBV replication targets are the basis for novel anti-HBV drug development strategies. Many of them are already in clinical trial phase 1 or 2, while others with promising results are still in preclinical stages. As research intensifies, potential HBV curative therapies and modalities in the pipeline are now on the horizon.

Keywords

Hepatitis B; Core inhibitor; cccDNA; DAA—directly acting antiviral; Immune therapy; Hepatocellular carcinoma

rschina@emory.edu .

Declaration of Competing Interest Drs. Schinazi and Bassit along with Emory University are entitled to equity and royalties related to anti-HBV products licensed to Aligos Therapeutics, Inc., being further evaluated in the research described in this review. The terms of this arrangement have been reviewed and approved by Emory University in accordance with its conflict of interest policies.

5.1 Introduction

Chronic hepatitis B virus (HBV) infection affects approximately 300 million people worldwide [1], and while prophylactic vaccines and antiviral therapies are currently in use, they do not provide a cure. Therefore, safe antiviral agents that target the HBV replication cycle and sites of virus persistence are urgently needed to prevent the nearly one million human deaths annually due to liver diseases associated with hepatitis B. HBV is a hepadnavirus that replicates its DNA in the liver through two main steps: formation of covalently closed circular DNA (cccDNA) and the reverse transcription of a pregenomic RNA (pgRNA).

With current available antiviral therapies for chronic hepatitis B, it is possible to control HBV replication. However, treatment is non-curative and therefore requires long-term continued use which has resulted in concerns for the development of antiviral resistance and adverse events, such as renal impairment or gastrointestinal disorders (important issue when considering adherence to treatment) [2–4]. The clinical endpoints now are focused on suppressing viral replication and alanine aminotransferase (ALT) normalization. This desirable endpoint of a functional cure (loss of HBsAg) is unlikely with current nucleoside analogs or pegylated interferons [5]. This may be due to cccDNA that persists in the nuclei of infected hepatocytes where it forms the template for all viral transcripts and HBV integration. New HBV targets and immune therapies are being sought, and we aim to review them according to their stage in clinical development, focusing on medicinal chemistry and/or biochemistry/molecular biology [6]. In addition, this review focuses on the outcomes of antiviral drugs newly developed or in clinical evaluation, as well as novel experimental drugs.

5.2 HBV Pathogenicity (Immunological Background)

HBV is a hepatotropic virus and most of the time does not cause a cytopathic effect [7]. The host immune response determines whether the virus persists (chronic infection) or not (cleared infection). In the natural history of chronic hepatitis B infection, initially there is an immunotolerant phase characterized by the presence of HBeAg, high rates of HBV DNA replication, and absence of inflammatory liver disease progression [3]. In this phase, the innate immune system is poorly activated due to an intrinsic ability of the virus to escape recognition [8].

In contrast, a persistent immune response to HBV-infected hepatocytes is the determinant of chronic liver disease, with inflammation (with or without HBeAg) leading to progression of fibrosis and cirrhosis, and ultimately hepatocellular carcinoma [3, 9, 10]. Individuals who have resolved HBV infection, with HBsAg clearance with or without HBs antibody, undetectable HBV DNA, and normal levels of ALT, are in the so-called functional cure phase [11]. In this phase, HBV is not fully eliminated, with a few hepatocytes remaining with the cccDNA form under a repressed translational control by innate and adaptive immune mechanisms [9].

In this regard, several immune pathways with the potential to suppress HBV replication in infected hepatocytes are currently under consideration as targets for the development of new therapeutic strategies for chronic hepatitis B infection. For example, retinoic acid-inducible gene-I (RIG-I) and apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) [9] are among other pathways that will be discussed below.

5.3 HBV Replication

HBV is a 3200 bp partially double-stranded DNA (rcDNA, relaxed circular DNA) from *Hepadnaviridae* family. Difference greater than 8% in nucleotide sequence across the complete HBV genotype determines ten major genotypes (A to J) with differences in replication, natural history, pathogenesis, and treatment response [12, 13]. HBV genome encodes four overlapping genes. The HBV RNA transcripts are translated into seven proteins: HBsAg (surface large [preS1+preS2+S domains], middle [preS2+S domain], small [S domain]), HBeAg, HBcAg (core), RT-polymerase, and X protein. The HBV virion particles have an outside envelope composed with three forms (large, middle, and small) of surface proteins that encloses the capsid with the double-stranded DNA genome (Fig. 5.1). An important intermediate form (occurring in the nucleus of infected cells) is the covalently closed circular DNA (cccDNA) that is the template for pregenomic RNA (pgRNA) transcription and produces the template for reverse transcription and viral genome replication [14].

5.3.1 Replication Cycle

HBV binds to the hepatocyte at the sodium taurocholate cotransporting polypeptide (NTCP) receptor and enters into the cells. HBV attachment is believed to be mediated through the preS1 domain [15]. After entry, the viral particles containing the relaxed circular DNA (rcDNA) are uncoated, and the nucleocapsid particle must be directed into the cellular nucleus. HBV rcDNA is converted to an episomal cccDNA (see detailed information below). HBV cccDNA is the transcription template for all four viral RNAs (Fig. 5.1):

1. A 2.4-kb mRNA for the large (L) envelope protein, a 2.1-kb mRNA for the middle (M), and major surface (S) proteins
2. A 0.7-kb mRNA for the X protein
3. A 3.5-kb pre-core mRNA that encodes the pre-core protein
4. A 3.5-kb pregenomic RNA (pgRNA) that encodes the core and the polymerase

The pgRNA, upon being exported to the cytoplasm, is encapsidated together with viral polymerase and subsequently reverse-transcribed into viral minus strand DNA. Then, the plus-stranded DNA is synthesized to form the partially double-stranded relaxed circular DNA. The mature nucleocapsid can either be recycled back to the nucleus to maintain the pool of cccDNA or packed with envelope proteins and exported as infectious virions to infect other cells [14, 16] (Fig. 5.1).

5.3.2 Role of cccDNA

Intrahepatic cccDNA is the episomal virus template in the nucleus of HBV-infected hepatocytes. It is considered an important cause of viral persistence and a key obstacle for a cure of chronic hepatitis B [17]. This is especially true because current antiviral therapies including nucleoside analogs do not eliminate HBV mini-chromosome (cccDNA) or integrated HBV; therefore, continued virus gene expression from these templates will drive pathogenesis toward hepatocellular carcinoma, one of the main complications of chronic hepatitis B. Another currently used treatment for chronic hepatitis B, interferon alpha, upregulates the expression of APOBEC3 nuclear deaminase resulting in a modest reduction in cccDNA copy number via deamination [18].

Because cccDNA elimination is a major goal for the future HBV antiviral agents for the treatment of chronic hepatitis B, it is important to monitor and study this particular HBV form. However, the amount of cccDNA compared to pgRNA is very low (median 1.5 copies and 6.5 per cell, respectively) [19]. Therefore, to detect HBV cccDNA unambiguously is a great challenge [17]. Southern blotting is the gold standard test for detection and quantification of HBV intermediates and cccDNA; however, few samples can be tested at a time, and it requires high amounts of infected cells to detect cccDNA. Because it is not a high-throughput system, other tests including cccDNA-specific PCR have been assessed using specific primers located at each side of the gap region of rcDNA together with the appropriate HBV DNA purification or nucleus enrichment and the use of appropriate enzymes to selectively remove HBV rcDNA without degrading cccDNA [20]. Because liver biopsy is required to quantify cccDNA in vivo, measurements of HBV RNA and HBcAg in the serum may serve as surrogate biomarkers for cccDNA [11].

5.4 Overview of Current Therapies

Interferon alpha 2b (FDA approved in 1991) and peginterferon alpha-2a (approved in 2005) are immunomodulators administered subcutaneously, but due to adverse effects treatment duration varies up to 48 weeks (Table 5.1) [26, 27]. There are reports that HBV genotype A may present a higher response rate considering HBeAg seroconversion [28, 29].

Lamivudine (approved in 1998), adefovir (approved in 2002), entecavir (approved in 2005), telbivudine (approved in 2006), tenofovir (approved in 2008), and tenofovir alafenamide (approved in 2016) are nucleoside analogs used orally, with fewer adverse events compared to immunomodulators and very efficient to reduce viral load (Table 5.1 and Fig. 5.2) [27]. However, a functional cure (loss of HBsAg) is rarely seen with these therapies. Duration of treatment varies, most of the time lasting several years. Because of the long-term need for these medications, adhesion to treatment is a concern, together with the development of drug resistance [21, 30].

Other nucleoside analogs approved are clevudine (approved for HBV in South Korea and the Philippines) and besifovir (nucleotide approved in South Korea) [31]. Although clevudine was approved as an antiviral agent for HBV without significant toxicity during the six-month clinical trial, longer therapy (14 months) was found to cause reversible mitochondrial

myopathy [32]. This nucleoside analog was one of the few drugs that seemed to have an impact on HBV cccDNA in a woodchuck model (Tennant, personal communication).

Thymosin alpha-1 (Zadaxin) is an immune modulator, administered subcutaneously with minimal side effects approved as monotherapy for chronic hepatitis B in Asian countries [33]. The activity is via an enhancement of T cell differentiation and maturation and is especially effective in settings where there is a reduction in T cell number and/or function [33].

5.5 Drugs in the Pipeline

There are several novel antiviral agents being developed for chronic hepatitis B. The drugs can be divided according to their strategies to eradicate chronic HBV infection (Table 5.2) [34]:

1. Virologic (direct-acting agents or DAAs)
2. Host immune approaches (indirect-acting agents or immune therapy)

5.5.1 Direct-Acting Antiviral Agents (DAAs)

Virologic antiviral agents or DAAs are new therapies that could directly target HBV replication steps without killing infected cells [35]. Nucleoside analogs target the viral reverse transcriptase enzyme, thus inhibiting HBV replication. Several nucleoside analogs are approved for chronic hepatitis B treatment as mentioned above, but because they require long-term use and do not completely clear HBV from hepatocytes, new DAAs are being developed, and next we will discuss different strategies used.

5.5.1.1 Capsid Assembly Effectors or Modulators (CAM)—The HBV nucleocapsid plays an essential role in the viral replication cycle that includes HBV genome packaging, reverse transcription, intracellular trafficking of relaxed circular DNA (rcDNA) into the nucleus, and maintenance of chronic infection. Capsid assembly modulators (CAM) are characterized by two types (Table 5.3 and Fig. 5.3): (1) class I or heteroarylpyrimidines (HAP) are core protein allosteric modulators (CpAM) that upon binding to HBV capsids promote their misassembled to aberrant non-capsid core polymers, and (2) class II or phenylpropanamides (PP), sulfamoylbenzamides (SBA), or derivatives are capsid assembly modulators that upon binding to the capsid form normal but empty nonfunctional capsids devoid of pgRNA/rcDNA.

Both classes of HBV capsid effectors can interfere with several steps of HBV replication cycle including pre- and post-capsid formation, prevention of capsid assembly, perturbation of capsid integrity of incoming virus particles, entry of HBV capsid and core particles into the cell nucleus, pregenomic RNA encapsidation, and consequently its reverse transcription. All these changes in the HBV replication cycle may ultimately prime inhibition of cccDNA formation and/or amplification (Fig. 5.1).

There are five capsid effectors in phase 2 clinical trials. Morphothiadin (GLS4) is a class I HAP compound developed from Bay41–4109 that has shown potent in vitro inhibition of

HBV DNA replication; nevertheless, in vivo studies with health volunteers have shown that GLS4 needs an extra-booster (ritonavir) to increase its plasma concentration and achieve effective antiviral activity in humans [36]. Two CpAM ABI-H0731 and ABI-H2158 are in phase 2 clinical trials. ABI-H0731 has shown a decline in HBV RNA that correlated with HBV DNA decline in a 4-week therapy [37], and several phase 2 clinical trials are being conducted with this compound in combination with nucleoside analogs, including entecavir or tenofovir. ABI-H2158 has shown in vivo decline of HBV DNA and pgRNA by ~2 $\log_{10}$ IU/ml and is in phase 2 clinical trial in combination with entecavir [38]. JNJ56136379 is an inducer of empty nonfunctional HBV capsids (CAM-N) that was well tolerated by healthy volunteers in phase 1 and has shown reduced HBV DNA and RNA levels; in a 4-week phase 1b monotherapy study, baseline polymorphisms or enrichment of substitutions did not show an impact on virological response, though the emergence of resistance to longer treatments are underway in phase 2 studies [39]. QL-007 (Qilu, PR China) is in phase 2 clinical trials with entecavir or tenofovir for both safety and efficacy evaluation (Table 5.3).

Four capsid effectors are in phase 1 clinical trial including RG7907, EDP-514, ABI-H3733, and ZM-H1505R. RG7907 (RO7049389), a class I CpAM, reduced both HBV DNA and RNA levels at the end of 28-days treatment, with favorable PK profiles [40]. EDP-514 is a class II core inhibitor that has shown to prevent de novo formation of cccDNA in human primary hepatocytes, and it is in phase 1a/1b study with healthy volunteers [41]. ABI-H3733 is a class II capsid inhibitor that has shown to be a potent inhibitor of HBV DNA (EC_{50} = 5 nM) and cccDNA formation (EC_{50} = 125 nM) in vitro [42]. ZM-H1505R is a new pyrazole compound that inhibits HBV DNA replication by inhibiting pgRNA encapsidation and cccDNA formation.

Three main capsid effectors are in preclinical studies: GLP-26, ALG-000184, and CB-HBV-001. GLP-26 (Emory University) is a novel class II CAM, with a unique glyoxamidopyrrolo backbone. It showed substantial in vitro effects in HBV DNA replication and HBe antigen with low nanomolar ranges (EC_{50} = 3 nM for both markers), with >1 log reduction in cccDNA, and no apparent cytotoxicity. Sustained decreases in HBeAg and HBsAg levels were also observed in HBV-infected humanized mouse model treated with GLP-26 in combination with entecavir up to 3 months after drug cessation [43–46]. ALG-000184 (Aligos Therapeutics/Emory University) is the prodrug of ALG-001075, another potent class II CAM that has shown picomolar activity in vitro and substantial effects in HBV DNA replication in mouse model, with no apparent signs of toxicity and markedly improved solubility [47]. This drug is now entering phase 1a/1b clinical trial in New Zealand, Hong Kong and Republic of Moldova. CB-HBV-001 is a new oxazolidinone, pyrazole capsid inhibitor that is being evaluated in preclinical trials (AASLD 2018).

5.5.1.2 Entry Inhibitors—HBV enters the cell by attaching the receptor binding region of pre-S1 to the NTCP receptor at the membrane of the hepatocyte [48] (Fig. 5.1). Bulevirtide (Myrcludex B) binds irreversibly to NTCP inhibiting the HBV entry into the hepatocyte [49]. This drug is administered subcutaneously and is being studied for chronic hepatitis B and delta in phase 2b with or without peginterferon (PEG-IFN) alfa-2a (Table 5.4) [49]. Preliminary results showed that 12/30 (40%) of individuals treated with bulevirtide plus PEG-IFN for 48 weeks had alanine aminotransferase (ALT) normalization and HDV

RNA negative. In the follow-up of 24 weeks of treatment, 4 out 15 individuals treated with 2 mg bulevirtide plus PEG-IFN had undetectable HBsAg, and three out four had HBsAg seroconversion [50]. Bulevirtide was well tolerated, with some drug-related adverse events primarily caused by an increase in total bile salts [50]. This is explained because the drug binding to NTCP prevents infection but also inhibits hepatic bile salt uptake leading to the transiently elevated bile salt level [51].

5.5.1.3 Small Interfering RNA (siRNA)—RNA interference (RNAi) is the mechanism through which double-stranded RNAs silence cognate genes (Fig. 5.1). It is characterized by the presence of RNAs about 22 nucleotides homologous to the gene that is being suppressed. Dicer is the cellular nuclease that cleaves double-stranded RNAs and can produce putative guide RNAs or small interfering RNA (siRNA) [52]. After the sense strand is removed and the antisense strand is loaded on the RNA-induced silence complex (RISC), it hybridizes to a complementary region of a target mRNA, which results in its degradation [53]. This phenomenon provides effective agents for inhibiting infectious, metabolic, cancer, and genetic diseases [53]. A critical issue in the development of siRNA-based drugs is to avoid toxicity such as (1) immunogenic reactions to dsRNA (2′-O-methyl base modifications have largely avoided this issue), (2) toxicity of excipients (work continues on developing potent and nontoxic nanoparticles), (3) unintended RNAi activity (avoided by detailed screening target sites against human genome sequences), and (4) on target RNAi activity in nontarget tissues (selection of highly diseased selective genes and delivery routes which reduce accumulation in nontarget tissues) [54]. Previous studies showed that siRNA could significantly inhibit HBV transcripts and cccDNA in vitro in HepG2 cells and in vivo in mice [53, 55, 56]. Currently, several siRNAs are being evaluated in preclinical and phases 1, 1/2, and 2 clinical trials shown in Table 5.5. VIR-2218 has shown dose-dependent HBsAg reductions (mean decline of 1.0 $\log_{10}$) in HBeAg negative or positive patients virally suppressed on nucleos (t)ide analogs without significant fibrosis [58]. Another siRNA drug, JNJ-3989 (ARO-HBV) that is in a phase 2a study, has demonstrated a $\geq\log_{10}$ reduction in HBsAg at nadir was achieved in 98% of patients [59]. In total, 15/38 (39%) of patients who were responders throughout the study were sustained responder at day 392 [59].

5.5.1.4 Nucleic Acid Polymers (NAPs)—NAPs are phosphorothioate oligonucleotides (PS-ONs) that inhibit HBV via a post-entry mechanism blocking the assembly/release of HBV subviral particles (Fig. 5.1). The universal model for NAP pharmacology is based on the interaction of the amphipathic protein domain and the hydrophobic side of NAPs, preventing the conformational changes in the target or its interaction with other amphipathic helices [60]. In this class of antivirals, there are the HBsAg inhibitors and the STOPs (s-antigen transport inhibiting oligonucleotide polymers).

HBsAg Inhibitors: Aside from the ability of HBsAg to sequester anti-HBs from the blood system, HBsAg has direct immunoinhibitory action against both innate and adaptive immune responses (Fig. 5.1). HBsAg loss is infrequently achieved with the current therapy; therefore, antivirals targeting the inhibition of HBsAg are being developed. NAPs have the ability to interact with hydrophobic surfaces of proteins and have emerged as the first therapy to be able to achieve rapid HBsAg loss [61].

REP 2139 is a phosphorothioate oligodeoxyribonucleotide (PS-ONs) with the sequence $(dAdC)_{20}$ (Table 5.6 and Fig. 5.4) [62]. Clinical studies of REP 2139 in combination with thymosin or PEG-IFN was well tolerated and resulted in liver flares (without liver dysfunctions) following initial reductions of serum HBsAg and HBV DNA [62]. REP 2165 is a version of REP 2139 which has been shown preclinically to retain antiviral activity with lower accumulation in the liver. The results of phase 2 randomized trial showed that addition of NAPs to tenofovir and PEG-IFN increased functional cure after therapy without altering tolerability [63].

STOPs (s-Antigen Transport Inhibiting Oligonucleotide Polymers): STOPs are oligonucleotide aptamers (protein binding) comprised of a repeating poly AC sequence (Fig. 5.1). STOPs share the structural similarity with NAPs but contain several novel chemical features. STOPs can reduce HBsAg secretion by affecting protein trafficking from the infected cell resulting in its degradation [64]. In HepG2.2.15 cells, ALG-10133 reduced HBsAg secretion in nanomolar range and with synergistic effects when combined with class II CAMs [65]. ALG-10133 has been selected as the lead candidate, starting clinical trials on 2020 with projected human efficacious dose of 30–75 mg delivered SC weekly (Table 5.7) [66].

5.5.1.5 Antisense Molecules—Antisense oligonucleotides (ASO) (Table 5.8.) are small single-stranded nucleic acid sequences that bind with high selectivity to their target RNAs. This triggers degradation via an RNAse H-dependent pathway [68]. GSK 3228836 is a 2′-O-methoxyethyl free ASO currently in development for the treatment of chronic hepatitis B. It has been tested as a subcutaneous injection in doses up to 120 mg, and no safety concerns were identified [69]. GSK3389404 (GSK404) is a second-generation ASO that showed an acceptable safety profile [70]. GSK3389404 presented platelet dose-dependent declines that plateaued on treatment and started to recover after dose completion [70, 71]. RO7062931 is an N-acetylgalactosamine (GalNAc) conjugated single-stranded oligonucleotide (SSO) with locked nucleic acid (LNA) that is complementary to messenger RNAs (mRNAs) of the HBV genome [72, 73]. Gal-Nac conjugation should reduce ASO renal and platelet toxicities. It was well tolerated in healthy volunteers. Phase 1 studies showed a mean nadir of HBsAg of -0.5 $\log_{10}$ IU/mL, with treatment emergent ALT elevations with transient concurrent HBsAg decline (0.6–0.8 $\log_{10}$ IU/mL) with no changes in liver function [74].

5.5.1.6 Nucleoside Analogs—ATI-2173 is a novel phosphoramidate prodrug of clevudine in preclinical studies for chronic hepatitis B (Table 5.9 and Fig. 5.4) [67]. Long-term use of clevudine was found to exhibit reversible skeletal myopathy in a small group of individuals and therefore subsequently discontinued from development. ATI-2173 was designed by modifying clevudine that bypasses the first phosphorylation step where the 5′-monophosphate is converted to the active 5′-triphosphate in the liver [67]. ATI-2173 activity was decreased by 25 viral polymerase mutations associated with entecavir, lamivudine, and adefovir resistance, but not capsid inhibitor resistance mutations [67]. It has been claimed that this compound could behave as a non-nucleoside antiviral agent.

5.5.1.7 RNAseH Inhibitors—RNAseH is one of the two enzymatically active domains on HBV polymerase and destroys the HBV RNA after it has been copied into DNA by the reverse transcriptase [75]. RNAseH is a potential target for antiviral drugs, and over 150 RNAseH inhibitors are divided in four compound classes: (1) α-hydroxytropolones (αHT), (2) N-hydroxyisoquinolinediones (HID), (3) N-hydroxypyridinediones (HPD), and (4) and N-hydroxynapthyridinones [76–81]. Novel amide αHT were studied with EC_{50} values from 0.31 to 54 μM [79]. Studies in chimeric mouse showed that an HPD and an αHT suppressed HBV replication to up to 1.4 log_{10} after two weeks of treatment followed by a rebound in the viral titers [82].

5.5.2 Indirectly Acting Antiviral Agents (Immune Therapy)

Specific immune therapy can maintain the HBV replication under control of a functional host antiviral response [9] (Fig. 5.1). An example of approved immune therapy for chronic hepatitis B is interferon alpha (pegylated or not). Pegylated interferon alpha alone or in combination therapy can achieve sustained off-treatment control but in only a small portion of individuals [26].

Therapeutic restoration of protective immunity is a strategy that can be considered to achieve the functional cure of HBV [83]. Several approaches are being considered such as therapeutic vaccines, innate immune stimulation (TLR-8 and TLR-7 agonists), host acting pathway (apoptosis inducer and cyclophilin inhibitor), gene editing, and many other mechanisms.

5.5.2.1 Therapeutic Vaccines—There is a renewed interest in therapeutic vaccines with the development of novel formulations, suitable immunization routes for designed adequate antigens, and adjuvant strategies (Table 5.10). In addition, it is important to consider adequate strategies, including combination therapy with other antivirals, either concomitant or sequential strategies.

5.5.2.2 Innate Immune Stimulation—The host immune responses to HBV determine if the individuals will clear (functional cure) or fail to clear the virus (chronic hepatitis B). Toll-like receptor (TLR) family and its functions are one way to modulate the immunological host responses [96]. TLR8 and TLR7 are endosomal TLRs members with a high degree of sequence and function similarity. They recognize pathogen-associated molecular patterns (viral single-stranded RNA fragments) and trigger innate and adaptive immune responses[96, 97]. Agonist ligands of Toll-like receptors 7 and 8 have immune-stimulating activity allowing to intervene several diseases and to be valuable vaccine adjuvant candidates [96].

Selgantolimod (formerly GS-9688) is a small molecular agonist of Toll-like receptor 8 (TLR8) [98]. It sustained reduced intrahepatic RNA and DNA of woodchuck hepatitis virus (WHV) in animal model. With a finite, short duration treatment, the serum WHsAg level reduced with half of animals with levels below the limit of detection [97]. Selgantolimod is an oral drug under phase 2 clinical trial (Table 5.11 and Fig. 5.4). RO7020531 (RG7854) is an oral prodrug of a TLR-7 agonist in phase 2 clinical trial (Table 5.11). Carboxylesterase (mainly CES2) and oxidation by aldehyde oxidase converts RO702053 into the active

metabolite RO7011785 [99]. Preclinical data showed that a combination of HBV locked nucleic acid antisense oligonucleotide (HBV-LNA ASO) with RO7020531 reduced HBsAg and HBV DNA with delayed rebound off-treatment in mice [100].

5.5.2.3 Host Acting Pathway—Cellular inhibitor of apoptosis proteins (cIAPs) impairs clearance of hepatitis B virus (HBV) infection by preventing TNF-mediated killing/death of infected cells. Animal studies showed that drug inhibitors of cIAPs were able to reduce serum HBV DNA, hepatitis B surface, and core antigens [101]. APG-1387 is an apoptosis inducer; it is a second mitochondria-derived activator of caspase (SMAC) mimetic, and it targets inhibitors of apoptosis proteins (IAPs) [102]. Currently, APG-1387 is under clinical trial phase 1 study for chronic hepatitis B (Table 5.9 and Fig. 5.4).

CRV-431 is an oral cyclophilin inhibitor, non-immunosuppressive analog of cyclosporine A. CRV 431 is a small molecule under clinical development for the treatment of liver diseases including fibrosis and hepatocellular carcinoma [103]. Preclinical studies showed antiviral activity against hepatitis B reducing HBV DNA and HBsAg levels in transgenic mice and a phase 1 is ongoing (Table 5.12 and Fig. 5.4) [104].

5.5.2.4 Gene Editing—Clustered regularly interspaced short palindrome repeats (CRISPR)/Cas9-based antiviral strategy is one of the most versatile gene-editing tools, discovered as a bacterial adaptive immune system [105]. The CRISPR/Cas9 system can specifically destruct HBV genomes in vitro and in vivo, mediating specific cleavage of cccDNA [106–108] (Fig. 5.1). Several optimal targets in HBV genome have been described, such as the surface and polymerase overlap region; the YMDD RT motif and the HBV enhancer I, II, X protein; and pre-core regions with high efficacy [109]. However, CRISPR/Cas system inevitably targets integrated HBV DNA and induces double-strand breaks (DSBs) of host genome, raising concerns of genome instability and carcinogenicity [108, 110]. To avoid DSBs of the host genome, recently it was described a permanently Cas9-mediated base editors that effectively introduced nonsense mutations that generated premature stop codons of surface gene in both integrated and cccDNA reducing HBsAg secretion [110]. EBT107 is a gene-editing CRISPR/Cas 9 drug that uses a duplex gRNA excision knockout as a candidate for HBV in preclinical studies (Table 5.13) [111].

ARCUS genome-editing technology is another platform of gene editing being developed for chronic hepatitis B [112]. The ARCUS technology is based on the properties of a naturally occurring gene-editing enzyme – the homing endonuclease I-CreI—and reduces the risk of additional off-target DNA edits [113].

5.5.2.5 Other Mechanisms

Recombinant hepatitis B human monoclonal antibody: Lenvervimab (GC1102) is a recombinant hepatitis B human monoclonal antibody expected to improve sustained virological response reducing HBsAg levels in individuals with chronic hepatitis B infection [114]. It is under study for HBV-related liver transplant recipients (Table 5.14).

Farnesoid X receptor (FXR) agonist: HBV enters the hepatocyte by binding to NTCP, the genome of which contains two active farnesoid X receptor (FXR)α response elements that

participate in HBV transcriptional activity [115]. In vitro studies showed that FXR agonists inhibited viral mRNA, DNA, and protein production and reduced the cccDNA pool size [115]. Vonafexor (EYP001) is a farnesoid X receptor (FXR) agonist with anti-HBV effects [116, 117]. It is under study in combination with PEG-IFN, nucleoside analogs in double or triple therapy (Table 5.14).

PD-L1 pathway: The programmed cell death protein 1 (PD-1)/programmed death-ligand 1(PD-L1) pathway is a key immune checkpoint regulator that controls the induction and maintenance of immune tolerance in chronic hepatitis B infection [118]. ASC22 (KN035) is a novel fusion anti-PDL1 antibody being studied for the treatment of solid tumors and in clinical trials for chronic hepatitis B phase 2a (Table 5.14).

T cell immunotherapy: LTCR-H2–1 (Table 5.14) is a preclinical drug that boosts adaptive immune response through T cell receptor (TCR) gene transfer [119]. It is engineered to target virus-derived peptides presented on MHC class I on the surface of virus-infected cells. This technology is based on leukapheresis to isolate white blood cells, followed by T cell expansion; HBV targeting TCR are introduced into the activated T cells by viral transduction or electroporation, and then after phenotypic and functional validation, the TCR-engineered T cells are infused back into the individual [120].

5.6 Conclusions

Currently, nucleoside analogs and peginterferon are available for chronic hepatitis B treatment and are quite effective and safe. They can prevent progression of disease, but even persons treated with these drugs can develop hepatocellular carcinoma. The treatments can achieve inhibition of HBV replication; however, few individuals achieve “functional cure” status (HBsAg clearance with or without surface antibody). Several novel drugs are in the pipeline for treatment and elimination of chronic hepatitis B. The drugs are at different stages of development from preclinical to phase 2 clinical trials, and some of them are considered for combination strategies. These drugs will be instrumental for a sustained HBV DNA undetectability with sustained clearance of HBsAg and for preventing liver cancer. Elimination of cccDNA and integrated HBV DNA will be key to eradicate chronic hepatitis B infection. Currently, there are numerous drugs that have the potential to cure HBV, but most do not have the necessary potency to clear all cccDNA. We now know that the half-life of cccDNA (several months and not decades) is shorter than was previously reported [121]. Thus, it may be possible to eliminate cccDNA in approximately 1 year with more potent agents or more likely a combined modality (e.g., capsid effector plus STOPs). As expounded above, a great number of approaches are being tried to eliminate HBV, and it is clear that we are beginning to turn the tide.

Acknowledgments

This work was supported by the funding from NIH (1-RO1-AI-132833 to RFS) (1-RO1-AI-148740 to JC Gumbart) and Emory Center for AIDS Research (5P30-AI-50409 to RFS). SKO would like to thank Fundação de Amparo à Pesquisa de São Paulo (FAPESP 2017/50042-2) and Brazilian Council for Development of Science and Technology (CNPq) for grant PQ 308609/2018-2. The authors thank Dr. James J Kohler (Emory University) for editing the review and Dr. Zhe Chen (Emory University) for drawing the chemical structures.

Abbreviations

Ad	Adenovirus
ALT	Alanine aminotransferase
APOBEC	Apolipoprotein B mRNA editing catalytic polypeptide-like
ARCUS	Gene-editing platform
ASO	Antisense oligonucleotide
CAM	Capsid assembly effectors or modulators
CAS	CRISPR associated
cccDNA	Covalently closed circular DNA
CES	Carboxylesterase
cIAPS	Cellular inhibitor of apoptosis proteins
CpAM	Core protein allosteric modulators
CRISPR	Clustered regularly interspaced short palindrome repeats
DAAs	Direct-acting agents
DS	Double stranded
DSBs	Double-strand breaks
EC_{50}	Median effective concentration to inhibit HBV DNA replication
ENV	Envelope
FXR	Farnesoid X receptor
GalNAc	N-acetylgalactosamine
GLS4	Morphothiadin
HAP	Heteroarylpyrimidines
HBcAg	HBV core antigen
HBeAg	HBV e antigen
HBsAg	HBV surface antigen
HBV	Hepatitis B virus
HID	N-hydroxyisoquinolinediones
HPD	N-hydroxypyridinediones
IAPs	Inhibitors of apoptosis proteins

ID	Intradermal
IM	Intramuscular
IV	Intravenous
L	HBV large surface protein
LNA	Locked nucleic acid
M	HBV middle surface protein
MHC	Major histocompatibility complex
mRNA	Messenger RNA
n/a	Not applicable
NAPs	Nucleic acid polymers
nM	Nanomolar
NTCP	Sodium taurocholate cotransporting polypeptide
PD-1	Programed cell death protein 1
PD-L1	Programed death ligand protein 1
PEG-IFN	Peginterferon
pgRNA	Pregenomic RNA
PK	Pharmacokinetics
POL	Polymerase
PP	Phenylpropanamides
PS-ONs	Phosphorothioate oligonucleotides
rcDNA	Relaxed circular DNA
RIG-I	Retinoic acid-inducible gene-I
RISC	RNA-induced silence complex
RNAi	RNA interference
RT	Reverse transcriptase
S	HBV small surface protein
SBA	Sulfamoylbenzamides
SC	Subcutaneous
siRNA	Small interfering RNA

SMAC	Second mitochondria-derived activator of caspases
SSO	Single-stranded oligonucleotide
STOPs	s-Antigen transport inhibiting oligonucleotide polymers
TCR	T cell receptor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
VLV	Virus-like vesicles
WHsAg	WHV surface antigen
WHV	Woodchuck hepatitis virus
YMDD RT	Tyrosine, methionine, aspartate, motif aspartate reverse transcriptase motif
αHT	α-hydroxytropolones
μM	Micromolar

References

1. Razavi H (2020) Global epidemiology of viral hepatitis. Gastroenterol Clin North Am 49:179–189 [PubMed: 32389357]
2. De Fraga RS, Van Vaisberg V, Mendes LCA et al. (2020) Adverse events of nucleos(t)ide analogues for chronic hepatitis B: a systematic review. J Gastroenterol 55:496–514 [PubMed: 32185517]
3. Easl (2017) EASL 2017 clinical practice guidelineson the management of hepatitis B virus infection. J Hepatol 67:370–398 [PubMed: 28427875]
4. Zhang X, Chen X, Wei M et al. (2019) Potentialresistant mutations within HBV reverse transcriptase sequences in nucleos(t)ide analogues-experienced patients with hepatitis B virus infection. Sci Rep 9:8078 [PubMed: 31147594]
5. Block TM, Locarnini S, Mcmahon BJ et al. (2017) Use of current and new endpoints in the evaluation of experimental hepatitis B therapeutics. Clin Infect Dis 64:1283–1288 [PubMed: 28200098]
6. Anonymous (2020) Drug watch - compound in development for chronic hepatitis B. Hepatitis B Foundation. https://www.hepb.org/treatment-and-management/drug-watch/
7. Zhang YY, Hu KQ (2015) Rethinking the pathogenesis of hepatitis B virus (HBV) infection. J Med Virol 87:1989–1999 [PubMed: 25989114]
8. Maini MK, Gehring AJ (2016) The role of innateimmunity in the immunopathology and treatment of HBV infection. J Hepatol 64:S60–S70 [PubMed: 27084038]
9. Bertoletti A, Le Bert N (2018) Immunotherapy forchronic hepatitis B virus infection. Gut Liver 12:497–507 [PubMed: 29316747]
10. Lin J, Wu JF, Zhang Q et al. (2014) Virus-related livercirrhosis: molecular basis and therapeutic options. World J Gastroenterol 20:6457–6469 [PubMed: 24914367]
11. Cornberg M, Lok AS, Terrault NA et al. (2020) Guidance for design and endpoints of clinical trials in chronic hepatitis B - report from the 2019 EASLAASLD HBV treatment endpoints conference. J Hepatol 72:539–557 [PubMed: 31730789]

12. Madejón A, Romero M, Hernández Á et al. (2016) Hepatitis B and D viruses replication interference: influence of hepatitis B genotype. World J Gastroenterol 22:3165–3174 [PubMed: 27003993]
13. Sozzi V, Shen F, Chen J et al. (2018) In vitro studiesidentify a low replication phenotype for hepatitis B virus genotype H generally associated with occult HBV and less severe liver disease. Virology519:190–196 [PubMed: 29734042]
14. Fanning GC, Zoulim F, Hou J et al. (2020) Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat Rev Drug Discov 19:291
15. Eller C, Heydmann L, Colpitts CC et al. (2018) Thefunctional role of sodium taurocholate cotransporting polypeptide NTCP in the life cycle of hepatitis B, C and D viruses. Cell Mol Life Sci 75:3895–3905 [PubMed: 30097692]
16. Hu J, Cheng J, Tang L et al. (2019) Virological basisfor the cure of chronic hepatitis B. ACS Infect Dis 5:659–674 [PubMed: 29893548]
17. Nassal M (2015) HBV cccDNA: viral persistencereservoir and key obstacle for a cure of chronic hepatitis B. Gut 64:1972–1984 [PubMed: 26048673]
18. Lucifora J, Xia Y, Reisinger F et al. (2014) Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 343:1221–1228 [PubMed: 24557838]
19. Laras A, Koskinas J, Dimou E et al. (2006) Intrahepatic levels and replicative activity of covalently closed circular hepatitis B virus DNA in chronically infected patients. Hepatology 44:694–702 [PubMed: 16941694]
20. Qu B, Ni Y, Lempp FA et al. (2018) T5 exonucleasehydrolysis of hepatitis B virus replicative intermediates allows reliable quantification and fast drug efficacy testing of covalently closed circular DNA by PCR. J Virol 92:e01117
21. Ono SK, Kato N, Shiratori Y et al. (2001) The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J Clin Invest 107:449–455 [PubMed: 11181644]
22. Lada O, Benhamou Y, Cahour A et al. (2004) In vitrosusceptibility of lamivudine-resistant hepatitis B virus to adefovir and tenofovir. Antivir Ther 9:353–363 [PubMed: 15259898]
23. Idenix Pharmaceuticals I (2006) NDA 22–011 TYZEKA™. https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/022011lbl.pdf
24. Gilead (2016) NDA 208464. https://www.accessdata.fda.gov/drugsatfa_docs/nda/2016/208464Orig1s000MedR.pdf
25. Tillmann HL (2008) The treatment of chronic hepatitis B: Focus on adefovir-like antivirals. Ther Clin Risk Manag 4:797–802 [PubMed: 19209262]
26. Kim V, Abreu RM, Nakagawa DM et al. (2016) Pegylated interferon alfa for chronic hepatitis B: systematic review and meta-analysis. J Viral Hepat 23:154–169 [PubMed: 25967226]
27. Schinazi RF, Ehteshami M, Bassit L et al. (2018) Towards HBV curative therapies. Liver Int 38 (Suppl 1):102–114
28. Janssen HL, Van Zonneveld M, Senturk H et al. (2005) Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 365:123–129 [PubMed: 15639293]
29. Moucari R, Martinot-Peignoux M, Mackiewicz Vet al (2009) Influence of genotype on hepatitis B surface antigen kinetics in hepatitis B e antigennegative patients treated with pegylated interferonalpha2a. Antivir Ther 14:1183–1188 [PubMed: 20032548]
30. Abreu RM, Bassit LC, Tao S et al. (2019) Long-termvirological and adherence outcomes to antiviral treatment in a 4-year cohort chronic HBV study. Antivir Ther 24:567–579 [PubMed: 31799942]
31. Kasl (2019) KASL clinical practice guidelines formanagement of chronic hepatitis B. Clin Mol Hepatol 25:93–159 [PubMed: 31185710]
32. Park SH, Park KS, Kim NH et al. (2017) Clevudineinduced mitochondrial myopathy. J Korean Med Sci 32:1857–1860 [PubMed: 28960041]
33. Naylor PH, Mutchnick MG (2018) Immunotherapyfor hepatitis B in the direct acting antiviral era: reevaluating the thymosin α1 efficacy trials in the light of a combination therapy approach. J Viral Hepat 25:4–9 [PubMed: 29052304]

34. Peters MG, Locarnini S (2017) New direct-actingantiviral agents and immunomodulators for hepatitis B virus infection. Gastroenterol Hepatol 13:348–356
35. Martinez MG, Villeret F, Testoni B et al. (2020) Canwe cure hepatitis B virus with novel direct-acting antivirals? Liver Int 40(Suppl 1):27–34 [PubMed: 32077597]
36. Zhao N, Jia B, Zhao H et al. (2020) A first-in-human trial of GLS4, a novel inhibitor of hepatitis B virus capsid assembly, following single- and multipleascending-oral-dose studies with or without ritonavir in healthy adult volunteers. Antimicrob Agents Chemother 64:e01686
37. Yuen MF, Agarwal K, Gane EJ et al. (2020) Safety,pharmacokinetics, and antiviral effects of ABI-H0731, a hepatitis B virus core inhibitor: a randomised, placebo-controlled phase 1 trial. Gastroenterol Hepatol 5:152–166
38. Yuen MF, Agarwal K, Gane EJ et al. (2019) Thesecond-generation hepatitis B virus (HBV) core inhibitor (CI) ABI-H2158 is associated with potent antiviral activity in a 14-day monotherapy study in HBeAg-positive patients with chronic hepatitis B (CHB). Hepatology 70:1497A–1498A [PubMed: 31661168]
39. Verbinnen T, Hodari M, Talloen W et al. (2020) Virology analysis of chronic hepatitis B virus–infected patients treated for 28 days with JNJ-56136379 monotherapy. J Viral Hepat 27:1127–1137 [PubMed: 32579776]
40. Gane E, Yuen MF, Bo Q et al. (2019) RO7049389, acore protein allosteric modulator, demonstrates robust decline in HBV DNA and HBV RNA in chronic HBV infected patients. J Hepatol 70:e491
41. Vaine M, Dellisola V, Clugston S et al. (2019) EDP-514, a novel HBV core inhibitor with potent antiviral activity both in vitro and in vivo. J Hepatol 70:474–475
42. Huang Q, Haydar S, Zhou Y et al. (2019) Preclinicalprofile of HBV core protein inhibitor, ABI-H3733, a potent inhibitor of cccDNA generation in HBV infected cells. J Hepatol 70:e48
43. Amblard F, Boucle S, Bassit L et al. (2020) Novelhepatitis B virus capsid assembly modulator induces potent antiviral responses in vitro and in humanized mice. Antimicrob Agents Chemother 64:e01701
44. Bassit L, Cox B, Ono SK et al. (2018) Novel andpotent HBV capsid modulator reduces HBeAg and cccDNA in core site directed T109I mutant in HepNTCP cells. J Hepatol 68:S16–S17
45. Bassit L, Verma K, Ono SK et al. (2019) Novel HBVcapsid assembly modulator inhibits pregenomic RNA encapsidation by accelerating capsid assembly kinetics and disrupting core protein dephosphorylation. J Hepatol 70:e457
46. Sari O, Bassit L, Gavegnano C et al. (2017) Synthesisand antiviral evaluation of 2',2',3',3'-tetrafluoro nucleoside analogs. Tetrahedron Lett 58:642–644 [PubMed: 28163339]
47. Debing Y, Jekle A, Vendeville S et al. (2019) Preclinical assessment of a novel CAPSID assembly modulator, ALG-001075, demonstrates best-in-class in vitro potency and in vivo antiviral efficacy. Hepatology 70:437A [PubMed: 30791105]
48. Yan H, Zhong G, Xu G et al. (2012) Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife 1:e00049
49. Smolders EJ, Burger DM, Feld JJ et al. (2020) Clinical pharmacology of current and investigational hepatitis B virus therapies. Aliment Pharmacol Ther 51:231–243 [PubMed: 31840863]
50. Wedemeyer H, Schöneweis K, Bogomolov P et al(2019) Final results of a multicenter, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of myrcludex B in with PEG-interferon Alpha 2a in patients with chronic HBV/HDV co-infection. J Hepatol 2019:e81
51. Donkers JM, Appelman MD, Graaf SFJ (2019) Mechanistic insights into the inhibition of NTCP by myrcludex B. JHEP Rep 1:278–285 [PubMed: 32039379]
52. Bernstein E, Caudy AA, Hammond SM et al. (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409:363–366 [PubMed: 11201747]
53. Thongthae N, Payungporn S, Poovorawan Y et al(2014) A rational study for identification of highly effective siRNAs against hepatitis B virus. Exp Mol Pathol 97:120–127 [PubMed: 24953337]
54. Setten RL, Rossi JJ, Han S-P (2019) The current stateand future directions of RNAi-based therapeutics. Nat Rev Drug Discov 18:421–446 [PubMed: 30846871]

55. Giladi H, Ketzinel-Gilad M, Rivkin L et al. (2003) Small interfering RNA inhibits hepatitis B virus replication in mice. Mol Ther 8:769–776 [PubMed: 14599810]
56. Li G, Jiang G, Lu J et al. (2014) Inhibition of hepatitisB virus cccDNA by siRNA in transgenic mice. Cell Biochem Biophys 69:649–654 [PubMed: 24569930]
57. Collier WH (2020) Arbutus announces positive preliminary phase 1a/1b clinical trial results for AB-729, a proprietary GalNAc delivered RNAi compound in development for people living with chronic hepatitis B. In: GlobeNewswire, https://www.globenewswire.com/news-release/2020/03/26/2007290/0/en/Arbutus-Announces-Positive-Preliminary-Phase-1a-1b-Clinical-Trial-Results-for-AB-729-a-Proprietary-GalNAc-Delivered-RNAi-Compound-in-Development-for-People-Living-with-Chronic-Hepa.html
58. Gane E, Lim Y-S, Tangkijvanich P et al. (2020) Preliminary safety and antiviral activity of VIR-2218, an X-targeting HBV RNAi therapeutic, in chronic hepatitis B patients. J Hepatol 73:S50–S51
59. Gane E, Locarnini S, Lim TH et al. (2020) Short-termtreatment with RNA interference therapy, JNJ-3989, results in sustained hepatitis B surface antigen suppression in patients with chronic hepatitis B receiving nucleos(t)ide analogue treatment. J Hepatol 73:S20
60. Vaillant A (2016) Nucleic acid polymers: broad spectrum antiviral activity, antiviral mechanisms and optimization for the treatment of hepatitis B and hepatitis D infection. Antiviral Res 133:32–40 [PubMed: 27400989]
61. Vaillant A (2019) REP 2139: antiviral mechanismsand applications in achieving functional control of HBV and HDV infection. ACS Infect Dis 5:675–687 [PubMed: 30199230]
62. Al-Mahtab M, Bazinet M, Vaillant A (2016) Safetyand efficacy of nucleic acid polymers in monotherapy and combined with immunotherapy in treatmentnaive Bangladeshi patients with HBeAg+ chronic hepatitis B infection. PLoS One 11:e0156667
63. Bazinet M, Pântea V, Placinta G et al. (2020) Safetyand efficacy of 48 weeks REP 2139 or REP 2165, tenofovir disoproxil, and pegylated interferon alfa-2a in patients with chronic HBV infection naïve to nucleos(t)ide therapy. Gastroenterology 158:2180–2194 [PubMed: 32147484]
64. Hong J, Pandey R, Rajwanshi VK et al. (2019) S-antigen traffic-inhibiting oligonucleotide polymers (STOPS) can effectively inhibit hepatitis B surface antigen (HBsAg) secretion from hepatitis B virus (HBV) cell lines. Hepatology 430A:70
65. Tan H, Hong J, Hyunsoon K et al. (2020) Combination drug interactions of hepatitis B virus (HBV) S-antigen transport - inhibiting oligonucleotide polymers in vitro. J Hepatol 2020:S868
66. Jobe A (2020) Aligos therapeutics doses first subject in phase 1 proof of concept study of oligonucleotide drug candidate ALG-010133. In: GlobeNewswire, https://www.globenewswire.com/news-release/2020/08/18/2080093/0/en/Aligos-Therapeutics-Doses-First-Subject-in-Phase-1-Proof-of-Concept-Study-of-Oligonucleotide-Drug-Candidate-ALG-010133.html
67. Squires KE, Mayers DL, Bluemling GR et al. (2020) ATI-2173, a novel liver-targeted non-chain terminating nucleotide for HBV cure regimens. Antimicrob Agents Chemother 64:e00836–20
68. Han K, Cremer J, Elston R et al. (2019) A randomized, double-blind, placebo-controlled, firsttime-in-human study to assess the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of GSK3389404 in healthy subjects. Clin Pharmacol Drug Dev 8:790–801 [PubMed: 30861337]
69. Han K, Cremer J, Elston R et al. (2019) A randomized, double-blind, placebo-controlled, firsttime-in-human study to assess the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of GSK3389404 in healthy subjects. Clin Pharmacol Drug Dev 8:790–801 [PubMed: 30861337]
70. Kelong H, Hiroshi I, Robert E et al. (2020) Pharmacokinetics of GSK3389404, an antisense oligonucleotide (ASO), is similar in subjects with chronic hepatitis B (CHB) across Asia-Pacific region. Hepatol Int 14:S66
71. Yuen MF, Heo J, Kumada H et al. (2019) Results after12 weeks treatment of multiple doses of GSK3389404 in chronic hepatitis B (CHB) subjects on stable nucleos(t)ide therapy in a phase 2a double-blind, placebo-controlled study. Hepatology 70:433A–434A

72. Feng S, Cheung TT, Luk AOY et al. (2020) Livertargeted single stranded (SSO) oligonucleotide RO7062931 is safe and well tolerated in Chinese healthy volunteers (HVs) with similar pharmacokinetic profile to non-Chinese HVs. Hepatol Int 14:S72
73. Gane EJ, Wat C, Das S et al. (2019) Interim results ofa phase 1 study of RO7062931, a novel liver-targeted single-stranded oligonucleotide (SSO) with locked nucleic acid (LNA) that targets HBV transcripts. Hepatology 70:436A
74. Yuen M-F, Gane E, Kim DJ et al. (2020) RO7062931 antisense oligonucleotide phase 1 study demonstrates target engagement in patients with chronic hepatitis B on established nucleos(t)ide therapy. J Hepatol 2020: S51
75. Tavis JE, Zoidis G, Meyers MJ et al. (2019) Chemicalapproaches to inhibiting the hepatitis B virus ribonuclease H. ACS Infect Dis 5:655–658 [PubMed: 29565562]
76. Edwards TC, Lomonosova E, Patel JA et al. (2017) Inhibition of hepatitis B virus replication by N-hydroxyisoquinolinediones and related polyoxygenated heterocycles. Antiviral Res 143:205–217 [PubMed: 28450058]
77. Edwards TC, Mani N, Dorsey B et al. (2019) Inhibitionof HBV replication by N-hydroxyisoquinolinedione and N-hydroxypyridinedione ribonuclease H inhibitors. Antiviral Res 164:70–80 [PubMed: 30768944]
78. Edwards TC, Ponzar NL, Tavis JE (2019) Sheddinglight on RNaseH: a promising target for hepatitis B virus (HBV). Expert Opin Ther Targets 23:559–563 [PubMed: 31084514]
79. Li Q, Lomonosova E, Donlin MJ et al. (2020) Amide-containing α-hydroxytropolones as inhibitors of hepatitis B virus replication. Antiviral Res 177:104777
80. Lomonosova E, Daw J, Garimallaprabhakaran AK et al. (2017) Efficacy and cytotoxicity in cell culture of novel α-hydroxytropolone inhibitors of hepatitis B virus ribonuclease H. Antiviral Res 144:164–172 [PubMed: 28633989]
81. Lomonosova E, Tavis JE (2017) In vitro enzymaticand cell culture-based assays for measuring activity of HBV RNaseH inhibitors. Methods Mol Biol 1540:179–192 [PubMed: 27975316]
82. Long KR, Lomonosova E, Li Q et al. (2018) Efficacy of hepatitis B virus ribonuclease H inhibitors, a new class of replication antagonists. Antivir Res 149:41–47 [PubMed: 29129708]
83. Boni C, Barili V, Acerbi G et al. (2019) HBV immune-therapy: from molecular mechanisms to clinical applications. Int J Mol Sci 20:2754
84. Hiasa Y, Yoshida O, Guillen GE et al. (2019) The HBvaccine containing HBS and HBC antigen (NASVAC) can effectively induce anti-HBS antibody in non-responders to the prophylactic vaccine. Hepatology 70:589A–590A
85. Brown W (2020) Altimmune announces IND clearance for a phase 2 trial of HepTcell™ immunotherapeutic for the treatment of chronic hepatitis B. In: Altimmune (ed) Globe newswire. Altimmune, Gaithersburg
86. Lim YS, Mutimer D, Heo J et al. (2019) A phase 1bevaluation of HepTcell HBV-specific immunotherapy in NUC-controlled, eAg negative chronic HBV infection. J Hepatol 70:e50–e51
87. Daniela P (2018) AIC649 Innate activation with inactivated parapoxviruses for HBV therapy. In: International HBV Cure Meeting. Toronto
88. Yoon SK, Seo YB, Im SJ et al. (2015) Safety andimmunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. Liver Int 35:805–815 [PubMed: 24620920]
89. Zoulim F, Fournier C, Habersetzer F et al. (2020) Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial. Hum Vaccin Immunother 16:388–399 [PubMed: 31373537]
90. McNally RT (2018) GeoVax and CaroGen to collaborate on development of therapeutic hepatitis B vaccine. https://www.geovax.com/news/geovax-and-carogen-to-collaborate-on-development-of-therapeutic-hepatitis-b-vaccine
91. George R, Ma A, Motyka B et al. (2020) A dendriticcell-targeted chimeric hepatitis B virus immunotherapeutic vaccine induces both cellular and humoral immune responses in vivo. Hum Vaccin Immunother 16:779–792 [PubMed: 31687875]
92. Su J, Kosinska A, Brunner L et al. (2019) ExploringTH1/TH2 adjuvants to improve the efficacy of the therapeutic vaccination against chronic hepatitis B. J Hepatol 70:e485–e486

93. Yarovinsky TO, Mason SW, Menon M et al. (2019) Virus-like vesicles expressing multiple antigens for immunotherapy of chronic hepatitis B. iScience 21:391–402 [PubMed: 31704650]
94. Tg (2018) Hookipa and Gilead ink US$400m deal.In: European Biotechnology - life science and industry magazine. https://european-biotechnology.com/up-to-date/latest-news/news/hookipa-and-gilead-ink-us400m-deal.html
95. Vaccines V (2019) VBI Vaccines and Brii Biosciences initiate phase 1b/2a study of BRII-179 (VBI-2601) in patients with chronic hepatitis B infection. https://www.vbivaccines.com/wire/vbi-and-brii-initiate-phase-1b-2a-study-of-brii-179-inchronic-hbv/
96. Patinote C, Karroum NB, Moarbess G et al. (2020) Agonist and antagonist ligands of toll-like receptors 7 and 8: ingenious tools for therapeutic purposes. Eur J Med Chem 193:112238 [PubMed: 32203790]
97. Daffis S, Balsitis S, Chamberlain J et al. (2020) Tolllike receptor 8 agonist GS-9688 induces sustained efficacy in the woodchuck model of chronic hepatitis B. Hepatology 73:53–67 [PubMed: 32246499]
98. Mackman RL, Mish M, Chin G et al. (2020) Discovery of GS-9688 (Selgantolimod) as a potent and selective oral toll-like receptor 8 agonist for the treatment of chronic hepatitis B. J Med Chem 63:10188–10203 [PubMed: 32407112]
99. Luk A, Jiang Q, Glavini K et al. (2020) A single andmultiple ascending dose study of toll-like receptor 7 agonist (RO7020531) in Chinese healthy volunteers. Clin Transl Sci 13:985–993 [PubMed: 32268000]
100. Blaising J, Yu Y, Zhou X et al. (2019) Combinationtreatment of liver-targeted HBV locked nucleic acid antisense oligonucleotide and tlr7 agonist RO7020531 leads to prolonged off-treatment antiviral effect in the AAV-HBV mouse model. Hepatology 70:428A–429A
101. Ebert G, Allison C, Preston S et al. (2015) Eliminatinghepatitis B by antagonizing cellular inhibitors of apoptosis. Proc Natl Acad Sci U S A 112:5803–5808 [PubMed: 25902530]
102. Pan W, Luo Q, Yan X et al. (2018) A novel SMACmimetic APG-1387 exhibits dual antitumor effect on HBV-positive hepatocellular carcinoma with high expression of cIAP2 by inducing apoptosis and enhancing innate anti-tumor immunity. Biochem Pharmacol 154:127–135 [PubMed: 29679556]
103. Trepanier DJ, Ure DR, Foster RT (2017) In vitrophase I metabolism of CRV431, a novel oral drug candidate for chronic hepatitis B. Pharmaceutics 9:51
104. Gallay P, Ure D, Bobardt M et al. (2019) The cyclophilin inhibitor CRV431 inhibits liver HBV DNA and HBsAg in transgenic mice. PLoS ONE 14:e0217433
105. Lee C (2019) CRISPR/Cas9-based antiviral strategy: current status and the potential challenge. Molecules 24:1349
106. Dong C, Qu L, Wang H et al. (2015) Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. Antiviral Res 118:110–117 [PubMed: 25843425]
107. Liu Y, Zhao M, Gong M et al. (2018) Inhibition ofhepatitis B virus replication via HBV DNA cleavage by Cas9 from Staphylococcus aureus. Antiviral Res 152:58–67 [PubMed: 29458131]
108. Yang H-C, Chen P-J (2018) The potential and challenges of CRISPR-Cas in eradication of hepatitis B virus covalently closed circular DNA. Virus Res 244:304–310 [PubMed: 28627393]
109. Kennedy EM, Kornepati AV, Cullen BR (2015) Targeting hepatitis B virus cccDNA using CRISPR/Cas9. Antiviral Res 123:188–192 [PubMed: 26476375]
110. Yang YC, Chen YH, Kao JH et al. (2020) Permanentinactivation of HBV genomes by CRISPR/Cas9-mediated non-cleavage base editing. Mol Ther 20:480–490
111. Excision (2020) Technology - a novel approach toCRISPR. https://www.excision.bio/technology
112. Haskins B (2020) Precision biosciences, Gilead sciences pull plug on potential $445M deal. WRAL TechWire. https://www.wraltechwire.com/2020/07/07/precision-biosciences-gilead-sciences-pull-plugon-potential-445m-deal/
113. Biosciences P (2020) ARCUS genome editing. https://precisionbiosciences.com/our-approach/arcus-genome-editing/
114. Lee HW, Park JY, Hong T et al. (2019) A prospective,open-label, dose-escalation, single-center, phase 1 study for Lenvervimab (GC1102), a new and safe human monoclonal antibody drug for chronic hepatitis B patients. Hepatol Int 13:S48

115. Radreau P, Porcherot M, Ramière C et al. (2016) Reciprocal regulation of farnesoid X receptor α activity and hepatitis B virus replication in differentiated HepaRG cells and primary human hepatocytes. FASEB J 30:3146–3154 [PubMed: 27251172]

116. Darteil R, Joly S, Radreau P et al. (2019) In vitrocharacterization of EYP001 a novel, potent and selective FXR agonist entering phase 2 clinical trials in chronic hepatitis B. Hepatology 70(suppl 1):60A

117. Monteiro C, Bruezière L, Laheux S et al. (2019) Anin-silico disease model for the development of FXR agonist EYP001 as a therapy for HBV infection. Hepatology 70:441A–442A

118. Li B, Yan C, Zhu J et al. (2020) Anti-PD-1/PD-L1 blockade immunotherapy employed in treating hepatitis B virus infection-related advanced hepatocellular carcinoma: a literature review. Front Immunol 11:1037–1037 [PubMed: 32547550]

119. Ghany MG, Block TM (2018) Disease pathways andmechanisms of potential drug targets. Clin Liver Dis 12:12–18

120. LionTCR (2019) TCR T cell. http://www.liontcr.com/technology

121. Huang Q, Zhou B, Cai D et al. (2020) Rapid turnoverof HBV cccDNA indicated by monitoring emergence and reversion of signature-mutation in treated chronic hepatitis B patients. Hepatology 73:41–52 [PubMed: 32189364]

Key Points

- Current treatments do not completely clear HBV from hepatocytes leading to the establishment of lifetime chronic infection.
- Novel anti-HBV therapies targeting different steps of HBV replication cycle with the potential of curing individuals chronically infected are needed.
- Elimination of cccDNA from the nuclei of hepatocytes and clearance of HBV surface antigen (HBsAg) from blood are crucial to achieving a functional and complete cure.
- Drug-drug combinations synergistically targeting key steps of HBV replication cycle and immunomodulators boosting the host immune response may lead to a functional cure.
- Novel strategies including CRISPR and siRNA technologies which can inactivate persistent HBV cccDNA and also target integrated viral DNA may eliminate HBV from chronically infected human hepatocytes.

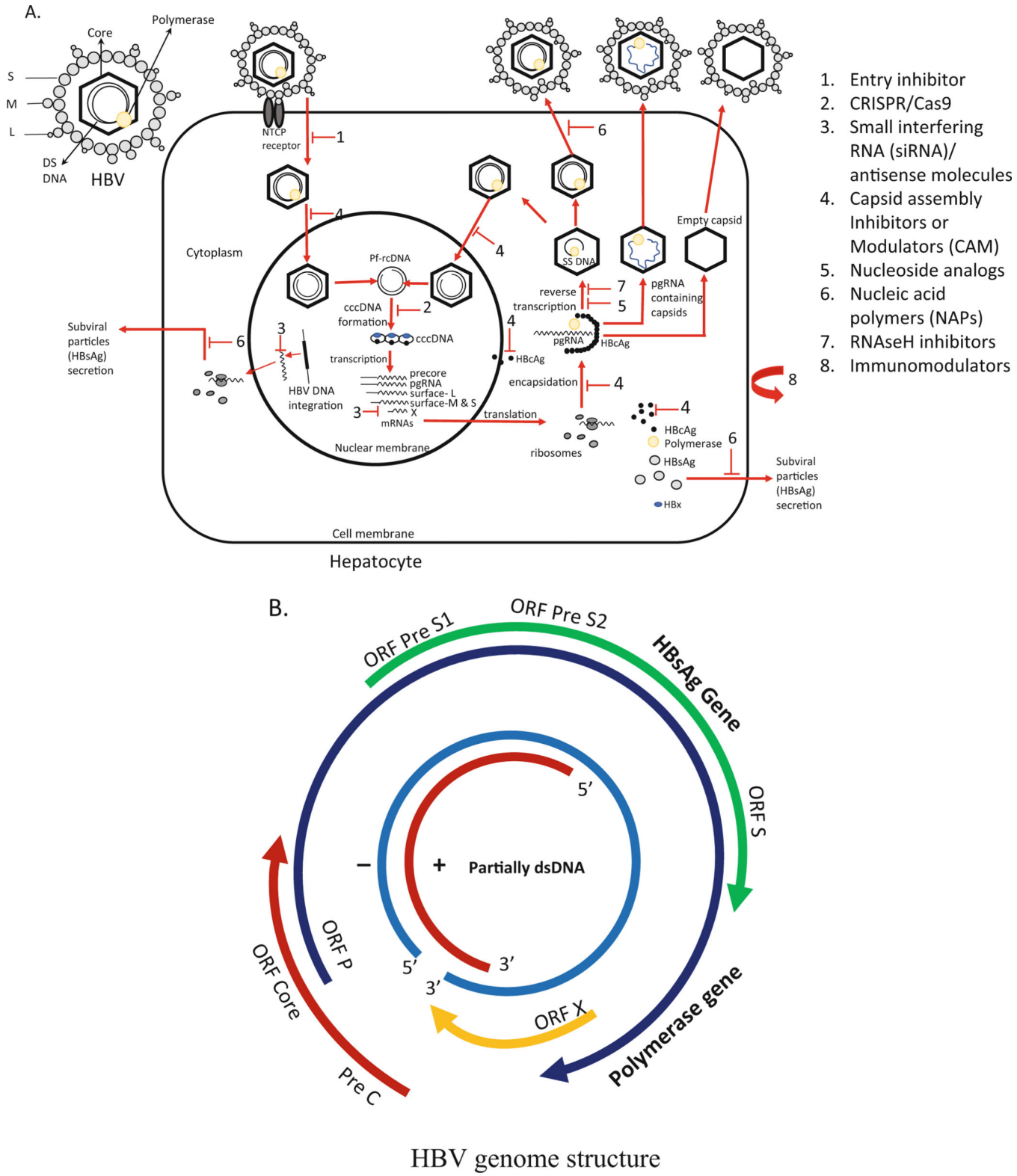

Fig. 5.1.

(**a, b**) HBV replication mechanism, genome structure, and schematic representation of inhibition sites. (**a**) The HBV has an envelope composed with three forms (large, middle, and small) of surface proteins that encloses the capsid with the double-stranded DNA genome. (**b**) Replication starts with HBV binding to the hepatocyte at the NTCP receptor. After entry, the viral particles are uncoated, and the nucleocapsid particle goes to the cellular nucleus. HBV protein free rcDNA (Pf-rcDNA) is converted to an episomal cccDNA, which is the transcription template for all four viral RNAs. The pgRNA is encapsidated together with viral polymerase and subsequently reverse-transcribed into viral minus strand DNA, followed by degradation of the RNA by RNAseH. Then, the plus-stranded DNA is synthesized to form the partially double-stranded relaxed circular DNA. Mature nucleocapsid can either be recycled back to the nucleus to maintain the pool of cccDNA or packed with envelope proteins and exported as infectious virions to infect other cells. pgRNA containing nucleocapsid and empty nucleocapsids are also packed with envelope proteins and released. *S* small, *M* medium, *L* large, *DS* double stranded, *NTCP* sodium taurocholate cotransporting polypeptide, *CRISPR* clustered regularly interspaced short palindrome repeats; *CAS9* CRISPR associated 9

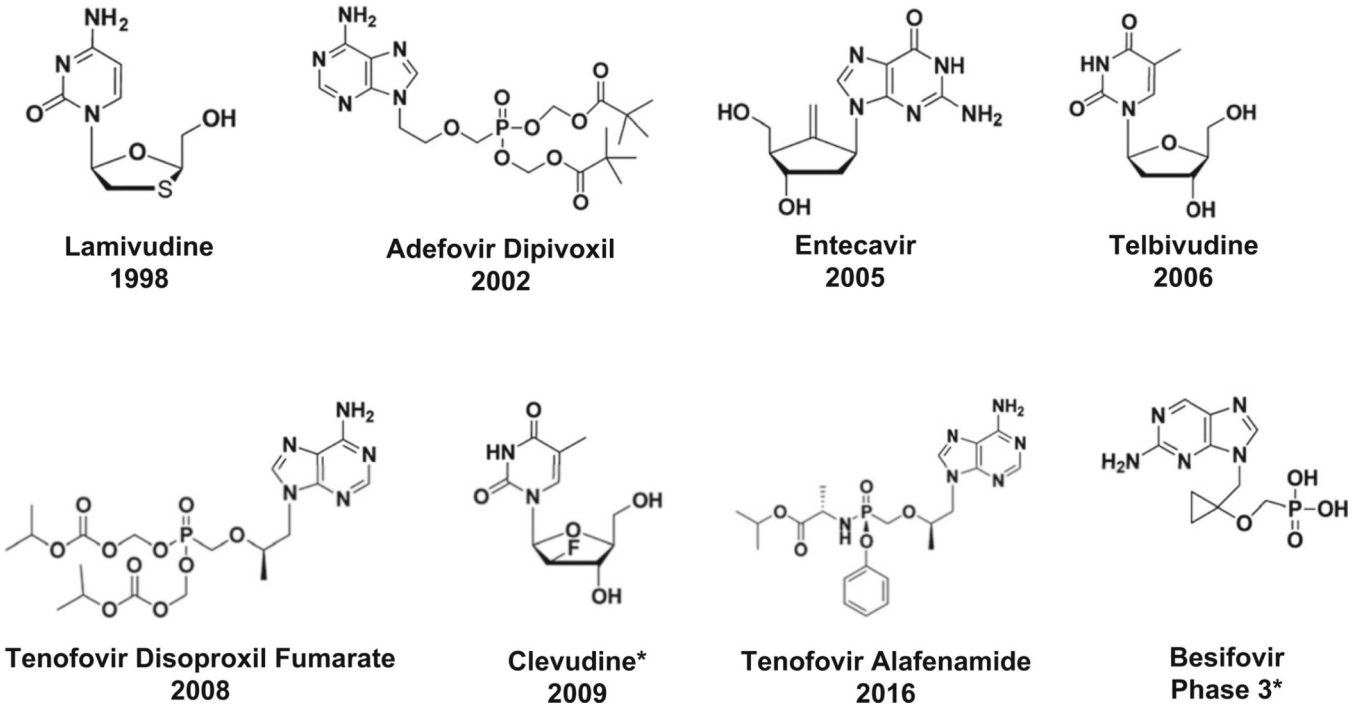

Fig. 5.2.

Chemical structures of nucleoside/nucleotide analogs and year of FDA approval. *Approved in South Korea

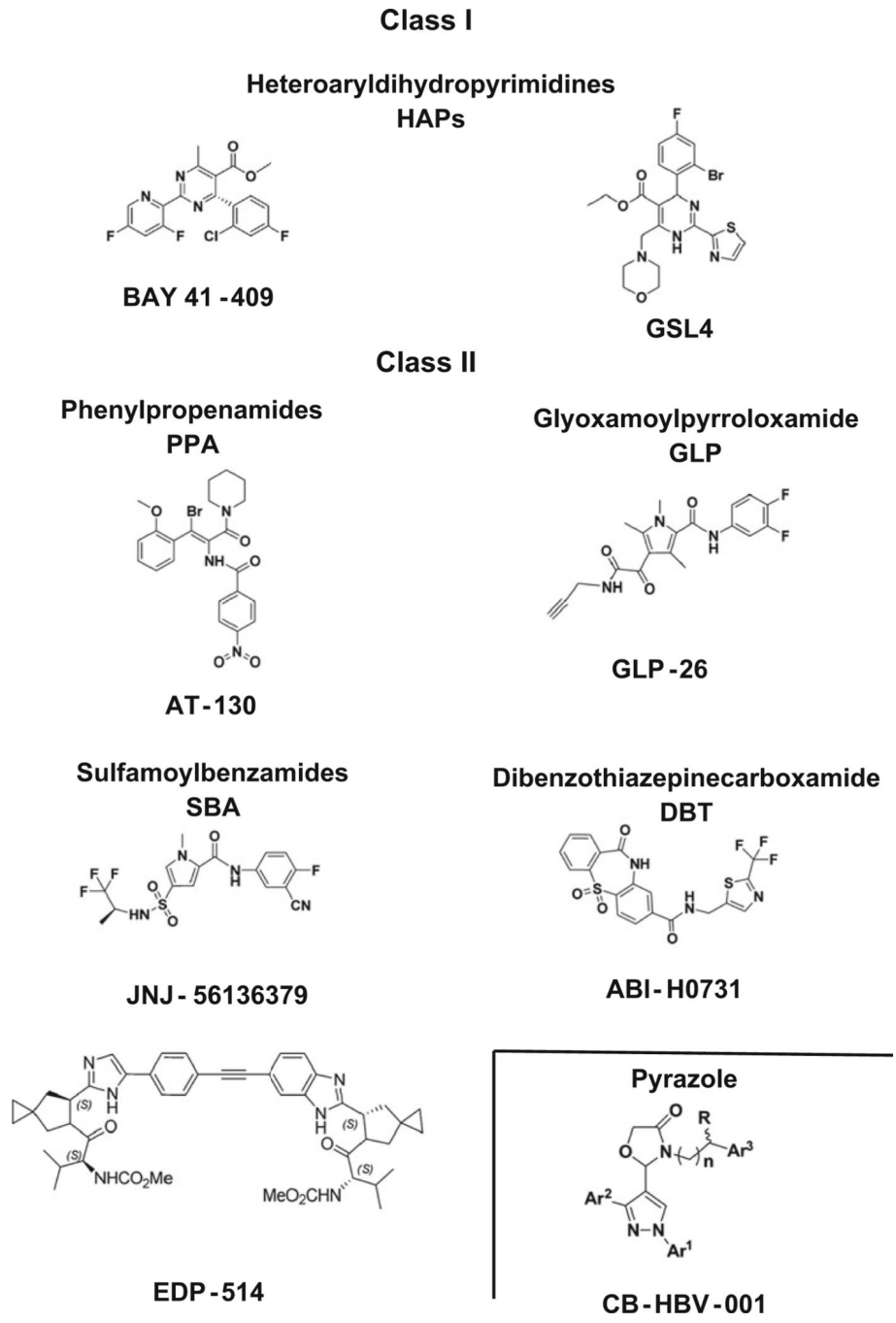

Fig. 5.3.

Chemical structures of prototypical capsid assembly effectors or modulators (CAM)

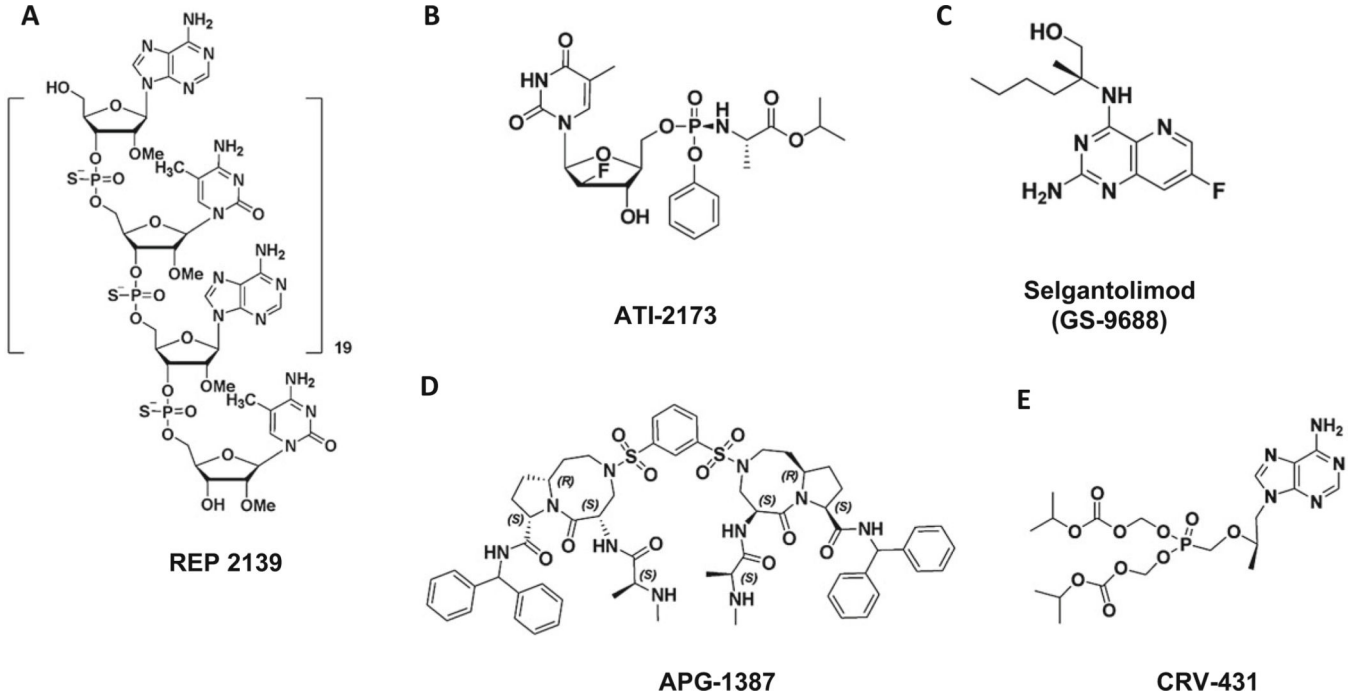

Fig. 5.4.

Chemical structures of HBsAg inhibitor (**a**), nucleoside analog (**b**), innate immune stimulation (**c**), cellular inhibitor of apoptosis proteins (cIAPs) (**d**), and cyclophilin inhibitor (**e**)

Table 5.1

Antivirals approved for chronic hepatitis B

Drug	Route	Class	HBV DNA EC_{50}, μM	Company	FDA approval year
Lamivudine	Oral	Nucleoside analog	0.56 [21]	GlaxoSmithKline	1998
Adefovir dipivoxil	Oral	Nucleotide analog	0.58 [21]	Gilead Sciences	2002
Entecavir	Oral	Nucleoside analog	0.00036 [21]	BMS	2005
Tenofovir disoproxil fumarate	Oral	Nucleotide analog	0.1 [22]	Gilead Sciences	2008
Telbivudine	Oral	Nucleoside analog	1.3 [23]	Novartis	2006
Tenofovir alafenamide	Oral	Nucleotide analog	0.0347 [24]	Gilead Sciences	2016
Interferon alpha 2b	Parenteral	Immunomodulator		Merck	1991
Peginterferon alpha 2a	Parenteral	Immunomodulator		Genentech	2005
Clevudine	Oral	Nucleoside analog	0.053 [21]	Bukwang/Esai Pharmaceuticals	2009
Besifovir (LB80380)	Oral	Nucleotide analog	0.5 [25]	LG Chem Ltd.	n/a
Thymosin alpha-1	Parenteral	Immunomodulator		SciClone Pharmaceuticals	

EC_{50} median effective concentration to inhibit HBV DNA replication, *n/a* not applicable

Table 5.2

Classes of antiviral agents in the pipeline for HBV

Direct-acting antiviral agents (DAAs)
Capsid assembly inhibitors or modulators (CAM)
Entry inhibitor
Small interfering RNA (siRNA)
Nucleic acid polymers (NAPs)
HBsAg inhibitors
s-Antigen transport inhibiting oligonucleotide polymers (STOPs)
Antisense molecules
Nucleoside analogs
Indirect-acting agents (immune therapy)
Therapeutic vaccines
Innate immune stimulation
TLR-8 agonist
TLR-7 agonist
Host acting pathway
Apoptosis inducer
Cyclophilin inhibitor
Gene editing
Gene-editing CRISPR/Cas 9
Gene-editing ARCUS platform
Other mechanisms
Monoclonal antibody
FXR agonist
Host targeting antisense (LNA)
PD-L1
Cell immunotherapy
MicroRNA
Nucleic acid-directed HBV cell killing

Adv Exp Med Biol. Author manuscript; available in PMC 2022 April 07.

Table 5.3

Clinical status of capsid assembly effectors or modulators

Drug/class	Route	HBV DNA Log reduction[a]	Company	Clinical trial phase	ClinicalTrials.gov identifier
Morphothiadin (GLS4)/I	Oral	2.3	HEC Pharma, PR China	2	NCT03638076/NCT04147208/NCT04147208
JNJ 56136379/II	Oral	2.9	Janssen, Ireland	2	NCT02662712
ABI-H0731/II	Oral	3.9	Assembly Biosciences, USA	2	NCT03109730/NTC03780543/NTC03577171/ NTC03576066/NTC04454567/NTC02908191
ABI-H2158/II	Oral	2.3	Assembly Biosciences, USA	2	NCT04398134
QL-007/	Oral	-	Qilu, PR China	2	NCT04157257/NCT04157699
RG7907 (RO7049389)/I	Oral	3–5	Roche, Switzerland	1	NCT02952924
EDP-514/II	Oral	> 4.0[b]	Enanta Pharma, USA	1	NCT04470388
ABI-H3733/II	Oral	5.0[c]	Assembly Biosciences, USA	1	NCT04271592
ZM-H1505R/pyrazole	Oral		ZhiMeng Biopharma, PR China	1	NCT04220801
ALG-000184/II	Oral	5.0[b]	Aligos Therapeutics, USA/Emory University	1	NCT04536337
GLP-26/II	Oral	1–3[b]	Emory University, Aligos Therapeutics	Preclinical	n/a
CB-HBV-001/pyrazole	Oral	12[c]	ZhiMeng Biopharma, PR China	Preclinical	n/a

N/A not applicable

[a] HBV DNA Log_{10} IU/ml in vivo (data obtained from clinical trials)

[b] Log_{10} decrease in HBV DNA (data obtained from mice models)

[c] HBV DNA, EC_{50} (nM) in vitro

Table 5.4

Entry Inhibitor in development

Drug	Route	Dose	Company	Clinical trial phase	ClinicalTrials.gov identifier
Bulevirtide (myrcludex B)	Parenteral	2–5 mg SC qd 48 weeks	Hepatera, Russia with MYR GmbH, Germany	2b	NCT02888106

Table 5.5

Small interfering RNA (siRNA) drugs in development

Drug	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
VIR-2218 (ALN-HBV02)	Parenteral (SC)	Alnylam and Vir Biotech, USA	2	NCT03672188
JNJ-3989 (ARO-HBV)	Parenteral (SC)	Arrowhead Pharma with Janssen, USA	2a	NCT03365947
RG6346 (DCR HBVS)	Parenteral (SC)	Roche, Switzerland	2/1	NCT03772249
AB-729 [57]	Parenteral (SC)	Arbutus Biopharma, USA	1	NA

NA not available

Table 5.6

HBsAg inhibitors in development

Drug	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
REP 2139	Parenteral	Replicor, Canada	2	NCT02726789
REP 2165	Parenteral	Replicor, Canada	2	NCT02565719

Table 5.7

STOPs (s-antigen transport inhibiting oligonucleotide polymers) in development

Drug	EC_{50} μM	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
ALG-10133	0.0032	Parenteral (SC)	Aligos Therapeutics, USA	1	NCT04485663

EC_{50} median effective concentration to inhibit HBV DNA replication

Table 5.8.

Antisense molecules in development

Drug	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
GSK 3228836 /IONIS-HBVRx/ISIS505358	Parenteral (SC) [67]	Ionis with GSK, USA	2a	NCT04449029/NCT02981602
GSK3389404/Ionis-HBV-LRx	Parenteral (SC)	Ionis with GSK	2a	NCT02647281
RO7062931	Parenteral (SC)	Roche	1a	NCT03505190/NCT03038113

Table 5.9

Nucleoside analog in development

Drug	EC_{50} μM	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
ATI-2173	0.0013	Oral	Antios Therapeutics, USA	1	NCT04248426

EC50 median effective concentration to inhibit HBV DNA replication

Table 5.10

Therapeutic vaccines in development

Drug	Platform	Route	Company	Clinical trial phase	ClinicalTrials.gov Identifier
NASVAC (ABX203)	HBs and HBc antigen mixed with carboxyl vinyl polymer [84]	Nasal	CIGB, Cuba	3	NCT01374308/ NCT02249988
HepTcell (FP-02.2)	Synthetic HBV-derived peptides formulated with IC31®, a TLR9based adjuvant [85]	IM [86]	Altimmune, USA	2	NCT02496897
AIC 649	Parapoxvirus (iPPVO) [87]	IV	AiCuris, Germany	1	
JNJ 64300535 (HB-110)	Plasmids encoding HBsAg, HBcAg, and IL12 [88]	IM Electroporation	Janssen, Ireland/Ichor Medical Genexine, USA	1	NCT03463369
TG1050	Ad5 which encodes truncated HBV core, POL, and two small domains from the ENV [89]	SC	Transgene, France	1	NCT04168333/ NCT02909023/ NCT04168333
MVA-VLP-HBV	Modified vaccinia Ankara-virus-like particle-hepatitis B virus [90]		GeoVax, USA	Preclinical	n/a
Chimigen HBV	Recombinant chimeric fusion protein comprising hepatitis B virus (HBV) S1 and S2 surface antigen fragments, core antigen, and a murine monoclonal antibody heavy chain fragment (Fc) [91]		Akshaya, Canada	Preclinical	n/a
TherVacB	HBsAg, HBcAg, and a boost using a modified vaccinia virus Ankara (MVA) vector [92]		Helmholtz Zentrum Muenchen, Germany	Preclinical	n/a
3xT2A and Mix2A	VLVs expressing polymerase (Pol), core (HBcAg), and MHBs [93]		CaroGen, USA	Preclinical	n/a
HBV	TheraT® and VaxWave® investigational arenavirus-based immunization technologies [94]		HOOKIPA Pharma, Austria, with Gilead	Preclinical	n/a
VBI-2601 (BRII-179)	Recombinant, protein-based immunotherapeutic [95]		VBI Vaccines, USA	1b/2a	

ID intradermal, *SC* subcutaneous, *Ad* adenovirus, *POL* polymerase, *ENV* envelope, *VLV* virus-like vesicles, *N/A* not applicable

Table 5.11

Innate immune stimulation in development

Drug	Class	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
GS9688 (Selgantolimod)	TLR-8 agonist	Oral	Gilead Sciences, USA	2	NCT03491553/NCT03615066
RO7020531 (RG7854)	TLR-7 agonist	Oral	Roche, Switzerland	2	NCT02956850/NCT03530917/NCT04225715

Table 5.12

Host-acting pathway

Drug	Class	Route	Company	Clinical trial phase	ClinicalTrials.gov identifier
APG-1387	Apoptosis inducer	IV	Ascentage Pharma, PR China	1	NCT03585322
CRV-431	Cyclophilin inhibitor	Oral [103]	Hepion, USA (formerly ContraVir)	1	NCT03596697

IV intravenous

Table 5.13

Gene editing

Drug	Class	Company	Clinical trial phase
EBT107	Gene-editing CRISPR/Cas 9	Excision Bio, USA	Preclinical
ARCUS nucleases	Gene-editing ARCUS platform	Precision Biosciences, USA	Preclinical

IV intravenous

Table 5.14

Drugs with other mechanisms in development

Drug	Route	Mechanisms	Company	Clinical trial phase	ClinicalTrials.gov identifier
Lenvervimab (GC1102)	IV	Monoclonal antibody	Green Cross, South Korea	2a	NCT03801798/NCT02304315
Vonafexor (EYP001)	Oral	FXR agonist	Enyo Pharma, France	1	NCT04365933
ASC22 (KN035)	SC	PD-L1 pathway	Ascletis Pharma, PR China	2a	NCT04465890
LTCR-H2–1	IV	T cell immunotherapy	Lion TCR, Singapore	1	NCT04745403

IV intravenous, *SC* subcutaneous, *N/A* not applicable