New Developments in the Treatment of Hepatitis B Virus Infection

Sunny Sandhu, MD,[1] Paul Martin, MD,[2] and Ira M. Jacobson, MD[3]

[1]Division of Gastroenterology and Hepatology, Stanford University, Palo Alto, California

[2]Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, California

[3]Division of Gastroenterology and Hepatology, NYU Langone Grossman School of Medicine, New York, New York

Corresponding author:
Sunny Sandhu, MD
Stanford Digestive Health
5565 W Las Positas Blvd, Suite 200
Pleasanton, CA 94588
Tel: (650) 736-5555
E-mail: ssandhu4@stanford.edu

Abstract: Chronic hepatitis B virus (HBV) infection remains a serious global public health concern and a leading cause of liver cirrhosis and hepatocellular carcinoma. Although currently available oral therapies can effectively suppress HBV DNA, there is no approved regimen that has shown to reliably achieve a functional cure. In recent years, various classes of novel therapy for HBV infection have undergone considerable research and advanced to clinical trials, targeting multiple stages of the viral life cycle and systemic immunomodulatory pathways. This article discusses the complex HBV life cycle and highlights emerging therapeutic advances on the horizon for HBV treatment.

Keywords
Chronic hepatitis B, antiviral therapy, capsid assembly modulator, small interfering RNA, antisense oligonucleotide, therapeutic vaccine

Chronic hepatitis B virus (HBV) infection remains a globally prevalent and underdiagnosed disease and is the primary driver of hepatocellular carcinoma (HCC) worldwide.[1] An estimated 254 million people currently have chronic hepatitis B (CHB)—defined as the presence of detectable hepatitis B surface antigen (HBsAg) in blood—and despite an effective vaccine, there are approximately 1.2 million new infections globally per year. In 2022, HBV infection resulted in more than 1 million deaths worldwide, predominantly from complications of cirrhosis and HCC.[2,3] In the United States, it is estimated that up to 2.4 million people have CHB.[4] Persons with CHB are usually said to have active infection if there is HBV DNA detectable in blood, whereas inactive infection refers to those in whom HBV DNA is low or undetectable.[5] HBV is an oncogenic virus, with an increasing HBV viral load and duration of infection implicated in an increased cumulative risk of HCC.[6] The World Health Organization has outlined specific targets to eliminate HBV as a public health threat by 2030.[7] Current goals of therapy are to achieve durable suppression of HBV replication, normalize liver enzymes, and prevent the development of cirrhosis and HCC. Although currently licensed therapies for CHB are effective at suppressing viral replication, they are typically ineffective at achieving a functional cure, which is defined as sustained loss of HBsAg with undetectable HBV DNA levels, with or without seroconversion to hepatitis B surface antibody (anti-HBs), after completion of a course of therapy.[8-11] The low likelihood of a

Table 1. Mechanisms of HBV Persistence

Mechanisms	Key features
Generation of cccDNA	• cccDNA behaves as a stable minichromosome that is not targeted or eliminated by host immune mechanisms • Continuously serves as a template for viral transcription even during therapy with antiviral agents
HBV DNA integration into host genome	• Aberrant reverse transcription yields duplex linear HBV DNA • HBV DNA is randomly integrated into the host genome • Occurs early during HBV infection • Implicated in the pathogenesis of HCC
Intracellular recycling of nucleocapsids	• Some mature viral nucleocapsids return to the hepatocyte nucleus and supply rcDNA, which forms more cccDNA • Increased intracellular cccDNA pool perpetuates HBV infection within the hepatocyte
Adaptive immune system exhaustion	• Vast numbers of subviral particles containing HBsAg are secreted from the hepatocyte • Persistent exposure to viral antigens induces T-cell exhaustion
Innate immune system evasion	• HBV DNA and RNA (concealed in the viral nucleocapsid) evade recognition by immune pathways including TLRs • Viral proteins suppress IFN production and inhibit dendritic and NK cell activity
Low immunogenicity of viral antigens	• HBV stimulates regulatory T cells, suppressing activity of effector T cells • Immunosuppressive cytokines (IL-10, TGF-β) are upregulated, attenuating immune responses
Noncytolytic persistence in hepatocytes	• Infected hepatocytes remain functional without significant cytopathic effect or lysis • HBV persists within hepatocytes and evades immune clearance

cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; IL, interleukin; NK, natural killer; rcDNA, relaxed circular DNA; TGF-β, transforming growth factor-beta; TLRs, Toll-like receptors.

cure for HBV infection reflects both the complexity of the HBV life cycle and the limitations of current HBV therapy. However, the development of novel agents targeting discrete components of its replicative cycle as well as immunomodulatory pathways holds promise for increasing the efficacy of available antiviral agents. This article discusses these pathways as well as recent developments and emerging therapies for HBV infection.

Hepatitis B Virus Life Cycle

HBV is a hepatotropic, partially double-stranded DNA virus and member of the *Hepadnaviridae* family. The 42-nm infectious virion consists of an outer lipid envelope, which surrounds a nucleocapsid containing the viral genome DNA in the form of relaxed circular DNA (rcDNA).[12] Hepatocyte infection is initiated by the binding of preS1 to glycoprotein on the viral envelope with the sodium taurocholate cotransporting peptide (NTCP), a bile acid receptor, on the basolateral membrane of the hepatocyte's surface, which enables entry of the viral nucleocapsid into the hepatocyte via endocytosis.[13] After entry, the rcDNA is released and transported to the hepatocyte nucleus, where cellular factors modify rcDNA and generate covalently closed circular DNA (cccDNA). The latter serves as a template for transcription of several RNAs that code for the viral proteins needed for viral replication and protein synthesis. The RNA transcripts produced are transported to the cytoplasm, where they are translated into several viral proteins, including hepatitis B core antigen (HBcAg), precore protein, e antigen (HBeAg), surface antigens, HBV X protein, and DNA polymerase, which includes a capacity for reverse transcription of pregenomic RNA (pgRNA) to generate new rcDNA molecules. Core proteins in the cytoplasm self-assemble into the viral nucleocapsid, the pgRNA and polymerase are packaged, and viral replication occurs in the intact nucleocapsid through reverse transcription. Mature viral nucleocapsids are then secreted from the infected hepatocyte, with some also returning to the hepatocyte nucleus where rcDNA forms more cccDNA. This latter mechanism perpetuates HBV infection within the hepatocyte. Additionally, vast numbers of subviral particles containing HBsAg but lacking viral DNA are secreted from the hepatocyte, greatly outnumbering complete virions. This abundance contributes to immune evasion by impairing effective clearance of circulating virus and infected hepatocytes. HBV also stimulates regulatory T

cells, suppressing activity of effector T cells, while upregulating immunosuppressive cytokines (interleukin-10, transforming growth factor-beta) and thus attenuating the host immune response. Finally, in a small proportion of replication cycles, aberrant reverse transcription occurs, yielding double-stranded linear DNA instead of rcDNA, which is integrated into random sites in the host genome. This process, involving recombination between viral and host DNA, begins very early during HBV infection and has been implicated in the pathogenesis of HCC, which may occur even in patients without cirrhosis.[8,12,14-17]

Immune Function in Acute and Chronic Hepatitis B Virus Infection

Acute Hepatitis B Virus Infection

In addition to having an intricate life cycle, HBV induces a complicated series of host immune responses in which both HBV-specific CD8+ cytotoxic T lymphocytes and CD4+ helper T cells play a prominent role. In acute HBV, the innate immune system is activated and naive CD8+ T cells proliferate with clonal expansion differentiating into cytotoxic CD8+ T cells, which mediate viral clearance through cytolytic as well as noncytolytic mechanisms. Direct cytolytic mechanisms represent an early response, which contributes to hepatocyte injury during acute infection. In contrast, noncytolytic mechanisms involve the generation of cytokines, including interferon (IFN)-γ and tumor necrosis factor–α, which suppress HBV replication in the hepatocyte and play an important role in facilitating clearance of HBV without inducing extensive hepatic inflammation. Although the innate immune response contributes to early viral control, effective clearance of HBV during acute infection ultimately depends on a robust adaptive immune response. The adaptive immune response is activated by CD4+ helper T cells, which produce cytokines necessary for further clonal CD8+ T-cell expansion, as well as activation of B cells and neutralizing antibodies to HBsAg and HBcAg.[12,18-20] An efficient and coordinated interplay between innate and adaptive immune responses is essential for HBV clearance during acute infection.

Chronic Hepatitis B Virus Infection

Despite the ability of HBV to elicit an immune response, it can impair immune recognition and evade both innate and adaptive immunity through multiple mechanisms, leading to chronic infection (Table 1). Persistent exposure to the abundant viral antigens induces T-cell exhaustion and prevents an effective immune response, facilitating HBV persistence. Moreover, cccDNA behaves as a stable minichromosome in hepatocytes that is not targeted or eliminated by host immune mechanisms, allowing it to continuously serve as a template for viral transcription even during therapy with antiviral agents.[21] These observations help explain why current antiviral therapies generally require lifelong administration and are unable to fully eradicate HBV in most patients with chronic infection.

Defining Goals of Treatment

At present, the aspirational goal for HBV therapy is to achieve a complete (ie, sterilizing) cure, characterized by the eradication of HBV DNA, cccDNA, and integrated HBV DNA in addition to HBsAg.[22] However, with the currently available oral therapies, although HBV DNA suppression can be reliably achieved, loss of HBsAg remains infrequent, occurring at an annual rate of only approximately 1%. Consequently, the current therapeutic goal is most often a functional cure, defined as sustained clearance of HBsAg and undetectable HBV DNA, with or without seroconversion to anti-HBs, for at least 6 months following a finite course of treatment.[9,23,24] However, advances in the understanding of the complex HBV life cycle, together with emerging therapeutic strategies, have created new opportunities to overcome previous barriers and move closer to the goal of increasing rates of functional cure and potentially achieving complete cure.

Current Available Therapies

There are currently 7 therapies approved by the US Food and Drug Administration (FDA) for the treatment of adults with CHB in the United States: 2 IFN formulations (IFN alfa-2b and peginterferon [PEG-IFN] alfa-2a) and 5 nucleos(t)ide analogues (NAs) (lamivudine, adefovir, entecavir, telbivudine, and tenofovir). The preferred first-line agents in treatment guidelines are PEG-IFN alfa-2a, entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide.[25] In the United States, most patients are currently treated with entecavir or 1 of the 2 forms of tenofovir, in large part owing to the adverse effect profile of IFN and the need for parenteral administration, although IFN has been studied in several trials in combination with NAs or novel antiviral agents.[11] Data accumulated since the advent and widespread use of oral NA therapies have supported the role of antiviral treatment in significantly decreasing the incidence of HCC and hepatic decompensation in treated patients.[26]

Interferon

IFN alfa was approved by the FDA for treatment of HBV infection in 1991.[27] Although detailed mechanisms of IFN are not completely understood, it is believed to exert both antiviral and immunomodulatory effects. Typically used for a finite treatment duration of 48 weeks, IFN

can achieve a durable seroclearance of HBsAg in approximately 5% of treated patients, an effect that appears higher in HBeAg-positive than -negative patients.[28] However, its subcutaneous administration, low antiviral efficacy, and overall poor tolerability owing to numerous adverse effects have limited its use.[29] With the advent of oral NAs, various strategies have been attempted, including combining therapies and the substitution of IFN for oral therapies to accelerate loss of HBsAg. However off-treatment flares and virologic relapse have remained concerns, while benefits are achieved only in a minority of patients.[30,31] Additionally, IFN is contraindicated in pregnancy as well as decompensated cirrhosis and cirrhosis with clinically significant portal hypertension owing to concerns of hepatic decompensation.[9]

Oral Nucleos(t)ide Analogues

Oral NA therapies block HBV DNA synthesis by reducing intrahepatic replication intermediates and circulating virions. Activated nucleos(t)ide inhibitors function as HBV polymerase inhibitors by competing with natural nucleotide substrates for incorporation into the growing viral DNA strand. Lamivudine, adefovir, and tenofovir function as chain terminators as they lack a 3′-OH group, thereby preventing further elongation. Entecavir functions as a delayed chain terminator, interrupting DNA synthesis after several nucleotides are added following the incorporation of entecavir monophosphate.[32] Active suppression of replication and a decrease in the infection of new hepatocytes in turn can diminish the quantity of cccDNA, its overall transcriptional activity, rates of HBV DNA integration, and degree of hepatic inflammation.[33] Long-term data have confirmed the benefit of antiviral treatment in reducing the incidence of HCC and hepatic decompensation.[34] However, NA therapy does not directly target cccDNA nor does it sufficiently restore the host's antiviral immune response against HBV. Thus, rates of HBsAg loss after 1 year are 1% to 3% in HBeAg-positive patients and 1% in HBeAg-negative patients.[23] Given the lack of meaningful reduction in HBsAg level even after years of continuous treatment, and risk of rebound of viral replication once therapy is discontinued, indefinite therapy is typically required.[35] Recent studies have evaluated the outcomes of stopping NA therapy if HBsAg falls to less than 100 IU/mL. RETRACT-B, a global 4-year follow-up study, demonstrated HBsAg seroclearance off therapy in roughly 33% of such patients. Although hepatitis flares occurred in roughly 1% of patients, these can result in fulminant hepatitis and the need for transplantation.[36] The 2025 American Association for the Study of Liver Diseases (AASLD)/Infectious Diseases Society of America guidelines recommend in general that treatment be continued until HBsAg seroclearance occurs.[11]

New Therapeutic Approaches in Chronic Hepatitis B Virus

Increasing appreciation of the complexity of the HBV life cycle and the interplay with host immune responses has led to the identification of numerous potential targets for the development of novel therapeutic strategies. These approaches either involve therapies that target specific stages of viral replication or target immunomodulatory mechanisms aiming to restore immune function and overcome the anergy associated with CHB (Table 2).

Direct-Acting Antiviral Agents

Several steps in the HBV life cycle may be amenable to disruption by antiviral therapy. These include viral entry into the hepatocyte, cccDNA silencing or elimination, viral transcription and translation, nucleocapsid assembly, HBV polymerase inhibition, and HBsAg secretion (Figure).

Entry Inhibition As outlined earlier, HBV entry to the hepatocyte occurs via interaction of the viral envelope preS1 glycoprotein and the hepatocyte NTCP bile acid receptor. Bulevirtide, a lipopeptide derived from the HBV preS1 region, competes with HBV for NTCP binding and thus inhibits viral entry. Its major clinical application has been in the treatment of hepatitis delta virus (HDV) infection, for which it is currently approved in Europe and several areas other than the United States, where approval is being sought.[37] Its effectiveness against HDV infection is largely owing to blockade of HDV-bearing viral particles enveloped by HBsAg into hepatocytes, thereby preventing the establishment of new hepatocyte infection as well as reinfection of cells already harboring HDV, which collectively decreases overall viral hepatic burden. Despite its potent effects on HDV viral entry, it has not been shown to reduce serum HBsAg by more than 1 log IU/mL or to cause HBsAg seroclearance.[38] Although it may have a mechanism of limiting de novo infection of previously uninfected hepatocytes in HBV monoinfection, its therapeutic role for HBV infection will likely be limited to combination treatment strategies, if any.

Covalently Closed Circular DNA Silencing or Elimination Persistence of cccDNA within the hepatocyte nucleus remains an obstacle to complete cure for HBV. Multiple novel approaches to eliminate or silence cccDNA are in preclinical stages. These include the use of gene editing technologies such as the clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) system, epigenetic modification, and small molecule inhibitors.[39-41] Utilizing these approaches,

Table 2. Novel HBV Targets and Therapies

Mechanisms	Agents
Antiviral agents	
Entry inhibitor	• Bulevirtide
cccDNA silencing or elimination	• Gene editing • CRISPR/Cas system • Epigenetic modifications • Small molecule inhibitors
Viral transcription/translation	
– *Antisense oligonucleotides*	• Bepirovirsen • AHB-137
– *Small interfering RNA agents*	• JNJ-3989 (daplusiran/tomligisiran) • VIR-2218 (elebsiran) • AB-729 (imdusiran) • RG6346 (xalnesiran) • RDB1016
Capsid assembly modulators	• JNJ-56136379 (bersacapavir) • ALG-000184 (pevifoscorvir sodium) • ABI-4334
HBsAg secretion inhibitors	• REP 2139 • REP 2165
Immunomodulators	
Innate immune system	
Interferon	• Interferon alfa-2b • Peginterferon alfa-2a
Toll-like receptor agonists	• Ruzotolimod • Vesatolimod • Selgantolimod
Adaptive immune system	
Therapeutic vaccines	• BRII-179 • VTP-300 • VRON-0200 • GS-2829 • GS-6779
Monoclonal antibodies	• Lenvervimab (GC1102) • Libevitug (HH-003) • Tobevibart (VIR-3434) • Brelovitug (BJT-778)
Checkpoint inhibitors (anti–PD-1, anti–PD-L1)	• Envafolimab • Nivolumab

cccDNA, covalently closed circular DNA; CRISPR/Cas, clustered regularly interspaced short palindromic repeats/CRISPR-associated; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PD-1, programmed death 1; PD-L1, programmed death ligand 1.

the potential to target both cccDNA and integrated HBV DNA increases the possibility of achieving complete cure.

Genome editing refers to altering genomic DNA at precise locations. This can involve addition, removal, or replacement of DNA sequences, ultimately allowing targeted genes to be disrupted or switched off. Currently, the major genome-editing technologies include zinc finger nucleases, transcription activator-like effector nucleases, and RNA-guided CRISPR/Cas systems.[42] These strategies are being explored to disable cccDNA by creating precise double-stranded DNA breaks, which subsequently undergo homologous repair, leading to mutations at the specified cleavage sites. A single administration of CRISPR-Cas9 ribonucleoproteins has been shown to eradicate greater than 98% of the cccDNA pool. Yet, solely targeting cccDNA may not be sufficient, as rebound HBV

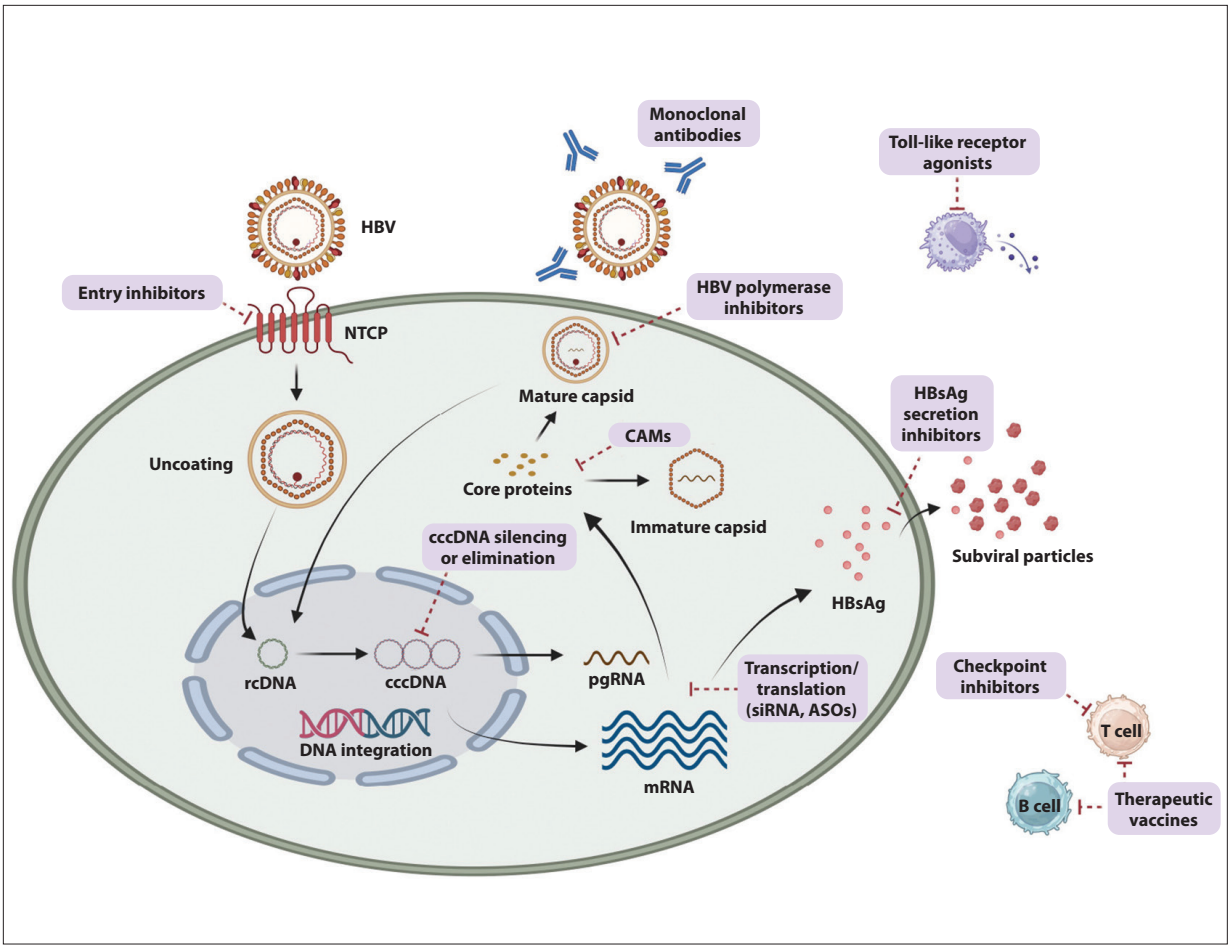

Figure. HBV life cycle with therapeutic drug targets. Created with BioRender.com.

ASOs, antisense oligonucleotides; CAMs, capsid assembly modulators; cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; mRNA, messenger RNA; NTCP, sodium taurocholate cotransporting peptide; pgRNA, pregenomic RNA; rcDNA, relaxed circular DNA; siRNA, small interfering RNA.

replication can occur owing to de novo cccDNA formation from its precursor rcDNA. Therefore, utilizing additional therapeutic strategies to eliminate rcDNA is likely needed.[43] Epigenetic modifications such as inducing DNA methylation or histone deacetylation or methylation are also being studied to target both cccDNA and integrated DNA. With these epigenetic modifications, downstream silencing of transcription activity can be achieved without disruption of the genome. Early data suggest these therapies can lead to strong and durable epigenetic silencing of cccDNA and integrated DNA.[44] Other strategies such as Cas9-mediated base editing to permanently inactivate integrated HBV DNA and cccDNA without inducing DNA breaks also offer potential approaches.[45] However, before any strategies can be implemented, several hurdles must be addressed, including the risk of off-target effects—both immediate and delayed—which raises concerns that will delay the clinical application of these strategies.

Early data have been reported from a phase 1 study conducted by Precision BioSciences using ARCUS, a gene editing technology based upon an enzyme in the homing endonuclease family that was derived from an algae species and can be used for sequence-specific targeting. The resultant product for HBV is a messenger RNA that encodes the ARCUS nuclease in a lipid nanoparticle.[46,47] The stated goal of the program is to eliminate cccDNA and inactivate integrated HBV DNA. Preliminary data from the phase 1 trial reported at the AASLD 2025 meeting included 3 patients in each of the 3 cohorts treated with 0.2 mg/kg, 0.4 mg/kg, or 0.8 mg/kg at 8-week intervals for 3 doses. All 3 participants in the initial arm experienced declines in HBsAg, 1 to a maximum of 69%. In 2 of the 3 patients, HBsAg had returned to baseline after the third dose but remained lower than baseline 7 months after initial treatment in the third patient. In the 0.4 mg/kg cohort, among patients who had just received

a third dose, 1 had an HBsAg decline of less than 50% while the other had a greater-than-50% decline. In the 0.8 mg/kg dose group, 2 patients within 1 to 2 weeks of their first dose and 1 who had recently received their second dose experienced a steep reduction in HBsAg.[46] These data appear to provide proof of concept of gene editing. Despite the small sample sizes and need for further evaluation, it appears possible from these data that higher doses were less likely to be associated with rebounds in HBsAg after each dose and potentially more sustained reductions for an unspecified duration. One participant did not complete dosing owing to a transient, reversible infusion-related reaction that resolved within minutes of initiating dosing.[46] Substantial amounts of additional data, including liver biopsy data, are expected from the cohorts already initiated at the time of the AASLD meeting as well as further cohorts with varied dosing schedules.

Viral Transcription and Translation Another approach to disrupting HBV replication is by blocking viral transcription with nucleotide sequences that are complementary to regions of the HBV genome, leading to posttranscriptional gene silencing. Such strategies are advantageous because they can reduce HBsAg production from both cccDNA and integrated HBV DNA. Two major RNA interference classes are in clinical trials: small interfering RNA (siRNA) and antisense oligonucleotides (ASOs). siRNA is a double-stranded RNA molecule that engages the RNA-induced silencing complex (RISC) in the cytoplasm and guides it to complementary viral RNA sequences. Once bound, the RISC mediates degradation of the targeted viral RNA and suppresses translation, thereby reducing the production of viral proteins. ASOs are single-stranded DNA molecules that bind complementary HBV RNA. This DNA-RNA hybrid leads to recruitment of RNase H, a cellular enzyme that selectively degrades the HBV RNA strand, thereby limiting translation and viral protein production, including HBsAg.[33,48] Both therapeutic strategies are designed to reduce HBV RNA levels and ultimately lower production of viral proteins such as HBsAg. By lowering HBsAg levels, some of these therapies are hoped to help facilitate partial restoration of antiviral immune function, although complete immune restoration has not been clearly demonstrated.

Small Interfering RNA Several siRNA agents, including JNJ-3989 (daplusiran/tomligisiran), VIR-2218 (elebsiran), AB-729 (imdusiran), RDB1016, and RG6346 (xalnesiran), are under evaluation and hold promise for a durable reduction in HBsAg with infrequent dosing regimens and acceptable tolerability. Following initial declines in HBsAg, however, there is typically a plateau without complete HBsAg seroclearance.[49-53] Limited data thus far show that these agents may successfully maintain low HBV DNA and HBsAg levels long term following discontinuation of NA therapy, with short-duration siRNA suppressing HBsAg for up to 6 years in some patients, potentially contributing to a functional cure.[54] However, large-scale data thus far have not shown the consistent ability of siRNA agents to achieve a functional cure; therefore, their role as monotherapy remains limited and combination therapies are likely needed.

Antisense Oligonucleotides Bepirovirsen is the ASO that has undergone the most extensive evaluation in clinical trials and is the first one to be evaluated in a phase 3 trial. In the phase 2 B-Clear trial, administration of bepirovirsen initially yielded HBV DNA and HBsAg levels below the limit of detection at the end of treatment (EOT) in approximately 26% of patients, with the primary outcome of sustained effect at 24 weeks following EOT met in 9% and 10% of NA-experienced and NA-naive patients, respectively. Nearly all sustained responders had baseline HBsAg less than 3000 IU/mL, with the highest efficacy in those with baseline HBsAg level no more than 3 log IU/mL. Additionally, alanine aminotransferase (ALT) flares have been observed to coincide with HBsAg reduction, suggesting a contributive immune-related mechanism.[27,55] Additional data have shown that sequential therapy with bepirovirsen followed by PEG-IFN is well tolerated and can reduce relapse rates compared with bepirovirsen alone, supporting the potential role of combination therapies with immunomodulating agents following HBV antigen suppression.[56] Bepirovirsen is currently the focus of 2 large international multicenter phase 3 studies, B-Well 1 and B-Well 2, evaluating its durability of functional cure rates following a finite course of treatment.[57,58] In another phase 2 study, bepirovirsen for 24 weeks is being evaluated following an initial 24 weeks of treatment with the siRNA agents daplusiran/tomligisiran.[59]

Another study recently evaluated the ASO AHB-137, given 300 mg weekly for 24 weeks to NA-suppressed HBeAg-negative patients with HBsAg levels less than 3000 IU/mL. More than 60% of patients lost HBsAg by week 24, and interim follow-up data presented at the AASLD 2025 meeting showed a 27% sustained HBsAg clearance rate at posttreatment week 24.[60] Further data on this drug, including rates of functional cure, are awaited.

Capsid Assembly Modulators As described previously, HBV core proteins self-assemble in the cytoplasm to form nucleocapsids that package pgRNA and polymerase, and following reverse transcription, mature nucleocapsids are either secreted as new virions or recycled back to the nucleus to produce more cccDNA. At a structural level, the capsid protein assembles into an icosahedral capsid

composed of 120 capsid protein homodimers, a process that is critically dependent on precise dimer–dimer contacts. Capsid assembly modulators (CAMs) exploit these interactions by either redirecting capsid protein into aberrant assembly pathways with misassembled capsids (CAM-A) or accelerating premature formation of incomplete capsids (CAM-E).[61] Consequently, CAMs reduce the production of mature virions and incomplete viral particles and, importantly, limit the nuclear recycling of rcDNA to cccDNA—an important second step for achieving substantial clearance of HBV from infected hepatocytes. As a result, HBV RNA suppression is also observed, which is predominantly a function of targeting nucleocapsids and not directly representing cccDNA transcriptional activity compared with other agents.

Several CAMs have been studied in early trials. JNJ-56136379 (bersacapavir), when combined with NAs for 24 weeks of therapy, was shown to significantly decrease HBV DNA up to 5.88 log IU/mL and HBV RNA up to 3.16 log IU/mL. However, despite successful suppression, viral rebound was nearly universal following discontinuation. Furthermore, there was a lack of significant effect on HBsAg and HBeAg levels, which was also a limitation of other early-generation CAMs.[62,63] More recently, early results with newer-generation and more potent CAMs have shown promise for reduction of HBsAg, HBeAg, and hepatitis B core-related antigen (HBcrAg). Various agents are currently being evaluated, including ALG-000184 (pevifoscorvir sodium) and ABI-4334.[64,65] In an ongoing trial of pevifoscorvir sodium, HBeAg-positive patients experienced a 0.8 log decline in HBsAg over 96 weeks, with larger declines in HBcrAg and HBeAg levels. This HBsAg decline does not occur with NAs. It is postulated that the decline with a potent CAM suggests silencing or clearance of cccDNA. In HBeAg-negative patients, profound declines in HBV DNA occur but HBsAg does not change significantly.[66] These observations are consistent with the suggestion that a higher proportion of HBsAg in HBeAg-negative patients is arising from integrated DNA rather than cccDNA; only the latter is targeted by CAMs. At present, a trial of this CAM vs NAs as monotherapy has been initiated in HBV-infected patients with HBV DNA greater than 20,000 IU/mL.[67] This and other potent CAMs may also be of interest in combination therapy with other novel agents.

Hepatitis B Surface Antigen Secretion Inhibitors Nucleic acid polymers (NAPs) inhibit the release of HBV subviral particles from infected hepatocytes into the circulation, which constitute greater than 99% of circulating HBsAg.[12,68] As discussed previously, prolonged exposure to these abundant subviral particles drives T-cell exhaustion and promotes HBV persistence.

Among NAPs, REP 2139 and REP 2165 are the most extensively studied and, when used in combination with PEG-IFN and NA therapies, have been shown to have high rates of HBsAg loss and functional cure, although studies thus far have been limited by small sample size. High rates of ALT flares have been described, reportedly subsiding without adverse clinical outcomes.[69] Larger-scale trials are needed to define the role of NAP-based regimens.

Immunomodulatory Approaches

As previously outlined, the relationship between HBV and the host immune system is characterized by a complex and dynamic interplay. Immunomodulatory therapies are designed to mitigate the underlying immune tolerance and T-cell dysfunction associated with chronic infection. PEG-IFN remains the sole immunomodulatory agent currently approved for the management of CHB. Nonetheless, its clinical use declined following the introduction of better-tolerated oral antivirals and its relatively low functional cure rates, although it may continue to have relevance within combination therapeutic approaches. Several strategies to activate both the innate (eg, IFN, Toll-like receptor [TLR] agonists) and adaptive immune systems (eg, monoclonal antibodies, therapeutic vaccines, checkpoint inhibitors) are currently being evaluated (Figure).

Toll-Like Receptor Agonists TLR agonists play a critical role in the innate immune system by recognizing pathogen-associated molecular patterns and activating downstream IFN and cytokine signaling pathways that contribute to the antiviral response. Various TLR7 and TLR8 agonists are in early-stage clinical trials. TLR7 agonists have demonstrated target engagement and activation of downstream immune pathways; however, HBsAg decline with monotherapy has been limited.[70] Agents such as ruzotolimod (RO7020531) have been shown to lead to mean HBsAg decline of up to 0.15 log IU/mL following 6 weeks of treatment, whereas other agents, including vesatolimod, fail to show any significant effects on HBsAg decline after 12 weeks of treatment.[71,72] TLR8 agonists are also being actively studied in clinical trials. Selgantolimod (GS-9688), which is currently in phase 2 clinical trials, was shown to elicit greater than 0.1 log IU/mL decline in 26% of patients, with 5% achieving HBsAg seroclearance 24 weeks after EOT.[73]

Monoclonal Antibodies Another approach to entry inhibition is monoclonal antibodies directed against HBV envelope proteins. Several monoclonal antibodies have been developed.

Lenvervimab (GC1102) is a recombinant human

immunoglobulin. Although the precise mechanism of action has yet to be fully elucidated, the drug is hypothesized to act through neutralization of HBsAg via immune complex formation, as well as inhibition of viral entry by binding to HBsAg. Although shown to decrease HBsAg titers in a dose-dependent manner when used in patients with HBsAg less than 1000 IU/mL, studies show a prompt rebound in HBsAg levels after treatment discontinuation.[74] Therefore, its role may be as part of a combination regimen.

Libevitug (HH-003) is a human monoclonal antibody that targets the preS1 region of the HBV envelope, thus blocking its binding to the NTCP receptor. In initial clinical trials in patients with CHB and coexisting chronic HDV infection, 24-week therapy was shown to reduce HDV RNA levels and normalize ALT.[75] Small studies have also supported its role in decreasing HBV DNA, HBsAg, and ALT levels.[76]

Tobevibart (VIR-3434), another human monoclonal antibody, targets an antigenic loop shared by small, medium, and large HBsAg proteins and has potent antibody-mediated neutralizing effects on serum HBsAg that help to prevent entry into hepatocytes. Antibody-dependent phagocytosis also facilitates clearance of HBsAg. In addition, tobevibart has been modified to have increased affinity for Fc receptors, which enhances antigen presentation and downstream immune activation effects. These modifications ultimately promote a T-cell response, inducing a vaccine-like effect.[77] In the recent phase 2 SOLSTICE study, tobevibart alone or with elebsiran (VIR-2218, a siRNA agent) was evaluated in patients coinfected with chronic HDV. At week 48, a combined response (defined as HDV RNA below the limit of detection or ≥2 log IU/mL decline from baseline together with ALT normalization) occurred in 56% with tobevibart plus elebsiran and 61% with tobevibart alone. Undetectable HDV RNA was observed in 66% vs 48%, ALT normalization in 56% vs 61%, and HBsAg levels below 10 IU/mL in 91% vs 21%, respectively.[78]

Brelovitug (BJT-778) is another high-affinity monoclonal antibody that targets an antigenic loop of HBsAg. By binding to surface antigens and subviral particles, brelovitug facilitates viral antigen internalization and presentation to support antispecific T-cell activation and antiviral immunity. In a recent phase 2 study for patients with chronic HDV infection and suppressed HBV DNA less than 100 IU/mL on NA therapy, monotherapy with brelovitug achieved 100% virologic response in patients at 48 weeks, with up to 82% of participants reaching the combined endpoint of virologic response and ALT normalization.[79] Further data from a global randomized, open-label, multicenter trial (NCT07298330) are awaited.[80]

Therapeutic Vaccines Therapeutic vaccination involves administering a noninfectious viral antigen to stimulate or enhance HBV-specific immune responses, with the goal of achieving long-term control of HBV infection.[81] Therapeutic vaccines have been an area of investigation for many years. Early HBsAg-based vaccines showed limited antiviral efficacy; however, incorporating non-HBsAg viral proteins appears to enhance responses, renewing interest in HBV therapeutic vaccines.[82] Although initial experience has documented reductions in HBsAg levels, the role of therapeutic vaccines remains to be determined.

BRII-179, a recombinant protein-based HBV therapy expressing preS1, preS2, and S HBV surface antigens to induce both B- and T-cell immunity, was shown to induce anti-HBs responses in greater than 30% of patients, although no significant decline in HBsAg was observed. It is currently being evaluated in the phase 2 ENSURE study utilizing sequential combination treatment strategies to improve functional cure outcomes.[83,84]

VTP-300 is a therapeutic vaccine designed to elicit immune responses against the polymerase, core, and S regions of HBV. In a phase 2 clinical trial, administration of VTP-300 induced both CD4+ and CD8+ T-cell responses and reduced HBsAg levels by more than 0.5 log IU/mL; however, sustained response was observed only in a subset of virally suppressed patients with baseline HBsAg levels below 50 IU/mL. VTP-300 is also under investigation in combination with low-dose nivolumab for its potential to achieve a functional cure in CHB.[85]

VRON-0200, a novel therapeutic vaccine that expresses a genetically encoded checkpoint modifier fused with HBV core and polymerase antigens, is designed to elicit CD8+ T-cell responses. In phase 1b studies, the addition of a single intramuscular VRON-0200 dose to standard of care led to HBsAg declines that persisted up to 1 year, with 47% of patients achieving greater-than-50% reduction.[86]

Other therapeutic vaccination strategies, including the utilization of nonreplicating arenavirus vectors such as GS-2829 and GS-6779, are also being actively studied.[87]

Checkpoint Inhibitors T-cell dysfunction is a key contributor to adaptive immune dysfunction and diminished rates of successful clearance of HBV. The programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) pathways inhibit T-cell function and have been shown to be upregulated in CHB.[88] Inhibition of these pathways utilizing PD-1 and PD-L1 inhibitors has been widely used in oncology, and more recently has become of interest in CHB.

Envafolimab, a subcutaneously administered anti–PD-L1, was shown in a phase 2 study to significantly reduce HBsAg levels with seroclearance in up to 43%

of virally suppressed patients on NAs who had HBsAg levels no more than 100 IU/mL at entry.[89] Nivolumab is another PD-1 inhibitor currently in phase 2 trials, including in combination treatment regimens. Immune-mediated damage to various organs, particularly but not only the thyroid, is an important part of the adverse event profile of this class of drugs, even at low doses, and careful monitoring is required in studies of these agents.[90]

Combination Regimens

The novel therapeutic agents described previously exhibit variable antiviral potencies; however, functional cure—defined as sustained HBsAg loss with or without seroconversion—is achieved in only a minority of treated patients. Pretreatment HBsAg levels have consistently emerged as a key predictor of the likelihood of HBsAg clearance, with lower baseline levels generally associated with a higher probability of seroclearance. This limitation has prompted considerable interest in combination regimens, which aim to leverage multiple mechanisms of action to enhance antiviral efficacy. By targeting multiple steps in the HBV life cycle and modulating the host immune response, such combinations have the potential to achieve deeper HBsAg reductions, increase rates of functional cure, and possibly reduce relapse after treatment cessation.

However, the efficacy of combination therapies remains difficult to predict and has often been limited. In the REEF-1 study, which assessed a 48-week regimen combining a siRNA agent with a CAM, the combination failed to demonstrate any additional therapeutic benefit and in fact appeared to confer inferior results for unclear reasons. This unexpected outcome suggests that novel therapeutic agents may not necessarily exhibit additive or synergistic effects when combined.[91]

PEG-IFN has also been evaluated in combination with several emerging therapeutic agents. Combining PEG-IFN with siRNA therapies has generally yielded favorable results, including greater reductions in HBsAg levels, higher rates of HBsAg loss, and potential decreases in relapse. In a phase 2 study, a combination of the siRNA therapy VIR-2218 (elebsiran) and PEG-IFN alfa led to greater mean HBsAg declines, with approximately 15.6% of patients on the combination achieving HBsAg seroclearance.[92] Limited data from additional studies indicate that the therapeutic benefit of combining siRNA therapies with PEG-IFN is greater with extended treatment durations of 24 weeks and among individuals with lower baseline HBsAg levels.[93] However, the tolerability of prolonged IFN-containing regimens may reduce their overall appeal. siRNA therapies are also currently being studied with other immunomodulatory agents, including TLR agonists, therapeutic vaccines, anti–PD-1/PD-L1 agents, and monoclonal antibodies.

Conclusion

Therapeutic options for HBV infection continue to expand, targeting multiple stages of the viral life cycle and immunomodulatory pathways to enhance treatment efficacies. RNA interference agents, including ASOs such as bepirovirsen, have emerged as front-runners in HBV treatment research, with the highest efficacy data reported to date. Although combination therapies also represent an important and promising area of investigation, several challenges in study design and interpretation remain across all therapeutic strategies. These include heterogeneity in study populations (eg, low vs high baseline HBsAg levels, presence or absence of cirrhosis), variability in the definition of clinical endpoints, uncertainties regarding optimal therapeutic combinations and treatment durations, and considerations of treatment burden, tolerability, and cost relative to once-daily NAs. Although challenges remain, combination strategies undoubtedly represent a promising path toward resolving the limitations of current monotherapies. Genetic modification strategies, including CRISPR-based approaches, represent a theoretically highly promising approach to HBV functional cure, although their development is still largely at the preclinical stage and requires careful evaluation of safety and long-term efficacy. As these novel HBV strategies continue to advance, the prospect of reliably achieving a functional cure for HBV infection is becoming increasingly feasible.

Disclosures

Dr Sandhu has no relevant conflicts of interest to disclose. Dr Martin is a consultant for Gilead. Dr Jacobson receives research support and grants from Gilead, Genfit, AbbVie, Bristol Myers Squibb, Enanta Pharmaceuticals, Assembly Biosciences, Atea, and Novo Nordisk; is a consultant for AbbVie, Bristol Myers Squibb, Intercept, Gilead, Merck, Janssen, Spring Bank Pharmaceuticals, Assembly Biosciences, and Aligos; and serves on the speakers bureau for Gilead and AbbVie.

References

1. Tu T, McQuaid TJ, Jacobson IM. HBV-induced carcinogenesis: mechanisms, correlation with viral suppression, and implications for treatment. *Liver Int*. 2025;45(1):e16202.
2. World Health Organization. Hepatitis B. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. Published July 23, 2025. Accessed May 2026.
3. Gao L, Ren Y, Hu S, et al. Global, regional, and national total burden related to hepatitis B and attributable risk factors in adults aged 65 years and older from 1990 to 2021 and projection to 2030. *Front Public Health*. 2025;13:1654356.
4. Wong RJ, Brosgart CL, Welch S, et al. An updated assessment of chronic hepatitis B prevalence among foreign-born persons living in the United States. *Hepatology*. 2021;74(2):607-626.
5. Sharma SK, Saini N, Chwla Y. Hepatitis B virus: inactive carriers. *Virol J*. 2005;2:82.
6. Chen CJ, Yang HI, Su J, et al; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA*. 2006;295(1):65-73.
7. World Health Organization. Global health sector strategies on, respectively,

HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030. https://www.who.int/publications/i/item/9789240053779. Published July 18, 2022. Accessed May 2026.
8. Tang LSY, Covert E, Wilson E, Kottilil S. Chronic hepatitis B infection: a review. *JAMA*. 2018;319(17):1802-1813.
9. Jeng WJ, Papatheodoridis GV, Lok ASF. Hepatitis B. *Lancet*. 2023;401(10381):1039-1052.
10. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*. 2018;67(4):1560-1599.
11. Ghany MG, Pan CQ, Lok AS, et al. AASLD IDSA practice guideline on treatment of chronic hepatitis B. *Hepatology*. 2026;83(4):974-997.
12. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. *N Engl J Med*. 2004;350(11):1118-1129.
13. Ni Y, Lempp FA, Mehrle S, et al. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology*. 2014;146(4):1070-1083.
14. Seto WK, Lo YR, Pawlotsky JM, Yuen MF. Chronic hepatitis B virus infection. *Lancet*. 2018;392(10161):2313-2324.
15. Asselah T, Loureiro D, Boyer N, Mansouri A. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. *Lancet Gastroenterol Hepatol*. 2019;4(11):883-892.
16. Li W, Han J, Wu H. Regulatory T-cells promote hepatitis B virus infection and hepatocellular carcinoma progression. *Chronic Dis Transl Med*. 2016;2(2):67-80.
17. Tsukuda S, Watashi K. Hepatitis B virus biology and life cycle. *Antiviral Res.* 2020;182:104925.
18. Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. *Nat Rev Immunol*. 2022;22(1):19-32.
19. Phillips S, Chokshi S, Riva A, Evans A, Williams R, Naoumov NV. CD8(+) T cell control of hepatitis B virus replication: direct comparison between cytolytic and noncytolytic functions. *J Immunol*. 2010;184(1):287-295.
20. Jung MC, Pape GR. Immunology of hepatitis B infection. *Lancet Infect Dis*. 2002;2(1):43-50.
21. Ortega-Prieto AM, Dorner M. Immune evasion strategies during chronic hepatitis B and C virus infection. *Vaccines (Basel)*. 2017;5(3):24.
22. Ghany MG, Buti M, Lampertico P, Lee HM; 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference Faculty. Guidance on treatment endpoints and study design for clinical trials aiming to achieve cure in chronic hepatitis B and D: report from the 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference. *J Hepatol*. 2023;79(5):1254-1269.
23. Yardeni D, Chang KM, Ghany MG. Current best practice in hepatitis B management and understanding long-term prospects for cure. *Gastroenterology*. 2023;164(1):42-60.e6.
24. Wong GLH, Gane E, Lok ASF. How to achieve functional cure of HBV: stopping NUCs, adding interferon or new drug development? *J Hepatol*. 2022;76(6):1249-1262.
25. Martin P, Nguyen MH, Dieterich DT, et al. Treatment algorithm for managing chronic hepatitis B virus infection in the United States: 2021 update. *Clin Gastroenterol Hepatol*. 2022;20(8):1766-1775.
26. Liu K, Choi J, Le A, et al. Tenofovir disoproxil fumarate reduces hepatocellular carcinoma, decompensation and death in chronic hepatitis B patients with cirrhosis. *Aliment Pharmacol Ther*. 2019;50(9):1037-1048.
27. Yuen MF, Heo J, Jang JW, et al. Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: a phase 2 randomized controlled trial. *Nat Med*. 2021;27(10):1725-1734.
28. Zhao Q, Liu H, Tang L, et al. Mechanism of interferon alpha therapy for chronic hepatitis B and potential approaches to improve its therapeutic efficacy. *Antiviral Res.* 2024;221:105782.
29. van Zonneveld M, Flink HJ, Verhey E, et al; HBV 99-01 Study Group. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. *Aliment Pharmacol Ther*. 2005;21(9):1163-1171.
30. Tamaki N, Kurosaki M, Kusakabe A, et al; Japanese Red Cross Hospital Liver Study Group. Hepatitis B surface antigen reduction by switching from long-term nucleoside/nucleotide analogue administration to pegylated interferon. *J Viral Hepat*. 2017;24(8):672-678.
31. van Bömmel F, Berg T. Risks and benefits of discontinuation of nucleos(t)ide analogue treatment: a treatment concept for patients with HBeAg-negative chronic hepatitis B. *Hepatol Commun*. 2021;5(10):1632-1648.
32. Menéndez-Arias L, Álvarez M, Pacheco B. Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance. *Curr Opin Virol*. 2014;8:1-9.
33. Dusheiko G, Agarwal K, Maini MK. New approaches to chronic hepatitis B. *N Engl J Med*. 2023;388(1):55-69.
34. Lok AS, McMahon BJ, Brown RS Jr, et al. Antiviral therapy for chronic hepatitis B viral infection in adults: a systematic review and meta-analysis. *Hepatology*. 2016;63(1):284-306.
35. Kim GA, Lim YS, An J, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. *Gut*. 2014;63(8):1325-1332.
36. Hirode G, Choi HSJ, Chen CH, et al; RETRACT-B Study Group. Off-therapy response after nucleos(t)ide analogue withdrawal in patients with chronic hepatitis B: an international, multicenter, multiethnic cohort (RETRACT-B study). *Gastroenterology*. 2022;162(3):757-771.e4.
37. Soriano V, Moreno-Torres V, Treviño A, et al. Bulevirtide in the treatment of hepatitis delta: drug discovery, clinical development and place in therapy. *Drug Des Devel Ther*. 2023;17:155-166.
38. Wedemeyer H, Schöneweis K, Bogomolov P, et al. Safety and efficacy of bulevirtide in combination with tenofovir disoproxil fumarate in patients with hepatitis B virus and hepatitis D virus coinfection (MYR202): a multicentre, randomised, parallel-group, open-label, phase 2 trial. *Lancet Infect Dis*. 2023;23(1):117-129.
39. Yao ZQ, Schank MB, Zhao J, et al. The potential of HBV cure: an overview of CRISPR-mediated HBV gene disruption. *Front Genome Ed*. 2024;6:1467449.
40. Ren J, Cheng S, Ren F, et al. Epigenetic regulation and its therapeutic potential in hepatitis B virus covalently closed circular DNA. *Genes Dis*. 2024;12(1):101215.
41. Fonseca SG, Cortés-Mancera FM, Rots MG, et al. The promise of epigenetic editing strategies in functionally curing chronic hepatitis B virus infections. *Epigenetics Commun*. 2026;6:4.
42. Xu Y, Li Z. CRISPR-Cas systems: overview, innovations and applications in human disease research and gene therapy. *Comput Struct Biotechnol J*. 2020;18:2401-2415.
43. Kostyushev D, Kostyusheva A, Brezgin S, et al. Depleting hepatitis B virus relaxed circular DNA is necessary for resolution of infection by CRISPR-Cas9. *Mol Ther Nucleic Acids*. 2023;31:482-493.
44. Cosgrove B. TUNE-401 is a novel first-in-class epigenetic silencer developed for treatment of chronic hepatitis B, with preclinical data showing strong, durable, and precise transcriptional repression of HBV DNA. Presented at: The Liver Meeting; November 15-19, 2024; San Diego, CA. Abstract 0152.
45. Yang YC, Chen YH, Kao JH, et al. Permanent inactivation of HBV genomes by CRISPR/Cas9-mediated non-cleavage base editing. *Mol Ther Nucleic Acids*. 2020;20:480-490.
46. Yuen M, Jucov A, Gane E, et al. PBGENE-HBV, a first-in-class gene editing therapy for chronic hepatitis B, demonstrates safety and antiviral activity in early cohorts. Presented at: The Liver Meeting; November 7-11, 2025; Washington, DC. Abstract 5017.
47. Precision BioSciences. ARCUS genome editing. https://precisionbiosciences.com/arcus-genome-editing/. Accessed May 2026.
48. Vaillant A. Oligonucleotide-based therapies for chronic HBV infection: a primer on biochemistry, mechanisms and antiviral effects. *Viruses*. 2022;14(9):2052.
49. Gane E, Yuen MF, Kakuda TN, et al. JNJ-73763989 pharmacokinetics and safety: liver-targeted siRNAs against hepatitis B virus, in Japanese and non-Japanese healthy adults, and combined with JNJ-56136379 and a nucleos(t)ide analogue in patients with chronic hepatitis B. *Antivir Ther*. 2022;27(3):13596535221093856.
50. Yuen MF, Locarnini S, Lim TH, et al. Combination treatments including the small-interfering RNA JNJ-3989 induce rapid and sometimes prolonged viral responses in patients with CHB. *J Hepatol*. 2022;77(5):1287-1298.
51. Zheng S, Cao H, Zhang M, et al. A potent GalNAc-siRNA drug, RBD1016, leads to sustained HBsAg reduction and seroconversion in mouse models of HBV infection. *Mol Ther Nucleic Acids*. 2025;36(3):102627.
52. Gane E, Lim YS, Kim JB, et al. Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: results from randomized clinical trials. *J Hepatol*. 2023;79(4):924-932.
53. Gane EJ, Kim W, Lim TH, et al. First-in-human randomized study of RNAi therapeutic RG6346 for chronic hepatitis B virus infection. *J Hepatol*. 2023;79(5):1139-1149.
54. Mak LY, Wooddell CI, Lenz O, et al. Long-term hepatitis B surface antigen response after finite treatment of ARC-520 or JNJ-3989. *Gut*. 2025;74(3):440-450.
55. Yuen MF, Lim SG, Plesniak R, et al; B-Clear Study Group. Efficacy and safety of bepirovirsen in chronic hepatitis B infection. *N Engl J Med*. 2022;387(21):1957-1968.
56. Buti M, Heo J, Tanaka Y, et al. Sequential Peg-IFN after bepirovirsen may reduce post-treatment relapse in chronic hepatitis B. *J Hepatol*. 2025;82(2):222-234.

57. ClinicalTrials.gov. Study of bepirovirsen in nucleos(t)ide analogue-treated participants with chronic hepatitis B (B-Well 2). https://clinicaltrials.gov/study/NCT05630820. Identifier: NCT05630820. Accessed May 2026.
58. ClinicalTrials.gov. Study of bepirovirsen in nucleos(t)ide analogue-treated participants with chronic hepatitis B (B-Well 1). https://clinicaltrials.gov/study/NCT05630807. Identifier: NCT05630807. Accessed May 2026.
59. ClinicalTrials.gov. A study of sequential therapy with daplusiran/tomligisiran (DAP/TOM) followed by bepirovirsen in participants living with chronic hepatitis B (CHB) (B-UNITED). https://clinicaltrials.gov/study/NCT06537414. Identifier: NCT06537414. Accessed May 2026.
60. Ding Y, Liang X, Gao Y, et al. High proportion of participants achieved sustained complete response 24 weeks after end of AHB137 treatment in HBeAg-negative chronic hepatitis B participants on NA therapy: pooled analysis of two phase 2 studies in China. Presented at: The Liver Meeting; November 7-11, 2025; Washington, DC. Abstract 5022.
61. Askari FS, Mohebbi A. The dynamic states of hepatitis B virus capsid monomers under the impact of different class of capsid-assembly modulators. *Sci Rep*. 2025;15(1):32731.
62. Janssen HLA, Hou J, Asselah T, et al. Randomised phase 2 study (JADE) of the HBV capsid assembly modulator JNJ-56136379 with or without a nucleos(t)ide analogue in patients with chronic hepatitis B infection. *Gut*. 2023;72(7):1385-1398.
63. Sulkowski MS, Agarwal K, Ma X, et al. Safety and efficacy of vebicorvir administered with entecavir in treatment-naïve patients with chronic hepatitis B virus infection. *J Hepatol*. 2022;77(5):1265-1275.
64. Liu K, Du S, Deng W, Qu Z, Hu X. Advances in the computational development of hepatitis B virus capsid assembly modulators. *Drug Discov Today*. 2025;30(10):104458.
65. Assembly Bio. Assembly Biosciences reports positive topline results from phase 1b clinical trial of next-generation investigational capsid assembly modulator ABI-4334 in chronic hepatitis B. https://www.assemblybio.com/news/assembly-biosciences-reports-positive-topline-results-from-phase-1b-clinical-trial-of-next-generation-investigational-capsid-assembly-modulator-abi-4334-in-chronic-hepatitis-b/. Published June 25, 2025. Accessed May 2026.
66. Yuen MF, Gane E, Agarwal K, et al. Sustained reduction of HBV antigen levels during the 8-week follow-up period in treatment-naïve (TN) or currently-not-treated (CNT) HBeAg-positive subjects with chronic hepatitis B virus infection after 96-week 300 mg ALG-000184, with or without entecavir (ETV) sustained reduction of HBV antigen levels during the 8-week follow-up period in treatment naïve (TN) or currently-not-treated (CNT) HBeAg-positive subjects with chronic hepatitis B virus infection after 96-week 300 mg ALG-000184, with or without entecavir (ETV). Presented at: The Liver Meeting; November 7-11, 2025; Washington, DC. Poster 1208.
67. ClinicalTrials.gov. A study evaluating the efficacy and safety of ALG-000184 compared with tenofovir disoproxil fumarate in untreated HBeAg-positive and HBeAg-negative adult subjects with chronic hepatitis B (B-SUPREME). https://clinicaltrials.gov/study/NCT06963710. Identifier: NCT06963710. Accessed May 2026.
68. Vaillant A. Nucleic acid polymers: broad spectrum antiviral activity, antiviral mechanisms and optimization for the treatment of hepatitis B and hepatitis D infection. *Antiviral Res*. 2016;133:32-40.
69. Bazinet M, Pântea V, Placinta G, et al. Safety and 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*. 2020;158(8):2180-2194.
70. Hui RW, Mak LY, Fung J, Seto WK, Yuen MF. Prospect of emerging treatments for hepatitis B virus functional cure. *Clin Mol Hepatol*. 2025;31(suppl):S165-S181.
71. Yuen MF, Balabanska R, Cottreel E, et al. TLR7 agonist RO7020531 versus placebo in healthy volunteers and patients with chronic hepatitis B virus infection: a randomised, observer-blind, placebo-controlled, phase 1 trial. *Lancet Infect Dis*. 2023;23(4):496-507.
72. Janssen HLA, Brunetto MR, Kim YJ, et al. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. *J Hepatol*. 2018;68(3):431-440.
73. Gane EJ, Dunbar PR, Brooks AE, et al. Safety and efficacy of the oral TLR8 agonist selgantolimod in individuals with chronic hepatitis B under viral suppression. *J Hepatol*. 2023;78(3):513-523.
74. Lee HW, Park JY, Hong T, Park MS, Ahn SH. Efficacy of lenvervimab, a recombinant human immunoglobulin, in treatment of chronic hepatitis B virus infection. *Clin Gastroenterol Hepatol*. 2020;18(13):3043-3045.e1.
75. Wang X, Chi X, Zhang Y, et al. LBP-24 - Safety and efficacy of anti-pre-S1 domain monoclonal antibody (HH-003) treatment in patients with co-infection of chronic hepatitis B virus (HBV) and hepatitis D virus (HDV): a single center, open-label, phase 2 trial. *J Hepatol*. 2023;78(suppl 1):S117.
76. Hu Y, Huang Y, Li G, et al. A first-in-patient phase Ib study of a hepatitis B virus (HBV) neutralizing antibody HH-003 in treatment-naïve participants with HBeAg-positive chronic HBV infection. Presented at: The Liver Meeting; November 4-8, 2022; Washington, DC. Poster 5063.
77. Lempp FA, Volz T, Cameroni E, et al. Potent broadly neutralizing antibody VIR-3434 controls hepatitis B and D virus infection and reduces HBsAg in humanized mice. *J Hepatol*. 2023;79(5):1129-1138.
78. Asselah T, Chattergoon MA, Jucov A, et al; SOLSTICE Trial Investigators. A phase 2 trial of tobevibart plus elebsiran in hepatitis D. *N Engl J Med*. 2026;394(4):343-353.
79. Agarwal K, Jucov A, Dobryanska M. Brelovitug (BJT-778) monotherapy achieved 100% virologic response in patients with chronic hepatitis D: on treatment week 48 phase 2 study results. Presented at: The Liver Meeting; November 7-11, 2025; Washington, DC. Abstract 0009.
80. ClinicalTrials.gov. A trial evaluating brelovitug vs delayed treatment for the treatment of chronic hepatitis delta infection (AZURE-4). https://clinicaltrials.gov/study/NCT07298330. Identifier: NCT07298330. Accessed May 2026.
81. Cargill T, Barnes E. Therapeutic vaccination for treatment of chronic hepatitis B. *Clin Exp Immunol*. 2021;205(2):106-118.
82. Vandepapelière P, Lau GK, Leroux-Roels G, et al; Therapeutic HBV Vaccine Group of Investigators. Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. *Vaccine*. 2007;25(51):8585-8597.
83. Ma H, Lim TH, Leerapun A, et al. Therapeutic vaccine BRII-179 restores HBV-specific immune responses in patients with chronic HBV in a phase Ib/IIa study. *JHEP Rep Innov Hepatol*. 2021;3(6):100361.
84. Wong GLH, Yuen MF, Lin B, et al. Elebsiran and PEG-IFNα for chronic hepatitis B infection: a partially randomized, open-label, phase 2 trial. *Nat Med*. 2026;32(1):151-159.
85. Tak WY, Chuang WL, Chen CY, et al. Phase Ib/IIa randomized study of heterologous ChAdOx1-HBV/MVA-HBV therapeutic vaccination (VTP-300) as monotherapy and combined with low-dose nivolumab in virally-suppressed patients with CHB. *J Hepatol*. 2024;81(6):949-959.
86. Wong G, Lim T, Currie S. HBsAg declines observed with VRON-0200 alone are rapidly enhanced with the addition of combination antiviral therapies: results from a phase 1b study for functional cure in chronically HBV-infected patients. Presented at: The Liver Meeting; November 7-11, 2025; Washington, DC. Abstract OP0196.
87. Gane EJ, Chen C-Y, Hu T-H, et al. Safety, tolerability, immunogenicity, and antiviral efficacy of GS2829 and GS6779, a novel arenaviral-vectored, therapeutic hepatitis B vaccine: results from a phase 1b study in virally suppressed patients with chronic hepatitis B. Presented at: The Liver Meeting; November 7-11, 2025; Washington, DC. Abstract 0197.
88. Peng G, Li S, Wu W, Tan X, Chen Y, Chen Z. PD-1 upregulation is associated with HBV-specific T cell dysfunction in chronic hepatitis B patients. *Mol Immunol*. 2008;45(4):963-970.
89. Qian J, Xie Y, Mao Q, et al. A randomized phase 2b study of subcutaneous PD-L1 antibody ASC22 in virally suppressed patients with chronic hepatitis B who are HBeAg-negative. *Hepatology*. 2025;81(4):1328-1342.
90. Brahmer JR, Lacchetti C, Schneider BJ, et al; National Comprehensive Cancer Network. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*. 2018;36(17):1714-1768.
91. Yuen MF, Asselah T, Jacobson IM, et al; REEF-1 Study Group. Efficacy and safety of the siRNA JNJ-73763989 and the capsid assembly modulator JNJ-56136379 (bersacapavir) with nucleos(t)ide analogues for the treatment of chronic hepatitis B virus infection (REEF-1): a multicentre, double-blind, active-controlled, randomised, phase 2b trial. *Lancet Gastroenterol Hepatol*. 2023;8(9):790-802.
92. Yuen MF, Lim YS, Yoon KT, et al. VIR-2218 (elebsiran) plus pegylated interferon-alfa-2a in participants with chronic hepatitis B virus infection: a phase 2 study. *Lancet Gastroenterol Hepatol*. 2024;9(12):1121-1132.
93. Yuen MF, Heo J, Nahass R, et al. Imdusiran (AB-729) administered every 8 weeks in combination with 24 weeks of pegylated interferon alfa-2a in virally suppressed, HBeAg-negative subjects with chronic HBV infection leads to HBsAg loss in some subjects at end of IFN treatment. Presented at: EASL Congress; June 5-8, 2024; Milan, Italy. Poster WED-371.