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Targeting HBV with RNA Interference: Paths to Cure

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Abstract

Chronic hepatitis B virus (HBV) infection affects millions worldwide despite the availability of effective vaccines. The stability of HBV's genomic minichromosome (cccDNA) within hepatocytes, the persistence of integrated viral sequences capable of producing viral antigens, and the virus's ability to evade immune control all contribute to the difficulty in achieving a functional cure. Existing antiviral treatments have minimal impact on HBV transcription, allowing persistent viral antigen production and immune dysfunction. Emerging RNA interference (RNAi) therapies targeting HBV RNAs reduce viral replication, antigen expression, and, in turn, cccDNA activity, providing a potential path to functional cure.

One sentence summary:

RNA-targeting therapies hold promise for contributing to HBV cure regimens, but challenges remain before that promise can be realized.

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Introduction

Hepatitis B virus (HBV) infection remains a global health challenge, affecting approximately 256 million people worldwide and causing nearly 1.1 million deaths annually (1–3). Chronic infection can lead to progressive liver damage, cirrhosis, and hepatocellular carcinoma (HCC). HBV is primarily transmitted through blood, sexual contact, or perinatally (mother to child), the latter being the most frequent route in high-prevalence regions.

HBV is a small (3.2 kb), enveloped DNA virus with a compact genomic organization (Fig. 1) and complex replication cycle (Fig. 2). HBV infects hepatocytes, the only cell type susceptible to infection. Cell entry involves initial low affinity binding to heparin sulfate (4), followed by high affinity binding to the hepatocyte-specific sodium (Na^+)-taurocholate co-transporting polypeptide (NTCP) receptor (5); the epidermal growth factor receptor (EGFR) also plays a role in viral internalization (6). This facilitates viral endocytosis and transport of the nucleocapsid to the cytoplasm. The nucleocapsid disassembles in the cytoplasm or at the nuclear pore, releasing the HBV genome as relaxed circular DNA (rcDNA) into the nucleus. The rcDNA is then repaired by the cellular DNA repair machinery, transforming it into supercoiled covalently closed circular DNA (cccDNA) (7). This episomal structure associates with histone and non-histone proteins and resembles a minichromosome (8).

The cccDNA minichromosome is the genomic reservoir for viral persistence and is the template for the production of all HBV RNAs transcripts needed for viral replication. Viral transcription is regulated in part by the viral X protein (HBx), which is recruited on the cccDNA (9). HBx is essential for cccDNA-driven viral transcription (10) and HBx promotes degradation of the structural maintenance of chromosomes 5/6 complex (SMC5/6), a host restriction factor that otherwise hinders cccDNA transcriptional activity by associating with the cccDNA (11, 12). Proteasomal degradation of SMC5/6, mediated by HBx, is critical for sustaining cccDNA transcriptional activity (10, 11).

HBV reverse transcription occurs in the cytoplasm within the viral capsid, converting the pregenomic RNA (pgRNA) into rcDNA. The pgRNA is central to the replication cycle, acting as a template for both viral protein production and the synthesis of new rcDNA (13). The mature rcDNA-containing nucleocapsids acquire the viral envelope, including viral proteins called hepatitis B surface antigens (HBsAg), within multivesicular bodies, before being secreted as infectious virions (14).

Reverse transcription of pgRNA produces double-stranded linear DNA in about 10% of the time that can be integrated into the host genome through non-homologous end joining (15). Although integration of viral sequences is a defective byproduct of HBV replication, it occurs frequently in the course of infection. Integrated HBV DNA can template HBsAg mRNA production, contributing to serum HBsAg levels even in the absence of cccDNA. This has substantial implications for therapy, as the loss of HBsAg is a key marker of functional cure. Moreover, HBsAg produced by HBV integrants may contribute to the spread of hepatitis D virus (HDV), which causes a more severe form of viral hepatitis, as HDV depends on HBV envelope proteins for formation of its virion and subsequent

infection of new cells (15). Infected hepatocytes also secrete the HBV e antigen (HBeAg) (14) and produce high concentrations of genome-free spheres and filaments, known as subviral particles (SVPs) consisting primarily of HBsAg that typically outnumber virions by 1000- to 100,000-fold. These SVPs contribute to immune dysfunction and HBV persistence due to the high concentrations of circulating HBsAg in patients with chronic infection.

Current Landscape of Approved Therapies for Chronic Hepatitis B (CHB)

Chronic hepatitis B (CHB) is a heterogeneous disease caused by HBV infection that is classified into stages based on serum HBeAg, HBV DNA, and alanine aminotransferase (ALT) concentrations, a marker of liver damage. Treatment eligibility is generally determined by viral load and ALT values, as liver damage is the primary driver of disease progression (16). The standard treatment for CHB involves nucleos(t)ide analogues (NAs), which inhibit the reverse transcription of HBV pgRNA into rcDNA (Fig. 2A). Taken daily, NAs effectively suppress viral replication and reduce liver damage; however, they rarely (less than 1% per year) achieve a functional cure, defined as undetectable concentrations of circulating HBV DNA and HBsAg for over six months without treatment (17, 18). NAs leave viral antigen production and the cccDNA template within infected hepatocytes largely unaffected (19). This means that most patients require indefinite NA therapy, as cessation commonly results in viral rebound, which can be accompanied by life-threatening liver damage (20). Despite stable, long-term NA therapy, patients with controlled disease still face an increased risk of cirrhosis and liver cancer, unlike those who attain a functional cure (21, 22). Achieving functional cure at a young age might be particularly important for minimizing long-term hepatic complications (23).

Pegylated interferon-alpha (peg-IFN- α) is also approved for CHB treatment but is rarely used outside of clinical studies due to its side effects and limited efficacy as monotherapy. Peg-IFN- α is administered weekly by subcutaneous injection over a 48-week period. When combined with NA therapy, IFN- α can lead to approximately 10% cure rates (24). Moreover, recent studies have demonstrated that peg-IFN- α can achieve up to 30% HBsAg loss after 48 weeks in patients with low (<1,000 IU/ml) baseline HBsAg concentrations (25, 26). However, whether therapeutic strategies to reduce HBsAg to less than 1,000 IU/ml may increase the efficacy of peg-IFN- α remains a question to be answered. The main mechanism by which IFN- α contributes to HBV functional cure remains uncertain; it can act directly on infected hepatocytes as an antiviral (27, 28) and it can exert immunomodulatory effects (29–31). As a cytokine, IFN- α also is the only currently approved immunomodulatory agent for CHB. Recently, it has been increasingly combined with novel direct-acting antivirals (DAAs) in clinical trials, including RNA interference (RNAi) therapies.

Emerging Therapeutics for CHB

HBV's complex replication cycle and variable clinical presentations suggest that achieving a functional cure will likely require combination therapies that target both the virus at different stages of its life cycle and cellular factors (32, 33). Leading strategies include entry inhibitors, capsid, assembly modulators (CAMs), monoclonal antibodies (mAbs), nucleic

acid polymers (NAPs), immunotherapies, genome and epigenetic modifiers, and therapeutic vaccines.

Entry Inhibitors

Novel entry inhibitors are under development to block HBV from infecting new hepatocytes. These include peptides that mimic the HBV envelope protein, which efficiently block the NTCP receptor (such as bulevirtide (34) – the only approved treatment in EU for people living with HDV), monoclonal antibodies (mAbs) targeting HBsAg (see below), and small molecules that inhibit HBV-NTCP binding (Fig. 2B). Entry inhibitors can block new HBV infection events, which occur even in patients treated with NAs (35). Moreover, such agents can be particularly effective during hepatic flares, where they can prevent reinfection of hepatocytes that may have lost the cccDNA during cell division (36) as hepatocytes regenerate following immune-mediated cell killing. However, because these inhibitors do not affect existing cccDNA in infected hepatocytes, their impact on persistent HBV reservoirs is limited and the long-term effect of agents blocking intrahepatic spreading in patients with CHB needs further study.

CAMs

CAMs are small molecules that interfere with formation of the HBV nucleocapsids (Fig. 2C), thereby reducing the production of new virions (37). Several classes of CAMs have been developed which accelerate capsid assembly and yield empty capsids with either normal morphology (CAM-E) or aberrant morphology (CAM-A). Highly potent CAMs may also inhibit capsid disassembly, thus preventing the formation of new cccDNA molecules (38). However, since CAMs do not target cccDNA directly, viral rebound tends to occur upon discontinuation of therapy, much like with NAs.

mAbs against HBsAg

Chronic exposure to high concentrations of HBV antigens (up to 1 mg/ml), especially HBsAg, drives immune dysfunction in CHB (39, 40). Anti-HBs mAbs were designed to eliminate HBsAg from the bloodstream and have demonstrated rapid antigen reduction in patients while preventing new infections in preclinical studies (41) (Fig. 2D). mAbs, modified to enhance Fc-mediated effector functions, may offer greater therapeutic efficacy by combining antigen clearance with immune system engagement (42). While promising, it remains unclear if HBsAg clearance alone is sufficient to restore immune function in patients with CHB (43). Additionally, the impact of immune complexes formed by anti-HBs mAbs and HBsAg on immune modulation is not well understood, and further studies are needed to determine whether these complexes contribute to immune restoration or influence disease progression.

NAPs

NAPs have been shown to inhibit the assembly and secretion of HBV subviral particles (Fig. 2E) (44). Although the results are promising, challenges remain in evaluating the role of NAPs in curing CHB due to the association of HBsAg decline with ALT elevations and uncertainties about the drug's precise mode of action. However, recent analysis from the

REP-401 study noted three different types of HBsAg decline kinetics that were associated with non-response, partial response, and functional cure, suggesting that the rate of HBsAg decline on therapy may be indicative of outcome (45). These observations may provide insight into the distribution of HBsAg coming from cccDNA versus integrated HBV DNA.

Immunotherapy and immune activation

The immune system plays a crucial role in clearing HBV-infected hepatocytes and suppressing HBV replication and cccDNA transcription through both cytolytic and non-cytolytic, IFN-mediated mechanisms (46–50). Accordingly, several immunotherapies have been explored to boost HBV-specific immunity. Preclinical models suggest that activation of pattern recognition receptors, such as Toll-like receptors (TLR7 and TLR8) and retinoic acid-inducible gene I (RIG-I), can suppress HBV replication by inducing expression of IFNs and other immune mediators, such as interleukin (IL)-12 and IL-18 (51–53). Although these agonists have shown induction of biomarkers consistent with their mode of action, they have yet to demonstrate substantial efficacy in reducing HBV replication in patients (54, 55). Other immunotherapeutic strategies that have demonstrated T cell reinvigoration in preclinical models include IL-2, 4–1BB agonists, and IL-27 (56–59); however, their efficacy and clinical utility in chronically infected patients remain to be established.

Genome and epigenetic modifiers

Strategies targeting the cccDNA minichromosome through mechanisms such as inducing lethal mutations, degradation, or functional silencing, may be crucial for curing CHB. Gene-editing technologies like nucleases (Zinc-finger nucleases, TALENs, and CRISPR-Cas9) have demonstrated the ability to generate double-strand breaks (DSBs), disrupting both cccDNA and integrated HBV DNA. However, outcomes vary, ranging from cccDNA degradation to repair and the formation of cccDNA variants. Challenges such as drug delivery, target specificity, risks of inducing chromosomal translocations through recombination between multiple HBV DNA integrants, and pre-existing immunity to Cas9 complicate their application, making human outcomes difficult to predict. Alternatively, base-editing approaches rewrite DNA without inducing cleavage, reducing the risk of host genome rearrangements, and have shown promising preclinical results (60). Recent advancements also focus on using epigenetic modifiers to irreversibly silence cccDNA and integrated HBV DNA (Fig. 2F). Epigenetic modifiers, such as TUNE-401 and CRMA-1001, delivered by lipid nanoparticles, have achieved potent and durable viral suppression in vitro and in vivo. These promising results have paved the way for TUNE-401 to advance toward clinical trials (61).

Therapeutic Vaccines

Historically, therapeutic vaccines have shown limited efficacy in reducing viral load in CHB as monotherapies. Initial vaccine strategies, including recombinant antigens, plasmid DNA, and peptides, were largely unsuccessful (62). More recent prime-boost strategies using highly immunogenic vectors, such as chimpanzee adenovirus or modified vaccinia Ankara (MVA), are promising but have limited efficacy in patients where HBsAg concentrations exceed 200 IU/mL (~1,000 ng/mL) (63–65). However, emerging data indicate that therapeutic vaccines may be effective in patients with lower HBsAg concentrations (<200

IU/mL), suggesting that initial antigen reduction may be able to enhance immunotherapeutic responses (65).

RNAi strategies for targeting HBV

The unique organization of the HBV genome makes RNA-targeting therapies an attractive approach for targeting chronic HBV infection. Host RNA polymerase II transcribes four HBV mRNAs with staggered 5' ends and a common 3' end from the cccDNA (Fig. 1). These mRNAs lack homology with human RNA sequences, allowing for specific therapeutic targeting. The 3' approximately 570 nt are shared among all HBV mRNAs enabling a single RNA-targeting agent to disrupt all cccDNA-derived transcripts (Fig. 2G) to reduce both viral replication and antigen production. This is a clear advantage over NAs, which cannot directly suppress viral protein production (66–69). Furthermore, HBV transcripts produced from integrated HBV DNA that have truncated HBV sequences, and terminate at a cryptic viral polyA signal or downstream of the integrant in the human genome (Fig. 1), can be suppressed by targeting an upstream sequence common to all HBV transcripts (15). Three primary RNA-targeting strategies are being developed to address CHB: small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), and polyadenylation inhibition, each with varying effects on circulating HBsAg concentrations (70).

siRNAs are 21 to 23 base-pair, double-stranded RNA molecules typically modified and conjugated to improve stability and cellular uptake (71). The guide strand of siRNA binds complementary mRNA sequences and directs the RNA-induced silencing complex (RISC) to degrade the target. This sequence-specific interaction is highly effective, but can be compromised by sequence variations in HBV strains.

ASOs are single-stranded DNA molecules, longer than siRNAs and often chemically modified or conjugated to increase stability and cellular uptake (72). ASOs bind their target RNAs to form DNA-RNA hybrids, triggering the following responses: (i) mRNA cleavage by RNase H1; (ii) interference with mRNA processing (splicing, 5' capping, or polyadenylation) through steric hindrance; or (iii) translation inhibition by blocking the mRNA 5' end. Beyond their RNA regulation roles, ASOs can also stimulate immune responses through pattern recognition receptor activation (73), broadening their therapeutic potential by combining antiviral activity with immunomodulation.

An additional RNA-targeting approach involves inhibiting the PABP5/7 poly-A polymerase complex, which typically binds HBV mRNA post-transcriptional regulatory elements (PRE) to synthesize poly-A tails, protecting the mRNA from degradation (74, 75). By shortening these poly-A tails, PABP5/7 inhibitors reduce mRNA stability, thereby lowering steady-state abundance of HBV transcripts. PABP5 provides primary activity, whereas PABP7 offers supplemental function, together creating a promising target for CHB therapy. PABP5/7 also participate in maturation of an array of human non-coding RNAs, including rRNA, splicing factors, snoRNA, and some microRNAs (76–78). Consequently, eliminating their function is likely to cause toxicity, but the degree of toxicity associated with the partial suppression likely to be achieved pharmacologically is not fully known.

To optimize delivery and effectiveness, RNAi compounds are chemically modified to increase their half-life, enabling lower dosing frequencies. Different delivery systems have been explored, which are based on distinct liver-targeting methods like lipid nanoparticles (LNP) (e.g., ARB-1467) and GalNAc (N-acetylgalactosamine) conjugation (e.g., RG-6346; AB729; VIR-2218; JNJ-3989) (79). GalNAc derivatives enhance binding to asialoglycoprotein receptors on hepatocytes, facilitating delivery of siRNAs to hepatocytes. Unconjugated ASOs are predominantly taken up by liver non-parenchymal cells, whereas GalNAc-conjugated ASOs concentrate in hepatocytes (80–82). GalNAc-conjugated ASOs, such as Roche's RO7062931 and GSK3389404 (83) have shown moderate HBsAg reductions in patients with CHB. The most advanced candidates to date, Bepirovirsen (GSK3228836) and AHB-137, are subcutaneously administered, unconjugated 20-mer ASOs with demonstrated efficacy in clinical trials (84, 85).

Efficacy and durability of RNAi therapies for CHB

Multiple phase 2 clinical trials have demonstrated consistent target engagement and effective mechanism of action both for siRNAs and ASOs. RNAi therapeutics generally induce a steady decline in HBsAg concentrations when administered as subcutaneous injections, though HBsAg reduction typically plateaus around six months of therapy (86). Approximately 90% of treated CHB patients achieve HBsAg concentrations below 100 IU/mL, a threshold associated with improved immunotherapeutic responses in patients with naturally low HBsAg concentrations. In particular, the ASO Bepirovirsen achieves HBsAg decline within approximately three months and may elevate ALT concentrations, which correlates with efficacy. Around 10% of patients in Phase 2 studies achieved undetectable HBsAg concentrations with Bepirovirsen (85).

Despite their efficacy in suppressing HBsAg, RNAi therapies generally lack durability once treatment is discontinued (87). Following withdrawal, HBV antigens gradually rebound, with some patients who reached HBsAg negativity reverting to HBsAg positivity within six months. Thus, although RNAi therapies effectively reduce HBV antigen production, combination therapies are likely to be necessary to deepen HBsAg declines and prolong durability of HBsAg loss. Early Phase 2 studies suggest that combining HBV antigen reduction by siRNAs with peg-IFN- α could further enhance HBsAg suppression. For instance, the PIRANGA study ([NCT04225715](#)) demonstrated that combining the siRNA xalnesiran (RO7445482) with peg-IFN- α for 24 weeks led to HBsAg loss in 17% of patients at the 48-week follow-up (88). Similarly, combining the siRNA imdusiran (AB-729) with peg-IFN- α resulted in a 33% HBsAg loss rate at week 24 ([NCT04980482](#)). Another study using the siRNA elebsiran (VIR-2218) combined with peg-IFN- α ([NCT03672188](#)) reported 31% of patients achieving HBsAg seroclearance at the end of treatment (89).

These findings support the concept that combining antigen reduction with peg-IFN- α could enhance treatment durability and improve cure rates over RNAi monotherapy, particularly in patients whose HBV replication is already suppressed by NA therapy. However, considerable questions remain due to the heterogeneity of trial designs, small patient cohorts, and lack of IFN- α monotherapy control arms. Key questions include: What is the effect of antigen reduction alone on HBV-specific immunity? Does IFN- α contribute

an immunomodulatory effect, or does it provide an additive antiviral effect by repressing transcription from the HBV cccDNA (90, 91)? Could an RNAi-based antigen reduction lead-in enhance the effectiveness of combination therapies by augmenting subsequent immune responses?

Effects of RNAi on HBV replication and maintenance

Targeting the HBV pgRNA through RNAi inhibits the formation of new virus particles, effectively suppressing viral replication but leaving the cccDNA reservoir intact (Fig. 2). Degrading subgenomic HBV RNAs halts the production of all viral proteins, reducing the concentrations of intracellular viral protein and of circulating antigens and SVPs. This in turn, could contribute to reinvigorating immune responses. Although RNAi therapies do not target cccDNA directly, an indirect effect on cccDNA activity was demonstrated through the reduction of HBx protein (91), which is crucial for promoting and sustaining cccDNA-driven transcription (10).

HBx binds the damaged DNA-binding protein 1 (DDB1), an adaptor for the cullin 4A RING (CUL4) E3 ubiquitin ligase complex, initiating degradation of the SMC5/6 complex, a critical host restriction factor (Fig. 3) (11, 12). The SMC5/6 complex maintains chromosomal stability and spatial organization through its multiple functions in DNA repair, replication, and chromosome segregation (92, 93). SMC5/6 binds to supercoiled DNAs, including cccDNA, and restricts transcription within subnuclear structures called nuclear domains 10 (ND10) by incompletely understood mechanisms (94). HBx degrades SMC5/6 to overcome this restriction and promote viral transcription.

The reliance of HBV replication on the degradation of SMC5/6 is underscored by studies in primary hepatocytes and humanized mouse models, where HBV infection markers are inversely correlated with SMC6 expression. Importantly, siRNA therapies targeting HBV RNAs reduce HBx-positive hepatocytes and restore SMC6 expression in both hepatocyte cultures and animals (91, 95). SMC6 was only detected in hepatocytes lacking active HBV transcription, and chromatin immunoprecipitation assays confirmed SMC5/6 binding to cccDNA (91), suggesting that RNAi targeting HBx can enable SMC5/6-mediated transcriptional silencing of cccDNA. Recent preclinical in vivo studies revealed that combining siRNA with peg-IFN- α enhanced epigenetic repression of the cccDNA minichromosome and further reduced its accessibility (94). The restoration of SMC5/6 during siRNA therapy may help explain the slow rebound of HBV replication upon therapy withdrawal. In humanized mice, cccDNA silencing was maintained after siRNA withdrawal for up to eight weeks only when new infection events were blocked using the entry inhibitor Bulevirtide (91). Without re-infection blockade, viral rebound was observed, underscoring the benefit of preventing viral spread to sustain RNAi's antiviral effects.

(94)Assessing the mechanisms of RNAi-mediated effects on SMC5/6 function in patients could inform new therapies aimed at either promoting SMC5/6-mediated silencing of cccDNA or preventing HBx-mediated degradation of SMC5/6. Although there is experimental evidence supporting these processes, questions remain regarding SMC5/6's role in the natural progression of CHB and during treatment. Additionally, the stability of

silenced cccDNA in patients is still unknown. Given SMC5/6's role in maintaining genomic stability, its restoration in HBV-infected livers could independently help limit liver disease progression and HBV-induced tumorigenesis (96). (97)

RNAi-Mediated Antigen Reduction and Immunomodulatory Strategies in CHB

Unlike acute HBV infection, which is typically resolved in adults through coordinated immune responses, chronic HBV infection induces dysfunction in HBV-specific T and B cells (57, 58, 98–106).

RNAi has shown promise in lowering hepatocellular HBV antigen expression and presentation in patients (98, 107), raising hopes that antigen lowering might reverse immune dysfunction. However, in most cases, antigen reduction alone is insufficient to trigger robust immune reactivation. Preclinical models confirm that decreasing hepatocellular antigen presentation does not restore immune function (43, 108). Evidence from individuals cured of hepatitis C virus (HCV) infection – in whom viral antigen is entirely cleared – suggest that persistent immune dysfunction can be maintained despite antigen removal, owing to stable epigenetic imprinting (109–111). Clinical trials for RNAi-based treatments in CHB, such as JNJ-3989 (86, 112) ([NCT03365947](#), [NCT03982186](#)), elebsiran (VIR-2218; [NCT03672188](#)) (89), xalnesiran (RO7445482; [NCT03772249](#), [NCT04225715](#)) (113, 114), imdusiran (AB-729; [NCT04980482](#)) (115, 116), Bepirovirsen ([NCT04449029](#)) (85), and AHB-137 ([NCT06115993](#)) have demonstrated substantial declines in HBsAg concentrations. However, anti-HBs seroconversion has occurred in only a minority of patients, typically those with low baseline HBsAg concentrations (117, 118). Isolated cases have shown that RNAi, when combined with NAs but without additional immunomodulators, can lead to deep virologic responses and partial immune restoration, accompanied by minimal ALT flares—suggesting that, under stringent conditions, antigen reduction alone may contribute to improved immune function (115, 116, 119).

These findings highlight the need for combination strategies that integrate antigen reduction with immunotherapeutic interventions to fully restore HBV-specific immunity. For example, a recent study showed that reducing HBV antigens with siRNA therapy enhanced B and T cell responses and improved infection control when combined with therapeutic vaccination (120). These preclinical data suggest that siRNA typically does not completely eliminate viral antigens in all cells, which could limit T cell receptor (TCR) engagement, essential for optimal immune activation (121).

Combination therapies that pair RNAi-mediated antigen suppression with peg-IFN- α have shown potential to deepen HBsAg declines and increase seroconversion rates in clinical trials (24, 25, 30, 122–124). Both peg-IFN- α 's immunomodulatory effects and its ability to augment RNAi-induced antigen suppression in hepatocytes may be key to achieve sustained immune control. Peg-IFN- α 's effect underscores the potential of combination approaches to address HBV-specific immune dysfunction more effectively than RNAi alone. This combinatorial synergy is further supported by the combined antiviral and

immunomodulatory properties of Bepirovirsen, where the ASO alone is able to achieve HBsAg loss.

In contrast to RNAi, mAb-mediated antigen reduction targets circulating HBsAg without necessarily impacting antigen presentation within hepatocytes. Neutralizing mAbs, such as VIR-3434 (41, 125), GC1102 (126) and BJT-778 clear HBsAg from the bloodstream, which may indirectly influence immune responses through immune complex formation and uptake in antigen presenting cells (127). However, these immune complexes may have limited capacity to activate HBV-specific T cells (43) unless modified to engage professional antigen-presenting cells. Fc modifications or conjugation with TLR agonists could enhance antigen-presenting cell uptake and activation, potentially promoting T cell priming (127). Additionally, IFN- α enhances the capacity of B cells to produce antibodies, which could be a key mechanism for HBsAg loss in siRNA + IFN- α combination studies (29, 128).

In summary, although RNAi and other antigen-reduction strategies reduce persistent antigenemia, complete immune restoration will likely require not only potent antiviral agents but also complementary immunomodulatory approaches or therapeutic vaccination. Moreover, current immunocompetent preclinical models offer only a limited approximation of the complex inflammatory environment seen in CHB, which can complicate early evaluation of combination strategies. Future research should focus on integrating suppression of HBV replication and antigen reduction with immune-stimulating therapies to optimize immune recovery and establish long-term viral control.

Efficacy of RNAi against HDV Infection

About twelve million individuals chronically infected with HBV are also co-infected with HDV, an RNA satellite virus that exacerbates HBV-associated liver disease. HDV employs HBsAg as its surface glycoproteins on its virion, making it absolutely dependent on HBV for virion assembly and transmission. The HDV genome is a circular, single-stranded RNA (HDV-G) approximately 1.7 kb in size (129). It forms a ribonucleoprotein (RNP) complex with two antigens, small (S-HDAg) and large (L-HDAg), and assumes a “quasi” double-stranded rod-like structure with bulges and loops (130). Replication occurs within the nucleus and involves rolling-circle mechanisms facilitated by S-HDAg, which promotes HDV genomic replication, whereas L-HDAg is essential for viral assembly (129, 131, 132). As HDV propagation is heavily reliant on HBsAg production, RNAi therapies targeting HBV mRNA can potentially inhibit HDV by curtailing the availability of HBsAg for virion assembly and release. Being an RNA virus, HDV, unlike HBV, does not establish a stable nuclear template, indicating that finite treatment regimens could be effective for HDV, potentially allowing for a more manageable therapeutic goal.

Recent clinical data from the REEF-D trial ([NCT04535544](#)) demonstrated that JNJ-3989, a siRNA targeting HBV RNAs, led to substantial reductions in both HBsAg and HDV RNA concentrations in patients' blood. A subset of patients achieved the primary efficacy endpoint (≥ 2 log decline in HDV RNA and normal ALT at 48 weeks). However, ALT elevations led to treatment discontinuation in many patients, highlighting a differential response in HBV/HDV co-infected individuals that warrants further investigation to

understand the underlying mechanisms of liver inflammation in this context. The SOLSTICE trial ([NCT05461170](#)) also assessed the efficacy of an siRNA targeting all HBV RNA transcripts (elebsiran) alone or in combination with a mAb binding to HBsAg and on HDV virions and observed a reduction in HDV RNA without ALT flares, indicating that siRNA-based combination treatment approaches may yield better safety profiles in co-infection contexts than monotherapies.

Early in vitro studies demonstrated that siRNAs targeting HDV mRNA effectively reduced HDV RNA in cell models, although siRNAs were less effective against the highly structured HDV-G and HDV anti-genome (HDV-AG) RNA strands present in the cell nucleus (133). In contrast, ASOs, which act both in the cytoplasm and nucleus, may offer a broader range of action by targeting cytoplasmic and nuclear HDV RNAs, including linear multimers formed during rolling-circle amplification and the HDV mRNAs. Future studies could explore ASO treatments targeting both HBV and HDV to achieve comprehensive inhibition of viral replication in individuals co-infected with HBV and HDV. The high genetic variation observed among HDV genotypes and variation that emerges during the course of infection raise the possibility that sequence-specific RNAi compounds may need to be tailored to each HDV genotype and that they may have a low barrier to resistance evolution.

Finally, RNAi therapies targeting HBV RNAs can reduce HBsAg produced not only from the cccDNA, but also from integrated HBV DNA sequences (15), thereby inhibiting HDV virion release but not HDV RNA replication. Although further studies are needed to elucidate the ability of HDV to persist in cells that do not express HBV envelope proteins, such strategies may in principle allow clearance of HDV despite the persistence of chronic HBV infection. This partial success could still offer substantial clinical benefits by slowing liver disease progression in co-infected individuals. Therefore, RNAi-mediated HBsAg reduction, perhaps in combination with other therapies, may offer a promising path forward for effectively managing HBV/HDV co-infection and reducing the burden of this aggressive form of viral hepatitis.

Concluding Remarks

RNAi therapeutics represent a major advancement in the treatment of chronic HBV and HDV infections. Although current approved therapies focus on suppressing viral replication and alleviating liver damage, RNAi-based approaches expand the treatment strategies by addressing the production of viral antigens, which facilitates silencing of cccDNA and opens a window for reinvigorating the immune response. However, the limitations of RNAi alone in achieving durable HBV-specific immunity highlight the necessity for combination therapies that integrate immunomodulatory agents or therapeutic vaccines.

The evolving field of siRNA and ASOs continues to expand our understanding of viral persistence and immune evasion, informing strategies for functional cures for chronic HBV and HDV infections. Yet, due to the paucity of liver samples available, data showing an improvement at the histological level for patients receiving RNAi-based treatments remain scant, and the long-term benefit of such therapies await further assessment. Moving forward, basic science and animal model studies are needed to help resolve the uncertainties around

how the molecules work. Clinical trials are essential to determine optimal dosing, patient selection, and combination regimens that maximize therapeutic efficacy and durability. As RNAi therapies progress, they promise not only to improve patient outcomes but also to bring us closer to achieving a functional cure for chronic HBV and HDV infections.

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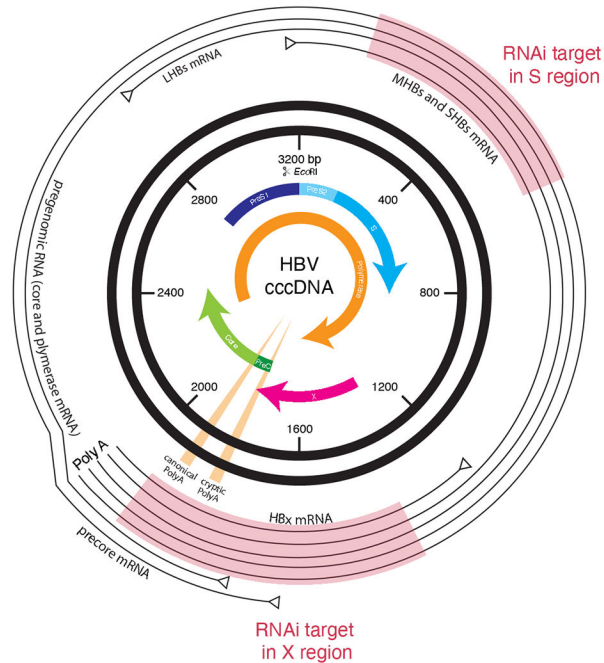
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A



B

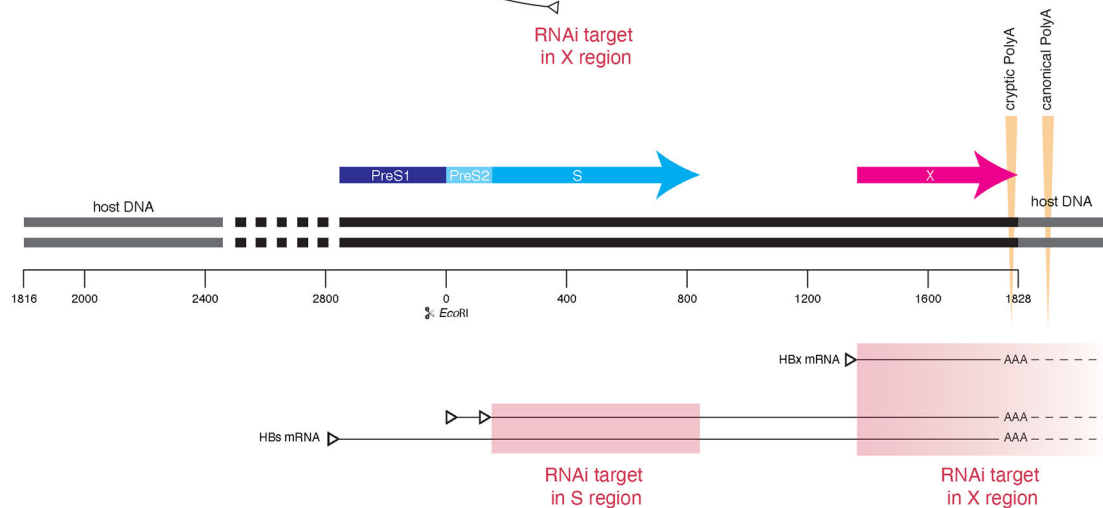


Fig. 1. The HBV Genome and Transcription Map.

(A) Shown is a circular map of the HBV genome as found in cccDNA. The black circles represent the viral DNA strands, and the colored arrows in the center denote the open reading frames (ORFs). The preC/C ORF has two in-frame initiation codons: the upstream codon encodes HBeAg, and the downstream codon encodes HBcAg. The preS1/preS2/S ORF contains three in-frame initiation codons, encoding L-HBsAg, M-HBsAg, and S-HBsAg from the 5', central, and 3' positions, respectively. The overlapping ORFs mean every nucleotide in the genome encodes for at least one protein, with about half encoding two proteins in different reading frames. The thin outer black lines represent the four classes of viral mRNAs, which share a common 3' polyadenylation site but start at different locations. Shaded regions indicate the two genomic targets of current RNAi therapeutics. As all four mRNAs share 3' sequences, RNAi targeting the HBx region can bind to all viral mRNA classes. **(B)** Transcription map of HBV DNA integrants in

the cellular genome. Integrants, typically derived from duplex linear failure products of reverse transcription, integrate randomly into the host genome. This integration separates the preC/C promoter from the preC/C and P ORFs but retains the intact preS1/preS2/S ORF and its two promoters, enabling continued production of HBsAg proteins. The HBx ORF is usually truncated at its 3' end but retains an intact promoter, allowing for the expression of C-terminally truncated HBxAg mRNAs. Integration results in the loss of the canonical HBV polyadenylation site, so mRNAs initiated within the integrant from the HBs or HBx promoters, or from upstream cellular promoters, are polyadenylated at cryptic sites in either HBV DNA (black) or cellular DNA (gray). These mRNAs contain 3' truncated HBV sequences, sometimes with additional human sequences. mRNAs initiated from upstream cellular promoters also contain human sequences at their 5' ends. The RNAi target regions, shown as red shaded areas, highlight how RNAi therapeutics can target transcripts originating from either cccDNA or integrated HBV sequences.

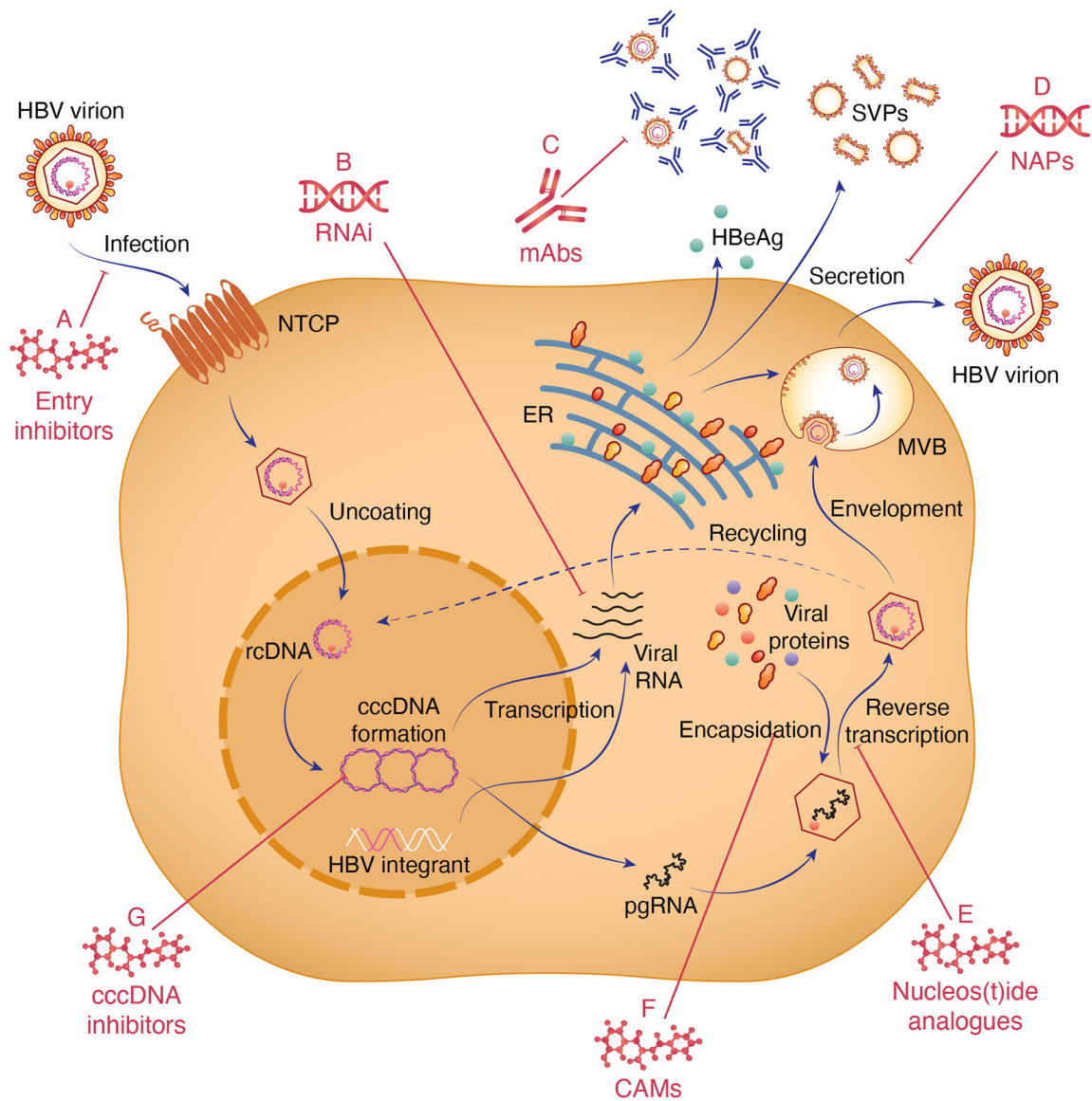


Fig. 2. Direct-acting antiviral drugs disrupt the HBV replication cycle.

(A) Entry Inhibitors, including peptides, antibodies, and small molecules, block new hepatocyte infection by preventing the interaction of large HBsAg with its receptor, NTCP, outside the cell. (B) RNAi therapies target the 3' end of HBV transcripts, promoting their degradation and halting HBV protein production in hepatocytes. (C) Anti-HBs mAbs bind circulating virions and subviral particles, forming immune complexes that clear antigens from the bloodstream. (D) NAPs specifically inhibit the secretion of HBV subviral particles from the Golgi complex, reducing circulating viral antigens. (E) The standard of care, nucleoside analogues, target reverse transcription just upstream of mature virion secretion. (F) Originally designed to block nucleocapsid assembly, which is essential for reverse transcription, CAMs may also block uncoating of virions during infection. (G) Strategies using CRISPR-based technologies directly target cccDNA to silence HBV transcription, turning off viral gene expression at the source. MVB: multivesicular body.

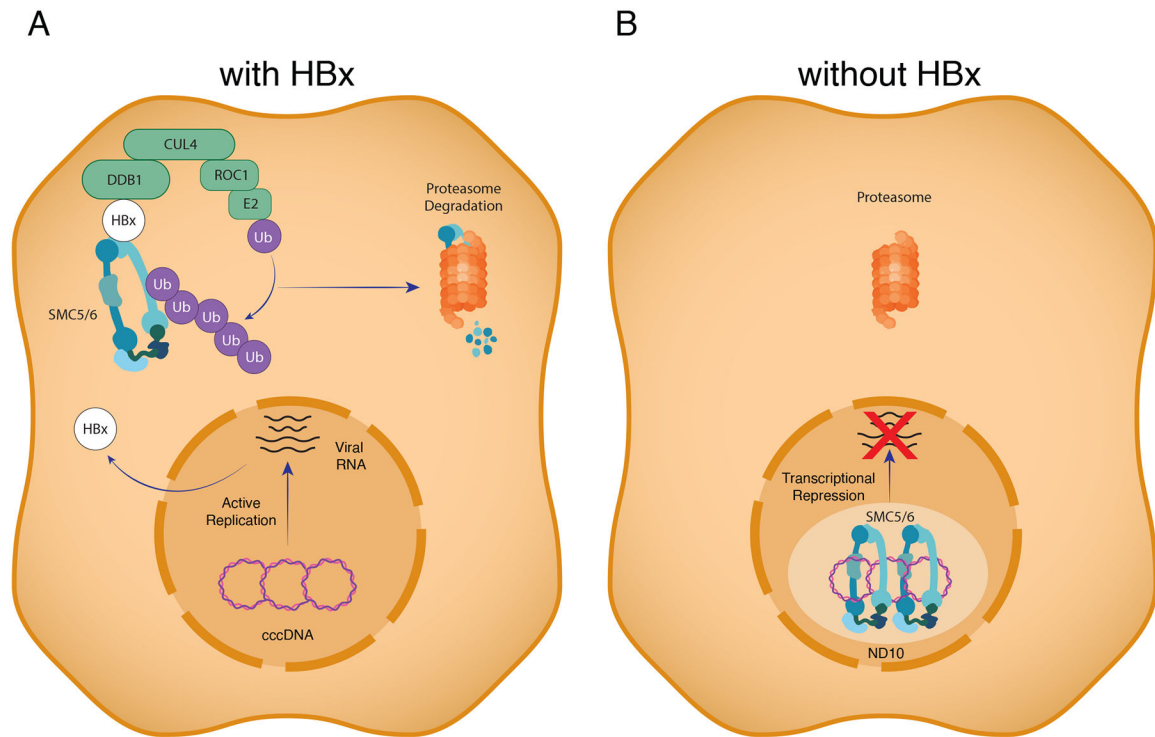


Fig. 3. SMC5/6 represses cccDNA transcription in the absence of HBx.

(A) All viral RNAs, including the short HBx mRNA that encodes the small regulatory HBx protein, are transcribed from the HBV cccDNA minichromosome. HBx interacts with DDB1, an adaptor protein that forms part of an E3 ubiquitin ligase complex with Cullin4 (Cul4). By recruiting the DDB1-E3 ligase complex to the host SMC5/6 complex, HBx facilitates the ubiquitination (Ub) and degradation of SMC5/6. (B) In the absence of HBx, including through RNAi, the SMC5/6 complex is no longer degraded. Instead, it associates with cccDNA, promoting its topological entrapment and inducing transcriptional silencing of viral genes. ND10: Nuclear Domain 10.