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Review of Expected Drug Targets and Targeted Drug Treatments for Hepatitis B

Zixi Li

Department of Pharmacy, National University of Singapore, Kent Ridge, Singapore
lizixi1224@gmail.com

Abstract: Chronic hepatitis B virus infection, which affecting over 300 million people globally, is strongly linked to hepatocellular carcinoma (HCC). HBV, partially double-stranded smal DNA virus, targets hepatocytes and will progress to liver cirrhosis and HCC. Although significant advancements have been made in antiviral therapy, a definitive cure remains elusive, primarily because of the persistence of covalently closed circular DNA (cccDNA). Significantly burdening patients and healthcare systems due to monitoring needs, drug resistance risks, and side effects. Current therapeutic options, including interferon-α and NRTIs, provide only partial viral suppression and require lifelong treatment. Emerging therapeutic strategies, such as entry inhibitors, polymerase inhibitors, capsid assembly modulators, and gene-editing tools, are being engineered to address various phases of the HBV life cycle. Notably, CRISPR/Cas9 has shown promising potential for cccDNA eradication in preclinical studies. Aiming for the "functional cure," combination therapies involving these novel agents are defined by the sustained loss of hepatitis B surface antigen (HBsAg) after treatment cessation. The review provides a comprehensive overview of the HBV life cycle, current information and emerging therapeutic strategies, and the potential of combination therapies to enhance treatment efficacy. However, achieving complete viral eradication remains a significant challenge, underscoring the need for continued research into novel therapeutic approaches.

Keywords: Hepatitis B, HBV, cccDNA, Myrcludex B

1. Introduction

HCC stands as the third most common cause of cancer-related deaths globally. Resulting in an estimated 555,000 fatalities annually. Hepatitis B, caused by HBV, is a significant contributor to liver cancer, with HBV responsible for about 23% of cirrhosis cases and 40% of HCC cases[1]. HBV, a small DNA virus, possesses a genome of approximately 3,200 base pairs and is characterized by its partially double-stranded structure. It predominantly targets human hepatocytes. The HBV genome is organized as a relaxed circular DNA with partial double-strandedness and is complexed with a multifunctional viral polymerase enzyme, which consists of four distinct domains.

In adults, HBV infection triggers an immune response that effectively manages viral replication and reduces the overall viral load by eliminating HBV DNA and HBsAg from the circulation. Nonetheless, cccDNA, a remnant of previous infection[2], may persist in some hepatocytes. In certain instances, this cccDNA can replicate, leading to a recurrent hepatitis B infection. The ongoing presence of cccDNA necessitates prolonged or even lifelong treatment, imposing substantial burdens

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on both patients and healthcare systems due to continuous monitoring, the risk of drug resistance, and cumulative side effects. Addressing this therapeutic gap is essential to improve patient outcomes and reduce the strain on healthcare resources.

Current FDA-approved therapies for hepatocellular carcinoma comprise interferon (IFN)-α and NRTIs, including oral tenofovir and entecavir. Nonetheless, these treatments are not curative, may necessitate lifelong administration, or can lead to adverse effects. Furthermore, they seldom lead to the loss of HBsAg, necessitating ongoing treatment to prevent viral resurgence.

The exploration of combination therapies, which include various antivirals and/or immunomodulators, aims to enhance therapeutic efficacy. The 'functional cure,'[3] characterized by the sustained absence of detectable serum HBsAg, is the primary goal of these treatments. Despite the cessation of drug therapy, HBsAg levels might still persist. Achieving a complete virus eradication, which necessitates the clearance of the cccDNA reservoir within infected hepatocytes, is the ultimate goal for HBV therapy[1].

2. HBV life cycle

Once inside, HBV is transported to plasma membrane compartments. The virus is then translocated within the cytoplasm via an endosomal network. Following this, Following the disassembly of the inner nucleocapsid through the nuclear pore complex, then HBV DNA is transported into the nucleus[4].

In the nucleus, HBV DNA is transformed into cccDNA, acting as a blueprint for the production of various RNA transcripts, such as pregenomic RNA (pgRNA) and mRNA for surface proteins. Following their synthesis, these RNA molecules are transported to the cytoplasm. There, they form complexes with the nuclear pgRNA and the viral polymerase. Within the capsid, the pgRNA is subsequently reverse-transcribed into relaxed circular DNA (rcDNA). The mRNAs are translated into proteins, including HBx proteins. Nucleocapsids formed by HBV can associate with the endoplasmic reticulum to produce HBV virions. The virions are released into the sinusoidal space, while excess surface proteins are assembled into non-infectious subviral particles (SVPs).

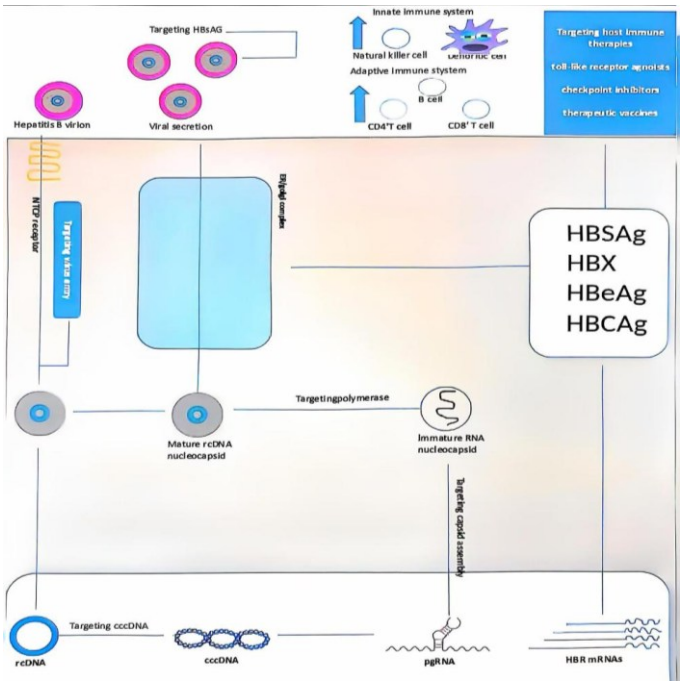

Figure 1: Key stages of the HBV life cycle offer multiple opportunities for targeted drug intervention. NTCP; rcDNA; cccDNA; CRISPR; Endoplasmic reticulum (ER)

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3. Therapies targeting the HBV virus life cycle

Table 1: Therapeutic strategies against HBV

Therapy	Stage	Mechanism
Targeting HBV Entry	Entry into Hepatocytes	Block HBV receptor interactions, preventing viral entry into liver cells.
Targeting HBsAg Release	Release of HBsAg	NAPs reduce HBsAg levels, enhancing immune response against HBV.
Targeting HBV Translation	Translation of Viral Proteins	RNA interference and antisense oligonucleotides degrade HBV RNA, inhibiting protein synthesis and replication.
Targeting HBV cccDNA	cccDNA Reservoir	Targets and disrupts cccDNA in hepatocytes, aiming to eliminate viral reservoirs.
Targeting Capsid Assembly	Assembly of Viral Capsid	Capsid assembly inhibitors like ABI-H0731 prevent virion production by disrupting the formation of the viral capsid.

3.1. Targeting HBV entry

Prevention strategies for HBV infection encompass the utilization of Innovative peptides, such as Myrcludex B, engineered from HBsAg, represent promising therapeutic candidates[5]. This 47-amino-acid myristoylated lipopeptide irreversibly binds to the pre-S1 domain of the large HBsAg (L-HBsAg). The NTCP, a key cellular transporter, facilitates HBV enter the hepatocytes.

Clinical studies have shown that the combination of bulevirtide, a therapeutic agent similar to Myrcludex B, with peginterferon exhibits dual efficacy against HBV and hepatitis D virus (HDV) in patients with delta hepatitis. CRV431[6], also known as rencofilstat.Individually. Preclinical studies have shown that lankofilstat alleviates fibrotic development and tumor formation in chronic liver disease models.

Small molecule derivatives, such as cyclosporine and various antiviral inhibitors, have been investigated across diverse experimental viral models. Certain of these compounds are known to inhibit viral entry mechanisms that are independent of the NTCP receptor. Concurrently, research efforts in Germany are focused on the development of neutralizing antibodies intended to prevent HBV entry. It is postulated that these antibodies may not only hinder the virus-NTCP receptor interaction but also potentially bolster the immune system's response.

3.2. Targeting HBsAg release

HBV secretion inhibitors primarily function by enhancing immune system capabilities rather than by directly inhibiting the release of viral particles. Nucleic acid polymers (NAPs), recognized for their exhibits broad-spectrum antiviral efficacy against various enveloped viruses, notably including HBV, possess distinctive mechanisms that involve the inhibition of viral budding and the downregulation of HBsAg levels.

REP 2139 and REP 2165 effectively block the assembly and secretion of hepatitis B virus SVPs[7], REP 2165, a derivative of REP 2139, has demonstrated preclinical efficacy while showing reduced liver accumulation. REP 2139 shown the best results for achieving an HBV functional cure.

Table 2: HbsAG and NTCP targeting drugs

Drug	Sponsor	Clinical stage	NCT	Mechanism
REP 2139 and REP 2165	Replicor Inc.	II	NCT02565719	HbsAG inhibitor
AB-729	Assembly Biosciences	II	NCT04820686	HbsAG inhibitor
ALG-010133	Aligos Therapeutics	I	NCT04485663	L-HbsAG inhibitor
Myrcludex B(bulevirtide)	Hepatera Ltd.	II	NCT02881008 NCT02888106	NTCP inhibitor

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3.3. Targeting HBV polymerase

Nucleos(t)ide analogues (NAs) are antiviral agents that target viral polymerase to inhibit retrotranscription and diminish the production and synthesis of viral DNA from pgRNA.

Besifovir was developed in South Korea and has demonstrated strong antiviral activity against HBV[8]. Tenofovir alafenamide (HS-10234)[9], a prodrug of tenofovir, is an orally active antiviral agent. Another prodrug, pradefovir, which combines tenofovir and adefovir, has also demonstrated comparable effectiveness to TDF observed in a Phase 3 clinical trial[10]. Additionally, a new lipid-conjugated formulation of tenofovir, known as Tenofovir exalidex, has completed Phase I clinical trials. This formulation is designed to enhance hepatic targeting.

3.4. Targeting HBV translator

Small interfering RNA (siRNA) molecules, a form of RNA interference (RNAi), are engineered to specifically target and degrade viral RNA transcripts through precise sequence recognition. siRNA prompts the destruction of these transcripts, thereby potentially inhibiting the production of viral antigens.

Nonetheless, there are significant concerns associated with drugs that target viral transcripts, such as potential toxicity arising from its delivery system, the possibility of off-target effects, along with the potential for viral resurgence because of inadequate covalent reduction of cccDNA. ARC-520[11] the inaugural RNAi therapeutic agent evaluated in a clinical trial for HBV, demonstrated an inability to eliminate HBsAg. However, it successfully diminished HBsAg levels to a point where the immune system could exert more effective control.

Antisense oligonucleotides (ASOs) are single-stranded nucleic acids that are in conjunction with their target RNA. Binding of ASOs to their target promotes RNA degradation. Recent advancements have led to the development of liver-specific and more stable ASOs, which are effective in reducing viral replication and HBsAg levels. In clinical studies, the combination therapy of ARO-HBV with NAs has demonstrated a reduction of at least 1.0 log IU/mL in HBsAg levels observed in 98% of cases with chronic HBV infections[10]. Additionally, GSK404[12] a liver-targeting antisense oligonucleotide, has been shown to inhibit viral protein synthesis, including that of HBsAg, and has successfully concluded Phase II clinical trials.

Table 3: RNA-related targeting drugs

Drug	Sponsor	Clinical stage	NCT number	Mechanism
ARC-520	Arrowhead Pharmaceuticals	II	NCT02577029	RNAi
JNJ-3989 (ARO-HBV)	Arrowhead Pharmaceuticals	II	NCT03365947	siRNA
RG6346	Hoffmann-La Roche	II	NCT04225715	HBV-RNAi
VIR-2218 (BRII-835)	Vir Biotechology	II	NCT04507269	siRNA

3.5. Targeting HBV cccDNA

The CRISPR/Cas9 system, along with alternative genome-editing technologies, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), presents a promising therapeutic strategy for HBV infections. Its simplicity and efficacy make the CRISPR/Cas9 system the most appealing option at present. Promising preclinical studies have demonstrated that Cas9 can cleave over 90% of HBV DNA, highlighting its potential in the treatment of HBV. Properly spaced guide RNAs can induce double-strand breaks, minimizing off-target risks. New gene editing technologies targeting HBV cccDNA might provide a cure without killing infected hepatocytes.

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Despite these advances, Gene editing approaches aimed at HBV DNA typically result in the degradation of viral DNA, which may result in irreparable mutations or the creation of defective viral genomes that prevent replication. Recent studies on CRISPR/Cas9 editing showed a 30-40% functional cure rate in preclinical models, but with off-target mutation rates ranging from 3-10%[13]. By comparison, epigenetic silencing does not eradicate HBV DNA but renders the intact viral genome transcriptionally silent within infected hepatocytes. Thus, only direct targeting of HBV cccDNA holds potential for a complete cure. Due to the challenges associated with assessing hepatic HBV cccDNA along with various biomarkers have been explored as proxies for viral genome quantification in HBV carriers.

The compound GS-5801[14] has shown to inhibit HBV transcription originating from cccDNA . Nonetheless, in a humanized mouse model of HBV infection, GS-5801 failed to demonstrate antiviral efficacy. Moreover, alpha-glucosidase, an inhibitor of the nuclear transcription factor Sp1 [15], has emerged as a promising candidate.

3.6. Targeting capsid assembly

The HBV core protein (HBc) plays a crucial role in the packaging of the viral genome. The HBV capsid facilitates the transport of rcDNA into the nucleus, thereby facilitating the establishment and maintenance of cccDNA levels. Additionally, the capsid protein is found in the nuclei of hepatocytes, where it interacts with host factors that play a role in regulating transcription.

Notable capsid assembly modulators include Morphothiadin GLS4, AB-423[16] and AB-506, an orally bioavailable inhibitor of HBV replication that targets the viral core protein. AB-506 binds to the HBV core protein, promoting capsid assembly while simultaneously inhibiting HBV pgRNA encapsulation, making it a promising candidate for CHB research. AB-506 as an HBV replication inhibitor targeting the viral core protein. Other modulators include JNJ-440 promoting vasodilation and exhibiting antihypertensive effects in mice Bersacapavir and Vebicorvir[17].

Lexibulin, also known as SRI-32007/CYT-997, a tubulin polymerization inhibitor, has recently been identified acting as a suppressant of HBV core promoter activity. Preclinical evaluations have shown that SRI-32007 inhibits tubulin polymerization, with an IC_{50} of 3 μM as determined by turbidimetric assays. In rat models, the compound exhibited favorable oral bioavailability—ranging from 50% to 70%. Furthermore, it displayed efficacy in murine cancer models when administered at a dosage of 10 mg/kg three times daily (t.i.d.). SRI-32007 was well tolerated at doses up to 202 mg/m² in Phase I [18].

Ciclopirox, a synthetic antifungal agent, has demonstrated efficacy in inhibiting HBV replication across cellular and murine models, attributed to its interference with HBV capsid assembly, this compound has been characterized by its crystal structure in complex with the HBV core protein, which illustrates an innovative binding mode at the dimer-dimer interface. The oral administration of ciclopirox has the potential to disrupt HBV capsid assembly, presenting a new therapeutic approach for managing chronic HBV infection.

Table 4: HBV capsid targeting drugs

Drug	Sponsor	Clinical stage	NCT	Mechanism
EDP-514	Enanta, Pharmaceuticals	I	NCT04008004	HBV capsid inhibitor
QL-007	Qilu Pharmaceutical Co., Ltd.	II	NCT04157699	HBV capsid modulators
SRI-32007 (CYT997)	Gilead Sciences	II	NCT00664378 NCT00650949	HBV capsid
GLS4 (morphothiadine)	Sunshine Lake Pharma Co.,Ltd	II	NCT04147208	HBV Nucleocapsid protein assembly inhibitor

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Table 4: (continued)

NVR 3-778 (AL-3778)	Novira Therapeutics, Inc.	I	NCT02112799 NCT02401737	Capsid assembly regulator
AB-506	Arbutus Biopharma Corporation	I	NCT03718080	HBV capsid protein inhibitor
AB-836	Arbutus Biopharma Corporation	I	NCT04775797	HBV capsid inhibitor
JNJ-440 (JNJ-64530440)	Alios Biopharma Inc	I	NCT03439488	HBV capsid inhibitor
JNJ-56136379 (JNJ-379)	Janssen Research & Development, LLC	II	NCT03361956	HBV capsid inhibitor

4. HBV combination treatment

Considering the challenges associated with eradicating cccDNA and integrated HBV DNA, as well as the complexities of HBV replication, the combination of novel agents may yield synergistic effects, especially in enhancing therapeutic outcomes.

Not all drugs are suitable for combination therapy, and the effectiveness of combining drugs of the same class may vary. Despite the numerous combination options available, the optimal drug combinations remain unclear. A promising strategy involves the use of virus entry blockers combined with maturation inhibitors, such as oral capsid assembly inhibitors. Another potential synergistic combination includes NAs paired with CAMs. However, whether such combinations will restore HBV-specific immune responses leading to viral clearance or provide additional immune system enhancement remains uncertain. Clinical outcomes with combinations of NAs and Peg-INFα have varied depending on the type of NA. For instance, lamivudine combined with Peg-INFα was less effective than monotherapy. In contrast, a recent randomized trial demonstrated that the combination of Peg-INFα, sequential vaccination, and entecavir (ETV) resulted in a higher rate of HBsAg loss compared to ETV monotherapy after 100 weeks[19].

The REEF-1 study evaluated the impact of adding JNJ-3989[20] and/or bersacapavir (CAM) to nucleoside analogues for chronic HBV. It found that while the combination slightly reduced HBsAg levels, the effect was milder than the 2.6 and 2.1 log reductions seen with monotherapy.

5. Conclusion

The primary goal of these treatments is to get the 'functional cure,' sustained HBsAg loss. Functional cure remains the current therapeutic goal, while eradication faces challenges due to cccDNA is not east to get clearance. But the replication mechanism of HBV is complex, and the eradication of cccDNA and integrated HBV DNA remains a substantial challenge. Not all pharmacological agents are appropriate for combination therapy, and the effects of drug combinations can vary significantly, even among similar drugs. Clinical outcomes of combination therapies exhibit considerable variability. Novel therapeutic agents, whether in combination with NAs or used independently, represent a promising strategy for achieving the functional cure of HBV infection. Ongoing progress in drug development hold the potential to significantly enhance strategies for eliminating HBV.

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