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Narrative Review

A Comprehensive Update on the Recent Advancements in the Treatment of Chronic Hepatitis B: Is it Feasible to Attain a Complete Cure-Practical Hurdles

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ABSTRACT

Background: Hepatitis B virus (HBV) discovery occurred over half a century ago; however, about 300 million patients with chronic hepatitis B (CHB) still live in the world, leading to approximately one million deaths every year. Despite the fact that presently recommended antivirals (for instance, nucleoside analogs) are efficacious at reducing HBV replication, they practically do not impact the present HBV covalently closed circular deoxyribonucleic acid (cccDNA) reservoir. HBV cccDNA is a key barrier to the total depletion of the virus via antiviral therapy. Having reviewed earlier HHBV and CHB cures, we have updated the latest advancements in this field.

Methods: A narrative review was performed with the search engines Pubmed, Google Scholar, and others, using the MeSH terms, for instance. "*Plausible Therapies*; *antivirals*"; "*NAs*"; "*pegylated-interferon-alpha (PEG-IFNa)*"; "*direct-acting antivirals (DAA's)*"; "*Core protein allosteric modulator (CpAM)*"; "*post-transcriptional regulation*"; "*Host targeted treatments (HTT)*"; "*agents hampering viral entry*"; "*targeting cccDNA directly*; *epigenetics*"; "*DNA methylation*"; "*Histone post-translational modifications*"; "*DNA methylation*"; "*Histone acetylation*"; "*Histone deacetylase*"; "*zinc finger nucleases*"; *(TALEN)*"; "*CRISPR/Cas9*"; "*Designer nuclease(s)*" from 2000 till date in 2024 February.

Results: We found a total of 900 articles, out of which we selected 142 for this review. No meta-analysis was done.

Conclusions: The true cure for HBV infection requires the elimination of viral cccDNA from HBV-infected cells; thus, the generation of new agents directly or indirectly targeting HBV cccDNA is the immediate requirement of restricting presently available drugs against HBV infection. Regarding this, it is the major focus of current anti-HBV research worldwide via separate modes to either inactivate or inhibit (functional cure) or eliminate (complete cure) BV cccDNA. Here we provide present advances and challenges for inactivating, silencing, or depleting viral cccDNA using anti-HBV agents from various sources, like small molecules (inclusive of epigenetic drugs), polypeptides, and proteins, small interfering ribonucleic acid (siRNA), or gene-editing strategies targeting or attenuating HBV cccDNA via several modes, and future directions that might be taken into account in efforts to truly cure chronic HBV infection. Till now, no breakthrough has been made in ameliorating HBV cccDNA, despite the fact that a plethora of candidates have advanced into the phase of clinical trials. Moreover, considerable substances work to indirectly target HBV cccDNA. No special substance possesses the capacity to directly target HBV cccDNA. Specifically, CCCC_R08, in addition to nitazoxanide, might be some of the most favorable compounds to clear HBV infection in small-molecule agents. Furthermore, CRISPR-Cas9 systems have the capacity to directly target HBV cccDNA for decay and illustrate significant anti-HBV activity. Consequently, gene-editing targeting HBV cccDNA might be one of the most attractive means for achieving the core goal of anti-HBV therapeutic approaches. Basic studies on HBV infection are required to get rid of these hurdles.

Keywords
Chronic hepatitis B; cccDNA; Direct-acting antivirals (DAA's); Host targeted treatments (HTT); Genome editing technologies.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection (CHB) is a remarkable problem for public health worldwide.[1] Although there is accessibility to the preventive vaccine, an individual succumbs to HBV-associated disease in each 30-second time period. According to a World Health Organization (WHO) determination, 250 million people globally are thriving with CHB. In addition, in view of the absence of potent epidemiological outcomes, this is broadly believed to be a considerable underdetermination.[2] CHB is correlated with long-term morbidity, including cirrhosis, and is intricately associated with the generation of hepatocellular carcinoma (HCC), which is the second-most common cancer resulting in mortality and is implicated in 600,000 deaths a year.[2]

CHB is a substantially dynamic event based on the crosstalk amongst the virus, hepatocytes, and host immune reactions. The natural history of CHB might be widely classified into 5 phases dependent on these clinical paradigms, for instance: i) alanine amino transferase (ALT); ii) serum HBV deoxyribonucleic acid (DNA). iii) Hepatitis B e antigen (HBeAg), and iv) Hepatitis B surface antigen (HBsAg). Whereas most subjects shift from one phase to the next, certain of them do not transit through every phase and might switch to a previous phase. Noticeably, it is significant that management planning is dependent on such phases.

The initial objective of the present treatment of the CHB is to repress the continuation of viral replication in addition to decreasing liver damage for the enhancement of survival; however, this treatment is not efficacious in the eradication of the HBV infection.[3] Nevertheless, recent advancements with regards to HBV research, in addition to success attained in the reference of the hepatitis C virus (HCV), have invigorated the generation of curing anti-viral therapies for CHB with the numerous attractive molecules presently going through clinical assessment.

Previously, we reviewed the Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system for the treatment of HBV in addition to part of epigenetic treatments for and in placental dysfunction, and reviewed the generation and epigenetic control of the covalently closed circular DNA (cccDNA) microchromosome, the manner in which host and viral factors impact transcription, and whether utilization of epigenome editing could be done for silencing HBV cccDNA forever and why persistence of HBV takes place, followed by 'targeting dysfunctional mitochondrial metabolism of hepatocytes caused by the HBV in the treatment of chronic HBV.[4-9]

Here we have summarized an exhaustive review concerning the most recent updated strategies for the treatment of CHB.

METHODS

Here we conducted a narrative review utilizing search engine results from Pubmed, Google Scholar, Web of Science, Embase, and the Cochrane review library, utilizing MeSH terms like *"CHB", "HBx", "HBc", "cccDNA minichromosome", "viral factors", "host factors", "viral replication", "HCC", "antivirals", "nucleos(t)ide analogues (NAs)", "pegylated interferon-alpha (PEG-IFNa)", "direct-acting antivirals (DAA's)", "core protein allosteric modulator (CpAM)",* and *"post-transcriptional regulation". "Host targeted treatments (HTT)"; "Agents hampering viral entry"; "targeting cccDNA directly"; "epigenetics"; "DNA methylation"; "histone post-translational modifications"; "DNA methylation"; "histone acetylation"; "histone deacetylase"; "zinc finger nucleases"; "(TALEN)"; "CRISPR/Cas9"; "designer nucleases"* from 2000 till date in 2024 February.

RESULTS

We found a total of 900 articles, out of which we selected 142 for this review. No meta-analysis was done.

AIMS OF THE TREATMENT OF THE CHB

Recently, an agreement has been reached with most researchers about the definitions of therapeutic cures regarding CHB. Three major definitions have been taken into account.[10]

i. Partial cure, by definition, is the stimulation of the continuously non-identifiable viral load and normalization of the ALT and determinable HBsAg quantities subsequent to the definitive treatment. This panorama, with the enhancement of virological and biochemical reactions, diminishes the propagation of cirrhosis and results in remarkable improvements in the quality of life (QOL) and survival of patients. Nevertheless, although there are clear clinical advantages, the risk of HCC continues.

ii. Functional cure possesses the properties of the sustenance of undeterminable viraemia with the elimination of HBsAg, with or without the serotransformation to the HBsAg. This status is inclusive of the continuation of the cccDNA, the template meant for transcription of the virus. A functional cure is considered to be the clinical end point in regard to possessing the safety of the NA therapy withdrawal in addition to the aims for the myriad of agents that are undergoing production. This specific kind of cure has been correlated with the reversion of the continuing liver damage in addition to further reducing the risk of the development of the HCC to a level that is practically akin to the subjects undergoing spontaneous clearance of the virus.

iii. Complete or total cure has been correlated with total elimination of circulating serum HBV DNA, depletion of circulating HBsAg with the generation of HBsAg antibodies, and complete elimination of cccDNA. Nevertheless, the present agreement states that acquisition of this complete cured status is tough in view of the continuation of the cccDNA.

iv. The sterilizing cure, by definition, is the total cure and elimination of incorporated HBV DNA fragments from the host chromosomes. The status simulating that of the vaccinated subjects, who have not been exposed to the virus ever, apparently, is substantially not plausible.

PLAUSIBLE THERAPIES

Subsequent to the invention of HBV in 1965, considerable ad-

vancements with regard to the acquisition of insights into HBV biology and pathogenesis have resulted in the approval of two classes of agents for the treatment of CHB: antivirals as well as immunomodulators, for instance.

Antivirals

i) **Nucleos(t)ide Analogues (NAs)** along with PEG-IFNα, respectively.[11] The most recent NAs that managed to gain regulatory approval, Entecavir (ETV), tenofovir disoproxil fumarate (TDF), and Tenofovir Alafenamide (TFA), are remarkably robust antivirals that possess remarkable genetic barriers towards resistance generation apart from a substantially good safety profile.[5] The reverse transcription activity of the HBV polymerase gets targeted by NAs and actually causes avoidance of biogenesis of the HBV DNA, which results in repression of viremia towards non-traceable quantities and normalization of the ALT.[12]

Sequentially, NAs stop the transmission of the HBV, decrease liver damage and fibrinogenesis, reduce the risk of decompensation, and prevent HBV-associated tumorigenesis, thus restricting the requirement for liver transplantation.[11,13] To our misfortune, the myriad advantages of the NAs are not inclusive of the elimination of HBsAg that is occasionally attained in subjects of CHB getting treatment or, of greater significance, the depletion of the cccDNA. cccDNA does not get targeted by NAs, aiding in the continuation of the cccDNA in the infected hepatocytes. Furthermore, the influence of the NAs over HBV replication is not complete. The existence of residual viremia facilitates "*de novo*" infection and replenishes the sustenance of the cccDNA pool. Thereby, a considerable incidence of virological and clinical relapses takes place in most subjects, resulting in the discontinuation of the NAs. Thereby, these processes dictate the utilization of such agents as treatment throughout life in view of their association with prohibitive cost,bad adherence, and plausible toxicity.[5] Lastly, despite a considerable reduction in the incidence of HCC on NAs, it does not totally get eradicated in view of the incorporation of the HBV DNA fragments in the host genome.

A broader variety of studies have displayed that for acquiring efficacious resolution of the HBV infection, immunological regulation is the requirement.[14-17] Long-term NAs treatment has been shown to modulate the immune reactions to HBV in a percentage of the subjects. Part recovery of the HBV-specifically inefficacious CD4+ and CD8+ T-cells in the case of CHB might take place as an inimical sequelae of the NAs-stimulated repression of viral replication; however, this continues to be incomplete and generally returns back in the case of discontinuation of the Nas.[15,18] Intriguingly, restoration of the antiviral working of natural killer cells (NK-cells) does not take place, and this might be significant regarding targeting for the production of robust therapies resulting in immunity for the long term.

ii) **PEG-IFNα**-dependent treatment might be an alternate option for the treatment of CHB. Noticeably, the time-based treatment course of PEG-IFNα can cause the elimination of HBeAg, which portrays a replacement marker regarding active viral replication and has the capacity to biogenesis the antibodies to HBeAg (HBeAg seroconversion). HBeAg seroconversion in subjects with the existence of a substantial replication phase of the HBV infection (HBeAg-positive (HBeAg+) CHB) is correlated with a lesser rate of viral replication in addition to enhancement of clinical results. Additionally, PEG-IFNα further possesses the capacity to cause the elimination of HBsAg and decrease cccDNA, resulting in a functional cure.

IFNα has been acknowledged to invoke the activation of NK-cells, which takes part in PEG-IFNα-stimulated regulation of the clearance of the HBV infection found in certain subjects. It has been illustrated that PEG-IFNα can facilitate the activation and proliferation of cytokines-generating CD56 bright NK-cells, which have the capacity to generate robust antiviral cytokines.[19] Nevertheless, the frequency of IFNγ-generating HBV-specific CD8+ T-cells is low, and no escalation of proliferation capability takes place at the time of the initial 6 months of treatment with PEG-IFNα utilization.[20] Actually, IFNα stimulates a drastic decrease in the total content of HBV-specific CD8+ T-cells and CMV-specific CD8+ T-cells.[19] Such an absence of influence on the HBV-specific CD8+ T-cells might explain the restricted effectiveness of IFNα therapy in the case of CHB. Nevertheless, the recovery of the HBV-specific CD8+ T-cell impact has been displayed in subjects where clearance of HBV infection takes place subsequent to the IFNα therapy, emphasizing the significance of activated adaptive immunity.[18,21] Clarification regarding the immunological modes behind the reactions to IFNα is not known, with the requirement for greater assessment for the maximization of the results of the therapy. PEG-IFNα is further acknowledged regarding its antiviral actions, including repression of the interferon-stimulated gene (ISG) that results in the blockade of the assembly of capsids, hampering HBV ribonucleic acid (RNA) formation, and causing dysfunctional viral transcript generation.[22-24] IFNα further results in stimulation of the breakdown of the cccDNA by the activation of cytidine deaminases from the Apolipoprotein B messenger RNA (mRNA) editing enzyme (APOBEC3) family and epigenetically controlling transcriptional activity.[25]

Total HBsAg clearance and seroconversion just take place in the case of 1/4th or 1/3rd of subjects that received PEG-IFNα.[26,27] This frustrating reaction rate might be reasoned out by the recent observations regarding pharmacokinetics getting negatively influenced by the generation of PEG-IFNα immunoglobulin (IgM) complexes in the liver and their segregation and elimination by Kupffer cells (KC's).[28]

Furthermore, PEG-IFNα therapy is generally correlated with the common experience of recurrent hepatic flares, limiting its utilization in cases of compensated liver disease.[26] Noticeably, of further significance is the remarkable inimical sequelae; numerous contraindications, in addition to the delivery mode (subcutaneous injection), are the main reasons reinforcing the non-acceptability of PEG-IFNα by the patients.[27]

As acknowledged, the discriminating modes behind NAs

as well as PEG-IFNα and combination approaches illustrate certain clinical advantages that are being evaluated, inclusive of de novo (simultaneously delivering NAs and PEG-IFNα). ii) add on (adding PEG-IFNα to the continuing NAs); iii) switch to (initiation with the NAs with subsequent PEG-IFNα). According to recent corroboration, concomitant treatment, with which to switch to or add on, might escalate the effectiveness of the combination therapies in patients with repression of viremia and lesser antigenemia and those who have undergone depletion of HBeAg on NAs treatment.[29-31] Nevertheless, greater assessment is required for the estimation of the most advantageous combination of approaches. Factors, for instance, i) time planning of therapy, ii) baseline quantities of HBsAg, and iii) repression of viremia, might be key to the effectiveness of this kind of treatment.

Stoppage of Long-Term NAs

The discontinuation of the NAs might be advocated in the case of occasional (<1%) CHB patients who do not have cirrhosis but have eliminated HBsAg. In the case of HBeAg+ CHB patients, interruption of NAs treatment is advocated in stable HBeAg seroconversion with the undeterminable viral load, and a minimal 12 months of combination treatment have been attained.[5] As compared to those cessation guidelines for CHB patients, not having cirrhosis has not been clearly defined.[32] Whereas according to certain guidelines, it is advocated that utilization of NAs be for an infinite time period, others advocate that cessation of the NAs might be done in the case of patients who have had 2 years of completed therapy with an undeterminable viral load that has been revealed for a minimum of 3 times at the gap of 3 months. This advocate has been evaluated and illustrated with a cumulative virological relapse (HBV DNA >2000 IU/ML) along with a clinical relapse (HBV DNA >2000 IU/ML) as well as an escalation of ALT rates of 70% and 43%, respectively.[32] The study by Hadziyannis et al,[33] a hallmark one, displayed biochemical and virological remission in 55% of HBeAg-negative patients who were not needing any treatment in view of a lack of liver disease.[33] What was more appreciable was the elimination of HBsAg observed in 33% of these subjects at the time of long-term follow-up, leading to a functional cure. Subsequent to this study, numerous studies have evaluated the stoppage of NAs in the cases of HBeAg-negative CHB patients.[34] Their outcomes demonstrated that numerous patients having a functional cure or transitioning to a stage of HBeAg-negative CHB did not need further therapy. The replenishment of long-term continuation of the immune reactions has been correlated with the promising results. Nevertheless, the precise modes behind this need further assessment.[35]

The problems involved in the utilization of such strategies might be correlated with robust, inimical sequelae, despite being usually safe. These sequelae might be as robust as ALT flares and hepatic decompensation and demise as well.[32] Sequentially,this kind of therapy might be evaluated in a selective group of patients, warranting intricate monitoring by those with expertise in the topic. Moreover, in the absence of guidelines that have been properly demarcated, there is a requirement for the presence of any of the advantages that are weighed against any kind of risk. A plausible disadvantage is the enhanced risk of the generation of HCC in view of the rebound of the virus, which takes place subsequent to the stoppage of the NAs. Nevertheless, no escalation of the risk of HCC has been found thus far; however, careful follow-up has been advocated.[36]

Isolation of the biomarkers having the capacity to predict successful NAs treatment withdrawal would be of considerable use, and a small pilot trial displayed plausible immune profiles; however, such work needs greater corroboration.[15] Hadziyannis et al[33] are performing a clinical trial with further assessment to determine if a small course of PEG-IFNα subsequent to cessation of the NAs possesses the capacity for activation of immune reactions and induction of elimination of HBsAg (nucleos(t)ide cessation in HBeAg-negative hepatitis B infection for facilitating HBsAg clearance-Nucleos(t)ide withdrawal in Hepatitis B virus infection (NUC-B)).

INNOVATIVE DIRECT-ACTING ANTIVIRALS

Assessment of newer classes of the direct-acting antivirals (DAA's) is getting done with the idea of targeting the variety of steps in the life cycle of HBV with the final aim of attaining the functional cure (Figure 1).[37]

HBV core particles possess a key role in the life cycle of HBV, being responsible for the following: i) liberation of the relaxed circular genomic DNA (RC-DNA) from the capsids, where their administration takes place into the nucleus; ii) packaging of the pregenomic RNA (pgRNA) for the reverse transcription into the RC-DNA. iii) the constitution of the Dane infectious viral particles; and iv) the restoration of the CccDNA pool.

CAPSID ASSEMBLY HAMPERING AGENTS

Two major classes of the core protein allosteric modulator (CpAM) are present.

i) Class I CpAM, referred to as heteroaryldihydropyrimidines (HAPS), changes the kinetics of the generation of capsids for forming the morphologically abnormal non-capsid polymers, resulting in the elimination of HBV core proteins.

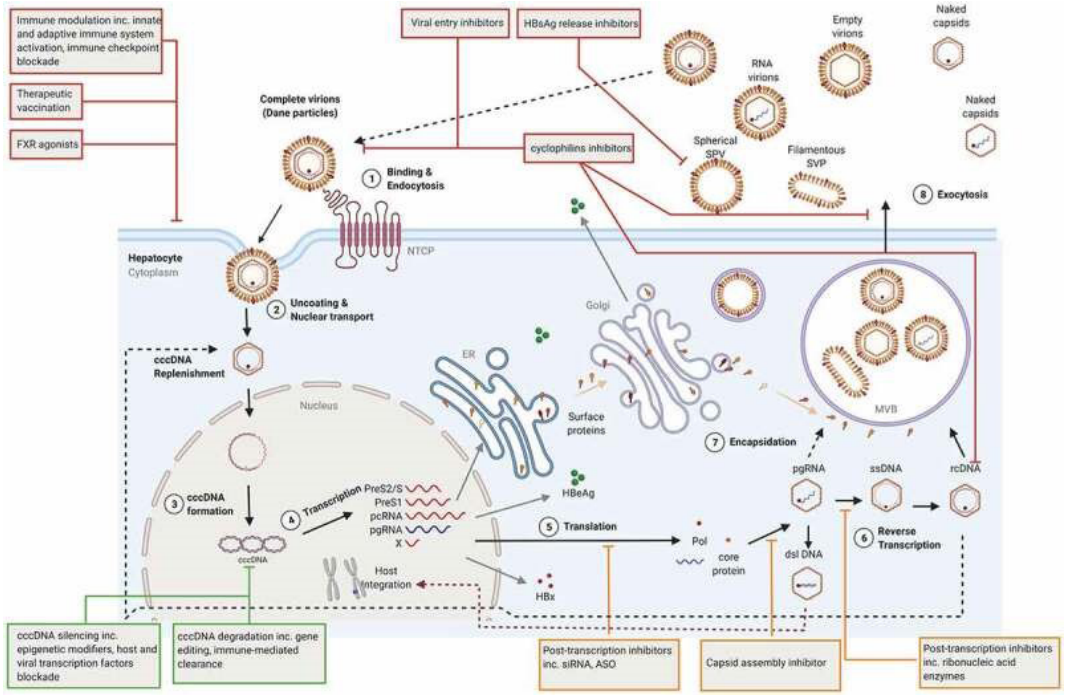
ii) Class II CpAM, referred to as phenylpropanamides (PPA) and sulphamoylbenzamides (SBA), cause augmentation of the assembly of mature capsids; b) avoidance of pgRNA encapsidation, and result in morphologically normal capsids having an absence of nucleic acid quantities.[38]

Of the maximum evaluated CpAMs, HAPS and SBA portray the most frequent ones. Nevertheless, innovative structural classes, for instance, sulphamoylpyrroloamides (SPA), glycoxamoylpyrroloxamides (GLP's), and (iii) dibenzo-thiazepine carboxamides, have been generated.[39]

HETEROARYLDIHYDROPYRIMIDINES

RO7049389, GLS4, and BAY41-4109 represent the HAPS family. RO7049389 is a small molecule whose assessment was performed

Figure 1. *HBV Life Cycle and Novel Therapeutic Targets*[37]



DAAs, HTT and cccDNA Direct-acting Agents are shown Orange, Red and Green, Respectively

recently in a multicenter, randomized, placebo-controlled phase 1 trial. RO7049389 therapy resulted in a 2.7-3.2 and 2.1-2.5 $\log_{10}$ reduction in HBV DNA and HBV RNA, respectively.[40] Nevertheless, no alterations were observed in the quantities of the HBsAg, and viral rebound towards pre-therapy quantities was observed at the time of post-treatment. At present, RO7049389 is undergoing clinical assessment, as is toll-like receptor 7 (TLR7) agonist RO7020531, in addition to NAs. Evaluation of GLS4 was attempted in a multiple ascending dose study and more recently in a multicenter, randomized, double-blind placebo-controlled phase 1a study.[41] Acknowledged that in combination with ritonavir, representing a metabolic enzyme hampering agent, which escalated the plasma quantities of GLS4, a 28-day course of GLS4 therapy stimulated a lesser aggravated reduction in HBV DNA, HBsAg, and HBeAg in contrast to lone ETV (HBV DNA-1.42-2.14 *vs.* 3.5 $\log_{10}$ IU/mL; HBsAg.06-0.14 *vs.* 0.33 $\log_{10}$ IU/mL; HBeAg 0.25-0.3 *vs.* 0.43 $\log_{10}$ IU/mL). Compared to that, the reduction of the pgRNA and HB core-related antigen (HBcrAg)-2 of the biomarkers of the transcriptional activity of the cccDNA was greater in the GLS4 combination and ritonavir (pgRNA: 0.75-1.88 *vs.* 0.96 $\log_{10}$ copies/ml; HBcrAg: 0.23-3.5 *vs.* 0.44 $\log_{10}$ U/mL). Nevertheless, post-treatment estimation of viral rebound occurred, and restoration of pgRNA quantities occurred to their baseline quantities. Evaluation of BAY41-4109 has been attempted in certain preclinical studies and illustrated the avoidance of assembly of the capsids, resulting in the induction of destabilization in hepatoma cell lines and humanized mice. In the case of primary hepatocytes, BAY41-4109 diminished HBV replication, intracellular HBV RNA, antigenemia, and generation of the cccDNA.[42,43]

SULPHAMOYLBENZAMIDES

The two SBA small molecules that are being analyzed at present are NVR-3-778 and JNJ-56136379.[44] NVR-3-778 was the initial molecule of this class in humans. Utilization of NVR-3-778 in a proof-of-principle study in a humanized mouse model in combination with PEG-IFNα illustrated that it had the capacity to induce greater repression of viremia and HBV RNA.[45] Yuen et al[46] later corroborated the superiority regarding antiviral activity, safety, and pharmacokinetics of such a combination in the case of a 28-day dose-ranging phase 1b trial with a 1.97 $\log_{10}$ IU/mL and 2.09 $\log_{10}$ copies/mL reduction in the HBV DNA and HBV RNA, respectively.[46] Nevertheless, no significant alterations were observed in HBsAg, HBeAg, and HBcrAg in the 28-day therapy over all the cohorts.

In a small double-blind study that was inclusive of healthy subjects, the delivery of JNJ-6379 as a monotherapy caused a reduction in viremia of 2.16-2.09 $\log_{10}$ IU/mL and a diminished 1.43-2.58 $\log_{10}$ copies/mL decrease in the case of HBV RNA.[47] To our misfortune, HBV DNA and RNA were back to baseline quantities subsequent to the stoppage of the therapy, and no significant alterations in the HBsAg were determined. At present, the assessment of JNJ-6379 is being performed in combination with NAs in a larger cohort. Although JNJ-6379 illustrated significant hampering actions against cccDNA *in vitro*, no such actions were observed *in vivo*.[44]

ABI-H0731

ABI-H0731 (vebicorvir), which portrays a 1st generation capsid hampering agent, has further been evaluated in combination with NAs. Initial outcomes obtained illustrated ecstatic viral repression, with certain patients being in agreement with the criteria for the cessation of the treatment, for instance, reduction of HBV DNA and pgRNA<20 IU/mL, seroconversion of HBeAg or HBeAg<5 IU/mL, for a minimal duration of 6 months. Nevertheless, in this group of patients, ABI-H0731 had an incapacity for sustenance of virological reactions subsequent to cessation of treatment; thereby, this clinical trial has been further stopped.[48] Currently, ABI-H0731 is being evaluated in combination with the other therapies.

Numerous CpAMs are being evaluated in clinical trials in the form of single or combination therapies.[41,47,49]

POST TRANSCRIPTIONAL REGULATION

An innovative therapeutic strategy for the treatment of CHB targeting the formation of HBV mRNA has assumed favorable status with the idea of hampering all five HBV mRNA transcripts using a single molecule.[50] Such HBV mRNA hampering agents work by breaking down mRNA or repressing their translation for the restriction of generation of the HBV protein, for instance, HBsAg, HBeAg, and HBcrAg, thus cessation of active replication with efficacy and liberation of viral or subviral particles. Variable post-transcriptional regulation approaches have been posited, inclusive of RNA interference (RNAi), antisense oligonucleotides (ASO), and ribonucleic acid enzymes.

RNAi Strategy

The RNAi strategy implicates using 20-25 base pairs of small RNAi (si RNA) molecules fashioned for binding overlapping areas that code for numerous mRNA transcripts. RNAi therapeutics got started in a phase II clinical trial with the assessment of ARC 520, which is composed of two cholesterol-conjugated siRNAs, si HBV-74 and si HBV-77, in combination with Nacetylgalactosamine (NAG) for targeted administration to hepatocytes. A robust reduction of HBeAg occurred in the case of HBeAg+ patients; however, there was a modest action in HBeAg- or HBeAg+ patients that had previously received NA's.[51] The variations in the reactions were revealed in HBeAg-chimpanzees, which confirmed that HBsAg was usually generated by incorporating HBV DNA in the absence of the ARC 520 binding regions. This resulted in the fashioning of the next generation of siRNAs, for instance, JNJ-3989 (previously ARO HBV), that target sequences upstream of such deletions. As compared to ARC 520, JNJ-3989 had better tolerability in the phase 2 dose-ranging study. In 39/40 patients, HBsAg was diminished, with the sustenance of HBsAg decreasing in 56% of the patients 9 months subsequent to the last dosage.52 JNJ-3989, in combination with TDF or ETV, stimulated a 0.73-0.84 log IU/ml HBsAg decrease in 98% of the patients with the sustenance of a decrease in HBV RNA, HBeAg, and HBcrAg.[52]

Initial safety and pharmacokinetic outcomes for repeat dosage of the NAG siRNA AB 729 delivered to CHB patients in a phase 2 study were reported recently by Yuen et al.[53] A significant reduction of HBsAg occurred and continued to be<100 IU/mL in 70% of the patients. Intriguingly, no HBsAg rebound was determined post-treatment. In an akin study, AB 729, as a single or repeat dosage study (6/7 doses), the total HBsAg (large, middle, and small HBsAg isoforms) diminished, which was associated with a decrease in HBV RNA and large and middle HBs protein regardless of the dosage regimen.[54] Additionally, this alteration in the T-cells was associated with HBV proliferative capability and the frequency of IFNγ-generating HBV-specific T-cells, which have a significant role in the regulation of the HBV infection. Such immunological processes took place prior to or at the time of mild to moderate ALT flares.[55] Administration of AB 729 was further done in the form of a single dose to HBeAg patients with lesser viremia and thereby no eligibility for the standard of care. It resulted in a significant reduction of the HBV DNA and HBeAg and undeterminable quantities of the HBV RNA and HBcrAg in all the cases up to 36 weeks after dosing.[56]

VIR-2218, an HBx targeting RNAi, was assessed in a small-ranging dose phase 2 study. The latest outcomes illustrated a dose-based decrease in HBsAg, their quantities diminishing by >1 log IU/mL in 71% of HBeAg+ and HBeAg-CCHB patients; however, the percentage of the patients who attained sustenance of the reactions was less, implicating just 4/20 patients. These outcomes corroborated the generation of the VIR-2218 with the PEG-IFNα. Interim evaluation displayed that co-delivery of these two agents stimulated a faster and greater ecstatic HBsAg decrease as compared to each lone agent.[57]

Antisense Oligonucleotides (ASO)

The modes behind ASO, for instance, RO-293, GSK-33389404, and GSK-3228836, vary from the siRNA's. Binding of the ASO takes place with complimentary sequences of HBV mRNA and generates hybrid DNA: RNA or RNA: RNA duplexes, leading to the breakdown of the RNA target through RNase H-based pathways. The replacement of GSK-33389404, which displayed minimum effectiveness, was made by GSK-3228836, whose assessment was conducted recently in a small phase 2a study. Intriguingly, GSK-3228836 demonstrated a minimal 3 log IU/mL decrease in the HBsAg quantities in the treatment-naive patients and CHB patients who had received and presently have further progressed to the phase 2b study.

Ribonucleic Acid Enzymes

Ribonucleic acid enzymes catalyze the cleavage of DNA and RNA complexes. For instance, HBV RNase H-breaks down pgRNA at the time of minus DNA strand generation amongst the nucleocapsids. Therefore, RNase H hampering would result in the accumulation of DNA and RNA complexes and halt the reverse transcription events, leading to the generation of defective, non-infectious virions. Nevertheless, RNase H hampering agents have not reached the stage of clinical assessment in view

of the technical problems encountered with the generation of the active enzymes.

HOST-TARGETED TREATMENTS

Like numerous other infectious substances, HBV is substantially dependent on the cellular host factors for practically each step of its replication life cycle.[58] Moreover, unattractive virus-host crosstalk is central to the immune escape and generation of the persistence of the HBV. Thereby, targeting of the host factors involved in the HBV replication life cycle and activation of the ones possessing the anti-HBV working are plausibly promising therapeutic strategies. Noticeably, since additional selective pressure is exerted by them directly on the virus,such factors make them promising targets by providing a greater barrier to resistance and plausibly pangenotypical anti-viral actions. Nevertheless, there is a requirement for vigorous evaluation of any toxic processes.

Agents Hampering Viral Entry

The recent isolation of a liver-specific bile acid transporter, sodium-taurocholate-co-transporting peptide (NTCP-SLC10A1), as a host entry factor for HBV has opened innovative therapeutic vistas for the blockade of entry and diminishing viral spreading.[59]

Burlevirtide: i) Burlevirtide, previously called myrcludex, comprises the preS1 domain of the large surface protein, which hampers NTCP and avoids viral entry. In a phase 2 dose-ranging study, assessment of the burlevirtide was performed in HBeAg-negative CHB patients. Despite HBV DNA diminishing by >1 log IU/mL in 32% of patients, it did not have any impact on HBsAg. In view of the majority of studies concentrating on coinfection with the Hepatitis D virus, a satellite virus of HBV that is based on HBsAg for infection, the latest interim outcomes obtained demonstrated that burlevirtide in combination with PEG-IFNα stimulated a steep decrease in HDV RNA to undetermined HDV RNA quantities, with certain cases displaying sustenance of undetermined HDV RNA quantities for 6 months subsequent to the cessation of the treatment. These outcomes stimulated the approval of burlevirtide by the European Medical Agency (EMA)[60] for the treatment of chronic HDV.

Cyclophilins: Additionally, blockade of HBV entry is feasible by small-molecule agents, for instance, Bi) Cyclophilins and CyclosporinA (CsA), a well-acknowledged immunosuppressant having the capacity to avoid attachment to NTCP without disrupting bile acid transport and working in experimental models.[61] CsA-obtained agents, having the absence of immunosuppressive actions, have been generated subsequently and are inclusive of SSY 446, CCY 450, and 27A. They have the capacity to avoid HBV interactions with NTCP with considerable effectiveness *in vitro*; however, they have not reached the clinical stage for CHB.[62]

Neutralizing Monoclonal Antibodies (NMAb's): At present, VIR 3434, a preS1 NMAb's, is undergoing assessment in a phase 1 study.[63] Initial outcomes in a small cohort of CHB demonstrated an average decrease of 1.3 log IU/mL in the HBsAg quantities. A combination phase 2 study with the utilization of RNAi VIR 3434 is in the enrollment phase. Whereas HBV entry-handling agents have their position in the therapeutic arsenal for the conferring of protection to the naïve hepatocytes, they don't target directly or cause the depletion of the cccDNA reservoir. The clinical utility of the entry-higging agents with regard to CHB cure might exist in combination regimens with the goal of avoiding *de novo* cccDNA generation and/or cccDNA breakdown.

HBsAg Liberation Hampering Agents

HBsAg plays a major role in the HBV life cycle. Lying buried in the lipid bilayer, HBsAg generates the surface of the viral genome possessing HBV virions and aids viral entry through the binding of the preS1 area to the NTCP receptor. HBsAg assembling further takes place around the newly generated nucleocapsids for the viral exit, along with getting liberated in the extra part in the form of the empty subviral particles (SVP). Actually, maximum enrichment of the HBsAg is existent as an HBV antigen, which is implicated in 99% of the circulating SVP. It has been acknowledged for a long time that this extra HBsAg aids in the repressive immune milieu present at the time of CHB. Thereby, the idea of blockade of HBsAg liberation lies in the plausibility of the avoidance of liberation of the enveloped viruses, infection spreading, and replenishment of the efficacious HBV-specific immune reactions, which possess the capacity to regulate the cccDNA along with stimulating the clearance of the virus. DNA-dependent nucleic acid polymers (NAP) (REP 2055 and REP 2031) or RNA-dependent NAPs (REP 2139 and REP 2165) portray single-stranded nucleotides. Their antiviral activity is independent of their sequences; however, it is substantially based on their length and amphipathic biochemical makeup. NAP's blockade of liberation of HBsAg is caused by disturbance of the assembly or liberation of SVP through its crosstalk with the host factors that have not been worked out. Small proof-of-concept clinical trials have illustrated that REP 2139, REP 2165, and REP 2055 have the capacity to avoid HBsAg liberation.[65] In a recent small clinical trial (REP301 and REP301LTF), REP 2139 monotherapy with the subsequent PEG-IFNα combination regimens with the repeat PEG-IFNα lone in case of HBV/HHDV co-infection led to a quick reduction in HBsAg and escalated anti-HBsAg antibody titres in 42% of the patients with continuous sustenance for greater than a year post-follow-up.[64] Further, this study displayed symptoms of intoxication with heavy metals in patients, which were correlated with plasma and liver accumulation of REP 2139. This has ensured the fashioning of the next generation of NAPs, with REP2165 having the properties of diminished liver accumulation and akin anti-HBsAg actions like REP 2139. A phase 2 pilot study (REP401), where the combination of REP 2139 and REP 2165 was done with TDF and PEG-IFNα, by cessation of therapy, 60% of the HBeAg-CHB patients attained HBsAg ≤0.05 IU/ml, with all having HBsAg seroconversion, and reactions to REP 2139 and REP 2165 were similar.[65] At the 48-week follow-up, virological regulation and functional cure took place in 32.5 and 35% of patients, respectively. Favorably, in HBeAg-CHB patients, HBsAg generated by the incorporated HBV DNA gets eliminated, pointing out that REP 2139 and REP 2165 can di-

minish the incorporated HBV DNA; however, the modes behind their action have not been evaluated. Furthermore, over 90% of patients had ALT flares, which illustrated greater intensity in contrast to the ones with the HBV DNA eliminated, pointing out that flares that were host-stimulated portrayed immune regulation of infection as the mode behind their action. Further assessment of the virological markers in the REP301, REP301LTF, and REP401 trials revealed recently that a functional cure might be attained in maximum patients having the properties of undeterminable HBV DNA and HBV RNA, HBcrAg under the minimal limit for the quantification, normalized ALT quantities, HBsAg reduction <0.005 IU/mL, and determinable HBsAg antibodies.[66] Nevertheless, these favorable outcomes need to be corroborated in larger studies.

In vitro studies that corroborated blockade of HBsAg liberation by NAPs further displayed a mild hampering action, believed to be due to disruption of HBV binding to heparan sulfate proteoglycans.[67] Nevertheless, such latter working does not hold good with most current agents, REP 2139 and REP 2165, which basically impact generation and SVP liberation. It is further believed that NAPs cause avoidance of viral exit and further facilitate antiHBV immunity, despite the need for corroborating modes.[68] Actually, stimulation of cytokine generation from healthy peripheral blood mononuclear cells (PBMC) that got treatment with NAPs was found. Conversely, in primary human hepatocytes and liver sinusoidal endothelial cells, resistance to immunomodulatory actions was found, pointing out that NAP's antiviral actions in patients were not in view of the induction of innate antiviral reactions. Overall, the modes behind immune NAP's actions need to be worked out.

Farsenoid X Receptor-α Agonists

Numerous transcription factors are implicated in the modulation of the HBV cccDNA. Of these factors, BA nuclear receptor Farsenoid X Receptor-α Agonists (FXR) binding takes place between two response elements on cccDNA and stimulates HBV transcription. FXR is further responsible for the generation and sustenance of CccDNA. The proviral actions of FXR might be disrupted if it gets engaged with agonists that display hampered cccDNA transcription.[69] Despite the fact that the modes behind FXR agonists still need to be worked out, it is believed that they might disturb the active kinds of FXR, change FXR working, or destabilize the crosstalk amongst FXR and cellular factors implicated in the transcription of pgRNA.[69] Interestingly, numerous studies have corroborated that FXR can facilitate cccDNA transcription. A phase 2 assessment of the FXR agonist EYP001 (vonafexor) is being conducted in combination with PEG-IFNα and ETV in treatment-naïve HBeAg+ and HBeAg-CHB patients.[70] The latest interim outcomes demonstrated ecstatic HBV DNA and HBsAg reduction in the HBeAg+ group; however, there were fewer reactions in the HBeAg- group (-3.7 *vs.* -0.4 1 log IU/mL; -0.91 log *vs.* -0.0 IU/mL). HBsAg decreased to a greater degree in the EYP001 and PEG-IFNα groups in contrast to EYP001 in combination with PEG-IFNα and ETV. Although ALT and AST flares were observed, their resolution occurred upon cessation of treatment.

Cyclophilins Hampering Agents

Cyclophilins are host proteins from the large family of immunophilins possessing the Peptidylprolyl isomerase (PPIase) enzymes actions. They catalyze the transformation of X prolyl bonds (X portraying any amino acids) from the cis to trans conformation, which is involved in protein folding.[71] Cyclophilins are further responsible for protein trafficking, cellular signaling, and immunomodulation. The human genome encodes for 16 Cyclophilin isoforms having their placement in various cellular chambers, of which 4 possess the capacity of getting liberated.[72] Variable cytophilins, including cytophilins A, B, and 40, are upregulated and involved in numerous pathologies, including cancer. Considerable corroborating proof is that cyclophilins take part in human immunodeficiency virus (HIV) infections and replication.[73,74] PPIase actions were further observed in the HCV replication life cycle and resulted in the generation of numerous cyclophilin hampering agents, for instance, NIM811, DEBIO-025 (alisporivir), and SCY-635, which have revealed antiHCV actions with a lack of cytotoxicity isolating cyclophilins as therapeutic targets of choice for chronic hepatitis C.[75] To our misfortune, patients with robust pancreatitis were revealed to have single deaths, resulting in the termination of clinical evaluation of these agents. All the generation of pancreatitis took place in the PEG-IFNα/HCV anti-viral ribavirin triple combination, but not with the lone DEBIO-025. These latter observations revealed that such robust, inimical sequelae were not correlated with DEBIO-025.

Cyclophilins are further responsible for the HBV replication life cycle. *In vitro* DEBIO-025 hampers viral replication further study, inclusive of, for instance, alisporivir regulation rates and diminished HBsAg generation.[76] Knockdown experiments displayed that Cyclophilin A and, to a lesser degree, C and D are actively implicated in the HBV replication life cycle, and DEBIO-025 possessed pan-cyclophilin blockade characteristics.[76] The antiviral actions of cyclophilins were further corroborated *in vitro* and in mice with NVP018, a sangamide cyclophilin hampering agent.[77] Till now, a 2nd generation cyclophilin hampering agent, CRV431, has illustrated greater effectiveness in mouse models of HBV infection without cytotoxic actions and is currently undergoing clinical assessment in healthy persons.[78] Furthermore, CRV431 is undergoing clinical assessment in non-alcoholic steatohepatitis (NASH) and HCC.[79] Outcomes from preclinical studies in animal models of NASH, in human cell cultures, and in tissue explants displayed the effectiveness of CRV431 as an antifibrotic and corroborated the assessment of CRV431 in phase 2 of a clinical trial.

TARGETING CccDNA DIRECTLY

HBV cccDNA continues to be a barrier to HBV cure.[80] This considerably stable minichromosome gets protection conferred on the nucleus of hepatocytes and guides the transcription of viral proteins continuously. Thereby, agents targeting cccDNA might be capable of attaining a functional and total cure. Hence, treatments fashioned for the silencing or breakdown of cccDNA have attracted considerable attention in the past decade. Nevertheless,

targeting cccDNA continues to be a main therapeutic problem, and sequentially, no therapeutic targets are undergoing clinical generation.

The transcription of HBV cccDNA is controlled by numerous host factors and viral proteins, for instance, HBx and serum HBcAg. Different studies have displayed the implications of the HBx protein in controlling cellular pathways, transcription factors, and tumor generation.[81] According to a recent study, HBx binding to the damaged-particular DNA binding protein 1 (DDB1) breaks down the structural maintenance of the chromosome 5/6 (Smc 5/6) complex, a restriction factor possessing the capacity to blockade HBV RNA transcription.[82] Intriguingly, nitazoxamide, an antiparasitic agent, was illustrated to halt the breakdown of the Smc 5/6) complex and sequentially hamper HBV replication intermediates, including cccDNA, *in vitro*.[83] In a pilot clinical trial, nitazoxamide was delivered for up to 48 weeks to nine treatment-naive CHB patients.[84] This possessed good tolerability, with inimical sequelae varying from mild to moderate. Intriguingly, HBV DNA became undeterminable with the elimination of HBsAg in 89% and 33% of patients, respectively. Additionally, HBeAg seroconversion took place in the case of two HBeAg+ patients. At the time of cessation of therapy, all the patients were delivered NA's according to the protocol of the study, which did not aid in the patients follow-up subsequent to Nitazoxamide antiviral actions post-treatment. No subsequent follow-up assessment of nitazoxamide has been performed until now as therapy for CHB.[85]

Acknowledged the chromosomal arrangement, cccDNA correlates with histones and non-histone proteins, inclusive of proteins that are of viral and host origin. Enrollment of numerous of these proteins takes place to control the HBV minichromosome at the level of epigenetics.[86,87] For instance, subsequent to its enrolment in cccDNA, HBx results in an escalation of the expression of DNA methyltransferase (DNMTs), whereas HBcAg crosstalks with the Histone acetylases (HAT's) for facilitation of cccDNA transcription.[87,88] Therefore, targeting pro-HBV epigenetic alterations with the aid of epigenetic modifiers might facilitate the silencing of cccDNA. Numerous epigenetic agents that target DNA methylation or histone acetylation are undergoing development for the therapy of cancers, including HCC, and might be repurposed for therapy for CHB.[89] For instance, AGK2, a histone acetylate sirtuin 2 (SIRT2) hampering agent, was illustrated to result in repression of replication in cell lines and transgenic mice.[90] GS-5801, a Lysine demethylase-5 hampering agent, illustrated considerable effectiveness on HBV in primary hepatocytes; however, no action in a phase 1 study was seen, therefore it was interrupted.[90] Nevertheless, although it's attractive and has robust plausibility, epigenetics treatment can induce inimical sequelae, in view of the implications of epigenetic enzymes in a variety of cellular events. Therefore, targeting the modes behind HBx and serum HBcAg modulation of cccDNA control might be a safe alternate strategy. Nevertheless, for obtaining these outcomes, the acquisition of deeper insights into the molecular modes behind controlling cccDNA biogeneration, transcription, and turnover is of tantamount significance.

The APOBEC3 enzyme possesses the capacity to target cccDNA directly. Their working is to deaminase cytidine into uridines in the c DNA at the time of reverse transcription, resulting in G to A hypermutation at the time of generation of the 2nd DNA strand. The A3 family, composed of seven deaminases (A3A, A3B, A3C, A3D, A3F, A3G, and A3H), plays a significant role in the innate immune defense modes. For instance, A3G can restrict HIV-1 infectivity by stimulating the breakdown of the hypermutated viral genome.[91] It has been illustrated in recent studies that IFNα, IFNγ, and IFNλ can upregulate A3A and A3G and stimulate the breakdown of cccDNA.[92] Akin to that, IFNγ and TNFα get generated by HBV, and particular T-cells result in cccDNA breakdown through A3-stimulated deamination.[93] Nevertheless, only partial clearance of the cccDNA was attained in these studies.

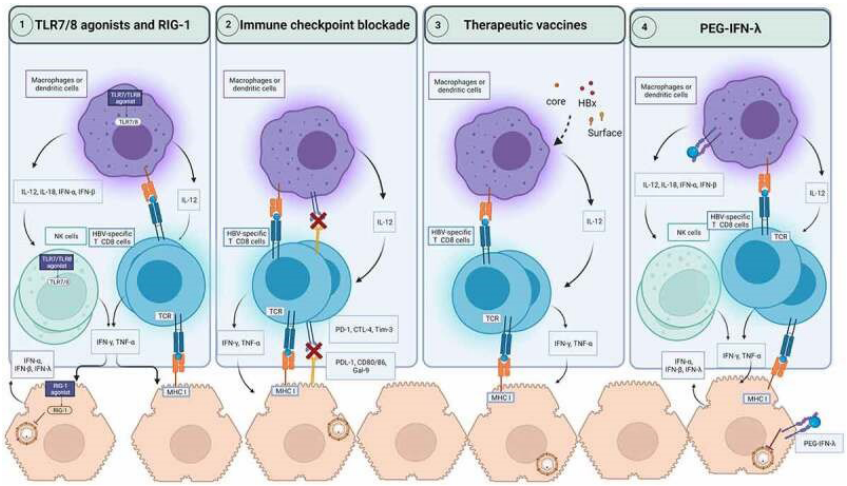
Genome editing technologies, for instance zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and more recently clustered regularly interspersed short palindromic repeats (CRISPR) RNA-guided nuclease(s)/CRISPR-associated 9 (Cas9), possess the capacity to halt the generation of cccDNA and restrict its accumulation in the nucleus. Such editing strategies generate DNA double-strand brakes (DBSs), which stimulate the activation of invalid healing of cccDNA by non-homologous end joining (NHEJ) pathways and stimulate the generation of insertions and deletions resulting in the breakdown of open reading frames (ORFs) of genes. Intriguingly, gene editing by itself can further breakdown the cccDNA.[94] A plethora of preclinical studies have illustrated that CRISPR/Cas9 possess the maximum efficacy of these platforms in cleavage and activation of cccDNA in hepatoma cell lines and hemodynamically injected mice with success.[95] Nevertheless, problems regarding genomic instability in view of host DNA cleavage and off-target actions and botherations—for instance, greater HBV genome heterogeneity, absence of administration specificity, and previously present immunity to Cas9—have resulted in the avoidance of gene editing from reaching the clinical generation.[96] Greater research is being conducted to get over these restrictions.[95,97] Intriguingly, an innovative CRISPR-derived base editor technology is evaluating introducing point mutations in cccDNA and incorporating HBV DNA fragments without the induction of the DBS.[98]

Greater research is the requirement for escalating insight into CccDNA biology and resulting in advancements in therapeutic approaches possessing curative probability.

IMMUNE DEPENDENT TREATMENTS

From the natural spontaneous rectification of HBV infection, we have learned that the generation of robust and collaborative reactions from the innate and adaptive arms of the immune system is key to the long-term, consistent control of HBV infection.[14,99] Conversely, the propagation of CHB arises from impaired and exhausted antiviral immune reactions.[8] Thereby, boosting the immune system for the rectification of dysfunctional HBV immunity is the foundation for the generation of immunomodulatory therapeutic approaches (Figure 2).[100]

Figure 2. *Immune-based Therapeutic Strategies for CHB*[37]



The activation of pathogens recognition receptors such as TLR7, TLR8 and RIG-I by agonists can induce the secretion of antiviral cytokines by hepatocytes and innate immune cells such as macrophages, dendritic cells, and NK cells. This activation of innate immunity can restore HBV-specific T cells to produce antiviral cytokines such as IFN-γ and TNF-α. RIG-I can also exerts direct antiviral activities against HBV. Immune checkpoint blockade prevents the engagement of inhibitory checkpoints express on HBV-specific T cells with their ligands on macrophages, dendritic cells, and hepatocytes. As a result, HBV-specific T cells functionality can be restored to produce antiviral cytokines and potentially cytotoxic response to clear infected hepatocytes. Therapeutic vaccines encoding for viral antigens, can enhance cross-presentation of these antigens and boost HBV-specific T cell response. PEG-IFN-λ therapy can modulate dendritic cells, and macrophages and mediate the cross talk with NK and HBV-specific T cells. In hepatocytes, PEG-IFN-dddd induces the engagement of signaling pathways which suppress HBV replication.

Innate Immune System Activation

HBV possesses the capacity for recognition by pattern recognition receptors (PRR), including the toll-like receptor (TLR) and retinoic acid-inducible-I (*RIG-I*) gene.[100] Occupation of PRR results in the activation of particular downstream signaling processes leading to type I and type III IFN reactions and other proinflammatory cytokines and chemokines, which might stimulate the activation of NK-cells and facilitate the replenishment of HBV-specific adaptive immune reactions.[101] Nevertheless, the virus has cleverly formed a plethora of ways of hampering PRRs signaling pathways in order to flee from immune surveillance.[102] In view of that, attempting pharmacological stimulation of PRRs is believed to be an approach for reactivation of both the innate and adaptive arms of the immune system for the clearance of viruses.[1,73]

Toll-like Receptor

Toll-like Receptor-7 (TLR7) and TLR8 portray endosomal sensors of single-stranded RNA molecules that are expressed by hemopoietic cells, for instance, Bcells, monocytes, macrophages, dendrite cells, NK-cells, and cytotoxic T-cells.[103,104]

Various TLR7-RO7020531, JNJ-4964, GS-9620 (alias Versatolimod), and TLR8-GS-9688 (alias Selgatolimod) agonists are currently undergoing assessment. RO7020531 possessed good tolerability and safety profiles in healthy persons taking part who have presently switched to a combination regimen with capsid assembly hampering agent RO7049389 in CHB patients.[105] GS-9620 (alias) stimulated robust antiviral actions in woodchucks and chimpanzees.[106] Nevertheless, although there was a robust preclinical reaction accompanied by noticeably enhanced NK-cells and HBV-specific T-cells, this was not correlated with a reduction of HBsAg.[107,108] A plausible reason for the elimination of the effectiveness of GS-9620 is the lesser dose delivered to patients as compared to chimpanzees (4 mg/patient *versus* 1 mg/kg in chimpanzees), with the idea of restricting inimical toxic sequelae to a minimum. TLR7 agonist JNJ-4964 was evaluated in AAV/HBV mice for a time period of 12 weeks and resulted in considerable repression of HBV DNA and HBsAg quantities and sustenance of generation of HBsAg.[109] Further evaluation of JNJ-4964 was conducted on healthy subjects. It displayed greater safety and tolerability as a single dose and stimulated transitory generation of IFNα, IP-10, monocyte chemoattractant protein 1 (MCP1), interleukin-1 receptor (IL-1RA), and ISG.[110] Administration of TLR8-agonist GS-9688, alias Selgatolimod, was done at a dose of 1.5 mg/3 mg to virally repress HBeAg+ CHB patients.[111] The latest outcomes obtained reported that none of the patients attained a greater than 1 log IU/mL reduction in HBsAg quantities, and just 6% attained ≥0.5 log IU/mL by the cessation of the treatment. Moreover, HBeAg seroconversion took place in just 16% of HBeAg+ patients. Immunological evaluation reported a transitory dose-based induction of circulating IFNγ in patients who got GS-9688 treatment, whereas there was a diminished frequency of circulating CD+3 T-cells plausibly in view of them relocating to the liver. Additionally, GS-9688 evaluation was performed in combination with TAF and illustrated that stimulation of healthy and CHB PBMC with GS-9688 caused activation of NK-cells, HBV-specific T-cells, and mucosal-associated invariant T-cells (MAIT), but diminished regulatory T-cells and monocytic monocyte-derived suppressor cells (MDSC), two cell subkinds acknowledged for immunorepressive features. On the other hand, GS-9688 escalated the immunorepressive working of MDSCs, which might explain its restricted effectiveness in certain patients.[112]

Retinoic acid-inducible gene I

Retinoic acid-inducible gene I (RIG-I), an intracytoplasmic double-stranded RNA sensor, is further a target for the pharmacological activation of immune reactions to CHB.[101] Akin to TLR"s once activation takes place, RIG-I results in signal transduction *via* intracellular signaling pathways that stimulate the generation of IFN and other cytokines. Furthermore, in a recent study, it was demonstrated that the recognition epsilon encapsidation signal observed in pgRNA was further observed in RIG-I, facilitating the generation of type III IFN instead of type I IFN.[113] This type III IFN induction, alias IFN (lambda), points to its robust antiviral actions, as this cytokine is acknowledged for hampering HBV replication directly and activating innate and adaptive immunity in CHB patients.[114,115] Furthermore, RIG-I can disrupt the epsilon HBV polymerase crosstalk, directly repressing HBV replication. Thereby, RIG-I apparently possesses a double role as an innate immune reaction modulator and a direct effector against HBV. The oral RIG-I agonist SB9200 (inarigivir) was delivered as monotherapy with a subsequent switch to TDF in HBeAg+ and HBeAg-CHB patients. Reduction of both HBV DNA and HBV RNA occurred in both groups in a dose-based fashion, whereas HBsAg was diminished by greater than 0.5 log IU/mL in 22% of the patients. Conversely, in a dose-ranging study with TAF, no dose-based alterations in viral load or HBsAg were seen.[116] Nevertheless, all the trials of the SB9200 have been discontinued in view of unanticipated inimical sequelae, inclusive of hepatocellular impairment and escalated quantities of ALT in 3 cases and 1 death in the phase 2 CATALYST trial. The modes behind these robust, inimical sequelae are being evaluated. This reemphasizes the significance of taking safety into account in the generation of innovative agents as therapeutic agents for the treatment of CHB. Noticeably, immune evasion modes utilized by HBV include the repression of expression of PRR's on hepatocytes, Kupffer cells (KC's), and hematopoietic cells, which might explain the diminished effectiveness of PRR's agonists in CHB.[117] What is more promising is that recent studies have revealed that antivirals, in particular PEG-IFNα, can restore the expression of TLRs, and this might corroborate the utilization of combination approaches with PEG-IFNα.[118]

BLOCKADE OF IMMUNE CHECKPOINT

Immune checkpoint receptors (ICRs) constitute master controllers of the immune system.[17,119] For the sustenance of self-tolerance, avoidance of autoimmunity, and modulation of an immune reaction that is sufficient in fighting not only infections but malignancies as well, there is a requirement of hampering and stimulatory ICRs. Modulation of ICRs, particularly hampering/inhibitory checkpoints (IC's), has brought about a revolution in the field of cancer. Regarding CHB having the characteristics of escalated quantities of antigens and proinflammatory cytokines, programmed cell death (PD-1), cytotoxic associated antigen 4 (CTLA4), T-cell immunoglobulin and mucin domain containing 4 (Tim 4), etc., chronically repressed immune working facilitates HBV infections becoming persistent. The overexpression of such IC's is attractive for an archetype where the active immune system stimulates pathogenesis and conserves the working and structural integrity of organs; however, it is incapable of stimulating an efficacious HBV-specific reaction. Evans et al[17] were the first to illustrate that, at the time of CHB, PD-1 upregulation was correlated with HBV-specific T-cell impairment.[17] Additionally, PD-1 had an association with viremia and HBeAg with a progressively decreasing relationship with the NA treatment and further so at the time of HBeAg seroconversion. Therefore, IC's blockade might reverse HBV's particular immune impairment and cause a replenishment of immune reactions with the capacity to clear CHB.

Thus far, studies assessing anti-PD-1 blockade have revealed outcomes that have not been favorable. In a small study evaluating the low dose of nivolumab in virally repressed HBeAg-CHB patients, a functional cure was attained in just 1/14 patients, and a minimum reduction in HBsAg was found.[120] Nevertheless, there was good tolerability. On immunological evaluation, no alterations in T-cell reactions were revealed over the time period. Taking into account the substantially broader IC's impairment on the immune cells and broader ranges of molecules, it is not astonishing that a single IC's blockade resulted in restricted effectiveness. Greater doses, concurrent blockade of the other IC's, and combination treatments with the innovative agents might be approaches for facilitation of T-cell replenishment. Nevertheless, it has to be kept in mind that robust immune reactions and considerable hepatotoxic processes have been displayed broadly as robust inimical sequelae correlated with IC's blockade at the time of anti-cancer immunotherapy.[121]

ACTIVATION OF THE ADAPTIVE IMMUNE SYSTEM DIRECTLY

The Interferon System

As detailed previously, recommendations for PEG-IFNα have been made in the form of the 2nd line of therapy in view of its correlation with inimical sequelae and restricted effectiveness in patients. Nevertheless, although there are these concerns, it is only recommended for a defined time period and is now teamed up with PEG-IFNα and the other antiviral agents in clinical development.

IFNλ, whose addition has been done in the IFN family, has been taken into account as the better alternate for CHB.[122] There is remarkable sharing of characteristics between IFNλ and IFNα. The viral infection possesses the capacity to induce these IFN's, causing activation of the antiviral immune reactions *via* stimulating signaling pathways, for instance, JAK/STATs, and induing the IFN-stimulated genes (ISG). The basic discriminating point amongst the two cytokines is as per the organization of their receptors, which determines the tropism of these cytokines-stimulated immune reactions. Whereas IFNα receptors have universal expression, that of IFNλ has restriction to just epithelial cells and immune cells, therefore being correlated with lesser inimical sequelae as compared to IFNα.

Assessment of PEG-IFN λ was performed in two studies. In the case of head-to-head contrasting of the PEG-IFNα

(LIRA-B 2a) in HBeAg-CHB patients, PEG-IFNλ illustrated significantly greater diminished viral loads, and that of HBsAg following treatment and HBsAg seroconversion was equivalent in both arms of the study.[116] Nevertheless, PEG-IFN λ did not illustrate superiority over PEG-IFNα-24 weeks post-treatment; however, it illustrated better safety profiles in view of its receptor-restricted organization. The immunological and molecular modes behind these discriminating actions are not clear, requiring further evaluation.

Another trial with a lead in ETV with subsequent ETV and PEG-IFN λa combination was performed parallelly (LIRA-B 2b). Longitudinal immunological surveillance was conducted, which illustrated that PEG-IFN-a possessed the capacity to facilitate potent NK-cells and HBV-specific T-cells working in patients who had a greater reduction in viremia and antigenemia.[114] This was as compared to PEG-IFNα, which possessed inimical actions on T-cells. To our misfortune, this study got interrupted prematurely in view of the fact that PEG-IFN λa did not meet the non-inferiority criteria for the LIRA-B 2a study. Currently, PEG-IFN λ has been repurposed for HDV treatment in the form of monotherapy or in combination with a farnesyl transferase-honoring agent or an antiretroviral, which illustrates a significant HDV RNA decline in cases of HBV/HDV co-infection.[123]

Clarification is existent from these studies that conclusions can be drawn regarding the antiviral actions of PEG-IFN λa against HBV that are substantially different from those of PEG-IFNα. Achieving insight into the modes behind these actions might aid in the garnering of IFNλa and get over these restrictions of this treatment.

THERAPEUTIC VACCINATION

Therapeutic vaccines need to vary from their prophylactic cousins in addition to restoring dysfunctional T-cell reactions at the time of priming a newer immune reaction, inclusive of the humoral arm. Robust preclinical outcomes obtained in animal models corroborated the utilization of therapeutic vaccines for CHB.[124] To our misfortune, this did not get corroborated in CHB patients. For instance, heat-inactivated yeast-based vaccines that encode for HBsAg, HBcAg, and HBx, GS4774, did not result in any extra diminishing of HBsAg as compared to the ones who got NA's.[125] Although there were unfavorable outcomes, GS4774 did result in the escalation of IFN γ, TNF-α, and IL-2 by HBV-specific CD8+T-cells; however, nothing was close to the reactions observed in cases of acutely restricted infections.[125] This restricted effectiveness might be explained by greater HBsAg circulating quantities in CHB patients.

ABX-203 (Heber Nasvac), an intranasalvaccine possessing HBsAg and HBcAg, was evaluated in a phase 3 study and contrasted to lone PEG-IFNα. At the time of the cessation of treatment, there was an equivalent repression of HBV DNA in both groups, whereas in the follow-up phase, 57.7% of patients having vaccination possessed a viral load under 250 copies per ml, in contrast to 35% in the PEG-IFNα group.[126] Furthermore, greater seroconversion took place more commonly in the group that had a vaccination. To our misfortune, no HBsAg quantification was tried; however, quantitative evaluation displayed no elimination of HBsAg in either group.

INO-1800 portrays a DNA-dependent vaccine that encodes for IL-12 in virally repressed CHB patients. A good safety profile and improvement in the IFN-γ HBV-specific T-cell reaction were declared recently.

The therapeutic vaccine BR II-179, a virus-like particle that encodes for the 3 HBsAg proteins assessment, was recently conducted in a phase 1b/IIa study, and multiple doses were delivered with or without IFNα in the case of NA-repressed CHB patients.[127] BR II-179 evoked a humoral reaction in all the ones having vaccination; however, maximum robustness was determined against S protein in 30% of the patients getting a single dose. A more moderate reaction was determined against pre-S1 and S2, however, just in the IFNα arm. An immunological subevaluation was performed in a smaller cohort. There was a significant escalation of IFN-γ-generating T-cells in 3/8 patients from the IFN combination arm; however, no significant alterations were found in the other study arm. Intriguingly, patients getting maximum vaccination doses illustrated greater potent immune reactions. Although in BR II-179 the activation of humoral and cellular reactions took place, no significant alterations in circulating HBsAg, HBV RNA, or HBcrAg took place, pointing out that induced immunity was not robust for action.

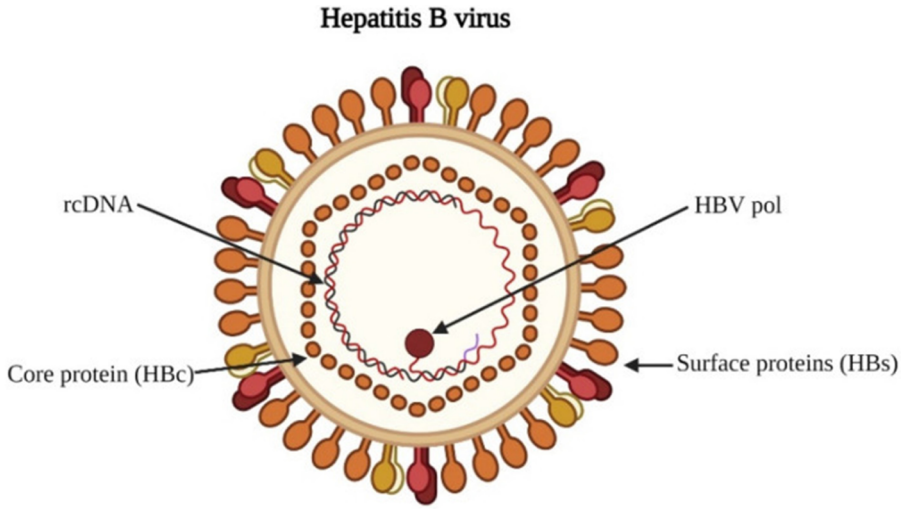
Non-replicative adenoviruses, for instance, TG-1050, encode a fusion protein comprised of HBV polymerase, HBcAg, and HBsAg. Virally repressed CHB patients got either a single dose or three doses of the vaccine. TG-1050 had greater tolerability; however, a minimum reduction of HBsAg took place. Immunologically, TG-1050 stimulated IFN-γ HBV-specific T-cell reactions against all three HBV virus antigens.

The single main restriction of therapeutic vaccines is immune reactions going against the vector backbone, which possesses the capacity to abrogate HBV-specific reactions and prohibits multiple dosages. A heterologous prime boost vaccine strategy might be the answer.

Hepatitis B virus (HBV) infection is a global public health problem that is intricately correlated with liver cirrhosis and HCC. The prevalence of acute and chronic HBV infection, liver cirrhosis, and HCC has significantly diminished since the introduction of purifying hepatitis B surface antigen (HBsAg) from the plasma of asymptomatic HBsAg carriers. Following that, recombinant DNA technology resulted in the generation of a recombinant hepatitis B vaccine. Despite the accessibility of various licensed vaccines for HBV infection, the persistence of research is imperative for the generation of further efficacious vaccines. Prophylactic hepatitis B vaccination has been significant in the avoidance of hepatitis B in view of its efficaciously generated protective immunity against hepatitis B viral infection. Prophylactic vaccines just have the requirement of invoking the produc-

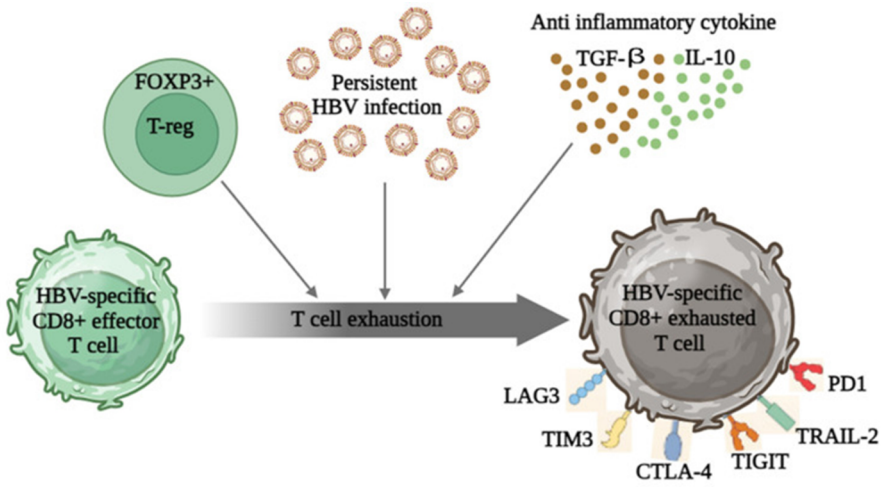
tion of neutralizing antibodies against HBV envelope proteins, while therapeutic vaccines have a greater probability of inducing exhaustive T-cell reactions and therefore need to be inclusive of other HBV antigens, for instance, HBV core and polymerase. The existing vaccines have been validated to be highly efficacious in avoiding HBV infection; however, the objective of continuing research is to enhance their effectiveness, the time period of protection conferred, and their availability. The routine delivery of the HBV vaccine is safe and well-tolerated worldwide. The idea behind this kind of immunization is to trigger an immunological reaction in the host, which will stop HBV replication. The clinical effectiveness and safety of the HBV vaccine are impacted by a plethora of immunological and clinical factors. Nevertheless, this success is now in peril in view of the breakthrough infections caused by HBV variants with mutations in the S gene, greater viral loads, and virus-induced immunosuppression. Mehmood et al[128] have detailed a recent update on the various kinds of available HBV vaccines and recent progress in the ongoing battle to produce new vaccines against HBV (Figures 3, 4, and 5).

Figure 3. *Basic Structure of HBV Virion*[128]



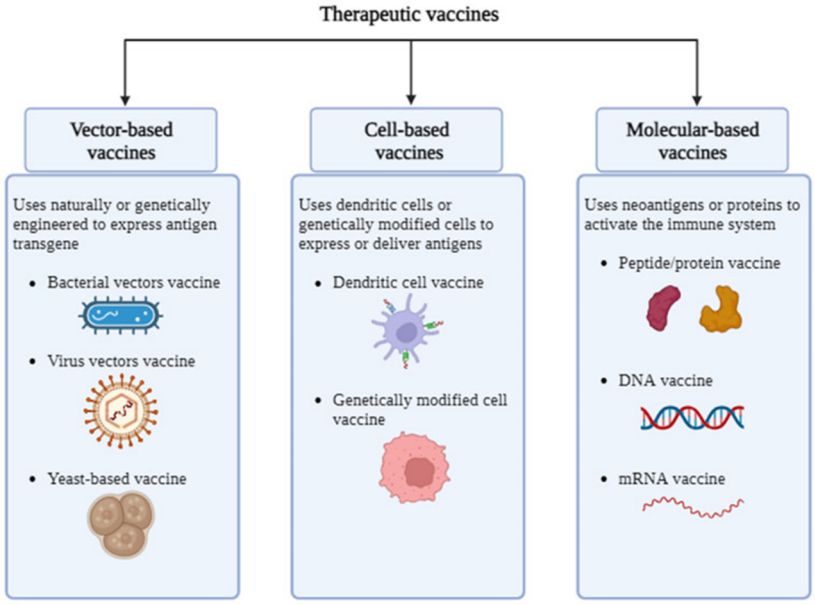
The inner protein shell of the virus, known as the main particle or "HBcAg," contains the viral DNA and enzymes responsible for replication, commonly referred to as "DNA polymerase." On the other hand, the outer membrane, composed of lipid and protein, is called the "surface antigen" or "HBsAg." rcDNA stands for "relaxed circular DNA" and HBV pol stands for "HBV polymerase".

Figure 4. *Dysregulation of Immune Response after HBV Infection*[128]



Persistent HBV infection causes HBV-specific CD8+ T cells to exhaust themselves. The death receptor TRAIL-2 is upregulated in exhausted CD8+ T lymphocytes, which show reduced proliferation and are more likely to undergo apoptosis. When CD8+ T cells run out, their effector activity diminishes, but they remain partially activated, leading to hepatocyte injury that persists, repeated DNA damage, genomic instability, mutation accumulation, and ultimately neoplastic transformation. Furthermore, a tumor-prone immunological milieu is produced when worn-out HBV-specific CD8+ T cells express a number of inhibitory molecules, including PD-1, TIGIT, CTLA-4, TIM3, and LAG3. These factors also impair CD8+ T cells' ability to perform immune surveillance.

Figure 5. *Schematic Presentation of Basis of Therapeutic Vaccines Development*[128]



There are various types of therapeutic vaccines, such as vector-based vaccines, molecular-based vaccines, and cell-based vaccines.

COMBINATION TREATMENT AND UPDATE ON RECENT THERAPIES

Recognizing how complicated HBV replication is, in addition to the considerable immune paralysis in the form of the properties of CHB, combination treatment with the substances having separate modes behind their actions is apparently a favorable therapeutic strategy for the acquisition of a cure. Synergism in actions with the standard of careNA's and PEG-IFNα are the ones most evaluated; however, innovative substances in clinical generation are further undergoing assessment with the further combination of the pharmaceutical companies for the amplification of their research endeavors. For instance, studies evaluating the safety in addition to effectiveness of the combination treatment of capsid hampering agent ABI-H0731 and RNAi AB 729 and TLR8 agonist and RNAi VIR-2218 are undergoing ongoing trials.

A phase 2 triple combination therapy capsid hampering agent JNJ-6379, siRNA JNJ-3989, and NAs were displayed to cause a 1.01-2.26 log IU/mL HBsAg decrease in all the patients correlated with significantly diminished HBV DNA, however, demonstrated minimal actions on HBeAg and HBcrAg.[129] Numerous other combination therapies are being evaluated (for instance, RO7049389 from the HAPS family and TLR7 agonists RO7020531 and NA).

Ogunnaike et al[130] recently updated the treatment of CHB, summarizing that the avoidance of end-stage liver diseases correlated with chronic HBV is dependent on antiviral therapy with the aim of achieving a cure, which is basically based on suppressing the pool of cccDNA as well as HBsAg. The HBV cure strategy can be widely defined into two kinds, as detailed previously: functional cure and sterile cure. A functional cure for HBV involves the elimination of HBsAg, with or without the generation of antibodies against HBsAg, in addition to serum DNA and the persistence of undeterminable cccDNA with low or nil transcriptional activity, enabling the cessation of treatment. In a functional cure, the activated immune system needs to have the capacity to regulate the remnants of the infected cells. Nevertheless, a sterile cure for HBV would require total depletion of the cccDNA.[131,132]

Attainment of HBV functional cure concentrates on depleting HBsAg as well as repression or depletion of cccDNA. Recommended IFN/PEG-IFN therapy mainly possesses immunostimulatory actions and has the capacity to induce HBsAg negativity; however, the success rate is minimal and inimical sequelae are frequent. Alternatively, HBsAg-liberating hampering agents might be further used to avoid the liberation of HBsAg. Taking into account that HBsAg might further use classical host-cell liberating pathways, this strategy may also be unsuccessful. Nevertheless, Rep2139, a hampering agent of HBsAg liberation, when combined with PEG-IFN-α, stimulates a significant decrease in viremia as well as seroconversion in favorable HBsAg responders.[133,134]

NAs goal is to hamper HBV DNA replication in addition to decreasing HBV DNA and ALT quantities, but rarely decrease the cccDNA pool in the liver. Although currently approved NAs do not possess the capacity to acquire a functional cure, by themselves, the chances of attaining a long-lasting functional cure for hepatitis B with no rebound subsequent to cessation of therapy would be higher using a combination of immunomodulators, such as IFN-α, and two or more antivirals that would

possess the capacity to target various steps in the virus replication cycle. A combination of virus entry blockers in addition to maturation-honoring agents, for instance, oral CAMs, might be considerably efficacious regarding this.[135] A combination of NAs as well as CAMs might be considerably more efficacious in their capability to target key steps in the HBV replication cycle,[136] as demonstrated in Figures 6 and 7.

When patients need treatments, the liver cccDNA pool is already generated. In view of its long half-life and the absence of massive liver regeneration, it has been mathematically modeled that long-term treatment with NAs would be imperative to significantly impact this pool.[137,138] The use of long-acting antiviral drugs might be beneficial in that patients would have a steady-state plasma quantity of antiviral drugs, allowing for easier compliance. Attaining a functional cure, for instance, through undeterminable serum HBV-DNA and HBsAg elimination, is correlated with excellent long-term results.[137] Preclinical studies displayed that LA medicine is considerably more efficacious for long-term viral suppression in contrast to oral medication.[139,140] Long-term viral repression using LA formulations can further provide a predictable panorama of cccDNA (Figure 7).

CONCLUSIONS

The road to acquiring a greater functional cure rate for HBV and avoiding end-stage liver diseases is challenging. It is apparent that neither antiviral medications nor immunomodulators possess the capacity to attain a functional cure on their own, indicating that further enhancements are essential for escalating the probability of a functional cure. These enhancements might include developing long-acting medications to provide sustained viral reppression, increasing the chances of efficaciously reducing HBsAg concentration, and silencing the cccDNA. Bao et al[141] reviewed as well as summarized recent advancements in addition to hurdles in their endeavors to inactivate, silence, or eliminate viral cccDNA using anti-HBV agents from sources such as small molecules (including epigenetic drugs), polypeptides or proteins, siRNA, or gene-editing approaches targeting or attenuating HBV cccDNA *via* several modes, as well as future directions that might be taken into account in efforts to truly cure chronic HBV infection. In conclusion,no breakthrough has been made yet in ameliorating HBV cccDNA, despite the plethora of candidates that have advanced into the phase of clinical trials. Moreover, a considerable number of substances work to indirectly target HBV cccDNA.

Figure 6. *(1) Therapeutic Interventions for the Prevention of End-stage Liver Diseases by Effective Suppression of HBV Replication and (2) Elimination of Viral DNA Products*[130]

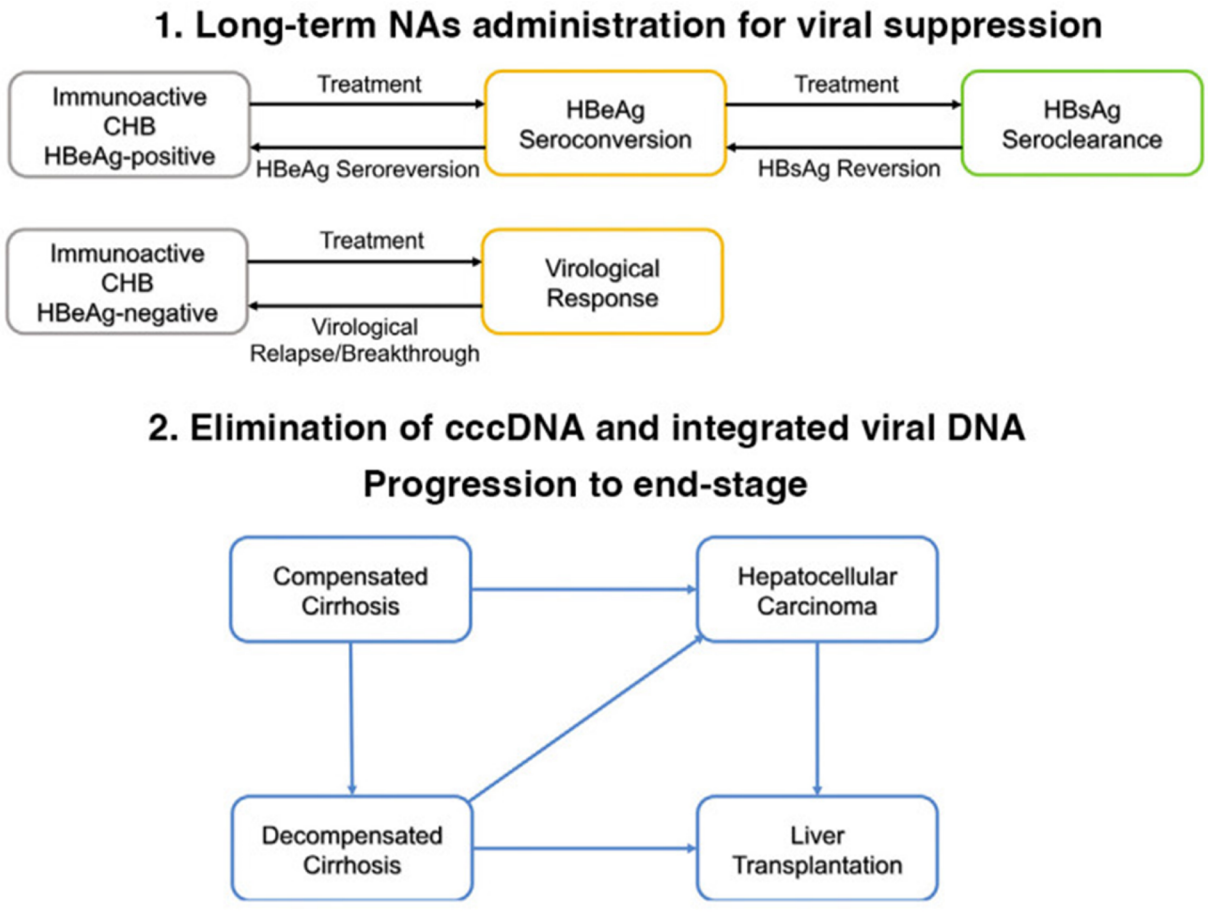
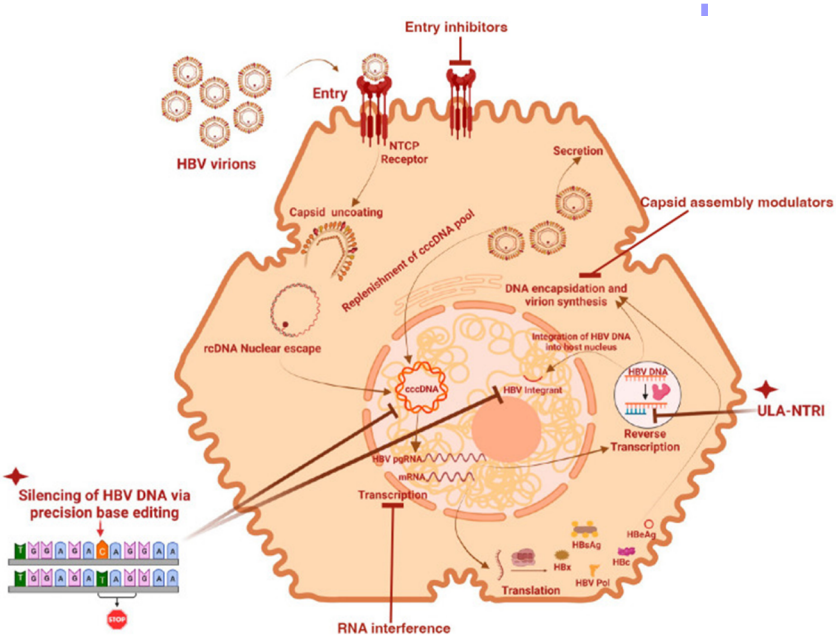


Figure 7. *Current Therapeutic Strategies for HBV Treatment Aim for a Comprehensive Approach to Eradicating the Virus and Cure*[130]



These include entry inhibitors that block the NTCP receptors, preventing viral entry into hepatocytes; base editing tools selectively rewrite persistent HBV cccDNA and integrants rendering them into dysfunctional phenotypes; RNA interference targets HBV RNA to prevent its translation into viral proteins; capsid assembly modulators block the encapsidation of Pol-pgRNA and cccDNA replenishment; and ultralong-acting nucleotide reverse transcriptase inhibitors (ULA-NTRI) directly inhibit reverse transcription of HBV RNA, thereby providing sustained suppression of HBV DNA synthesis and chromosomal DNA integration. Figure created with BioRender.

No outstanding substance possesses the capacity to directly target HBV cccDNA. In a general sense, CCC_R08, in addition to nitazoxanide, might be some of the most favorable compounds to clear HBV infection in small-molecule agents. Furthermore, CRISPR-Cas9 systems possess the capacity to directly target HBV cccDNA for decay as well as illustrate significant anti-HBV activity. Sequentially, gene-editing targeting HBV cccDNA might be one of the most attractive means for attaining the core goal of anti-HBV therapeutic approaches. Basic studies on HBV infection are required to be performed to get over these hurdles.[141]

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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