

REVIEW

# Novel approaches towards conquering hepatitis B virus infection

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## Abstract

Currently approved treatments for hepatitis B virus (HBV) infection include the immunomodulatory agent, IFN- $\alpha$ , and nucleos(t)ide analogues. Their efficacy is limited by their side effects, as well as the induction of viral mutations that render them less potent. It is thus necessary to develop drugs that target additional viral antigens. Chemicals and biomaterials by unique methods of preventing HBV replication are currently being developed, including novel nucleosides and newly synthesized compounds such as capsid assembling and mRNA transcription inhibitors. Molecular therapies that target different stages of the HBV life cycle will aid current methods to manage chronic hepatitis B (CHB) infection. The use of immunomodulators and gene therapy are also under consideration. This report summarizes the most recent treatment possibilities for CHB infection. Emerging therapies and their potential mechanisms, efficacy, and pitfalls are discussed.

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## INTRODUCTION

The hepatitis B virus (HBV) is a major world health problem, leading to 1.2 million deaths per year according to the World Health Organization (WHO)<sup>[1]</sup>. HBV

infection can result in acute, fulminant, or chronic disease, liver cirrhosis, and the development of hepatocellular carcinomas (HCC). There is a vaccine, but no 100% effective antiviral treatment available for patients with chronic hepatitis B (CHB). The response rate to IFN therapy, as measured by the loss of hepatitis B e antigen (HBeAg), is less than 40%<sup>[2]</sup>. This treatment is even less effective in Asian patients (primarily Chinese), particularly for those with below normal alanine transaminase (ALT) levels<sup>[3]</sup>. IFN therapy is also associated with many disabling side effects and is therefore only suitable for some patients.

Since HBV DNA replication occurs via reverse transcription<sup>[4]</sup>, the use of reverse transcriptase inhibitors is an attractive target for anti-HBV therapy. Nucleoside analogues are chemically synthesized drugs that mimic natural nucleosides. In China, three nucleoside/nucleotide drugs are used to manage chronic HBV infection: lamivudine (3TC), adefovir dipivoxil (ADV), and entecavir (ETV). Although all three are potent viral suppressors, none is able to permanently eradicate HBV<sup>[5]</sup>. As a result, the durability of the antiviral response is suboptimal once treatment is halted. In some patients, HBV DNA levels and ALT concentrations increase and result in a potentially life-threatening recurrence of disease<sup>[6,7]</sup>. Patients can only be safely withdrawn from nucleos(t)ide therapy if HBeAg seroconverts to anti-HBe or HBV DNA diminishes to undetectable levels<sup>[8,9]</sup>. To prevent disease recurrence, long-term polymerase inhibitor maintenance therapy is often required<sup>[10]</sup>. In addition, prolonged use of nucleoside/nucleotide is associated with the emergence of drug-resistant mutants<sup>[11,12]</sup>, and clinically characterized by increasing serum HBV DNA and ALT levels<sup>[10,13]</sup>. Each drug has a different profile of resistant mutations<sup>[14]</sup>, so it is essential that each is appropriately managed. These findings underscore a requirement for new and better-tolerated therapies for hepatitis B virus infection.

In this report, we review different strategies for drug design, and evaluate their effectiveness *in vitro*, in models of HBV replication *in vivo*, and in clinical trials.

## NUCLEOSIDE ANALOGUES

Orally applied nucleoside and nucleotide analogs have been important therapies against HBV infection throughout the last decade. The nucleoside analogs, lamivudine and entecavir, and the nucleotide analog, adefovir dipivoxil, are approved for use in humans. Many similar compounds are being tested in preclinical or clinical settings (Table 1).

Table 1 Anti-HBV nucleoside/nucleotide analogues under development

Phase	Drugs	Company
Phase III	Emtricitabine (FTC)	Gilead (California, USA)
	Tenofovir DF	Gilead (California, USA)
	Telbivudine (L-dT)	Idenix (Massachusetts, USA)
Phase II	Clevudine (L-FMAU)	Gilead/Triangle (California, USA)
	Elvucitabine ( $\beta$ -L-Fd4C)	Achillion (Connecticut, USA)
	Valtorcitabine (val-L-dC)	Idenix (Massachusetts, USA)
	Amdoxovir (DAPD)	Triangle (California, USA)
	Racivir [(+/-)-FTC]	Pharmasset (New Jersey, USA)
Phase I	LB80380	LG Life Sciences (Seoul, Korea)
	Alamifovir (purine nucleoside analogue)	Lilly/Mitsubishi (Indiana, USA/Osaka, Japan)
	MIV 210 (FLG prodrug)	Medivir/GSK (Huddinge, Sweden/Brentford, UK)
	Hepavir B (PMEA prodrug)	Ribapharm (California, USA)
Pre-clinical	$\beta$ -L-FddC	Biochem/GSK (Sante-Foy, Canada/Brentford, UK)
	6-[2-(phosphonomethoxy) alkoxy]-2, 4-diaminopyrimidines	Rega Institute for Medical Research, K.U.Leuven (Leuven, Belgium)
	2-benzenesulfonylalkyl-5-substituted-sulfanyl-[1, 3, 4]-oxadiazoles	National University of Singapore (Singapore)

### EMTRICITABINE (FTC)

Emtricitabine is a nucleoside analogue used for treatment against human immunodeficiency virus (HIV) and also has clinical activity against HBV. It has a similar structure to lamivudine, differing only in a fluorine at its 5' prime end. In a randomized double-blind study, patients received 200 mg of emtricitabine ( $n = 167$ ) or a placebo ( $n = 81$ ) once daily for 48 wk and underwent a pretreatment and end-of-treatment liver biopsy. Following treatment, 62% of patients who had received emtricitabine had improved liver histology, while only 25% of the placebo patients showed improvement ( $P < 0.001$ ). Significant improvement was also demonstrated between subgroups that were positive ( $P < 0.001$ ) and negative ( $P = 0.002$ ) for hepatitis B e (HBe) antigen. Serum HBV DNA levels were below 400 copies/mL in 54% ( $n = 167$ ) of the emtricitabine group and only 2% ( $n = 81$ ) of the placebo group ( $P < 0.001$ ), while alanine aminotransferase levels were normal in 65% (109/167) of the emtricitabine group and 25% (20/81) of the control group ( $P < 0.001$ ). At wk 48, 20 of 159 patients (13%) from the emtricitabine group in whom HBV DNA was detected at the end of treatment, had virus with resistance mutations (95% confidence interval, 8%-18%). The rate of seroconversion to anti-HBe (12%) and loss of HBe antigen were not different between arms, and the safety profiles of emtricitabine and placebo were similar during treatment. Forty-eight weeks of emtricitabine treatment resulted in significant histologic, virologic, and biochemical improvement in chronic HBV infected patients, regardless of whether HBe antigen was detectable<sup>[15]</sup>.

Phase III clinical trials are underway to determine the long-term safety and efficacy of emtricitabine, however its role as a monotherapy may be limited by its structural similarity to lamivudine and the corresponding risk of drug resistance.

### TENOFOVIR (VIREAD, PMPA)

Tenofovir was FDA approved in 2001 for use in HIV infected adults in combination with other antiretroviral agents. Lamivudine-associated and ADEFOVIR-resistant

mutations were not detected when tenofovir was used in a clinical trial. Thus, tenofovir may be a highly effective rescue drug in HBV-infected patients who show altered responsiveness to lamivudine and ADEFOVIR<sup>[16]</sup>. An additional double-blind, placebo-controlled trial showed that tenofovir may be a useful component of antiretroviral therapy for HIV/HBV co-infected patients. Importantly, tenofovir is equivalent to adefovir in its ability to reduce HBV DNA levels, and may, in fact, be superior<sup>[17]</sup>. If HBV treatment can be deferred until combination antiretroviral therapy for HIV infection is needed, the combination of tenofovir plus lamivudine or emtricitabine will be the potent HBV therapy and a solid backbone for HIV combination antiretroviral therapy, and a potent treatment for HBV and it likely decreases the emergence of HBV resistance. It will decrease the chance that HBV resistance will emerge as well<sup>[18]</sup>.

### CLEVUDINE (L-FMAU)

Clevudine is a nucleoside analog with an unnatural beta-L configuration, and *in vitro* studies suggest that it is effective against lamivudine-resistant HBV mutants. In the Woodchuck model, a daily clevidine dose of 10 mg/kg resulted in a 100 million copies' decrease in viral load. Interestingly, a delayed rebound in viral load was observed after drug cessation in a dose-dependent manner. No evidence of clevidine toxicity was observed in treated animals, however, further studies are being conducted to assess its long-term efficacy and safety<sup>[19]</sup>. Clinical trials show that clevidine is one of the most potent analogs available for treating HBV, and that its antiviral effects can last up to 6 mo after treatment, as illustrated by sustained normalization of ALT levels<sup>[18]</sup>. The mechanism by which clevidine elicits its anti-hepadna virus activity is distinct from other nucleoside analogs. It acts as a competitive inhibitor by binding to the catalytic site of HBV polymerase and inhibiting the priming of HBV DNA chain elongation. Nucleoside inhibitors, in general, interfere with viral polymerase activity through competitive inhibition and incorporation into the viral DNA strands<sup>[20]</sup>.

## TELIVUDINE (LdT)

Telivudine is a novel nucleoside analog that is being developed for the oral treatment of chronic HBV. It is a highly specific and selective inhibitor of replication *in vitro*, and specifically targets the HBV DNA polymerase. Unlike other nucleoside antivirals, telivudine does not act against other viruses or induce mitochondrial toxicity by targeting mammalian DNA polymerases. Telivudine preferentially inhibits HBV second-strand (DNA-dependent) DNA synthesis, in contrast to LdC and lamivudine, which are first-strand (RNA-dependent) DNA synthesis inhibitors<sup>[21]</sup>.

Telivudine has a significantly higher rate of response than the standard HBV treatment, lamivudine, as well as superior viral suppression capability. It is generally well tolerated, with a low adverse effect profile, and no toxicity at its effective treatment dose.

Preclinical and clinical studies show that telivudine has good pharmacokinetic properties that support once-daily dosing, and are not affected by gender, food intake, or liver health. Patients with moderate to severe renal impairment do require dose adjustment, however, which is also necessary for other drugs of this class.

Phase II b clinical trial results illustrate that patients with chronic HBV who are treated with telivudine have significantly greater virologic and biochemical responses than those treated with lamivudine. Combination therapies revealed similar results to those obtained using telivudine alone. These data support the ongoing phase III evaluation of telivudine as a treatment for patients with chronic HBV<sup>[22]</sup>.

## OTHER NUCLEOSIDE ANALOGS

Additional nucleoside analogs that have favorable toxicity profiles and a promise of increased effectiveness against HBV are in various stages of clinical development. The phase III trials of emtricitabine, clevudine, tenofovir, and telivudine will help define the efficacy and safety profiles of these drugs, while the profiles of newer and more potent drugs like LB80380 remain to be confirmed. It is important to recognize, however, that many of these compounds share cross-resistance profiles with existing nucleoside analogues such as lamivudine, adefovir, and entecavir<sup>[23,24]</sup>. As a result, these drugs may not offer much advantage over current treatment regimens. Current research efforts are focusing on the development of drugs that offer low rates of resistance or little cross-resistance with other nucleoside analogues.

## NOVEL MOLECULAR TARGETS OF HBV THERAPY

Because HBV pol carries out the enzymatic functions of reverse transcription and DNA synthesis, it is the primary target of HBV antiviral development<sup>[25]</sup>. Nucleoside and nucleotide analogues are the primary class of antiviral agents used for this purpose. In recent years, several compounds that specifically attack molecular targets other than HBV pol have been identified, including inhibitors of HBV encapsidation and HBcAg translation. Encapsidation

occurs when the viral RNA, pol, and core are assembled into the nucleocapsid prior to viral replication<sup>[26]</sup>.

## HETEROARYLDIHYDROPYRIMIDINE (HAP)

The heteroaryldihydropyrimidines (HAPs), including BAY41-4109, BAY38-7690, and BAY39-5493, are a new class of antivirals that inhibit production of HBV virions. HAPs show more favorable (50% and 90%) inhibitory concentrations (IC<sub>50</sub> and IC<sub>90</sub>) than lamivudine in a cell-based HBV replication assay. They act as allosteric effectors, binding the HBV core protein and resulting in its degradation, which subsequently inhibits nucleocapsid formation<sup>[27]</sup>. HAPs inhibit HBV replication in a transgenic mouse model with an efficacy similar to that of lamivudine<sup>[28]</sup>. Since these drugs destabilize preformed capsids, they may be used to treat blood products in order to lower the HBV transmission rates. Thus, HAPs may become a valuable addition to anti-HBV therapy. None of them has yet been tested in humans, but the clinical trial results of Bay 41-4109 are expected.

## PHENYLPROPENAMIDES

The phenylpropenamides represent another group of compounds that inhibit encapsidation<sup>[29]</sup>. The phenylpropenamide derivatives, AT-61 and AT-130, are synthesized and shown to inhibit HBV replication. These agents inhibit encapsidation by directly preventing nucleocapsid formation, a mechanism distinct from that used by HAPs. In a cell-based replication system, the phenylpropenamides are not as potent as lamivudine in inhibiting HBV replication (the IC<sub>50</sub> is approximately 10 times higher), but are active against the lamivudine-resistant YMDD mutant<sup>[29,30]</sup>. These drugs are specific for HBV and have no activity against related viruses such as woodchuck hepatitis virus (WHV) and DHB. Although this class of compounds has a favorable toxicity profile, clinical trials are still required.

## HELIOXANTHIN ANALOGUES

Helioxanthin was originally isolated from the shrub, *Taiwania ctyptomeroioides*, and its derivative, 5-4-2, was synthesized in the laboratory. Helioxanthin and 5-4-2 belong to a class of small molecules that inhibit the HBV DNA as well as the HBV RNA and viral protein expression. Their structures are different from other anti-HBV compounds, suggesting that they may have a unique mode of action. Cheng YC *et al* found that helioxanthin and 5-4-2 inhibited HBV mRNA levels in HepG2 2.2.15, as well as the HBV transcripts, 3.5 kb and 2.4/2.1 kb. The HBV core protein also decreased after treatment. Anti-HBV activity was evaluated *in vitro* using the HBV stably transfected hepatoma cell lines, W10 (adr, wt) and DM2 (adr, rtL180M/rtM204V, lamivudine-resistant), and helioxanthin and 5-4-2 inhibited both wild type and mutated HBV. Since the core protein activates the pregenomic/pre C promoters, it is possible that the decrease in 3.5 kb transcript results from a lack of transactivation by the core protein. Helioxanthin and 5-4-2 profoundly inhibited

pregenomic/preC and preS/S promoter activity using a gene reporter system, suggesting that they target multiple steps of the viral life cycle. The detailed mechanism of action by this class of compounds is being explored<sup>[31]</sup>, and clinical trials are still required.

## GLUCOSIDASE AND PEPTIDE INHIBITORS OF CAPSID ASSEMBLY

The heavy glycosylation of HBV envelope proteins is important for viral assembly. As a result, specific glucosidase inhibitors have been developed to inhibit the assembly process. N-nonyl-deoxyojirimycin (N-nonyl-DNJ) is an inhibitor of N-linked glycan processing and the endoplasmic reticulum (ER) glucosidase. Researchers show the N-nonyl-DNJ has antiviral activity in the woodchuck model of HBV infection<sup>[32]</sup>. Another glucosidase inhibitor, N-nonyl-deoxygalactojirimycin (N-nonyl-DGJ), exerts its antiviral activity prior to viral envelopment, thus may prevent proper encapsidation of the HBV pregenomic RNA. These agents show promise in inhibiting viral replication using the WHV model, but toxicity may limit their clinical efficacy. Using a molecular approach to screen a phage display library, Dyson *et al* identified peptide aptamers that specifically interfere with the interaction between core particles and envelop proteins during assembly<sup>[33]</sup>. These peptides bind specifically to the tip of the core protein shell that comprises conserved amino acid residues within the nucleocapsid<sup>[34]</sup>. This is important because of the risk of drug resistance. One candidate peptide inhibited HBV replication in a cell-based assay and exhibited no toxicity.

These promising approaches underscore the importance of identifying other molecular targets that may be used in combination therapies.

## IMMUNOMODULATORY AGENTS

A variety of immunomodulatory therapies have been developed over the last few decades to manage CHB. These therapies are designed to eliminate the virus by activating either nonspecific host immune responses or HBV-specific CD4+ T helper and CD8+ cytotoxic lymphocytes<sup>[35]</sup>. The nonspecific modalities include the use of TLRs, thymosin, IFN- $\alpha$ , and IFN- $\gamma$ , and the specific modalities include dendritic cell and cytotoxic T-lymphocyte (CTL)-based therapies. In recent years, the APOBEC family has shown promise as an anti-HBV drug.

## APOBEC3G

To replicate efficiently, viruses must overcome innate defense mechanisms. Human APOBEC3G is a cytidine deaminase that represents one such barrier by conferring broad intracellular antiretroviral protection. This enzyme is packaged in virions and acts during reverse transcription to deaminate deoxycytidine residues to deoxyuridine (dU) within the growing minus-strand of viral DNA. These dU-rich reverse transcripts are either degraded or result in proviruses that are largely nonfunctional due to a G-to-A hypermutation. Most lentiviruses escape APOBEC3G

inhibition by expressing a protein, Vif, which prevents deaminase incorporation into the virion and triggers its proteasomal degradation. However, APOBEC3G is capable of blocking a wide spectrum of distantly related retroviruses. Turelli *et al* show APOBEC3G-mediated inhibition of HBV and DHBV DNA production in human HuH-7 hepatoma cells and avian hepatoma cells<sup>[36]</sup>. Thus, the viral and cellular interaction partners required for anti-hepadnaviral APOBEC3G action are conserved among these species. Rosler C *et al* found that core-associated HBV RNA is not reduced in the presence of A3G, and that wild-type levels of pgRNA associate with HBV core protein in the presence or absence of A3G<sup>[37]</sup>. Yang DL *et al* showed a dose dependent decrease in the levels of intracellular core-associated HBV DNA, however, as well as a decrease in the extracellular production of HBsAg and HBeAg following APOBEC3G treatment. The levels of intracellular core-associated viral RNA also decreased, but the expression of HBcAg in transfected cells remained the same. Consistent with these *in vitro* results, levels of HBsAg in the sera of mice decreased dramatically. A larger 1.5-log<sub>10</sub> decrease in serum HBV DNA and liver HBV RNA levels were observed in APOBEC3G-treated versus control groups<sup>[38]</sup>. These findings suggest that APOBEC3G suppresses HBV replication and antigen expression both *in vivo* and *in vitro*, and is a promising advance in HBV therapy.

## THERAPEUTIC VACCINATION

HBV persistence is thought to result from poor HBV-specific T cell responses<sup>[39]</sup>. This has resulted in efforts to stimulate HBV-specific T cells using therapeutic vaccines<sup>[40]</sup>. Mancini-Bourguine *et al* conducted a phase I study to evaluate the effectiveness of an HBV DNA vaccine that encodes HBV envelope proteins in ten chronic HBV carriers who did not respond to current antiviral therapies<sup>[41]</sup>. Patients received four 1 mg intramuscular injections of the vaccine and an increased frequency of HBV specific T cell responses was observed. HBV DNA levels declined in five patients, and one patient successfully cleared the infection.

Yuan *et al* constructed a hepatitis B immunogenic complex therapeutic vaccine from a combination of yeast-derived recombinant HBsAg and human anti-HBs immunoglobulin (YIC)<sup>[42]</sup>. Its safety profile and the immune responses it elicited were examined in a phase I clinical trial. IFN- $\gamma$  levels were higher in all eight subjects studied ( $P = 0.015$ ) and IL-2 levels increased in seven of the eight subjects ( $P = 0.002$ ). These results show that the hepatitis B immunogenic complex therapeutic vaccine (YIC) can induce a potent anti-HBs response.

Wu *et al* also developed an innovative minovirus vaccine to induce hepatitis B virus specific cytotoxic T-lymphocyte responses<sup>[43]</sup>. They proved that their mimovirus could induce an HBsAg28-39-specific CTL response *in vivo*. This type of vaccine is now under the phase II clinical trial in China.

The promise of these approaches requires further examinations in a large randomized study.

## DENDRITIC CELL VACCINATION

Dendritic cells (DCs) function as antigen-presenting cells. Peripheral DCs phagocytose microbes and viruses, and migrate to the regional lymph nodes where they mature and present foreign protein peptides to naive T cells<sup>[44]</sup>. These T cells then become activated, and acquire direct antiviral function as well as the ability to produce a variety of cytokines, including IFN, IL-2, IL-12, and IL-18. Many viruses, including HBV, are able to escape immune surveillance and persist in the host without evoking an immune response. Zheng *et al* studied the functional defects of DCs in patients with CHB and showed that human leukocyte antigen (HLA) class II and B7 expression are not upregulated on these cells, leading to inadequate IL-12 levels to fight against infection<sup>[45]</sup>. Although DC vaccination shows promise, it is still in the preclinical phase. With advances in technology, DC-based therapy may be an important method of managing CHB<sup>[46]</sup>.

## TLR LIGANDS

TLRs play an important role in innate immune recognition and regulation<sup>[47]</sup>. They belong to a family of evolutionarily conserved receptors that recognize structural patterns on different pathogens<sup>[48]</sup>. After finding a particular virus or microbe, TLRs activate phagocytes and DCs to mount an immune response<sup>[49]</sup>. In an HBV transgenic mouse model, Isogawa *et al* showed that a single injection of a TLR ligand can inhibit HBV replication in hepatocytes by inducing the production of antiviral cytokines<sup>[50]</sup>. These data support the further development of this approach.

## CTL-BASED THERAPY

CTL-based immunotherapy is based on the concept that HBV-specific CTLs control infection by suppressing HBV replication in infected humans<sup>[51]</sup>. Vitiello *et al* developed a lipopeptide-based vaccine containing one CTL epitope from the HBV core region. This vaccine induced an HBV-specific CTL response in healthy volunteers in a phase I clinical trial that was comparable to CTL responses observed during acute HBV infection<sup>[52]</sup>. In a phase II trial in patients with chronic HBV, however, CTL-based therapy was much less effective for suppressing HBV DNA<sup>[53]</sup>. This therapeutic approach may still be clinically useful if it is designed to recognize multiple CTL epitopes.

## CYTOKINES

Cytokines play a major role in controlling viral infections<sup>[54]</sup>. In a transgenic mouse model, type 1 IFNs ( $\alpha$  and  $\beta$ ) were shown to inhibit HBV viral replication<sup>[55,56]</sup>. IFN- $\gamma$  also prevents HBV replication by activating natural killer T (NKT) cells and T cells<sup>[57]</sup>, however, clinical trials with IFN- $\gamma$  did not show much benefit in patients with CHB<sup>[58]</sup>. Robek *et al* reported that IFN- $\lambda$  inhibits HBV replication and induces IFN-stimulated gene expression using a mechanism distinct from that used by IFN- $\alpha$ , - $\beta$ , or - $\gamma$ .

Thus, IFN- $\lambda$  may be useful as a therapeutic agent in the management of CHB<sup>[59]</sup>.

Because of its ability to induce T cell proliferation, IL-2 is hypothesized to be an important immunostimulatory molecule, especially during chronic viral diseases<sup>[60]</sup>. IL-2 downregulates HBV gene expression in a transgenic mouse model and in patients with HIV, intermittent rIL-2 therapy prolongs CD4 T cell survival<sup>[61]</sup>. As a result, rIL-2 may be used as an adjunct therapy to prime other forms of immunomodulation such as therapeutic vaccination<sup>[62]</sup>.

Cavanaugh *et al* demonstrated the antiviral efficacy of IL-12 in an HBV transgenic mouse model<sup>[63]</sup>, however the overall reduction in viral titers was modest compared to other anti-HBV treatments<sup>[64]</sup>. Kimura *et al* showed that IL-18 also inhibits HBV replication in a transgenic mouse model<sup>[65]</sup>, but its efficacy in humans remains to be tested.

While many of these cytokines may not be potent as single agents they may help understand the mechanisms used by various immunomodulatory strategies to control HBV infection<sup>[62]</sup>.

## ADOPTED CELL THERAPY

Sun *et al* isolated peripheral blood mononuclear cells from patients and activated them by anti-CD3 monoclonal antibody, interleukin-2 and interferon- $\gamma$  *in vitro* for 10 d to produce multifactor activated immune cells (MAICs)<sup>[66]</sup>. When the cells have expanded and activated effectively 10 d later, these patients were transfused with these cells. Significant HBV inhibition was observed in 8 out of 14 until 1 year after transfusion. These findings strongly suggest that MAICs transfusion can effectively inhibit the replication of hepatitis B virus.

## GENE THERAPY

Researchers are developing novel nucleic acid-based interventions against HBV. These tools for manipulating gene expression are an attractive means of targeting HBV at different stages of its life cycle, with the ultimate goal of completely eradicating the virus<sup>[67]</sup>. Although this approach is not realistic for clinical use at this time, tremendous advances in this field have been made over the past few years. There are three gene therapy approaches: the use of antisense oligodeoxyribonucleic acids (ODNs), ribozymes, and short interfering RNAs (siRNAs). Some researches have shown significant results using these treatments, but the mode of delivering nucleic acid-based therapies remains a problem. Since HBV primarily replicates in hepatocytes, it is important that these compounds target the liver in order to reduce the required dose and minimize nonspecific effects<sup>[68]</sup>. Safety is also a potential concern with this therapeutic approach<sup>[69]</sup>, as is the issue of host enzymes biodegrading these compounds and rendering them ineffective. Nonspecific activation of the immune system is further noted as a risk of administering nucleic acid-based compounds<sup>[70]</sup>. Advances in delivery strategies and an improved understanding of the mechanisms of these technologies should lead to safer and more efficacious nucleic acid-based therapeutic approaches.

## CONCLUSIONS

Treatment of chronic HBV requires inhibiting hepatitis B virus replication or eliminating the virus from cells. The major problem with current treatments is the emergence of drug resistant variants over time. Novel therapies that target unique molecules or require shorter treatment time are still in demand. Several new anti-HBV nucleoside analogues are in different stages of clinical trials, and in the next decade we should see an increase in the use of agents designed to target specific molecules. The greatest challenge in the future of HBV treatment is the achievement of a safe, cost-effective, and durable regimen that takes advantage of novel therapeutic modalities.

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