Future prevention and treatment of chronic hepatitis B infection

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Abstract

Vaccination for hepatitis B virus (HBV) infection and treatment for chronic hepatitis B (CHB), while effective for primary prevention and control of the disease, still have their limitations. Global coverage of HBV immunization needs improvement. Several patient populations are noted to have suboptimal seroprotective rates after HBV vaccination. There are currently several potential new vaccines undergoing animal and human studies, most notably vaccines containing immunostimulatory DNA sequences. Long-term nucleoside analogue therapy is necessary in achieving permanent virologic suppression. Potential new treatments explore new mechanisms of action, including the inhibition of hepatitis B surface antigen release, targeting anti-fibrotic mechanism, and immunomodulation through novel interferons and therapeutic vaccines. The clinical application of potential new vaccines and therapies would enhance the prevention of HBV infection and treatment of CHB.

(126 words)

Introduction

It is estimated that 2 billion people worldwide have been exposed to the hepatitis B virus (HBV) (1). Four hundred million patients are infected with chronic hepatitis B (CHB), with an estimated 600,000 deaths annually from its associated complications (2). The last few decades has witnessed remarkable progress in the treatment and prevention of CHB. The introduction of universal HBV vaccination has markedly reduced the prevalence of HBV infection (3, 4). Recent years have also seen a drastic increase in therapeutic options in CHB. Permanent virologic suppression, and sometimes even hepatitis B surface antigen (HBsAg) seroclearance, can now be achieved (5). Nevertheless, both vaccination and treatment in CHB still have their limitations. Future potential improvements in vaccination and therapy will be discussed thoroughly in this review.

Vaccination – current standards and limitations

The first HBV vaccines being introduced in 1982 were plasma-derived, and with improvements in recombinant DNA technology, were gradually replaced by recombinant-based vaccines (6). Serum antibody to the hepatitis B surface antigen (anti-HBs) of ≥ 10 mIU/mL is defined as a protective level. Currently, 162 countries have already implemented universal HBV vaccination programs (7), although a World Health Organization report in 2006 found actual implementation of vaccination among newborns to be only 26 to 36% (8).

A study in Taiwan by Ni et al followed up 18,779 vaccinated newborns for 20 years. They found the rate of HBsAg positivity to be only 1.2% (4). Other Taiwanese

studies established the efficacy of universal vaccination in reducing fulminant hepatic failure (9, 10) and hepatocellular carcinoma (HCC) (11). Studies in Hong Kong also confirmed the efficacy of both plasma-derived and recombinant-based vaccines without booster doses up to 22 years of follow-up, with highly effective anamnestic responses in patients with low anti-HBs titers i.e. a significant increase in anti-HBs titers indicating exposure to HBV without development of HBV infection. (12-15). Studies in Italy and Alaska, regions with an intermediate endemicity of CHB, also found universal HBV vaccination to achieve a similar efficacy (16, 17). A meta-analysis of 42 separate cohorts found the cumulative incidence of HBV breakthrough infection in immunocompetent subjects to be 0.7% (18). The occurrence of vaccine-escape HBV mutants, initially a concern when discovered (19), was noted to be of limited prevalence, probably due to the replicative weakness of the mutant virus (20, 21).

Factors associated with failure of the HBV vaccine are listed in Table 1. Identified risk factors for vaccine failure among newborns include hepatitis B e antigen (HBeAg)-positivity and a high viral load in HBsAg-positive mothers (4, 22). In addition, current HBV vaccines are noted to be suboptimal among adult populations with impaired immunity. Rates of seroprotection are lower with increasing age, obesity, smoking, diabetes and renal disease (23-25). In patients with end-stage renal disease (ESRD) receiving hemodialysis, response rates could be as low as 50 to 70% (26, 27), especially among patients with co-existing hepatitis C infection (28) and poor nutritional status (29). Outbreaks of HBV infection were still reported among hemodialysis units in developed countries (30). The response rates among patients with human immunodeficiency virus (HIV) infection are also lower (31, 32); <u>using additional booster</u>

doses and increasing the vaccine dose in such subjects only attained 1-year response rates of 58.8% to 63.0% (33, 34). Other patient groups prone to vaccine failure include chronic alcoholics with overt liver disease (35) and recipients of liver (36) and renal (37) transplant.

Hence, while universal HBV vaccination has a profound impact in the prevention of HBV transmission, there are two areas needing improvement: increasing global vaccination coverage, and enhancing response rates among the suboptimal response groups mentioned above. The first aspect requires international collaborative efforts by governments of endemic countries, the World Health Organization and different non-profit organizations. The following sections will concentrate on the second aspect.

Enhancing vaccination response

A recent study analyzed data from 2,356 children born to HBsAg-positive mothers in Taiwan found children born to HBeAg-positive mothers to have a higher prevalence of HBsAg-positivity compared to children born to HBeAg-negative mothers (9.26% and 0.23% respectively, p <0.001) (22). The authors suggested utilizing serum HBeAg in addition to HBsAg as screening tools to identify vaccinated children at high risk of developing CHB. Prospective trials would be needed to justify its cost-effectiveness.

Nucleoside analogue therapy in HBeAg-positive mothers has been shown to reduce perinatal transmission. In two recent studies, telbivudine treatment in HBeAg-positive mothers, starting in the second or third trimester, was associated with a significant reduction in rates of perinatal transmission up to 28 weeks after delivery (38,

39). There are also studies showing the administration of hepatitis B immunoglobulin (HBIG) to either HBsAg-positive mothers (40) or newborns (two-dose injections) (41) is able to improve vaccine responsiveness. Long-term follow-up results of the above studies would be needed to ascertain the efficacy of both antenatal nucleoside analogue therapy and perinatal HBIG administration.

Various strategies have also been employed to improve vaccine responsiveness in high-risk adults (Table 1). Intradermal vaccination, when given to patients with ESRD, is able to achieve higher rates of anti-HBs positivity (42, 43), although an improved response was not reproduced among patients with HIV infection (44, 45). Increasing the vaccine dosage among patients with ESRD can also improve vaccine response (46). Other suggested approaches include improving the vaccine adjuvant formula (47, 48), using plasmid DNA vaccines that encode HBsAg (49, 50), and administrating HBsAg-pulsed blood dendritic cells (51). Many such suggested methods are still undergoing in animal studies.

There is currently one promising vaccine that has already undergone multiple clinical trials – the hepatitis B surface antigen-1018 ISS adjuvant containing vaccine (HELIPSAV).

Hepatitis B surface antigen-1018 ISS adjuvant containing vaccine (HELIPSAV)

Immunostimulatory DNA sequences (ISS) are unmethylated cytosine phosphoguanosine (CpG) motifs that are found in various viruses, including HBV, but are rare in mammalian cells (52). These CpG motifs are recognized by toll-like receptor 9, resulting in the preferential activation of type 1 (TH1) immune response that

subsequently amplifies adaptive immune responses (53). Several cytokines, including interleukin-12 and type I interferon (IFN) secreted by dendritic cells, are involved in this immune process (54). As a result, synthetic ISS are attractive vaccine adjuvants that stimulate specific pathways critical to immune response regulation, resulting in minimal toxicity (55).

The ISS contained in HELIPSAV is a 22-mer phosphorothioate oligonucleotide, which is mixed with 20 µg of yeast (56). Excellent protective anti-HBs titers and good tolerability were noted in initials phase I studies (57). This was followed by a phase II comparative study using a licensed recombinant HBV vaccine among 99 healthy subjects aged 18 to 28 years. A greater proportion of HELIPSAV-treated recipients had a seroprotective anti-HBs levels at week 28 after when compared to those given recombinant HBV vaccine (100% and 64% respectively, p <0.001). HELIPSAV-treated group also had higher geometric mean titers than the recombinant vaccination group (2,074 versus 32 mIU/mL) (58).

The phase III results of HELIPSAV have been recently published (59). The first study, involving 2,415 healthy subjects aged 11 to 55 compared two doses of HELIPSAV versus three doses of recombinant HBV vaccination. HELIPSAV was able to achieve a higher seroprotective rates when compared to recombinant vaccine at the primary immunogenicity endpoint (95.1% and 81.1% respectively). Another phase III study investigated the efficacy of HELIPSAV in 420 healthy subjects aged 40 to 70 years and seronegative to HBsAg, anti-HBs and antibody to the hepatitis B core antigen (anti-HBc) (60). The seroprotective rate of HELIPSAV was significantly higher at week 50 when compared to recombinant vaccine (100% versus 68.6%). A third study

involving 2,449 participants aged 40-70 found HELIPSAV to achieve a seroprotective rates of 94.6%, 94.7% and 95.6% in males, obese subjects and smokers respectively. The seroprotective rates in the recombinant vaccine group among these three subject populations were only 67.8%, 65.4% and 65.3% respectively (61). In all studies, HELIPSAV was well-tolerated with similar safety profiles when compared to the recombinant vaccine.

The report of a case of Wegener's granulomatosis 171 days after the second dose of HELIPSAV (59) led to the vaccine being put on hold by the United States Food and Drug Administration in 2008 (62). However, clinical hold on HELIPSAV has been lifted since September 2009 after the provision of additional safety data by the manufacturing pharmaceutical company.

Other vaccine adjuvants

Other clinical trials in human subjects are summarized in Table 2. CPG 7909 is another CpG motif-based HBV vaccine adjuvant that has been found effective in phase I studies (63) and phase II studies involving HIV-infected subjects (64). Vaccine adjuvants systems that stimulate both cellular and antibody immune responses have been recently proven to be effective against malaria (65), and a similar system known as AS02, using monophosphoryl lipid A and *Quillaja saponaria* as vaccine adjuvants is also effective against HBV (66) , including in patients with renal insufficiency (67). There are also oral (49) and intranasal (68) HBV vaccines undergoing clinical evaluation.

Information on HBV vaccines still in the phase of animal studies has been described in detail elsewhere (69, 70). Therapeutic vaccines aimed at CHB treatment would be described in a subsequent section.

Treatment of chronic hepatitis B – current standards

Treatment of CHB has been revolutionized in the last two decades. Sustained and profound virologic suppression is now possible with continuous nucleoside analogue therapy (71, 72). These can result in a reduction in the incidence of cirrhotic complications and HCC (73, 74), as well as in reversing biopsy-proven cirrhosis (75). Certain CHB populations also respond satisfactorily to pegylated IFN therapy (76).

Despite the favorable responses achieved with current available therapy, there are definitely aspects for improvement. Pegylated IFN therapy is still limited by its suboptimal response rate in certain CHB populations and its side-effect profile (77). Concerning nucleoside analogue therapy, HBsAg seroclearance, the ultimate treatment endpoint, is rarely seen (78). A prolonged treatment duration with potent nucleoside analogue therapy is needed in order to achieve histologic regression of fibrosis or cirrhosis (75). The reduction in the incidence of HCC, though significant, is not absolute (79). Lastly, no treatment can totally eradicate HBV in infected individuals. This ultimate objective may not be achievable since HBV forms highly stable covalently closed circular DNA (cccDNA) in the hepatocyte nuclei, as well as integrates into the host genome from the early stage of the infection.

An exhaustive summary of novel anti-HBV drugs undergoing human and animal trials can be found elsewhere (80). This review concentrates on therapies with the

potential to be approved for widespread use in the foreseeable future. These include novel nucleoside analogues, HBsAg release inhibitors, novel IFNs, anti-fibrotic agents and therapeutic vaccines (Table 3).

Novel reverse transcriptase inhibitors

Given the established efficacy of nucleos(t)ide analogues in controlling CHB infection, there are several novel drugs that inhibit the reverse transcriptase involved in HBV DNA replication for both wild-type and drug-resistant HBV. These include besifovir and lagociclovir.

Besifovir (*LB-80380)*

Besifovir is an acyclic nucleotide phosphonate with its chemical structure similar to that of adefovir and tenofovir (81). It is a prodrug which is converted to LB-80331 through deacetylation in both the liver and intestine, then further oxidized to LB-80317, the active metabolite with antiviral effect towards HBV (Figure 1). LB-80317, unlike the prodrugs of adefovir and tenofovir, uses guanine instead of adenine as its base moiety, which contributes to an improved drug efficacy due to the lower intracellular concentrations of potentially competing guanine nucleotides compared to adenine nucleotides (82). Preclinical studies have found besifovir to show potent antiviral efficacy against both wild-type and drug-resistant HBV, with little reduction in mitochondrial DNA or lactic acid accumulation. Animal studies also found besifovir , when used in a similar dose as adefovir, to be 45 times less nephrotoxic (81).

A randomized placebo-controlled phase Ib dose escalation study of besifovir was performed in 29 Asian HBeAg-positive CHB patients for 4 weeks with a 12-week follow-up period (83). The maximum median HBV DNA reductions were 3.05, 4.20, 3.67 and 3.68 log copies/mL for doses 30, 60, 120 and 240 mg respectively. All reductions in viral loads were significantly greater when compared to placebo (p = 0.011).

A phase II, open-label, multicenter, dose escalation study was performed in 65 lamivudine-resistant HBeAg-positive CHB patients (84). Besifovir was given together with lamivudine for 4 weeks, then followed by 8 weeks of besifovir monotherapy, subsequently followed by 24 weeks of adefovir. The mean HBV DNA reductions from baseline were 2.81, 3.21, 3.92, 4.16 and 4.00 log copies/mL for doses 30, 60, 90, 150 and 240 mg respectively. The degree of HBV DNA suppression at week 12 was dose-dependent (p <0.001). The drug was also well-tolerated with no drug-related adverse events reported. Another phase IIb multicenter study compared the efficacy and safety of besifovir with entecavir up to 48 weeks (85, 86). One hundred and fifteen CHB patients were randomized in a ratio of 1:1:1 to receive either besifovir 90 mg, besifovir 150 mg or entecavir 0.5 mg daily. After 48 weeks, besifovir 90 mg and 150 mg were found to have similar rates of virologic suppression when compared with entecavir (67.7%, 81.8% and 80.0% respectively achieving HBV DNA <60 IU/mL, p >0.05). Rates of HBeAg seroconversion were also comparable (21.1%, 16.7% and 14.3% respectively, p >0.05). Full sequencing of the HBV polymerase region during follow-up did not detect any resistant mutations. Both doses of besifovir were significantly

associated with the lowering of L-carnitine levels, which were normalized in all patients with carnitine replacement. No other drug-related adverse events were reported.

With its high potency, besifovir may find its place in the future as a third first-line agent, both for treatment-naïve as well as for lamivudine-resistant patients.

Lagociclovir

Lagociclovir valactate (also known as MIV-210) is a prodrug of 3'-fluoro-2', 3'-dideoxyguanosine (FLG). Animal studies have found FLG to be a potent inhibitor of hepadnavirueses, which includes HBV (87), and is active against lamivudine-, adefovir- and entecavir-resistant HBV (88).

A woodchuck model involving different doses of lagociclovir found serum HBV DNA to decrease by more than 7 log after 10 weeks of therapy. There was also a 2 log decrease in intrahepatic covalently closed circular DNA (cccDNA). The reduction in HBV DNA, when compared to previous woodchuck models, was comparable to entecavir and better than lamivudine or adefovir (89). Phase I and II clinical studies are currently ongoing in Europe and Asia (80).

HBsAg release inhibitors

All currently available oral anti-HBV medication are nucleos(t)ide analogues, which target the activity of reverse transcriptase in viral replication. There has been research aimed at developing novel drugs targeting other areas in the biology of HBV. The inhibition of HBsAg release is one such potential target, especially since the

therapeutic reduction of serum HBsAg is associated with a corresponding decline in intrahepatic cccDNA (90).

The HepG2 and HepA2 cell lines transfected by HBV have been applied as *in vitro* models in evaluating the efficacy of novel anti-HBV drugs. A study reported artesunate-containing herbal extracts were able to effectively inhibit serum HBsAg release in HepG2 cells (91). Serum HBsAg production was also similarly suppressed by pyranocoumarin analogues in HepA2 cells based on extracts isolated from the medicinal plant *Clausena excavate* (92). An aromatically substituted tetrahydro-tetrazolo-(1, 5-a)-pyrimidine known as HBF-0259 was able to suppress HBsAg production in HepG2 cells without affecting HBeAg or HBV DNA synthesis (93). A chemically improved version of HBF-0259 was able to achieve potent HBsAg inhibition in HBV-transgenic mice and was effective against both wild-type and drug-resistant HBV. No signs of toxicity through serum chemistry analysis were noted (94).

REP 9AC

REP 9AC is a nucleic acid-based amphipathic polymer, which belongs to a new class of antiviral compounds based on the sequence-independent activity of phosphorothioated oligonucleotides. Similar amphipathic molecules have been found to exhibit potent antiviral activity against human immunodeficiency virus (HIV) (95), hepatitis C virus (96) and cytomegalovirus (97). REP 9AC facilities innate immunity against HBV by inhibiting release of subviral particles, including HBsAg, from infected hepatocytes. Both HBsAg and HBeAg have been reported to abrogate the toll-like

receptor induced innate response against HBV, resulting in the persistence of HBV infection (98).

Phase I/II studies of REP 9AC are currently ongoing. Interim reports found the intravenous infusion of REP 9AC resulted in >99.5% reduction in HBsAg in 7 of 8 patients within 7 days to 32 weeks of treatment, with a corresponding reduction in serum HBV DNA also noted. After stopping therapy, 3 patients maintained serum HBV DNA <500 copies/mL and HBsAg <10 IU/mL, while the other patients had >90% reduction in HBsAg and 2 to 7 log reductions in HBV DNA (99, 100). These preliminary results indicate HBsAg release inhibitor could become an important new tool in the control of CHB in the future.

Interferon-lambda (IFN-λ)

IFN-based therapy in CHB, while offering the advantage of a finite duration of therapy and the slightly higher rate of HBsAg seroclearance, is associated with high rates of virologic rebound after treatment cessation and an unfavorable side-effect profile (101). The multiple side-effects seen are related to the abundant IFN-α receptor distributed throughout the whole human body. IFN-λ (Figure 2), discovered in 2003 (102), induces antiviral response via a pathway similar to IFN-α and -β. IFN-λ receptors, although expressed in high amounts in hepatocytes(103), are not found in other human cells including fibroblasts, monocytes, adipocytes or primary central nervous system cells, and is thus associated with less systemic side-effects when administered (104, 105).

IFN-λ, despite its molecular difference, shares similar biological characteristics with IFN-α and –β. The stimulation of IFN-λ, similar to IFN-α and –β, involves toll-like receptors (102, 106). All three IFNs activate IFN-stimulated regulatory factor 3 and induces the expression of genes containing IFN-stimulated response elements (106). Nevertheless, IFN-λ does not bind to the IFN-α receptor complex, but triggers its cellular activity through a receptor consisting of two subunits: an interleukin-10 receptor and an IFN-λ receptor 1 (107).

IFN-α inhibits HBV replication by preventing the assembly of viral RNA-containing capsids in the cytoplasm (108). IFN-λ operates through a similar molecular mechanism as demonstrated by a study using murine hepatocytes (107). Subsequent studies using human hepatocytes also demonstrated a similar efficacy in inhibiting HBV replication (109, 110).

Phase I and II studies in CHB patients are currently ongoing. From published data on pegylated IFN-λ in chronic hepatitis C patients, IFN-λ was well-tolerated with minimal adverse effects (111). The efficacy of IFN-λ was reduced in chronic hepatitis C patients with prior IFN-α treatment (105) Its effect against HBV will have to await the results of current clinical trials.

FG-3019 – novel antifibrotic agent

Hepatic fibrogenesis is a common pathway of liver damage seen in many chronic liver diseases, including CHB. The development of anti-fibrotic agents aimed at regressing liver fibrosis has thus aroused great interest. Fibrogenic mechanisms are

dependent on the interplay of many pro- and anti-fibrotic cytokines, of which transforming growth factor beta (TGF-β) has been viewed as the "master" cytokine crucial for the advancement of fibrosis (112). Important regulators of these cytokines are the connective tissue growth factors (CTGFs). Initially discovered in 1991 in the conditioned medium of human umbilical vein endothelial cells (113), CTGF consists of four domains (Figure 3), and up-regulates the majority of pro-fibrotic cytokines, including TGF-β, hence promoting hepatic fibrosis (114). CTGF expression has been noted in hepatic stellate cells (HSCs), the main producers of extracellular matrix proteins related to fibrosis, in both experimental models and human patients (115, 116). Hepatocytes infected by HBV are also noted to have increased CTGF and TGF-β up-regulation (117). Fibrogenesis is likely triggered by the X protein of HBV, with human and rat HSCs exposed to the X protein showing an increased expression of CTGF, TGF-beta and other fibrogenic cytokines (118).

FG-3019 is an anti-CTGF monoclonal antibody that is designed to inhibit to inhibit TGF-β related fibrosis. The second domain of CTGF, which is homologous to the von Willebrand factor type C repeat, is the binding site of FG-301 (Figure 3). FG-3019 has the potential to be used in multiple disease entities in which fibrogenesis is a major pathophysiological element, including pulmonary fibrosis, pancreatic cancer, kidney disease and liver fibrosis. Animal studies have found FG-3019 able to improve histologic signs of liver fibrosis by reducing the accumulation of extracellular matrix in the liver and reduce the number of activated HSCs (119). Hydroxyproline:proline (Hyp:Pro) ratios, which quantitatively scores organ fibrosis by measuring the collagen content relative to total organ protein, are increased by the co-administration of CTGF

and TG-Fβ. Hyp:Pro ratios are however significantly reduced in the liver through the administration of FG-3019 (120).

Phase I and II studies are currently ongoing in South East Asia, investigating the effect of FG-3019 or placebo with entecavir in CHB. FG-3019 is well-tolerated with no drug-related adverse effects, as shown in clinical trials involving pancreatic cancer and diabetic kidney disease (121, 122).

Therapeutic vaccines

A key element in the chronicity of HBV infection is the weakened innate immunity system resulting in the failure of viral clearance. Hepatocytes infected by HBV inhibit CD8 T cells, leading to the impairment of T-cell function and reduction of anti-viral cytokines (e.g. IFN-γ and tumor necrosis factor-α) (123). Therapeutic vaccination is thus a potential strategy which could theoretically eradicate HBV by strengthening the patient's immune response (124), although the results of such trials have not been satisfactory.

A double-blind placebo controlled phase II trial used a therapeutic vaccine containing HBsAg–anti-HBs immune complexes in HBeAg-positive CHB. Although increased rates of HBeAg seroconversion were seen in the therapeutic vaccine arm, there was no difference in the rates of virologic suppression (125). Moderate levels of serum HBV DNA and HBsAg levels were still seen 24 weeks post-treatment (126). DNA vaccines are gaining in popularity given their ability to induce both humoral and cellular immune responses. Two phase I studies investigated therapeutic plasmid DNA vaccines based on recombinant HBsAg proteins, which, although well-tolerated and

immunologically effective, did not result in significant HBV DNA suppression (127, 128). Another study found combining a DNA vaccine containing recombinant interleukin-12 with lamivudine did not improve rates of virologic suppression (129). DNA vaccines also fail to maintain virologic remission after cessation of nucleoside analogue therapy (130).

One promising therapeutic vaccine currently undergoing clinical trials is DV-601. This vaccine comprises of recombinant HBsAg and hepatitis B core antigen (HBcAg), and is engineered to stimulate a broad spectrum of T cells. In a phase I dose-escalation study, 14 CHB patients were started on concurrent entecavir and DV-601. A preliminary analysis showed all patients to have substantial reductions in both serum HBV DNA and HBsAg titers; HBeAg titers were also reduced in HBeAg-positive patients. Anti-HBs and antibody to the hepatitis B e antigen (anti-HBe) developed in higher dose groups. The vaccine was also well-tolerated (131).

The problem with therapeutic vaccines is that most CHB patients, especially those from Asia, Africa and certain Mediterranean countries, have been exposed to the virus since early childhood when the immune system is not yet fully matured. These carriers have also been exposed to large amounts of HBsAg for long periods of time. With the virus forming cccDNA inside the hepatocyte nuclei as well as viral integration into the host genome, CHB patients might not mount a satisfactory immune response to therapeutic vaccination.

Future directions

CHB is now both preventable and treatable due to the remarkable advances achieved in the last three decades. Yet, eliminating and eradicating HBV totally remains

difficult. In the era of universal immunization, HBV vaccination can be further improved in three areas: increasing global coverage, recognizing HBsAg-positive mothers with increased risk of mother-to-infant transmission, and improving responsiveness among high-risk individuals. The use of nucleoside analogues in high-risk HBsAg-positive mothers, while effective, requires long-term data of affected infants for validation, especially if universal implementation is planned. HELIPSAV can achieve high seroprotective rates in hyporesponsive individuals, and its future global availability would be crucial in the prevention of HBV among high-risk subjects.

Concerning CHB treatment, long-term nucleoside analogue therapy is needed to maintain permanent virologic suppression. Potential new therapies explore new methods of HBV control, including inhibiting HBsAg release, improving immunomodulation and targeting mechanisms of fibrosis. Different mechanisms of action could signify the potential for combination therapies in the future. Yet it is still uncertain if these methods could achieve HBsAg seroclearance, or more importantly, reduce and eradicate HBV cccDNA from infected hepatocytes. To elevate the aim of HBV therapeutics from virologic suppression to eradication, future studies on intrahepatic virologic kinetics are needed, with the focus on investigating mechanisms of intrahepatic cccDNA decline in patients achieving spontaneous or treatment-related HBsAg seroclearance. Until then, long-term nucleoside analogue therapy would remain the best treatment option for CHB.

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Figure legends

Figure 1. The molecular structure of besifovir (LB-80380), and its two metabolites LB-80331 and LB-80317.

Figure 2. The molecular structure of interferon-lambda.

Figure 3. The 4 domains of connective tissue growth factor (CTGF), with the second domain being the binding site of FG-3019.

Figure 1
Click here to download high resolution image

LB80317

LB80331

LB80380
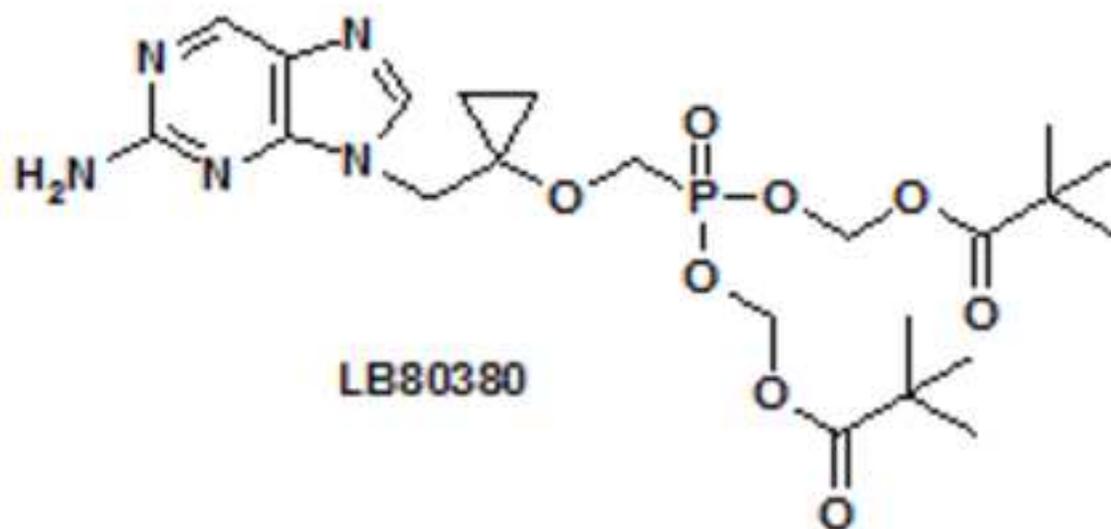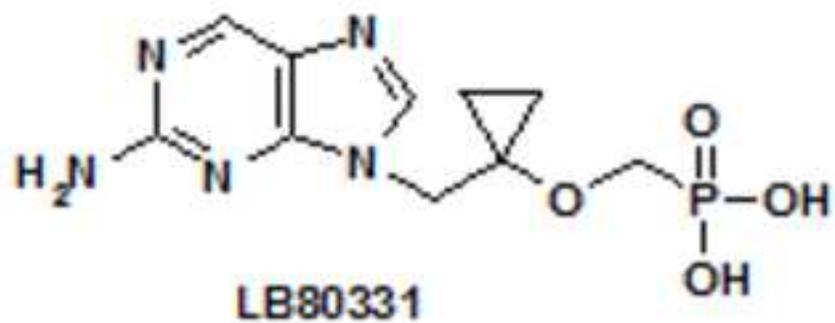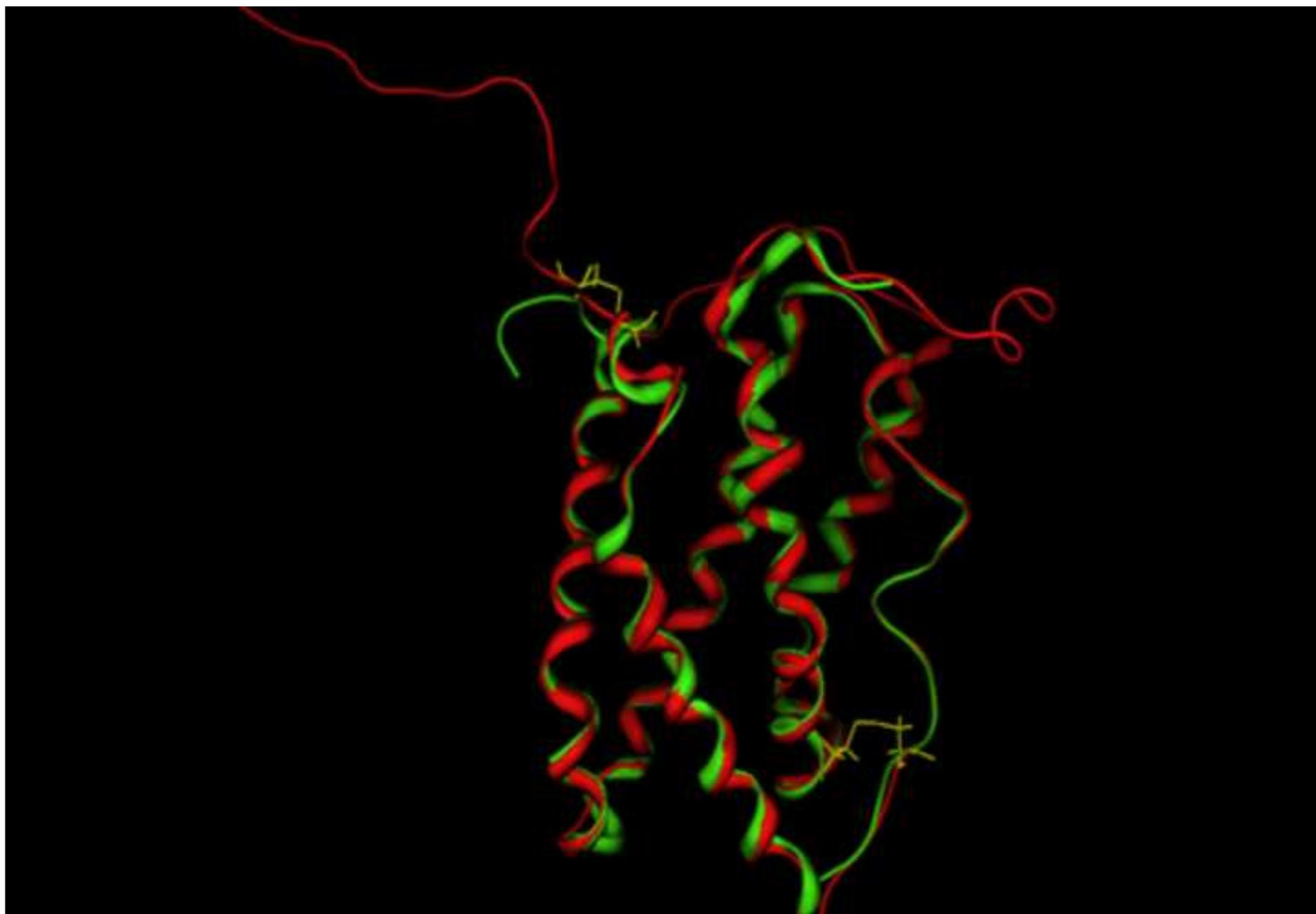
Figure 2

Figure 3
Click here to download high resolution image

N-Terminus

FG-3019
binding site

C-Terminus

Domain 1
IGF Binding Protein
Domain

Domain 2
Von Willebrand Factor
Type C Repeat

Domain 3
Thrombospondin
Type-1 Repeat

Domain 4
"Cys Knot"
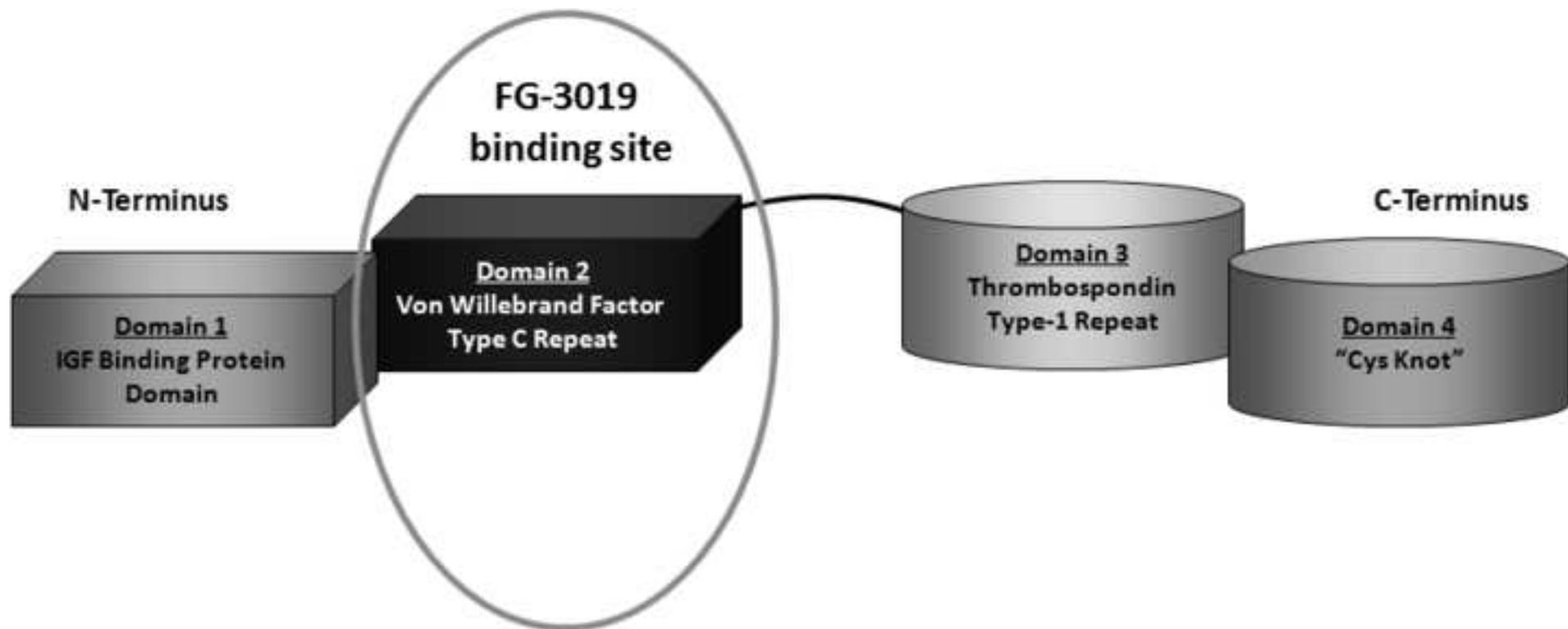
Table 1. Factors associated with HBV vaccination failure

Subjects	Factors	Methods to improve vaccine response
Newborn	HBsAg-positive mothers (especially HBeAg-positive) Mothers with high viral load	• HBsAg / HBeAg screening • Nucleoside analogue therapy for high-risk mothers • HBIG for high-risk mothers / infants
Adult	Increasing age Obesity Smoking Diabetes End-stage renal disease HIV infection Alcoholics with overt liver disease Recipients of liver / kidney transplantation	• ISS-based vaccination (e.g. HELIPSAV) • Intradermal vaccination • Increasing vaccination dose • Improving vaccine adjuvant formula • Using plasmid DNA vaccines • Using HBsAg-pulsed dendritic cells

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; ISS, immunostimulatory DNA sequences; HIV, human immunodeficiency virus.

Table 2. Promising HBV vaccine adjuvants undergoing clinical trials in human subjects

		Stage of research	Efficacy
HELIPSAV	Immunostimulatory CpG	Phase III	Effective in normal and hyporesponsive subjects
CPG 7909	Immunostimulatory CpG	Phase II	Effective in HIV-infected individuals
AS02	Monophosphoryl lipid A and QS 21	Phase II	Effective in patients with renal insufficiency
Plasmid DNA pWRG7128	DNA vaccine taken orally	Phase I	Humoral and cell-mediated responses induced in hyporesponsive subjects
NASVAC	Recombinant HBsAg and HBcAg taken intranasally	Phase I	Seroprotective up to 90 days

CpG, cytosine phosphoguanosine; QS, *Quillaja* saponaria; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen

Table 3. Promising therapeutic options for chronic hepatitis B

Drug	Category	Phase of clinical trial	Preliminary results
Besifovir	Nucleotide analogue	Phase II	High rates of virologic suppression in both treatment-naïve and lamivudine-resistant CHB
Lagociclovir	Nucleoside analogue	Phase I	Significant reduction in cccDNA in woodchuck model
REP 9AC	HBsAg release inhibitor	Phase II	Significant serum HBsAg reduction
Interferon-λ	Interferon	Phase II	Inhibit HBV replication in animal studies
FG-3019	Connective tissue growth factor	Phase II	Reduces liver fibrosis in animal studies
DV-601	Therapeutic vaccine	Phase I	Significant reductions in serum HBV DNA, HBsAg and HBeAg