Hepatitis B Cure: From Discovery to Regulatory Approval

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Abbreviations:

ALT – alanine aminotransferase

Anti-HBe – hepatitis B e antibody

Anti-HBs – hepatitis B surface antibody

cccDNA – covalently closed circular DNA

HBcrAg – hepatitis B core related antigen

HBeAg – hepatitis B e antigen

HBsAg – hepatitis B surface antigen

HBV – hepatitis B virus

HCC – hepatocellular carcinoma

hNTCP – human sodium taurocholate cotransporting polypeptide

IFN - interferon

NA – nucleos(t)ide analogue

pgRNA – pregenomic RNA

rcDNA – relaxed circular DNA

siRNA – small interfering RNA

U.S. FDA – United States Food and Drug Administration

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ABSTRACT

The majority of persons currently treated for chronic hepatitis B require long-term or lifelong therapy. New inhibitors of hepatitis B virus entry, replication, assembly or secretion, and immune-modulatory therapies are in development. The introduction of these novel compounds for chronic hepatitis B necessitates a standardized appraisal of the efficacy and safety of these treatments, and definitions of new or additional endpoints to inform clinical trials. To move the field forward, and to expedite the pathway from discovery to regulatory approval, a workshop with key stake holders was held in September 2016 to develop a consensus on treatment endpoints to guide the design of clinical trials aimed at hepatitis B cure. The consensus reached was that a complete sterilizing cure i.e. viral eradication from the host is unlikely to be feasible. Instead, a functional cure characterized by sustained loss of HBsAg with or without anti-HBs seroconversion, which is associated with improved clinical outcomes, in a higher proportion of patients than is currently achieved with existing treatments is a feasible goal. Development of standardized assays for novel biomarkers towards better defining HBV cure should occur in parallel with development of novel antiviral and immune modulatory therapies such that approval of new treatments can be linked to the approval of new diagnostic assays used to measure efficacy or to predict response. Combination of antiviral and immune modulatory therapies will likely be needed to achieve functional HBV cure. Limited proof-of-concept monotherapy studies to evaluate safety and antiviral activity should be conducted prior to proceeding to combination therapies. The safety of any new curative therapies will be paramount given the excellent safety of currently approved nucleos(t)ide analogues.

INTRODUCTION

The advent of several novel antiviral and immune modulatory therapies for chronic hepatitis B now necessitates a standardized appraisal of the efficacy and safety of these therapies, and definitions of new or additional endpoints to inform clinical trials. To move the field forward, and to expedite the pathway from discovery to regulatory approval, the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver jointly organized a Hepatitis B Treatment Endpoints Workshop on September 8-9, 2016 in Alexandria, Virginia. The primary goal of this workshop was to assemble key stake holders from regulatory agencies (United States Food and Drug Administration (U.S. FDA) and European Medicines Agency), biopharmaceutical and biotechnology companies engaged in development of diagnostic tests and therapeutic agents for hepatitis B, and academia, in order to develop a consensus on treatment endpoints to guide the design of clinical trials aimed at hepatitis B cure.

Sixty-six (33%) of 202 participants completed a pre-meeting survey, including 4 from regulatory agencies, 31 from industry, 28 from academia, and 3 from other healthcare sectors. During the workshop, experts reviewed the natural history of chronic hepatitis B virus (HBV) infection, efficacy of currently approved treatments, potential antiviral targets and approaches to restore immune responsiveness to HBV, and pre-clinical and early phase clinical trial data on novel antiviral and immune modulatory therapies for chronic HBV. The workshop concluded with a session on definition of HBV cure; efficacy endpoints, safety assessments, target populations and design of clinical trials; and diagnostic assays needed to support development of curative therapies. These topics were further discussed during a closed session involving 23 experts (including the four authors) representing all constituent groups. This report summarizes the discussions and consensus opinions of the 2-day meeting.

DEFINTION OF HBV CURE

The goal of developing new therapies is to achieve HBV cure, i.e. elimination of HBV, thereby allowing treatment to be stopped with no risk of virological relapse and no risk of liver disease progression. However, a true cure may not be feasible because HBV DNA is integrated into the host genome; even among persons who recovered from acute HBV, viral covalently closed circular DNA (cccDNA) can be detected in the liver explaining the reactivation of HBV replication when these "recovered" persons are profoundly immunosuppressed. However, the observation that hepatitis B surface antigen (HBsAg) may become undetectable in serum after clinical recovery from acute hepatitis B, spontaneously during the course of chronic HBV infection, and during or after nucleosid(t)e analogue (NA) or interferon (IFN) therapy, despite the likelihood of persistent integrated HBV genomes, argues for the feasibility of achieving undetectable levels of HBsAg.

A key objective of the meeting was to establish a definition of cure. Three definitions of HBV cure were proposed at this meeting: (1) Complete sterilizing cure with undetectable HBsAg in serum and eradication of HBV DNA including intrahepatic cccDNA and integrated HBV DNA. (2) Functional cure with sustained, undetectable HBsAg and HBV DNA in serum with or without seroconversion to hepatitis B surface antibody (anti-HBs) after completion of a finite course of treatment, resolution of residual liver injury and a decrease in risk of HCC over time. Several levels of functional cure including complete shut-down of cccDNA transcription, elimination of cccDNA, complete resolution of liver damage, and elimination of risk of HCC were discussed. (3) Partial cure with detectable HBsAg but persistently undetectable HBV DNA in serum after completion of a finite course of treatment.

The vast majority (87.9%) of survey respondents selected functional cure (sustained HBsAg loss) as the goal for new HBV therapies. This selection was endorsed by other participants and the expert panel as a feasible goal. In addition, functional cure offers several other advantages: it is easy to assess

and tests are widely available, it is associated with improved clinical outcomes and lower rates of disease reactivation and once achieved, there is no further requirement for therapy.

There was less consensus regarding the necessity of achieving anti-HBs seroconversion because it is unclear whether durability of HBsAg loss and clinical benefits of HBsAg loss are dependent on development of anti-HBs. Importantly, few of the participants believed elimination of cccDNA was a mandatory criterion for functional cure and less than half required cccDNA be rendered transcriptionally inactive reflecting uncertainties whether new therapies in development can silence or clear cccDNA, as well as pragmatic difficulties in measuring cccDNA.

Some members of the expert panel considered partial cure (sustained suppression of HBV replication off treatment but persistent presence of HBsAg) an acceptable intermediary step toward functional cure because partial cure is more achievable in the short-term, has been shown to lead to a reduction in clinical outcomes,[1] and could expedite drug development.

NATURAL HISTORY OF CHRONIC HBV INFECTION

The natural history of chronic HBV infection is variable and dependent on a complex interplay between the host immune response and the virus. Chronic HBV infection comprises four phases defined by three clinical parameters: serum alanine aminotransferase (ALT) concentrations, serum HBV DNA levels and hepatitis B e antigen (HBeAg) status (Figure 1). The first phase is characterized by the presence of HBeAg and high serum HBV DNA but normal ALT levels. It has been called the "immune tolerant" phase though recent studies have challenged the concept of immune tolerance. HBV-specific T cell responses have been observed in patients in the immune tolerant phase with similar frequency as in patients in the immune active phase. This finding has led some to propose that the immune response is better characterized as low inflammatory during the immune tolerant phase (as opposed to inflammatory during the immune active phase).[2-4] The immune tolerant phase is followed by the "HBeAg-positive immune active" phase, when ALT levels become elevated. After varying intervals,

seroconversion from HBeAg to HBe antibody (anti-HBe) occurs and a majority of patients transition to the "inactive carrier" phase during which ALT levels return to normal and serum HBV DNA levels are low or undetectable. In some patients, serum HBV DNA and ALT levels become elevated again, after years or decades. These patients are considered to be in the "HBeAg-negative immune active" phase which is characterized by fluctuating HBV DNA and ALT levels. The annual incidence of HBeAg-negative immune active hepatitis among inactive carriers is estimated to be 0.37%.[5] Some patients do not fit into any of these conventional phases. Serum HBsAg levels are highest during the immune tolerant phase and lowest during the inactive carrier phase. Consequently, quantification of serum HBsAg levels may help in determining the phase of infection particularly for HBeAg-negative persons[6] and to predict the risk of disease progression and hepatocellular carcinoma (HCC) in HBeAg-negative patients with low viremia (HBV DNA <2000 IU/mL).[7] Because phases of chronic HBV infection are defined based on clinical and not immunologic measures, the recent European Association for the Study of Liver Diseases proposed to describe these four phases as phases 1, 2, 3, and 4 [ref]. Patients in phases 1 and 2 would be HBeAg-positive and those in phases 3 and 4 would be HBeAg-negative and those with inactive disease (phases 1 and 3) would be considered to have chronic infection while those with active disease (phases 2 and 4) would be considered to have chronic hepatitis.

Some patients spontaneously clear HBsAg but this event is rare, occurring at a rate of 0.5 to 1% per year. These patients remain positive for hepatitis B core antibody and some may develop anti-HBs. The majority of patients who clear HBsAg have undetectable HBV DNA in serum but HBV DNA persists in the serum in some, and in the liver in all patients. These patients are considered to have occult HBV infection. While the risk of cirrhosis and end-stage liver disease is greatly diminished, the risk of HCC after HBsAg loss remains substantial particularly if HBsAg loss occurred after the age of 50 or after development of cirrhosis.[8-10] Importantly, HBV can be reactivated upon immunosuppression suggesting that eradication of HBV from the host is rarely achieved.[11]

Identifying individuals at greatest risk for development of cirrhosis and HCC is an important goal in the management of chronic HBV infection. Recent studies have highlighted the importance of viral load in predicting risk of cirrhosis and HCC.[12,13] However, many other host (sex, age, family history of HCC, obesity, diabetes), viral (HBV genotype and variants, coinfection with other viruses: hepatitis C, hepatitis D, HIV), and environmental (alcohol, smoking, carcinogens) factors contribute to liver disease progression.

Several risk models have been developed to predict risk of HCC.[14-18] Most of these models were derived from data in Asians. The applicability of these models to all racial/ethnic groups and HBV genotypes, patients with or without cirrhosis, and untreated patients as well as those receiving antiviral therapy has not been confirmed. Cirrhosis is the major risk factor for HCC. In the past decade, non-invasive assessment – serum marker panels and liver stiffness measurements - have largely replaced liver biopsies in staging of liver fibrosis. These non-invasive tests have also been shown to predict survival and HCC in patients with chronic HBV infection because they have high accuracy in diagnosing cirrhosis.[19-23]

CURRENT STATUS OF HBV TREATMENT

Goals of treatment and efficacy assessment

The goals of HBV therapy are to prevent the development of cirrhosis, hepatic decompensation, HCC and death from HBV-related liver disease. The eventual clinical outcomes of chronic hepatitis B, requires years if not decades of surveillance; therefore, biochemical (normalization of ALT), virological (suppression of serum HBV DNA), serological (HBeAg and/or HBsAg loss with or without seroconversion to anti-HBe and anti-HBs) and histological (decrease in hepatic necroinflammation with or without improvement in fibrosis) markers have been used as surrogates for clinical outcomes: cirrhosis, hepatic

decompensation, HCC and HBV-related mortality and to assess indications for treatment, response and prognosis. The durability of responses after treatment discontinuation is variable.

Indications for treatment

The decision to treat is based upon clinical assessment of the risk of disease progression which is related to phase of disease. This risk assessment is based primarily on HBV DNA and ALT levels and the stage of disease, as assessed by liver biopsy or non-invasive staging of hepatic fibrosis. Current guidelines recommend treatment for patients with cirrhosis or decompensated liver disease and, for patients without cirrhosis, evidence of modest to high viremia and biochemical or histological evidence of hepatic necro-inflammation. [24-26] Continued high levels of HBV replication together with hepatic inflammation increases the risk of cirrhosis and HCC; however, currently available therapies have lower efficacy in patients in the immune tolerant phase and treatment is not recommended for these patients. [27,28]

Approved therapies

Two classes of antiviral therapies have been approved for treatment of hepatitis B: IFNs and NAs. An advantage of IFN is it results in higher rates of HBeAg and HBsAg loss (particularly in patients with genotype A infection) compared to NAs. Pegylated IFN administered for 48-52 weeks results in HBeAg seroconversion in 24% to 27% and HBsAg loss in 3% to 7% of patients compared to 12% to 22% HBeAg loss and 0% to 3% HBsAg loss after the same duration of NA therapy. [29] Response to IFN is also more durable and HBeAg and HBsAg loss may occur after cessation of treatment, while virological relapse, even after HBV DNA has become undetectable, is frequent after cessation of NA. [30] However, IFN is less effective at suppressing viral replication compared to NAs, requires parenteral administration, is associated with numerous adverse effects, and is contraindicated in patients with decompensated

cirrhosis or severe exacerbations of hepatitis and those with autoimmune or psychiatric illnesses. NAs are administered orally and have negligible adverse effects. The recommended first-line NAs – entecavir and tenofovir have low risk of drug resistance but the requirement for indefinite therapy in the majority increases the cost and risk of non-adherence and adverse effects.

Various combinations of IFN and NA have been evaluated, but most studies have not shown an added benefit compared to monotherapy. A recent study showed that combination of pegylated IFN and tenofovir increased the rate of HBsAg loss to 9% at week 72 but the benefit was mainly observed in patients with genotype A.[31] Therefore, there is an urgent need to develop new therapies for HBV that can result in durable suppression of HBV replication, and an ensuing decrease in hepatic inflammation and fibrosis after a finite course of therapy. In addition, more research is needed to identify which patients can safely stop therapy. Importantly HBsAg loss at an older age and after the development of cirrhosis does not eliminate the risk of HCC; however, the benefit of further treatment for preventing HCC has not been proven. [32] Treatment that can result in a functional cure at an earlier stage of disease might have a greater impact in preventing HCC.

Lack of impact of NA on covalently closed circular DNA (cccDNA)

A major hurdle to HBV "cure" is the presence of cccDNA in the hepatocyte nucleus in a non-integrated form or episome. cccDNA serves as the template for transcription of all viral RNAs including pregenomic RNA and thus plays a key role in the viral lifecycle. There are two sources of cccDNA: incoming virions and re-cycling of encapsidated DNA from the hepatocyte cytoplasm. The half-life of cccDNA is long thus explaining why it is difficult to cure HBV infection and why HBV can reactivate either spontaneously or following immune suppression, many years after clearance of HBsAg. Chain terminating NAs block the reverse transcription of pregenomic RNA (pgRNA) to HBV DNA but they have marginal effect on cccDNA production, stability or transcription. Continued transcription from cccDNA

and integrated viral genomes may explain the relatively minor decrease in serum HBsAg levels during NA therapy despite undetectable serum HBV DNA levels.[33] Unfortunately, current assays for circulating HBsAg cannot distinguish the transcription of HBsAg from cccDNA versus integrated HBV DNA.

Impact of antiviral treatment on clinical outcomes

Most but not all long-term follow-up studies and meta-analyses indicate that IFN and NA treatment decrease the risk of HCC and liver-related mortality.[34] A landmark randomized controlled trial showed that the first-generation NA, lamivudine, decreased the risk of disease progression and HCC in patients with advanced fibrosis or cirrhosis and high viremia.[1] Several studies have shown that maintained viral suppression during NA therapy is associated with regression of fibrosis and reversal of cirrhosis and reduction in rates of hepatic decompensation.[35,36] The risk of HCC is also diminished although not eliminated, making it the only major complication during NA treatment. The observed reduction in the incidence of HCC appears to be more evident in persons with cirrhosis and after several years of continued treatment.[34]

NOVEL ANTIVIRAL THERAPIES

Recent major scientific discoveries have allowed a better understanding of the HBV lifecycle, including the identification of the cellular receptor for HBV entry, information on the key nuclear enzymes involved in cccDNA formation and on its epigenetic control, the observation of partial cccDNA degradation induced by IFN or NF-κB signaling pathways, and identification of the role of the HBx protein in HBV transcription. Improved cellular and animal models have also enhanced the in vitro and in vivo assessment of the antiviral activity of novel compounds, and their potential toxicity. These major advances in hepatitis B basic research have paved the way for the identification of multiple new therapeutic targets (Figure 2), essential progress toward a “cure” for hepatitis B. A list of novel antiviral therapies tested in clinical trials is provided in supplementary table 1.

HBV entry inhibitors

The pathway by which HBV enters hepatocytes involves virus attachment to Heparan Sulfate Proteoglycans, allowing the binding of HBV preS1 to human sodium taurocholate cotransporting polypeptide (hNTCP), followed by membrane fusion and nucleocapsid release in the cytoplasm of infected cells.

Entry inhibitors can be classified according to their modes of action: (1) Neutralizing antibodies target the antigenic loop of HBV S-domain or N-terminal epitopes in the preS1-domain. These antibodies are highly specific but require parenteral administration and large quantities of antibodies are necessary to neutralize circulating subviral envelope particles. (2) Attachment inhibitors are negatively or positively charged drugs that bind the virus (e.g. heparin) or cellular heparan sulphate proteoglycans (e.g. poly-Lysin). They are efficient but not specific. (3) Substrates of NTCP including conjugated bile salts (e.g. taurocholate, or ezetimibe) or other small molecules that are transported by NTCP; requirement for high concentrations and short half-time at the receptor limit their clinical application. (4) Irreversible NTCP inhibitors: Myrcludex B (myristolated pre-S1 peptide), Cyclosporin A and derivatives are allosteric inhibitors of NTCP. They irreversibly block receptor function at non-saturating concentrations and have long half-time at the receptor but they can block transport of bile salts and other NTCP substrates at higher concentrations.

Entry inhibitors may prevent *de novo* cccDNA formation in non-infected hepatocytes, and might be more effective in preventing mother-to-child transmission or reinfection after liver transplantation than in eliminating HBV in chronically-infected persons.

Clinical trials of Myrcludex B with or without pegylated IFN in chronic HBV and chronic HDV infection are underway.[37,38]

Targeting covalently closed circular DNA

Damage and destruction

A number of studies demonstrate that it might be possible to target cccDNA despite its location in the protected sanctuary of the hepatocyte nucleus. Studies in cultured hepatocytes showed that several cytokines (IFN-α, lymphotoxin-β receptor agonists, IFN-γ and tumor necrosis factor-α) can modulate pathways leading to the up-regulation of APOBEC3A/B deaminases, which in turn induced non-hepatotoxic degradation of cccDNA. However, only partial degradation was achieved in these tissue culture studies.[39] DNA cleavage enzymes, including homing endonucleases or meganucleases, zinc-finger nucleases, TAL effector nucleases, and CRISPR-associated (cas) nucleases, specifically targeting cccDNA are being explored in experimental models.[40] Some studies suggest that editing of HBV DNA by CRISPR-associated (cas) nuclease cleavage is more efficient than APOBEC-mediated cytosine deamination following treatment of infected cells with IFN.[41] Efficient delivery of these gene editing approaches to HBV-infected hepatocytes without unintended off-target effects will need to be addressed before they can be tested in clinical trials.

Functional silencing

Viral cccDNA is organized into a chromatin-like structure and is subject to epigenetic regulation.[42] Epigenome modifiers can potentially block cccDNA transcriptional activity and shut-down viral protein expression; however, if not specific, they may induce harmful effects on host cells. Some cytokines including IFN-α have been shown to decrease cccDNA transcription via epigenetic modifications in preclinical models.[43] HBx has been shown to be required for cccDNA transcription and viral replication via degradation of the Smc5/6 restriction factor; thus, HBx may be an attractive viral target to silence not only cccDNA transcription[44] but also several other HBx-dependent virus-related

cellular interactions. Current assays to detect cccDNA lack specificity and a standardized method to measure cccDNA or cccDNA transcription is needed.

Targeting viral transcripts

The use of RNA interference to inhibit replication of HBV has been extensively evaluated *in vitro* and validated in animal models. Small interfering RNA (siRNA) can be designed to target any viral transcript and induce their degradation by the RISC/Ago2 complex resulting in gene silencing. The potential limitations are the need for intravenous administration, the risk of off-target binding, the potential toxicity of the vehicle, and the risk of immune activation by pattern recognition receptors. Three siRNA formulations with different modes of delivery are under pre-clinical evaluation and/or early phase clinical trial. Preliminary results of a phase 2 trial showed a single dose of ARC-520 in combination with entecavir resulted in profound and durable decrease in serum HBV DNA in both HBeAg-positive and HBeAg-negative patients and decrease in HBsAg level in HBeAg-positive but not in HBeAg-negative patients.[45,46] The siRNAs were designed to target the co-termini of all transcripts from cccDNA and the lack of effect on HBsAg level in HBeAg-negative patients was postulated to be due to altered viral transcript sequences derived from integrated HBV DNA. Further studies are needed to determine whether the decline in HBsAg production alone might be sufficient to restore HBV-specific T cell response, or if addition of other antiviral or immune modulatory therapies is needed.

Antisense oligonucleotides targeting viral transcripts can block viral protein expression via steric blockade of protein translation and/or RNA degradation by RNAseH cleavage. In vitro and in vivo pre-clinical evaluation have shown their potential to inhibit viral replication and decrease viral antigen load but optimal delivery remains a challenge.

Nucleocapsid assembly and Pregenomic RNA (pgRNA) packaging

Nucleocapsid formation and packaging of the pregenomic RNA (the template for viral replication) are critical steps of the viral lifecycle. Therefore, developing inhibitors or modulators of this process are attractive therapeutic approaches. The HBV core protein is involved in many aspects of the viral lifecycle including transport of viral genome to the nucleus, uncoating to release relaxed circular DNA (rcDNA) in the nucleus, packaging of polymerase protein and pgRNA, capsid assembly, modulation of reverse transcription, and interaction with envelope proteins for virus assembly. It may have additional functions including modulation of cccDNA chromatin and stability, nuclear export of viral RNAs and modulation of innate immunity.

The HBV precore/core protein has recently emerged as a promising direct antiviral target. With the knowledge of the 3-dimensional structure of the core protein, several classes of non-nucleoside small molecules called core protein assembly modulators have been developed, including phenylpropenamide and heteroaryldihydropyrimidine derivatives. These molecules can strengthen protein-protein interaction, inhibit pgRNA encapsidation, and block plus strand DNA synthesis.[47,48] Results of the first dose ranging phase 1b study of NVR3-778 showed a decline in serum HBV DNA, HBV RNA and HBsAg, with more pronounced effect when combined with pegylated IFN.[49]

Targeting HBsAg

Strategies inhibiting viral gene expression either through cccDNA transcription or viral mRNA translation can decrease serum HBsAg levels. Owing to the large amount of circulating HBsAg in persons with chronic HBV infection, monoclonal antibodies aimed at neutralizing and/or depleting HBsAg from the bloodstream are unlikely to be effective unless used in combination with other approaches.

Nucleic acid polymers have been shown to decrease secreted HBsAg, through as yet unknown mechanisms. Clinical trials of REP 2055 and REP 2139 used in monotherapy followed by combination with pegylated IFN or thymosin alpha-1 induced a marked decline in circulating HBsAg levels and viremia

and anti-HBs seroconversion in some patients.[50] Similar results were observed in pilot studies of tenofovir and pegylated IFN in combination with REP 2139 or REP 2165.[51] These results need to be replicated in larger studies and the potential for cytotoxicity resulting from intracellular retention of HBsAg must be resolved.

IMMUNE RESPONSE TO HBV AND IMPLICATIONS FOR IMMUNE MODULATORY THERAPIES

An orchestrated innate and adaptive immune response is necessary to sense and control HBV infection. The induction of innate pro-inflammatory responses and cytokine activation are weak in chronic HBV infection. [52] IFN-γ production from NK cells is detectable in acute hepatitis, but deficient in chronic hepatitis. HBV-specific T cells necessary for HBV control (and may also play a role in hepatic inflammation) are dysfunctional and may be depleted or deleted in chronic HBV.[53,54] Their exhausted phenotype has been attributed to persistent antigen exposure and increased expression of T cell inhibitory responses, [3,52,55] providing the basis for the focus on reducing HBsAg production. B cell dysfunction in chronic hepatitis B is less well characterized though the high rate of HBV reactivation in patients receiving anti-CD20 suggests that B cells play a role in immune control of chronic HBV. Accordingly, several potential target mechanisms for immune modulation to engender or restore HBV-specific immune responses in conjunction with profound inhibition of HBV replication and HBsAg production to attain immunological control have been suggested (Figure 3). The main concerns of immune modulatory therapy are the induction of uncontrolled hepatitis flares or autoimmunity.

Interferons

IFN-α have been used to treat chronic HBV and chronic HDV. IFN-α induces expression of IFN sensitive genes encoding intracellular or secreted effector proteins with antiviral properties. Recent studies showed that IFN-α also inhibits pgRNA encapsidation, enhances cccDNA degradation, and exerts

epigenetic modification of cccDNA transcription.[39,56] Response to IFN-α (HBeAg seroconversion) is durable in >70% of patients.[57,58] Understanding the mechanisms for IFN non-responsiveness and the higher rates of response in genotype A infection could lead to new targets for antiviral and/or immune modulatory therapies.

Pathogen recognition receptors

Pathogen recognition receptors are an important gateway to sensing by the innate immune system. Pharmacological activation of the intrahepatic innate immune response with toll-like receptors 7, 8, or 9 have been studied. GS-9620, an oral agonist of toll-like receptor 7, induced a decline in serum HBV DNA and HBsAg levels as well as hepatic HBV DNA in HBV-infected chimpanzees.[59] Similar effects were also observed in woodchucks[60] but not in humans.[61,62] The discrepancies between results in animal models and humans highlight the importance of testing new therapies in humans at an early stage in drug development.

Stimulator of interferon genes (STING) agonists

Stimulator of interferon gene is the adapter protein of multiple cytoplasmic DNA receptors and a pathogen recognition receptor recognizing bacterial second messenger, and may be a potential target of pharmacologic activation of the innate immune response.[63] Stimulator of interferon gene agonists can also be used as adjuvants to therapeutic vaccination. Retinoic-acid-inducible protein 1 had been shown not only to induce IFN and cytokine production but also inhibit HBV replication through sensing of the epsilon structure of pgRNA.[64] SB 9200, an oral prodrug of the dinucleotide SB 9000, is thought to activate retinoic-acid-inducible protein 1 and nucleotide-binding oligomerization domain-containing protein 2, resulting in IFN-mediated antiviral immune responses in virus-infected cells, and decreased serum woodchuck hepatitis virus DNA and surface antigen levels.[65] Clinical trials are ongoing.

Check point modulation

The lack of a T-cell mediated response in chronic HBV is partly due to overexpression of co-inhibitory receptors including programmed cell death (PD-1), cytotoxic T lymphocyte associated antigen-4 lymphocyte activation gene and mucin domain to impair T cell effector function. T cell function may also be impaired by immunosuppressive cytokines including IL-10. Recent cancer therapies have indicated the potential of blockade of these co-inhibitory receptors with antibodies. Such inhibition may reverse immune dysfunction in hepatitis B as demonstrated in studies in woodchucks and ex vivo studies in humans.[4,66-68] The main concerns of this approach are induction of uncontrolled hepatitis flares and autoimmunity which can lead to fatal organ damage.[69]

Therapeutic vaccine

The goal of therapeutic vaccination is to stimulate or boost the host immune response to restore immune control resulting in sustained suppression of HBV replication and ultimately HBsAg loss. Therapeutic vaccination strategies including conventional HBsAg vaccine +/- potent adjuvants, T-cell vaccine, immune complexes of HBsAg and human anti-HBs, apoptotic cells that express HBV antigens, DNA vaccines or viral vectors expressing HBV proteins have been evaluated with limited success. Patients with high viral loads may be less responsive and patients with cirrhosis may be at higher risk for immune-mediated hepatitis flares. Pre-treatment with NAs to suppress HBV replication may enhance HBV-specific T cell response and prevent flares. GS-4774, a heat-inactivated yeast-based vaccine expressing HBV S/C/X fusion protein induced HBV-specific T cell responses in healthy volunteers but HBsAg decline and HBsAg loss was not observed in chronic hepatitis B patients virally suppressed on NA as well as those who were NA-naive .[70-72] A list of therapeutic vaccines tested in clinical trials is provided in supplementary table 1.

COMBINATION OF ANTIVIRAL AND IMMUNE MODULATORY THERAPIES

Combinations of antiviral therapy targeting multiple steps in the HBV lifecycle to suppress viral replication and viral antigen production and immune modulatory therapy to restore immune response to HBV will likely be needed to achieve the goal of HBV "cure" but which specific combination of agents will be needed is not certain since most antiviral or immune modulatory drugs in clinical evaluation are entering either Phase 1b or Phase 2 trials at this time. The possibility that HBV-specific T cell function can be partially restored has been demonstrated in patients with chronic HBV who had spontaneous or antiviral (IFN or NA)-mediated HBeAg or HBsAg loss.[73,74] With the availability of novel approaches such as siRNA or combination of antiviral agents targeting different steps in HBV lifecycle, marked reduction and even shut-down of HBsAg production may be achieved in a higher proportion of patients. The reduction in antigen load might facilitate the restoration of HBV-specific T-cell responses. Historically, immune modulatory therapies have focused on patients in the immune active phase but recent studies suggest that patients in the "immune tolerant or high-replication non-inflammatory" phase should also be considered, as HBV-specific T cells are present in this phase of disease.[3,75] The ability to "cure" hepatitis B at earlier stages of liver disease would theoretically have a greater impact on reducing risk of HCC.

CLINICAL TRIALS AIMED AT HBV CURE

Efficacy endpoints for clinical trials

Initial trials of IFN and NA used biochemical, virological, serological and histological endpoints to assess efficacy while more recent trials have focused on virological and serological endpoints because these endpoints have been shown to correlate with improved clinical outcomes.[35,76-78] The use of a biochemical endpoint is problematic because of the lack of a standardized definition of normal ALT.

Furthermore, with increasing prevalence of obesity and non-alcoholic fatty liver disease, failure to normalize ALT may not necessarily indicate ongoing liver inflammation induced by HBV. Indeed, phase 3 clinical trials of NA have consistently found a lower percent of patients achieving a biochemical versus a virological response. Non-invasive assessments of liver fibrosis have replaced liver biopsies in assessing liver disease in clinical practice and a histological endpoint would no longer be practical or necessary for assessing functional cure. However, liver biopsies may be required in proof-of-concept studies to confirm a novel mode of action and/or to validate noninvasive surrogate markers of antiviral activity.

For both antiviral and immune modulatory therapy trials, survey respondents ranked suppression of serum HBV DNA to undetectable and loss of HBsAg as the most important primary efficacy endpoint for phase 2 and phase 3 trials, respectively and the expert panel agreed. The difference in ranking of primary efficacy endpoint for phase 2 vs. phase 3 trials reflects a desire to have an earlier read-out in phase 2 trials and the potential difficulty in achieving HBsAg loss after shorter treatment. HBV DNA suppression will not be an appropriate endpoint for trials enrolling patients who are virally suppressed on NA. Decline in serum HBsAg level has been used as an endpoint in some trials but there is no consensus whether the kinetics of decline in HBsAg level can predict ultimate HBsAg loss.

There was general consensus (~65%) that the most appropriate time to assess the primary efficacy endpoint for phase 3 trials of novel antiviral or immune modulatory therapies should be month 6 post-treatment. The choice reflected the intent to achieve a durable response (“cure”) after a finite course of treatment. There was less consensus on the optimal time for assessing efficacy in phase 2 trials with the responses divided between month 6 on-treatment versus month 6 post-treatment. The expert panel indicated that the most appropriate time to assess efficacy endpoints may depend on mechanism of action and half-life of the drug. For example, drugs that inhibit reverse transcription of HBV pregenomic RNA to HBV DNA are expected to result in rapid decline in serum HBV DNA levels while drugs aimed at restoring immune response may take longer to have any measurable effects on viral

load. The expert panel also emphasized the need for long-term follow-up to confirm durability of responses and impact on clinical outcomes.

Diagnostic assays for new markers to determine therapeutic efficacy

There was consensus on the need for standardized assays to provide mechanistic insights into the effects of novel antiviral or immune modulatory agents and to have new surrogate markers to assess HBV "cure" (Table 1).

Serum HBsAg assays

HBsAg loss was ranked as the most important efficacy endpoint for phase 3 trials of both novel antiviral and immune modulatory therapies. Some experts suggested the need for more sensitive HBsAg assays. Others indicated that the persistence of low levels of HBsAg in serum may not stem from persistent cccDNA transcription but from integrated HBV DNA genomes. Assays that can differentiate HBsAg originating from integrated HBV DNA versus cccDNA are required. Failure to detect HBsAg in serum may not represent shut-down of HBsAg production as circulating HBsAg may be complexed to anti-HBs. HBV produces a surplus of empty envelope particles which can outnumber virions by >1,000-fold. Assays that can differentiate surface proteins in virions versus subviral particles (large, middle versus small HBsAg proteins) would be informative.

Serum HBsAg decline may be used as a surrogate to explore efficacy in early phase trials although its accuracy in predicting HBsAg loss has not been established. Quantitative HBsAg assays can also be used to categorize disease stage and to prognosticate. Several standardized quantitative HBsAg assays have been available in Europe and in Asia in the last decade and the consensus is that they will be essential tools for new drug development.

cccDNA quantification and transcriptional activity

Many new antiviral agents in development aim to enhance elimination or degradation of cccDNA or to epigenetically modify cccDNA transcription. Assays that reliably measure concentrations of cccDNA and/or its transcriptional activity would directly assess efficacy of these agents and would be invaluable in proof-of-concept studies. Currently, there are no standardized assays for intrahepatic cccDNA. However, there was consensus that an adequate sample of liver tissue would be necessary and appropriate measures to ensure specificity are needed particularly in the presence of abundant rcDNA.

Assays for serum markers that are reliable surrogates of intrahepatic cccDNA would be desirable for clinical trials. Several studies have shown that serum HBsAg level correlates better with hepatic cccDNA than serum HBV DNA level suggesting that it is an indirect marker of cccDNA.[79,80] However, while the correlation is satisfactory in HBeAg-positive patients it is suboptimal in HBeAg-negative patients.[79,81] HBsAg can be translated from subviral RNAs transcribed from cccDNA or from integrated HBV DNA. It has been suggested that HBsAg is predominantly derived from cccDNA in HBeAg-positive patients while an increasing proportion is derived from integrated HBV DNA in HBeAg-negative persons. Recent studies suggest that serum HBV RNA and hepatitis B core-related antigen (HBcrAg) levels may be more reliable surrogates of hepatic cccDNA than HBsAg levels.

Serum HBV RNA assays

The vast majority of circulating HBV virions contain partially double stranded rcDNA; however, circulating "virions" containing HBV RNA had been reported.[82] These RNA-containing "virions" are more abundant in patients receiving NA therapy probably because inhibition of reverse transcription of pgRNA by NA leads to accumulation of encapsidated pgRNA, some of which may be enveloped and secreted. Thus the detection of HBV RNA in serum in the absence of detectable serum HBV DNA in patients receiving NA therapy could infer ongoing cccDNA transcription, and has been shown to be a predictor of

viral relapse after discontinuation of NA therapy.[83] Assays for serum HBV RNA levels, particularly if they are specific for pgRNA, may provide a useful surrogate for transcriptionally active cccDNA.

Serum hepatitis B core related antigen (HBcrAg) assays

The HBV precore/core gene is translated into the core protein, precore/core precursor and HBeAg; these proteins can be collectively measured. HBcrAg can assemble into defective, enveloped particles that that do not contain HBV RNA or HBV DNA. A core related antigen immunoassay measuring all these forms of precore/core gene expression has been developed. Serum HBcrAg levels have been shown to correlate with hepatic HBV DNA and to predict viral relapse after discontinuation of NA therapy.[84] Assays for serum HBcrAg levels may provide indirect evidence for transcriptionally active cccDNA.[85]

Immune response to HBV

Restoration of immune response to HBV is a key step towards HBV "cure". Most immune modulatory therapies have focused on restoring T cell response. While markers of response to antiviral therapies can be used to evaluate immune modulatory therapies, standardized assays to measure improvement in specific immune responses to predict viral clearance would be informative in early phase drug development. The development of standardized assays to assess restoration of immune responses to HBV will require consensus on the requisite assays, their methodology, sensitivity and specificity, their correct interpretation, reproducibility in different laboratories and cross-validation of the assays.

Approval of HBV diagnostic assays

Development of standardized assays for surrogate endpoints for HBV cure should occur in parallel with development of novel antiviral and immune modulatory therapies to expedite research. During the closed session, the U.S. FDA and European Medicines Agency representatives proposed consideration of simultaneous testing of multiple intermediate secondary endpoints in all phase 2 and 3 trials. The research assays must be standardized, and data and assay platforms shared to facilitate identification of the appropriate surrogate markers of efficacy. It is important that the approval of new treatments is linked to the development and approval of new diagnostic assays used to measure efficacy or to predict response.

Assessment of safety and stop rules

The remarkable safety profile of current NAs imposes a stringent requirement for the safety of new HBV therapies at all stages of the disease. A unique concern in the development of hepatitis B therapies is the risk of severe hepatitis flares, which can result in hepatic decompensation and death. The U.S. FDA has explicit recommendations on managing drug induced liver injury; however, these recommendations do not apply to patients with underlying liver disease. Furthermore, transient hepatitis flares are not always harmful and may portend immune clearance of infected hepatocytes. Hepatitis flares may be due to direct drug-induced liver injury, drug-induced immune mediated hepatitis (e.g. checkpoint inhibitors), the underlying disease (incomplete viral suppression) or immune clearance of infected hepatocytes (successful viral suppression and accompanying restitution of the host immune response). The timing and course of the flare, and the associated chronological changes in serum HBV DNA levels can help in differentiating the cause of most but not all hepatitis flares for example flares related to drug-induced liver injury may by idiosyncratic and occur at any time. There was no consensus on definition of hepatitis flares (magnitude of ALT increase, absolute value or fold-change compared to baseline); however, a majority of survey respondents and the expert panel agreed that flares

accompanied by increase in bilirubin or prothrombin time and flares in patients with cirrhosis should be considered severe. Other adverse events can also occur, e.g. off-target effects of siRNA or epigenetic modification, autoimmunity associated with check-point inhibitors, hepatotoxicity from retention of dysfunctional viral particles. There was no consensus on when a trial or development of a new agent should be stopped due to safety concerns; however, survey respondents and the expert panel indicated that any death or liver transplantation, hepatic decompensation, irreversible autoimmunity, or incidence of severe hepatitis flare in >5% of patients could prompt a halt. Two-thirds of respondents to the survey and most of the expert panel recommended that safety and efficacy should be assessed for at least 6 months after stopping therapy.

Design of clinical trials for HBV cure

A combination of antiviral and immune modulatory therapies will most likely be needed to increase the likelihood of HBV cures. There was consensus that the antiviral activity and safety of individual new agents used as monotherapy, and infrequent or insignificant drug interactions should be first established before progressing to clinical trials of combination therapy. However, demonstration of efficacy as a monotherapy need not be required.

New therapies are most needed for patients at high risk of HBV-associated mortality (cirrhosis) and those in whom current therapies are less effective (immune tolerant, HDV infection). However, in designing early phase clinical trials, the target patient populations will necessarily be those who are most likely to respond and able to tolerate possible exacerbations of hepatitis. The consensus was to initially conduct trials in treatment-naïve HBeAg-positive patients with active disease or in HBeAg-positive or -negative patients virally suppressed on NAs. Proof of principle and safety data would first be established in patients without cirrhosis.

A particular challenge with designing phase 2 studies is to identify a target population with sufficient heterogeneity to be representative of the population with chronic hepatitis B but not so diverse that it hinders analysis because of multiple subgroups. Among the survey respondents, 59% felt that patient sub-populations should be studied separately. If multiple patient populations were included in the same trial, patients could be potentially stratified for cirrhosis, HBeAg status, HBsAg level, HBV DNA level, treatment history, and HBV genotype. Standardized criteria for ascertaining cirrhosis by non-invasive serum markers and/or liver elastography are required.

Given the heterogeneity of the natural course of chronic hepatitis B, there was consensus that randomized controlled trials are needed to establish efficacy, and comparison with placebo is feasible and ethical in trials for patients in the immune tolerant or inactive phases, since current guidelines do not recommend treatment for these patients. For patients with active disease or cirrhosis, investigational new agents can be compared to NA or IFN. Alternatively, the new drug can be tested against placebo as an additional therapy to NA. There was consensus that the trials should aim to demonstrate superiority of the investigational therapy.

SUMMARY AND RECOMMENDATIONS

A summary of the recommendations on endpoints and design of clinical trials for HBV cure is presented in Table 2. Improved understanding of the HBV lifecycle and host immune response to HBV have facilitated discovery and design of antiviral therapies directed against multiple steps of the HBV replication cycle, as well as potential immunomodulatory therapies to restore immune response to HBV infection. Development of novel HBV therapies will be further aided by the availability of improved cellular and animal infection models. While this progress has raised the possibility of a cure for hepatitis B, a complete sterilizing cure i.e. viral eradication from the host may be unrealistic due to presence of integrated HBV DNA. Although HBsAg clearance is infrequent, it does occur with NA and IFN therapy and

is associated with improved clinical outcomes. Therefore, a functional cure (characterized by sustained loss of HBsAg with or without anti-HBs seroconversion) after a finite course of novel antiviral and immune modulatory therapies in a higher proportion of patients than is currently achieved with existing treatments, is an attainable goal.

Cooperation between academia, industry and regulatory agencies to standardize and validate surrogate markers for cure, in order to facilitate the development of curative therapies and to facilitate the path from discovery to regulatory approval is required. Limited proof-of-concept monotherapy studies to evaluate safety and antiviral activity should be conducted prior to proceeding to combination therapy. The safety of any new curative therapies will be paramount given the excellent safety of currently approved NAs. Continued collaboration among the stakeholders will make HBV "cure" a reality for patients with chronic HBV.

Conflicts of interest:

ASL has received research grant funding from Bristol-Myers Squibb and Gilead

FZ has received research grants and consulting fees from Arbutus, Assembly, Gilead, Janssen, Roche, Sanofi

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MGG has no conflict to declare

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Figure Legends

Figure 1. Phases of chronic HBV infection.

1) Immune tolerant. HBeAg positive, high serum HBV DNA but normal ALT levels.
2) Immune clearance/HBeAg-positive chronic hepatitis. HBeAg positive, high serum HBV DNA and elevated ALT levels. HBeAg seroconversion to anti-HBe occurs after varying duration.
3) Inactive carrier. HBeAg-negative, serum HBV DNA low (generally <2000 IU/mL) or undetectable.
4) Reactivation/HBeAg-negative chronic hepatitis. HBeAg-negative, elevated levels of HBV DNA and ALT in serum, HBV precore and/or basal core promoter variant often present.

Traditionally phases of chronic HBV infection are defined by HBeAg status, serum HBV DNA and ALT levels. Quantitative HBsAg levels are different in each phase and are generally highest in immune tolerant phase and lowest in inactive carrier phase. While most patients progress from one phase to the next, not all patients go through each phase, and reversion to an earlier phase can occur.

Abbreviations: ALT, alanine aminotransferase; anti-HBe, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen

Figure 2. HBV lifecycle and antiviral targets.

1) HBV entry. Lipopeptides mimicking pre-S1 domain competing with Dane particle for binding to NTCP (e.g. Myrcludex B). Other small molecule inhibitors are in development.
2) Targeting cccDNA. Prevention of cccDNA formation. Damage and destruction via cytokines or cccDNA sequence-specific nucleases. Functional silencing via modulation of host cellular epigenetic-modifying enzymes by cytokines or inhibition of viral protein function.

3) HBV mRNAs. small interfering RNA approaches or anti-sense oligonucleotides to block viral replication and viral protein expression.

4) HBV Polymerase. Reverse transcriptase inhibitors include approved nucleos(t)ide analogues. RNAseH inhibitors are in preclinical evaluation.

5) Nucleocapsid assembly and pgRNA packaging. Capsid assembly modulators can affect nucleocapsid assembly, pgRNA encapsidation, and may affect the nuclear functions of HBc (cccDNA regulation and interferon stimulated gene expression).

6) Targeting HBsAg. Phosphorothioate oligonucleotides (NAPs) inhibiting HBsAg release and monoclonal antibodies to decrease circulating HBsAg load are under evaluation.

Abbreviations: HBc, hepatitis B core protein; HBsAg, hepatitis B surface antigen; HBx, hepatitis B x protein; pgRNA, pregenomic RNA; NTCP, sodium taurocholate co-transporting polypeptide; cccDNA, covalently closed circular DNA.

Figure 3. The immune liver microenvironment and immunotherapeutic targets

1) Innate immune responses. IFN-α exhibits antiviral activity in infected cells, but also contributes to cell-mediated immunity in vivo. TLR (TLR-7 and others) agonists to boost antiviral cytokine production and activation of NK cells, B cells and T-cells are in clinical evaluation. Drugs antagonizing cIAP can sensitize HBV-infected cells to TNF-mediated apoptosis.

2) HBV-specific T-cell exhaustion. Approaches to block inhibitory pathways (check point inhibitors: PD-1 blockade and others) and immunosuppressive cytokines (IL-10 and TGF-β) to achieve recovery of HBV-specific T cells and NK cells from chronic hepatitis B patients are currently in evaluation.

3) Engineering of redirected T cells via i) transfer of HBV-specific T-cell receptors or HBV-specific chimeric antigen receptors ex vivo in patient's T cells, or ii) retargeting of immune effector cells towards HBV-infected cells using bispecific antibody constructs.

4) Therapeutic vaccines. Antigenic stimulation by diverse approaches are currently being evaluated in phase I/II clinical trials in association with nucleos(t)ide analogues to promote CD4+ and CD8+T cell antiviral activity and antibody responses.

Abbreviations: cIAP, cellular inhibitor of apoptosis; NK, natural killer cells; HBsAg, hepatitis B surface antigen; PD-1, programmed cell death protein 1; TLR, Toll-like receptor; TNF, tumor necrosis factor.

Table 1 Potential Diagnostic Tools/Surrogate Markers for HBV Cure

HBV Marker	Purpose	Rationale / comments
HBsAg - ultrasensitive qualitative or quantitative assay	To detect minimal residual HBsAg, lower limit of detection of Lumipulse assay 0.004 IU/mL compared to current assays 0.05 IU/mL	HBsAg loss is considered the most reliable indicator for functional cure
HBsAg fragments – epitope mapping	To determine whether residual HBsAg is translated from cccDNA transcripts or integrated HBV DNA transcripts To detect antiviral-resistant / immune escape HBsAg variants	Persistent detection of HBsAg may be from integrated HBV DNA and not cccDNA. Integrated HBV DNA is often fragmented with deletions and re-arrangements while cccDNA translates into full length HBsAg. HBsAg variants may give rise to false negative results or inaccuracy in quantification in current assays.
Large (L) vs. Middle (M) vs. Small (S) surface protein	To differentiate complete virions from empty envelope particles	Complete virions are coated with L, M & S surface proteins but empty envelope particles comprise mostly S surface proteins
HBsAg-anti-HBs immune complex	To detect residual HBsAg masked by anti-HBs in immune complex	HBsAg loss is considered the most reliable indicator for functional cure
Quantitative HBsAg level	To facilitate differentiation of inactive carriers from HBeAg-negative chronic hepatitis	Serves as an intermediary measure of HBsAg loss

	To predict outcome in HBeAg-negative patients with low serum HBV DNA	HBsAg level declines before it becomes undetectable but accuracy in predicting HBsAg loss is low
HBV RNA level	To predict viral relapse when treatment is stopped.	Serves as a surrogate for transcriptionally active cccDNA particularly if assay is specific for pgRNA. Encapsidated pgRNA can be enveloped and secreted, levels higher in patients on NA because reverse transcription of pgRNA to HBV DNA is blocked. Shown in some studies to predict viral relapse after discontinuation of NA. Specificity of current assays for pgRNA versus subgenomic RNAs is unknown.
Hepatitis B core related antigen (HBcrAg)	To correlate levels with intrahepatic HBV DNA. To predict viral relapse when treatment is stopped.	Translated from HBV precore/core gene, can assemble into defective particles that are secreted. Shown in some studies to correlate with intrahepatic HBV DNA, ccc DNA transcriptional activity and to predict viral relapse after discontinuation of NA. Limitation: lack of sensitivity, composite biomarker.
cccDNA quantification and	To quantify cccDNA from treated and	cccDNA serves as template for transcription

transcriptional activity	untreated patients To assess transcriptional activity: pgRNA/cccDNA ratio	of HBV RNA and translation of HBV antigens Most direct measure of HBV cure Adequate sample of liver tissue and stringent protocol to ensure specificity is required.
Immune response to HBV antigens	To assess role as a biomarker for treatment response	Restoration of immune response is considered a necessary prerequisite for HBV cure Unclear what types of immune response are critical, what methods to use, and criteria for improvement or restoration of response that will translate to cure

Table 2 Summary of Recommendations on Endpoints and Design of Clinical Trials for HBV Cure

Definition of functional HBV cure

- Sustained HBsAg loss with or without anti-HBs seroconversion and undetectable HBV DNA in serum after completion of a finite course of treatment

Main objective

- To demonstrate superiority of the investigational therapy

Control arm

- Placebo or no treatment for patients in the immune tolerant or inactive phase
- NA or IFN as comparator therapy or add-on therapy to investigational therapy for patients with active disease or cirrhosis (IFN as comparator only if no cirrhosis or compensated cirrhosis with no portal hypertension)

Patient profile for initial trials

- HBeAg-positive patients with active disease, not currently on treatment
- HBeAg-positive or HBeAg-negative patients with baseline active liver disease, who are virally suppressed on NAs
- Factors to consider for stratification
    - HBeAg status
    - HBsAg level
    - Cirrhosis
    - HBV DNA level
    - Treatment history
    - HBV genotype

Primary efficacy endpoint

- Phase 2 trials: Suppression of HBV DNA in serum to undetectable month 6 on-treatment or month 6 post-treatment, decline in serum HBsAg level may be used as exploratory endpoint in trials enrolling patients virally suppressed on NAs and in trials of compounds targeting HBsAg
- Phase 3 trials: HBsAg and HBV DNA not detectable in serum month 6 post-treatment, must include long-term follow-up to confirm durability of response after completion of treatment

Safety issues

- Evidence of safety of new drugs as monotherapy, and evaluation of infrequent or insignificant drug interactions needed before new drugs are combined
- Hepatitis flares: need to differentiate flares due to immune clearance versus other causes
- Severe flares: hepatitis flares accompanied by increase in bilirubin or prothrombin time or occurring in patients with cirrhosis
- Any adverse event leading to death or liver transplantation, hepatic decompensation, irreversible autoimmunity, or incidence of severe hepatitis flare in >5% patients may prompt consideration for stopping trial

References:

1. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-31.
2. Bertoletti A, Kennedy PT. The immune tolerant phase of chronic HBV infection: new perspectives on an old concept. Cell Mol Immunol 2015;12:258-63.
3. Kennedy PT, Sandalova E, Jo J, et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143:637-45.
4. Park JJ, Wong DK, Wahed AS, et al. Hepatitis B Virus--Specific and Global T-Cell Dysfunction in Chronic Hepatitis B. Gastroenterology 2016;150:684-95 e5.
5. Wu JF, Chiu YC, Chang KC, et al. Predictors of hepatitis B e antigen-negative hepatitis in chronic hepatitis B virus-infected patients from childhood to adulthood. Hepatology (Baltimore, Md) 2016;63:74-82.
6. Liu J, Yang HI, Lee MH, et al. Serum Levels of Hepatitis B Surface Antigen and DNA Can Predict Inactive Carriers With Low Risk of Disease Progression. Hepatology 2016;64:381-9.
7. Tseng TC, Liu CJ, Yang HC, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012;142:1140-9 e3; quiz e13-4.
8. Kim GA, Lee HC, Kim MJ, et al. Incidence of hepatocellular carcinoma after HBsAg seroclearance in chronic hepatitis B patients: a need for surveillance. J Hepatol 2015;62:1092-9.
9. Yuen MF, Wong DK, Fung J, et al. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology 2008;135:1192-9.
10. Gounder PP, Bulkow LR, Snowball M, et al. Nested case-control study: hepatocellular carcinoma risk after hepatitis B surface antigen seroclearance. Aliment Pharmacol Ther 2016;43:1197-207.
11. Perrillo RP, Martin P, Lok AS. Preventing hepatitis B reactivation due to immunosuppressive drug treatments. JAMA 2015;313:1617-8.
12. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
13. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-86.
14. Yang HI, Yuen MF, Chan HL, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011;12:568-74.
15. Papatheodoridis G, Dalekos G, Sypsa V, et al. PAGE-B predicts the risk of developing hepatocellular carcinoma in Caucasians with chronic hepatitis B on 5-year antiviral therapy. J Hepatol 2016;64:800-6.
16. Lee MH, Yang HI, Liu J, et al. Prediction models of long-term cirrhosis and hepatocellular carcinoma risk in chronic hepatitis B patients: risk scores integrating host and virus profiles. Hepatology 2013;58:546-54.
17. Yuen MF, Tanaka Y, Fong DY, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol 2009;50:80-8.
18. Yang HI, Sherman M, Su J, et al. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Clin Oncol 2010;28:2437-44.
19. Jung KS, Kim SU, Ahn SH, et al. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatology 2011;53:885-94.

20. Masuzaki R, Tateishi R, Yoshida H, et al. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. Hepatology 2009;49:1954-61.
21. de Ledinghen V, Vergniol J, Barthe C, et al. Non-invasive tests for fibrosis and liver stiffness predict 5-year survival of patients chronically infected with hepatitis B virus. Aliment Pharmacol Ther 2013;37:979-88.
22. Kim SU, Lee JH, Kim DY, et al. Prediction of liver-related events using fibroscan in chronic hepatitis B patients showing advanced liver fibrosis. PLoS One 2012;7:e36676.
23. Kim JH, Kim JW, Seo JW, Choe WH, Kwon SY. Noninvasive Tests for Fibrosis Predict 5-Year Mortality and Hepatocellular Carcinoma in Patients With Chronic Hepatitis B. J Clin Gastroenterol 2016;50:882-8.
24. Terrault NA, Bzowej NH, Chang KM, et al. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016;63:261-83.
25. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1-98.
26. European Association For The Study Of The L. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-85.
27. Lok AS, Wu PC, Lai CL, et al. A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. Gastroenterology 1992;102:2091-7.
28. Chan HL, Chan CK, Hui AJ, et al. Effects of tenofovir disoproxil fumarate in hepatitis B e antigen-positive patients with normal levels of alanine aminotransferase and high levels of hepatitis B virus DNA. Gastroenterology 2014;146:1240-8.
29. Yapali S, Talaat N, Lok AS. Management of hepatitis B: our practice and how it relates to the guidelines. Clin Gastroenterol Hepatol 2014;12:16-26.
30. Papatheodoridis G, Vlachogiannakos I, Cholongitas E, et al. Discontinuation of oral antivirals in chronic hepatitis B: A systematic review. Hepatology 2016;63:1481-92.
31. Marcellin P, Ahn SH, Ma X, et al. Combination of Tenofovir Disoproxil Fumarate and Peginterferon alpha-2a Increases Loss of Hepatitis B Surface Antigen in Patients With Chronic Hepatitis B. Gastroenterology 2016;150:134-44 e10.
32. Cheng HR, Kao JH, Wu HL, et al. Clinical significance of circulating miR-122 in patients with dual chronic hepatitis B and C virus infection. Hepatol Int 2015;9:35-42.
33. Seto WK, Wong DK, Fung J, Huang FY, Lai CL, Yuen MF. Reduction of hepatitis B surface antigen levels and hepatitis B surface antigen seroclearance in chronic hepatitis B patients receiving 10 years of nucleoside analogue therapy. Hepatology 2013;58:923-31.
34. Lai CL, Yuen MF. Prevention of hepatitis B virus-related hepatocellular carcinoma with antiviral therapy. Hepatology 2013;57:399-408.
35. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381:468-75.
36. Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52:886-93.
37. Blank A, Markert C, Hohmann N, et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. J Hepatol 2016;65:483-9.
38. Urban S, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. Gastroenterology 2014;147:48-64.
39. Lucifora J, Xia Y, Reisinger F, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 2014;343:1221-8.

40. Ely A, Moyo B, Arbuthnot P. Progress With Developing Use of Gene Editing To Cure Chronic Infection With Hepatitis B Virus. Mol Ther 2016;24:671-7.
41. Seeger C, Sohn JA. Complete Spectrum of CRISPR/Cas9-induced Mutations on HBV cccDNA. Mol Ther 2016;24:1258-66.
42. Tropberger P, Mercier A, Robinson M, Zhong W, Ganem DE, Holdorf M. Mapping of histone modifications in episomal HBV cccDNA uncovers an unusual chromatin organization amenable to epigenetic manipulation. Proc Natl Acad Sci U S A 2015;112:E5715-24.
43. Belloni L, Allweiss L, Guerrieri F, et al. IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest 2012;122:529-37.
44. Decorsiere A, Mueller H, van Breugel PC, et al. Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature 2016;531:386-9.
45. Gish RG, Yuen MF, Chan HL, et al. Synthetic RNAi triggers and their use in chronic hepatitis B therapies with curative intent. Antiviral Res 2015;121:97-108.
46. Yuen M, Chan HLY, Liu K, Given B, Schleup et al. Differential reductions in viral antigens expressed from cccDNA vs integrated DNA in treatment naïve HBeAg positive and negative patients with chronic HBV after RNA interference therapy with ARC-520. Journal Hepatol 2016;64:213.
47. Klumpp K, Lam AM, Lukacs C, et al. High-resolution crystal structure of a hepatitis B virus replication inhibitor bound to the viral core protein. Proc Natl Acad Sci U S A 2015;112:15196-201.
48. Venkatakrishnan B, Katen SP, Francis S, Chirapu S, Finn MG, Zlotnick A. Hepatitis B Virus Capsids Have Diverse Structural Responses to Small-Molecule Ligands Bound to the Heteroaryldihydropyrimidine Pocket. J Virol 2016;90:3994-4004.
49. Yuen M, Kim, DJ, Weilert, F, Chan H L-Y, Lalezari J et al. NVR 3-778, a first-in-class HBV core inhibitor, alone and in combination with peg-interferon (PegIFN), in treatment-naive HBeAg-positive patients: early reductions in HBV DNA and HBeAg. Journal Hepatol 2016;64:LB-06.
50. Al-Mahtab M, Bazinet M, Vaillant A. Safety and Efficacy of Nucleic Acid Polymers in Monotherapy and Combined with Immunotherapy in Treatment-Naive Bangladeshi Patients with HBeAg+ Chronic Hepatitis B Infection. PLoS One 2016;11:e0156667.
51. Bazinet M, Pantea, V, Placinta, G, Moscalu, I, Cebotarescu, V, Cojuhari L et al. Preliminary safety and efficacy of REP 2139-Mg or REP2165-Mg used in combination with tenofovir disoproxilfumarate and pegylated interferon alpha 2a in treatmentnaïve Caucasian patients with chronic HBeAg negativeHBV infection. Hepatology 2016;64:LB-7.
52. Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012;61:1754-64.
53. Tan A, Koh S, Bertoletti A. Immune Response in Hepatitis B Virus Infection. Cold Spring Harb Perspect Med 2015;5:a021428.
54. Maini MK, Boni C, Lee CK, et al. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med 2000;191:1269-80.
55. Mason WS, Gill US, Litwin S, et al. HBV DNA Integration and Clonal Hepatocyte Expansion in Chronic Hepatitis B Patients Considered Immune Tolerant. Gastroenterology 2016.
56. Liu F, Campagna M, Qi Y, et al. Alpha-interferon suppresses hepadnavirus transcription by altering epigenetic modification of cccDNA minichromosomes. PLoS Pathog 2013;9:e1003613.
57. Lau DT, Everhart J, Kleiner DE, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;113:1660-7.
58. Buster EH, Flink HJ, Cakaloglu Y, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135:459-67.

59. Lanford RE, Guerra B, Chavez D, et al. GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 2013;144:1508-17, 17 e1-10.
60. Menne S, Tumas DB, Liu KH, et al. Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. J Hepatol 2015;62:1237-45.
61. Gane EJ, Lim YS, Gordon SC, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J Hepatol 2015;63:320-8.
62. Janssen HL, Brunetto, MR, Kim, YJ, Ferrari, C, Benedetta M et al. Safety and Efficacy of GS-9620 in Virally-suppressed Patients with Chronic Hepatitis B. Hepatology 2016;64:1851.
63. Guo F, Han Y, Zhao X, et al. STING agonists induce an innate antiviral immune response against hepatitis B virus. Antimicrob Agents Chemother 2015;59:1273-81.
64. Sato S, Li K, Kameyama T, et al. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity 2015;42:123-32.
65. Korolowicz KE, Iyer RP, Czerwinski S, et al. Antiviral Efficacy and Host Innate Immunity Associated with SB 9200 Treatment in the Woodchuck Model of Chronic Hepatitis B. PLoS One 2016;11:e0161313.
66. Tzeng HT, Tsai HF, Liao HJ, et al. PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model. PLoS One 2012;7:e39179.
67. Liu J, Zhang E, Ma Z, et al. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. PLoS Pathog 2014;10:e1003856.
68. Fisicaro P, Valdatta C, Massari M, et al. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. Gastroenterology 2010;138:682-93, 93 e1-4.
69. Johnson DB, Balko JM, Compton ML, et al. Fulminant Myocarditis with Combination Immune Checkpoint Blockade. N Engl J Med 2016;375:1749-55.
70. Gaggar A, Coeshott C, Apelian D, et al. Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vaccine, in healthy subjects: a randomized study. Vaccine 2014;32:4925-31.
71. Lok AS, Pan CQ, Han SH, et al. Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B. J Hepatol 2016;65:509-16.
72. Janssen HL, Yoon, SK, Yoshida, EM, Trinh, HN, Rodell, TC et al. Safety and efficacy of GS-4774 in Combination with TDF in Patients with Chronic Hepatitis B. Hepatology 2016;64:231.
73. Rehermann B, Lau D, Hoofnagle JH, Chisari FV. Cytotoxic T lymphocyte responsiveness after resolution of chronic hepatitis B virus infection. J Clin Invest 1996;97:1655-65.
74. Boni C, Laccabue D, Lampertico P, et al. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. Gastroenterology 2012;143:963-73 e9.
75. Hong M, Sandalova E, Low D, et al. Trained immunity in newborn infants of HBV-infected mothers. Nat Commun 2015;6:6588.
76. Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1997;26:1338-42.
77. Kim GA, Lim YS, An J, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut 2014;63:1325-32.
78. Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422-7.
79. Thompson AJ, Nguyen T, Iser D, et al. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. Hepatology 2010;51:1933-44.

80. Li W, Zhao J, Zou Z, et al. Analysis of hepatitis B virus intrahepatic covalently closed circular DNA and serum viral markers in treatment-naive patients with acute and chronic HBV infection. PLoS One 2014;9:e89046.
81. Manesis EK, Papatheodoridis GV, Tiniakos DG, et al. Hepatitis B surface antigen: relation to hepatitis B replication parameters in HBeAg-negative chronic hepatitis B. J Hepatol 2011;55:61-8.
82. van Bommel F, Bartens A, Mysickova A, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. Hepatology 2015;61:66-76.
83. Wang J, Shen T, Huang X, et al. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol 2016;65:700-10.
84. Jung KS, Park JY, Chon YE, et al. Clinical outcomes and predictors for relapse after cessation of oral antiviral treatment in chronic hepatitis B patients. J Gastroenterol 2016;51:830-9.
85. Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol 2009;81:27-33.

Figure 1. Phases of chronic HBV infection

338x190mm (96 x 96 DPI)

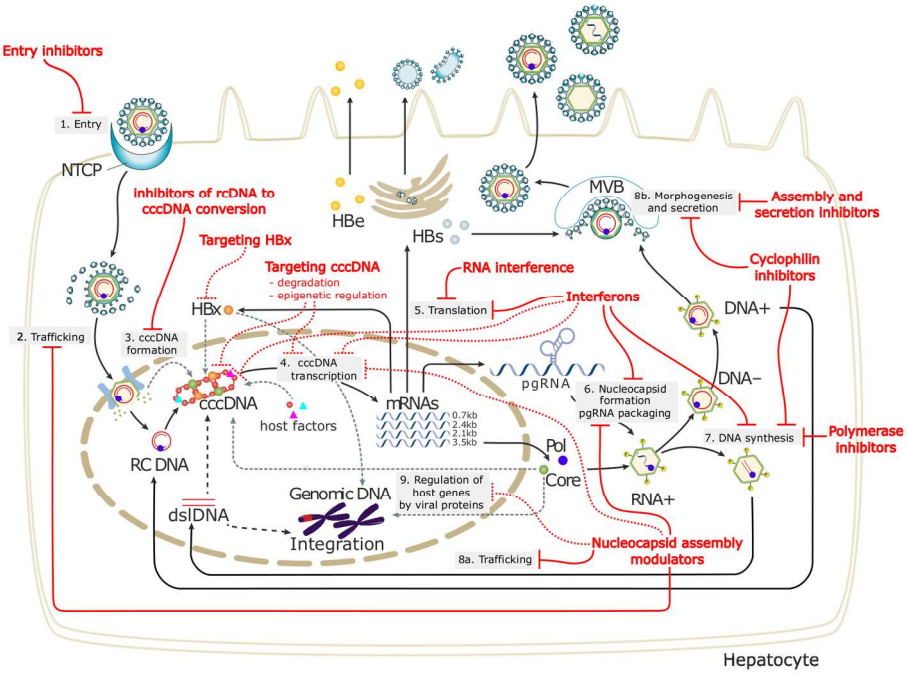

Figure 2. HBV lifecycle and antiviral targets.

162x113mm (300 x 300 DPI)

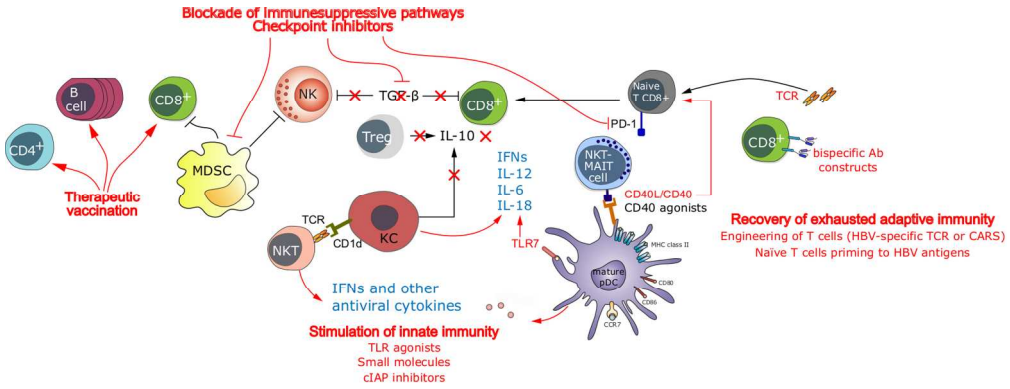

Figure 3. The immune liver microenvironment and immunotherapeutic targets

149x56mm (300 x 300 DPI)

	Targets	Compounds	Developer	Stage of development	ClinicalTrials.gov identifier
Direct acting antivirals	HBpol	GS-7340; Tenofovir Alafenamide Fumarate (prodrug of tenofovir)	Gilead	Approved	NCT01940471 and NCT01940341
	HBpol	AGX-1009 (prodrug of tenofovir)	Agenix/ Cinkate Pharmaceutical	Phase 3	No identifier found
	HBpol	LB80380; Besifovir	IlDong Pharmaceutical	Phase 3	NCT01937806
	HBpol	CMX-157 (prodrug of tenofovir)	Contravir	Phase 2a	NCT02710604
	HBc	GLS-4 (Morphothiadine mesilate)	HEC Pharm/SUnshine	Phase 2	China-CFDA
	HBc	NVR 3-778	Novira Pharmaceuticals/JnJ	Phase 1a	NCT02112799 & NCT02401737
	HBc	JNJ56136379	JnJ Janssen	Phase 1	NCT02662712
	HBc	Core protein Allosteric Modifier (CpAM)	Assembly Biosciences	Phase 1	
	HBs	REP-2139 & 2165 (Nucleic acid polymers)	Replicor	Phase 2 for both HBV and HDV	NCT02565719 and NCT02233075
	Unknown	RG7834	Roche	Phase I	New Zealand
	Viral RNAs	siRNA: ARC-520/ARC-521	Arrowhead	Discontinued	NCT02604212 and NCT02604199
		ALNHBV	Alnylam	Phase 1/2	NCT02826018
		ARB-1467	Arbutus	Phase 2	NCT02631096
		Lunar HBV	Arcturus /Janssen	Preclinical	
		BB HB 331	Benitec	Preclinical	
	Viral RNAs	siRNA: Ionis HBV_{Rx}	Ionis pharmaceuticals/GSK	Phase 1	
	NTCP	Myrcludex	Hepatera	Phase 2 for both HBV and HDV	NCT02881008/NCT02637999
	Promotion of apoptosis in infected cells	Birinapant	Tetralogic	Phase 1	NCT02288208
	Prenylation/farnesylation	Lonafarnib	Eiger BioPharmaceuticals	Phase 2 for HDV	NCT02430181, NCT02430194, NCT02511431
	Immune stimulation	Thymosin alpha	Seoul National	Phase 4	NCT00291616

Host targeting agents			University Hospital		
	Cyclophilins	CPI431	Contravir/ Ciclofilin Pharmaceuticals	preclinical	
	Cyclophilins	NVP018	Neurovive/ OnCore Biopharma	preclinical	
	pDC stimulation	GS-9620 (TLR7 agonist)	Gilead	Phase 2	NCT02579382
	Immune stimulation	INO-1800 (therapeutic vaccine)	Inovio Pharmaceuticals	Phase 1	NCT02431312
	Immune stimulation	Cyt-107 (IL-7)	Cythesis	Phase 1/2 (discontinued)	NCT01027065
	Immune stimulation	SB9200	SpringBank	Phase 2	NCT 02751996
	Immune stimulation	IFN-lambda	BMS	Phase 2 (discontinued)	NCT01204762
	Adaptive responses	ABX-203	Abivax	Phase 2/3	NCT02249988
	Adaptive responses	CVI-HBV-002 (S)	CHA Vaccine Institute	Phase 1/2	NCT02693652
	Adaptive responses	Engerix-B + NA	Chang Gung Memorial Hospital	Phase 4	NCT02505009 NCT01817725
	Adaptive responses	Euvax B (S) + PEG IFN + ETV	Seoul National University	Phase 4	CT02097004
	Adaptive responses	Nivolumab (Anti-PD1 mAb)	Ono Pharmaceuticals/BMS	Phase 1/2 for HCC	NCT01658878
	Adaptive responses	pPDPSC18	Powder Med		NCT00277576
	Adaptive responses	IFN + IL2 + HepB vaccine	Tongji Hospital		NCT02360592
	Adaptive responses	HBsAg-activated MoDC	Sun Yat-Sen University		NCT01935635
	Therapeutic DNA vaccine	HB110	Ichor/Janssen	Phase 1 (completed)	NCT01641536
	Therapeutic vaccine	Tomegavax HBV	TomegaVax	Phase 1	
	Therapeutic vaccine	HepTcell FP-02.2 (Peptide vaccine)	Altimmune	Phase 1 Phase 1	UK NCT02496897
	Therapeutic vaccine	GS-4774	Gilead	Phase 2	NCT02174276
	Therapeutic vaccine	TG-1050	Transgene	Phase 1	NCT02428400
	Therapeutic vaccine	DV-601 (S and C)	Dynavax	Phase 1	Completed
	Therapeutic vaccine	HB-110E	Genexine	Phase 1	NCT01641536
	Unknown	RG7795 (ANA773)	Roche	Phase 2	
	Unknown	RO6864018	Roche	Phase 2	NCT02391805

NA: nucleos(t)ide analogue; PEG IFN: pegylated interferon; TLR: toll-like receptor

Supplementary Table 1: List of HBV antiviral and immune modulatory therapies in clinical trials