

Hepatitis B Cure: From Discovery to Regulatory Approval

Anna S. Lok,¹ Fabien Zoulim,² Geoffrey Dusheiko,³ and Marc G. Ghany⁴

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 stakeholders 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 hepatitis B surface antigen with or without hepatitis B surface antibody 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 toward better defining hepatitis B virus 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 hepatitis B virus 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. (HEPATOLOGY 2017;66:1296-1313).

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 the Hepatitis B

Treatment Endpoints Workshop on September 8-9, 2016, in Alexandria, Virginia. The primary goal of this workshop was to assemble key stakeholders from regulatory agencies (US Food and Drug Administration 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.

Abbreviations: ALT, alanine aminotransferase; 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; HBx, hepatitis B x protein; HCC, hepatocellular carcinoma; IFN, interferon; NA, nucleos(t)ide analogue; NTCP, sodium taurocholate cotransporting polypeptide; pgRNA, pregenomic RNA; siRNA, small interfering RNA.

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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 health care 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 preclinical and early-phase clinical trial data on novel antiviral and immune modulatory therapies for chronic HBV. The workshop concluded with a session on the 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 4 authors) representing all constituent groups. This report summarizes the discussions and consensus opinions of the 2-day meeting.

Definition 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 have 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 hepatocellular carcinoma (HCC) over time (several levels of functional cure including complete shutdown 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 the development of anti-HBs. Importantly, few of the participants believed elimination of cccDNA was a mandatory

ARTICLE INFORMATION:

From the ¹Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI; ²Cancer Research Center of Lyon–INSERM U1052, Hospices Civils de Lyon, Lyon University, Lyon, France; ³University College London Medical School and Kings College Hospital, London, UK; ⁴Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Anna S. Lok, M.D.
Division of Gastroenterology and Hepatology,
University of Michigan Health System
1500 East Medical Center Drive

3912 Taubman Center, SPC 5362
Ann Arbor, MI 48109
E-mail: aslok@med.umich.edu

criterion for functional cure, and less than half required that cccDNA be rendered transcriptionally inactive, reflecting uncertainties over 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 (Fig. 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 hepatitis B e antibody 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

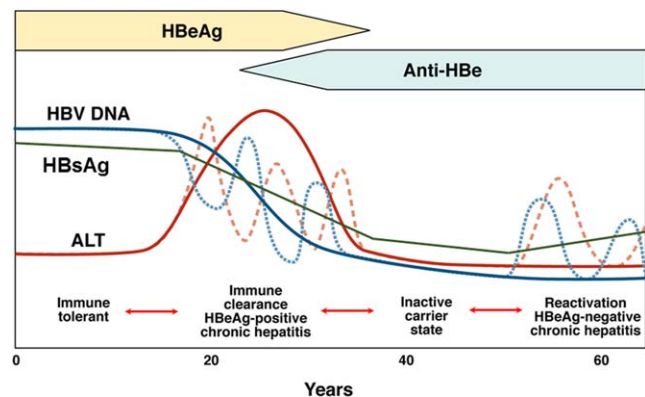


FIG. 1. Phases of chronic HBV infection. *Immune tolerant:* HBeAg-positive, high serum HBV DNA but normal ALT levels; *immune clearance/HBeAg-positive chronic hepatitis:* HBeAg-positive, high serum HBV DNA and elevated ALT levels, HBeAg seroconversion to hepatitis B e antibody occurs after varying duration; *inactive carrier:* HBeAg-negative, serum HBV DNA low (generally <2000 IU/mL) or undetectable; *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 the immune-tolerant phase and lowest in the 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.

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 HCC in HBeAg-negative patients with low viremia (HBV DNA <2,000 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.⁽⁸⁾ Patients in phases 1 and 2 would be HBeAg-positive, those in phases 3 and 4 would be HBeAg-negative, 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%-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.⁽⁹⁻¹¹⁾ Importantly, HBV can be reactivated upon immunosuppression, suggesting that eradication of HBV from the host is rarely achieved.⁽¹²⁾

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.^(13,14) 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, human immunodeficiency virus), and environmental (alcohol, smoking, carcinogens) factors contribute to liver disease progression.

Several risk models have been developed to predict risk of HCC.⁽¹⁵⁻¹⁹⁾ Most of these models were derived from data on 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, noninvasive assessment—serum marker panels and liver stiffness measurements—have largely replaced liver biopsies in staging of liver fibrosis. These noninvasive tests have also been shown to predict survival and HCC in patients with chronic HBV infection because they have high accuracy in diagnosing cirrhosis.⁽²⁰⁻²⁴⁾

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 require 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 hepatitis B e antibody 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 noninvasive 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 necroinflammation.⁽²⁵⁻²⁷⁾ 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.^(28,29)

APPROVED THERAPIES

Two classes of antiviral therapies have been approved for treatment of hepatitis B: IFNs and NAs. An advantage of IFN is that 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%-27% and HBsAg loss in 3%-7% of patients compared to 12%-22% HBeAg loss and 0%-3% HBsAg loss after the same duration of NA therapy.⁽³⁰⁾ 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.⁽³¹⁾ 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 the risk of nonadherence 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.⁽³²⁾ 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.⁽³³⁾ 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 NAs ON cccDNA

A major hurdle to HBV “cure” is the presence of cccDNA in the hepatocyte nucleus in a nonintegrated form or episome. cccDNA serves as the template for transcription of all viral RNAs including pregenomic RNA (pgRNA) and thus plays a key role in the viral life cycle. There are two sources of cccDNA: incoming virions and recycling 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 pgRNA to HBV DNA, but they have a 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.⁽³⁴⁾ 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.⁽³⁵⁾ 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, reversal of cirrhosis, and reduction in rates of hepatic decompensation.^(36,37) 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.⁽³⁵⁾

Novel Antiviral Therapies

Recent major scientific discoveries have allowed a better understanding of the HBV life cycle, including 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 nuclear factor- κ B signaling pathways, and identification of the role of the hepatitis B x protein (HBx) 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 (Fig. 2), essential progress toward a “cure” for hepatitis B. A list of novel antiviral therapies tested in clinical trials is provided in [Supporting Table S1](#).

HBV ENTRY INHIBITORS

The pathway by which HBV enters hepatocytes involves virus attachment to heparan sulfate proteoglycans, allowing the binding of HBV pre-S1 to human sodium taurocholate cotransporting polypeptide (NTCP), 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

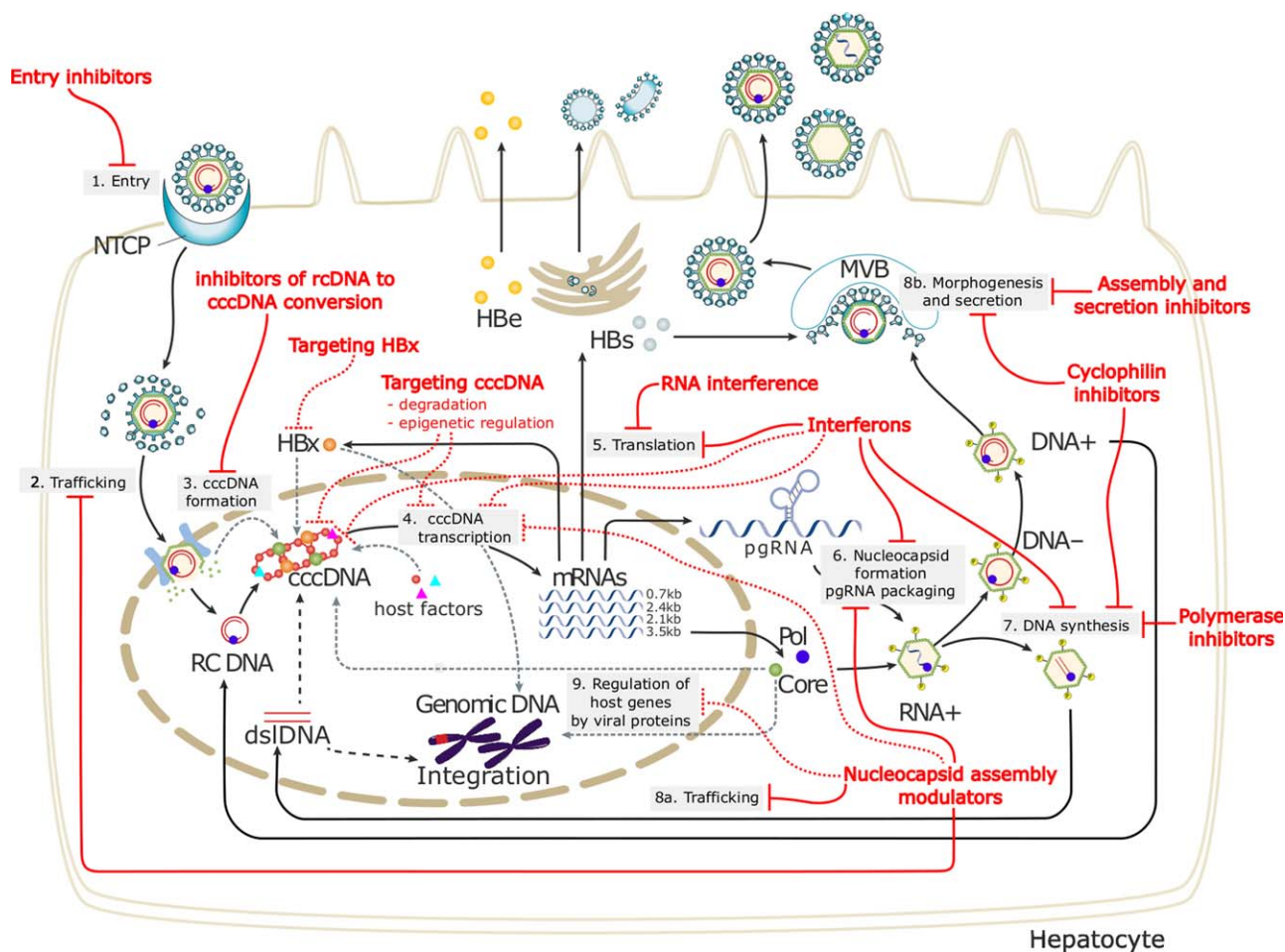


FIG. 2. HBV life cycle and antiviral targets. *HBV entry*: Lipopeptides mimicking pre-S1 domain competing with Dane particle for binding to NTCP (e.g., Myrcludex B). Other small molecule inhibitors are in development. *Targeting cccDNA*: Prevention of cccDNA formation. Damage and destruction through cytokines or cccDNA sequence-specific nucleases. Functional silencing through modulation of host cellular epigenetic-modifying enzymes by cytokines or inhibition of viral protein function. *HBV mRNAs*: siRNA approaches or antisense oligonucleotides to block viral replication and viral protein expression. *HBV polymerase*: Reverse transcriptase inhibitors include approved NAs. Ribonuclease H inhibitors are in preclinical evaluation. *Nucleocapsid assembly and pgRNA packaging*: Capsid assembly modulators can affect nucleocapsid assembly and pgRNA encapsidation and may affect the nuclear functions of hepatitis B core protein (cccDNA regulation and IFN-stimulated gene expression). *Targeting HBsAg*: Phosphorothioate oligonucleotides inhibiting HBsAg release and monoclonal antibodies to decrease circulating HBsAg load are under evaluation. Abbreviations: dsDNA, double-strand linear DNA; MVB, multivesicular body; rcDNA, relaxed circular DNA.

antigenic loop of HBV S-domain or N-terminal epitopes in the pre-S1 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 sulfate proteoglycans (e.g., poly-L-lysine). They are efficient but not specific. (3) Substrates of NTCP include conjugated bile salts (e.g., taurocholate, ezetimibe) and other small

molecules that are transported by NTCP; the requirement for high concentrations and short half-time at the receptor limit their clinical application. (4) Irreversible NTCP inhibitors: myrcludex B (myristoylated pre-S1 peptide), cyclosporin A, and derivatives are allosteric inhibitors of NTCP. They irreversibly block receptor function at nonsaturating 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 noninfected hepatocytes and might be more effective at preventing mother-to-child transmission or reinfection after liver transplantation than at eliminating HBV in chronically infected persons.

Clinical trials of myrcludex B with or without pegylated IFN in chronic HBV and chronic hepatitis D virus infection are under way.^(38,39)

TARGETING cccDNA

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 nonhepatotoxic degradation of cccDNA. However, only partial degradation was achieved in these tissue culture studies.⁽⁴⁰⁾ 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.⁽⁴¹⁾ 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.⁽⁴²⁾ 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.⁽⁴³⁾ 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 through epigenetic modifications in preclinical models.⁽⁴⁴⁾ HBx has been shown to be required for cccDNA transcription and viral replication through degradation of the Smc5/6 restriction factor; thus, HBx may be an attractive viral target to silence not only cccDNA transcription⁽⁴⁵⁾ 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 its 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 preclinical evaluation and/or early-phase clinical trial. Preliminary results of a phase 2 trial showed that a single dose of ARC-520 in combination with entecavir resulted in a profound and durable decrease in serum HBV DNA in both HBeAg-positive and HBeAg-negative patients and a decrease in HBsAg level in HBeAg-positive, but not in HBeAg-negative, patients.^(46,47) The siRNAs were designed to target the cotermini 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 through steric blockade of protein translation and/or RNA degradation by ribonuclease H cleavage. *In vitro* and *in vivo* preclinical evaluations have shown their potential to inhibit viral replication and decrease viral antigen load, but optimal delivery remains a challenge.

NUCLEOCAPSID ASSEMBLY AND pgRNA PACKAGING

Nucleocapsid formation and packaging of the pgRNA (the template for viral replication) are critical steps of the viral life cycle. Therefore, developing inhibitors or modulators of this process is an attractive therapeutic approach. The HBV core protein is involved in many aspects of the viral life cycle including transport of viral genome to the nucleus, uncoating to

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 proinflammatory responses and cytokine activation are weak in chronic HBV infection.⁽⁵³⁾ IFN- γ production from natural killer cells is detectable in acute hepatitis but deficient in chronic hepatitis. HBV-specific T cells necessary for HBV control (which may also play a role in hepatic inflammation) are dysfunctional and may be depleted or deleted in chronic HBV.^(54,55) Their exhausted phenotype has been attributed to persistent antigen exposure and increased expression of T-cell inhibitory responses,^(3,53,56) 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-cluster of differentiation 20 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 (Fig. 3). The main concerns of immune modulatory therapy are the induction of uncontrolled hepatitis flares and autoimmunity.

INTERFERONS

IFN- α has been used to treat chronic HBV and chronic hepatitis D virus. IFN- α induces expression of IFN-sensitive genes encoding intracellular or secreted effector proteins with antiviral properties. Recent studies have shown that IFN- α also inhibits pgRNA encapsidation, enhances cccDNA degradation, and exerts epigenetic modification of cccDNA transcription.^(40,57) Response to IFN- α (HBeAg seroconversion) is durable in >70% of patients.^(58,59) Understanding the mechanisms for IFN nonresponsiveness 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 receptor 7, 8, or 9 has 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.⁽⁶⁰⁾ Similar effects were also observed in woodchucks⁽⁶¹⁾ but not in humans.^(62,63) 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 IFN GENE AGONISTS

Stimulator of IFN 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.⁽⁶⁴⁾ Stimulator of interferon gene agonists can also be used as adjuvants to therapeutic vaccination. Retinoic acid-inducible protein 1 has been shown not only to induce IFN and cytokine production but also to inhibit HBV replication through sensing of the epsilon structure of pgRNA.⁽⁶⁵⁾ 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.⁽⁶⁶⁾ Clinical trials are ongoing.

CHECKPOINT MODULATION

The lack of a T cell-mediated response in chronic HBV is partly due to overexpression of coinhibitory receptors including programmed cell death, 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 interleukin 10.

Recent cancer therapies have indicated the potential of blockade of these coinhibitory receptors with antibodies. Such inhibition may reverse immune dysfunction in hepatitis B as demonstrated in studies in woodchucks and *ex vivo* studies in humans.^(4,67-69) The main concerns of this approach are induction of uncontrolled hepatitis flares and autoimmunity, which can lead to fatal organ damage.⁽⁷⁰⁾

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 with or without potent adjuvants, T-cell vaccine, immune complexes of HBsAg and human anti-HBs, apoptotic cells that express HBV antigens, DNA vaccines, and 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. Pretreatment 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 were not observed in chronic hepatitis B patients virally suppressed on NAs as well as those who were NA-naive.⁽⁷¹⁻⁷³⁾ A list of therapeutic vaccines tested in clinical trials is provided in [Supporting Table S1](#).

Combination of Antiviral and Immune Modulatory Therapies

Combinations of antiviral therapy targeting multiple steps in the HBV life cycle 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 because 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.^(74,75) With the availability of novel approaches such as siRNA or combination of antiviral agents targeting different steps in the HBV life cycle, marked reduction and even shutdown 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 noninflammatory” phase should also be considered as HBV-specific T cells are present in this phase of disease.^(3,76) 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.^(36,77-79) 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 nonalcoholic fatty liver disease, failure to normalize ALT may not necessarily indicate ongoing liver inflammation induced by HBV. Indeed, phase 3 clinical trials of NAs have consistently found a lower percent of patients achieving a biochemical versus a virological response. Noninvasive 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 versus phase 3 trials reflects a desire to have an earlier readout 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 NAs. Decline in serum HBsAg level has been used as an endpoint in some trials, but there is no consensus on 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 posttreatment. 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 posttreatment. The expert panel indicated that the most appropriate time to assess efficacy endpoints may depend on the mechanism of action and half-life of the drug. For example, drugs that inhibit reverse transcription of HBV pgRNA 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 stem not 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 shutdown 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 that appropriate measures to ensure specificity are needed, particularly in the presence of abundant relaxed circular DNA.

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.^(80,81) However, while the correlation is satisfactory in HBeAg-positive patients, it is suboptimal in HBeAg-negative patients.^(80,82) 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.

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 rearrangements, 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) versus middle (M) versus 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 To predict outcome in HBeAg-negative patients with low serum HBV DNA	Serves as an intermediary measure of HBsAg loss. 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.
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 and cccDNA transcriptional activity and to predict viral relapse after discontinuation of NA. Limitation: lack of sensitivity, composite biomarker
cccDNA quantification and transcriptional activity	To quantify cccDNA from treated and untreated patients To assess transcriptional activity: pgRNA/cccDNA ratio	cccDNA serves as a template for transcription 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 are 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.

Serum HBV RNA Assays

The vast majority of circulating HBV virions contain partially double-stranded relaxed circular DNA;

however, circulating “virions” containing HBV RNA had been reported.⁽⁸³⁾ 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.⁽⁸⁴⁾ Assays for serum HBV RNA levels, particularly if they are specific for pgRNA, may provide a useful surrogate for transcriptionally active cccDNA.

Serum 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 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.⁽⁸⁵⁾ Assays for serum HBcrAg levels may provide indirect evidence for transcriptionally active cccDNA.⁽⁸⁶⁾

Immune Response to HBV

Restoration of immune response to HBV is a key step toward 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 and 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 US Food and Drug Administration 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 US Food and Drug Administration 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, as well as 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 be 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 checkpoint inhibitors, and 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, hepatitis D virus 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 HBeAg-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 subpopulations 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 noninvasive 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 that comparison with placebo is feasible and

ethical in trials for patients in the immune-tolerant or inactive phases because 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.

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 posttreatment, 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 posttreatment, 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% of patients may prompt consideration for stopping trial

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 life cycle and host immune response to HBV has 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 the 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.

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Supporting Information

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