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Hepatitis B Treatment: What We Know Now and What Remains to Be Researched

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Abbreviations: ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; cccDNA, covalently closed circular DNA; EASL, European Association for the Study of the Liver; ETV, entecavir; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; NA, nucleos(t)ide analogue; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Abstract

Chronic hepatitis B virus infection remains a major global health burden. Currently, two types of treatment have been approved: interferons and nucleos(t)ide analogues. These treatments are effective in suppressing hepatitis B virus replication and in decreasing the risk of development of cirrhosis, liver failure, hepatocellular carcinoma, and death. However, these treatments do not eliminate the virus, and the risk of hepatocellular carcinoma remains. This review article summarizes current knowledge about hepatitis B treatment: safety, efficacy, and clinical indications. It also discusses limitations of existing treatment, gaps in knowledge, and feasibility of a hepatitis B cure.

Introduction

Chronic hepatitis B virus (HBV) infection remains a major global health burden affecting 292 million persons worldwide.[1] Treatments that are effective in suppressing HBV replication—interferons (IFNs) and nucleos(t)ide analogues (NAs)—have been available for nearly two decades, but these treatments do not eliminate the virus. IFNs and NAs have been demonstrated to prevent cirrhosis, liver failure, and hepatocellular carcinoma (HCC),[2] but the risk of HCC remains, even in virally suppressed patients. Thus, there is a need to develop "curative" therapies for hepatitis B. Equally important is the need to improve diagnosis and linkage to care. Globally, it is estimated that only 10% of persons chronically infected with HBV have been diagnosed, and only 5% of those who are eligible for treatment have received treatment.[1]

HBV biology and barriers to cure

HBV replication cycle

HBV is a hepatotropic DNA virus that replicates by means of reverse transcription of a pregenomic RNA. Figure 1 depicts the life cycle of HBV.[3] The circulating virion comprises an envelope and a nucleocapsid that contains a partially double-stranded relaxed circular DNA. The entry receptor of HBV has been identified to be sodium taurocholate co-transporting polypeptide (NTCP), which explains the hepatotropism of HBV. Upon entry into the hepatocyte, the envelope is shed, and the nucleocapsid is transported into the nucleus where the partially double-stranded relaxed circular DNA is repaired and the covalently closed circular DNA (cccDNA) is bound to chromatin. The cccDNA is transcribed into pregenomic RNA, which is then reverse transcribed into HBV DNA. The pregenomic RNA is also transcribed into viral messenger RNAs and in turn translated into viral proteins: surface (large, middle and small surface proteins), core (hepatitis B core antigen as well as precore/core from which hepatitis B e antigen [HBeAg] is derived), polymerase (which also functions as a reverse transcriptase), and X (unclear functions but may play a role in cccDNA transcription). The nucleocapsid is assembled in the cytoplasm and contains the pregenomic RNA as well as core and polymerase proteins. The pregenomic RNA is reverse transcribed into the (-) strand HBV DNA, which serves as a template for the (+) strand HBV DNA. However, the virion is enveloped and secreted before the (+) strand HBV DNA is completely synthesized. Nucleocapsids can be recycled from the cytoplasm back into the nucleus, and thus, cccDNA can be replenished without the need for entry of new virions. cccDNA has a long half-life, and its elimination in untreated patients appears to be predominantly mediated through loss of infected hepatocytes.

Mechanisms of action of NA and IFN

NAs inhibit reverse transcription of pregenomic RNA and synthesis of HBV DNA in the cytoplasm and have no direct effect on cccDNA. IFN has both antiviral and immunomodulatory activities, although the precise mechanisms of action remain unclear. Recent studies suggest that IFN may enhance cccDNA degradation and exert epigenetic modification of cccDNA transcription,[4] explaining its greater effect on viral protein production and, hence, higher rates of HBeAg and hepatitis B surface antigen (HBsAg) loss compared to NAs.

Barriers to eliminating HBV

Persistence of cccDNA and its ability to self-replenish and lack of direct effects of current therapies on cccDNA account for the difficulty in eliminating cccDNA. There are additional barriers to eliminating HBV. HBV DNA can be integrated into the host genome. Although integrated HBV DNA is often

rearranged and/or partially deleted and there is no evidence that it supports the full cycle of HBV replication, recent studies suggest that integrated HBV DNA can be sufficiently intact to support translation of viral proteins, e.g., HBsAg.[5] Elimination of integrated HBV DNA will likely require the removal of hepatocytes that harbor these DNA. Control of infections generally requires elimination of the infectious organisms coupled with activation of specific immune responses. Whereas patients who recovered from acute HBV infection display rigorous immune responses to multiple HBV epitopes, patients with chronic HBV infection manifest weak immune responses to very few HBV epitopes.[6]

Persistence of cccDNA, presence of integrated HBV DNA, and impaired innate and adaptive immune responses explain why it would be difficult to eliminate HBV from patients who are chronically infected. Indeed, HBV persists in the livers of persons who have recovered from acute HBV infection with HBsAg to hepatitis B surface antibody seroconversion. Thus, HBV reactivation can occur when "recovered" persons receive potent immunosuppressive therapy such as anti-CD20, and transmission of HBV infection can occur when livers from these persons are transplanted into seronegative recipients.

Phases of chronic HBV infection

The course of chronic HBV infection is characterized by fluctuations in HBV DNA and alanine aminotransferase (ALT) levels, reflecting variations in balance between HBV replication and host immune response. Traditionally, three clinical parameters—HBeAg status, HBV DNA level, and ALT level—are used to define the four phases of chronic HBV infection (Fig. 2).

During the first phase, patients are HBeAg-positive with high HBV DNA (>7 $\log_{10}$ IU/mL) but normal ALT levels. These patients are considered to be in the "immune tolerant" phase, although recent studies showed that HBV-specific T-cell responses during this phase are not significantly different from those in active phases, and this phase should be renamed as "low inflammatory" phase.[7] During the second "HBeAg-positive chronic hepatitis" phase, ALT becomes elevated. After varying durations, seroconversion from HBeAg to hepatitis B e antibody occurs. In some instances, this may be accompanied by ALT flares and even hepatic decompensation. After HBeAg loss, most patients enter the third "inactive carrier" phase. Patients in this phase are HBeAg-negative and have low (<2,000 IU/mL) or undetectable HBV DNA and normal ALT. Prognosis is generally favorable if there had not been significant liver injury prior to entry into this phase and patients remain in this phase. Some patients directly transition from the "HBeAg-positive chronic hepatitis" phase to the fourth "HBeAg-negative chronic hepatitis" phase, whereas most enter this phase after varying durations in the "inactive carrier" phase.

Patients in the "HBeAg-negative chronic hepatitis" phase are HBeAg negative and have moderate to high HBV DNA (3-7 $\log_{10}$ IU/mL) levels and elevated ALT. Although most patients progress from one phase to the next sequentially, reversion to the previous phase can occur. Furthermore, depending on the HBV DNA and ALT cut-off values used to define these phases, as many as 30% to 40% of patients do not fit into any phase. Many of these patients may be in transition from one phase to another, whereas some with low viremia and elevated ALT may have concomitant causes of liver injury, most commonly nonalcoholic fatty liver disease.

Recent studies showed that quantification of serum HBsAg levels can help in differentiating the phases of HBV infection—levels being highest in the "immune tolerant" phase and lowest in the "inactive carrier" phase. Quantitative HBsAg levels are most useful in differentiating whether an HBeAg-negative patient with low HBV DNA is truly in the "inactive carrier" phase or in the quiescent period of "HBeAg-negative chronic hepatitis" phase and in predicting risk of HCC.[8,9]

Eventually, some patients spontaneously clear HBsAg. HBsAg loss is reported to occur at the rate of 0.5% to 1% per year, but the incidence is not linear.[10] Risk of adverse clinical outcomes is greatly reduced after HBsAg loss, but risk of HCC remains if HBsAg loss occurred after age 50 or cirrhosis development or in the presence of hepatitis C or D coinfection.[11] Currently, HCC surveillance is recommended for those who lost HBsAg after age 50 or after cirrhosis development, but it is unclear if this needs to be continued for life because the risk may decrease over time to a level when HCC surveillance is no longer cost-effective.

Current treatment

Goals of treatment

The goals of hepatitis B treatment are to prevent the development of cirrhosis, liver failure, HCC, and HBV-related deaths. Proving that treatment improves clinical outcomes will require years if not decades of surveillance. Thus, surrogate markers have been used to measure efficacy of treatment: biochemical (normalization of ALT), virological (suppression of serum HBV DNA to undetectable using sensitive real-time polymerase chain reaction [PCR] assays with detection limits of 10-20 IU/mL), serological (HBeAg and/or HBsAg loss with or without seroconversion to hepatitis B e antibody and hepatitis B surface antibody), and histological (decrease in hepatic inflammation with or without reversal of fibrosis).

Indications for treatment

Chronic HBV infection, if acquired at a young age and if left untreated, is associated with a 20% to 40% lifetime risk of HBV-related mortality. However, as described above, spontaneous virological and clinical remissions with HBeAg and even HBsAg loss can occur. Thus, not all patients require treatment. Indications for treatment depend on the activity and severity of liver disease at presentation, and for those with no cirrhosis at presentation, the predicted risk of cirrhosis or HCC in the future. Various models have been developed to predict the risk of HCC. Variables included in each model differ but generally include demographics (sex, age), HBV markers (HBeAg status, HBV DNA level, and some also include HBV genotype, quantitative HBsAg), and liver disease parameters (ALT, platelet as a marker of cirrhosis/portal hypertension, cirrhosis status, or liver stiffness measurement as a marker of liver fibrosis). Although some of these models have been validated in external cohorts, prospective validation of these models in determining indications for treatment, intensity of monitoring, and need for HCC surveillance has not been performed.

The American Association for the Study of Liver Diseases (AASLD), the Asian Pacific Association for the Study of the Liver (APASL), and the European Association for the Study of the Liver (EASL) guidelines all recommend antiviral treatment for patients with decompensated cirrhosis regardless of HBV DNA or ALT levels.[12-15] Treatment is also recommended for patients with compensated cirrhosis and detectable serum HBV DNA regardless of ALT level. Patients with severe exacerbations of chronic hepatitis B (ALT flares with accompanying increase in bilirubin) and those with acute liver failure are also recommended to receive antiviral treatment. Although high-quality evidence to support these indications is not available, the potential benefits are undisputed, and the possible risks are small. Of note, the landmark double-blind randomized trial demonstrating a benefit of lamivudine versus placebo in reducing disease progression (defined as increase in Child-Turcotte-Pugh score by ≥2 points, clinical decompensation, HCC, or death) in patients with bridging fibrosis or cirrhosis enrolled patients with baseline serum HBV DNA >~140,000 IU/mL.[16] Since then, several studies have suggested a benefit of treatment in patients with compensated cirrhosis and lower HBV DNA levels (including those with HBV DNA <2,000 IU/mL).

For patients with no cirrhosis at presentation, guidelines recommend treatment for those with chronic hepatitis—HBeAg-positive or HBeAg-negative. Although HBV DNA levels are highest in the "immune tolerant" phase, current guidelines do not recommend treatment for patients in this phase because the likelihood of treatment-induced HBeAg loss is extremely low. In one study comparing tenofovir disoproxil fumarate (TDF) monotherapy versus TDF plus emtricitabine, HBV DNA was suppressed to <69 IU/mL in 65% of patients, HBeAg loss occurred in 3% of patients, and HBsAg loss occurred in none of the

patients at the end of 4 years of treatment, and relapse occurred in all patients when treatment was discontinued.[17]

Recognizing that persistently high HBV DNA levels and having a family history of HCC increase the risk of HCC,[18,19] guidelines recommend treatment if patients remain in the "immune tolerant" phase after the age of 30 to 40 years or if there is a family history of HCC. The AASLD guidelines used age 40 as the cutoff based on data from a study showing the risk of cirrhosis development increased from 1.1% to 4.1% to 28% in patients who lost HBeAg before age 30, between 30 and 39, and after age 40, respectively.[20] By contrast, the EASL guidelines selected age 30 as the cutoff. It is unclear which cutoff is more appropriate. Many experts have advocated for treatment early in the course of infection to minimize liver injury, integration of HBV DNA, and risk of HCC. However, it is unclear whether initiating treatment in the "immune tolerant" phase will prevent integration of HBV DNA because integration can occur early and can be detected in children. Similarly, although there is clear evidence that patients with a family history of HCC are at increased risk of HCC, it is unclear whether the risk is related to the number of family members affected, the biological relationships to the affected family members, and the age of family members when HCC was diagnosed. It is also unclear whether antiviral therapy initiated in the absence of standard indications will prevent HCC in patients who are genetically predisposed, are infected with an unusually virulent strain of HBV, or had been exposed to environmental carcinogens. Answers to these questions may be irrelevant if one day, simple, safe, highly effective curative treatments similar to direct-acting antivirals for hepatitis C become available when universal treatment would be recommended. In the meantime, studies that can provide answers to these nuances would be informative.

Another group of patients in the "immune tolerant" phase for which antiviral treatment is recommended are women with high viremia (>200,000 IU/mL).[13] Several studies showed that administration of antiviral therapy to these mothers in the third trimester of pregnancy further reduced the risk of mother-to-child transmission in newborns who received hepatitis B immune globulin (HBIG) and HBV vaccine at birth.[21,22] This benefit was not observed in a recent study where administration of HBIG and birth dose HBV vaccine occurred immediately after birth (median 1.2-1.3 hours) and a total of 5 versus 3 doses of HBV vaccine were administered.[23] Further studies to determine whether more timely administration of HBIG and birth dose HBV vaccine to newborns might obviate the need for antiviral therapy in highly viremic mothers are warranted. Some experts have indicated that TDF is more readily available and at lower cost than HBIG in resource-limited countries; however, HBV DNA testing is

also not readily available in these countries, making it difficult to differentiate high versus low viremic carrier mothers.

Antiviral therapy is also recommended as prophylaxis for HBsAg-positive patients as well as HBsAg-negative, hepatitis B core antibody–positive patients who require treatment with immunosuppressive therapies that are predicted to have moderate to high risk of HBV reactivation.[13] A major challenge is in assessing the risk of HBV reactivation associated with immunosuppressive or cancer therapies because new drugs and biologics come on the market every month, a combination of drugs are frequently used, and treatment may be modified if initial response is unsatisfactory.

<u>Efficacy and safety of available treatments</u>

Currently, two types of treatment are approved for chronic HBV infection: IFNs and NAs. Table 1 summarizes the virologic responses to these therapies.[13,24,25] Pegylated IFNs have more convenient dosing schedule (once vs. thrice weekly) and improved efficacy. Among the NAs, entecavir (ETV), TDF, and tenofovir alafenamide (TAF) are preferred because of their potent antiviral activity and high barrier to antiviral resistance. A 1-year course of pegylated IFN results in higher rates of HBeAg seroconversion (~30% vs. 10%-21%) and HBsAg loss (3% vs. <1%-3%) than the same duration of ETV, TDF, or TAF therapy in HBeAg-positive patients despite lower rates of undetectable HBV DNA (25% vs. 61%-76%). Similarly, in HBeAg-negative patients, a 1-year course of pegylated IFN results in a higher rate of HBsAg loss (4% vs. 0%-1%) than the same duration of ETV, TDF, or TAF therapy despite a lower rate of undetectable HBV DNA (63% vs. 90%-94%). Response to IFN is more durable, and rates of HBeAg and HBsAg loss continue to increase after cessation of treatment, whereas viral relapse is universal when NA is discontinued after 1 year of therapy.

Follow-up of patients for 3-4 years after completion of a 1-year course of pegylated IFN showed that HBsAg loss increased to 8%. By contrast, continuous treatment with NA for up to 5 years has not been shown to further increase the rate of HBsAg loss except for one study of TDF in HBeAg-positive patients where the rate of HBsAg loss increased to 10%. The low rate of HBsAg loss is related to the lack of direct effect of IFN and NA on cccDNA; thus, despite suppression of HBV DNA replication, cccDNA persists and transcription of pregenomic RNA and translation of viral protein continues. Furthermore, it is now recognized that integrated HBV DNA can also be a source of circulating HBsAg.

IFN has to be administered as injections and is associated with numerous adverse effects. It is contraindicated in patients with decompensated cirrhosis or severe exacerbations of chronic hepatitis

and those with autoimmune or psychiatric illnesses and must be used with caution in patients with compensated cirrhosis. By contrast, NAs are administered orally and have negligible adverse effects, and risk of antiviral drug resistance is ≤1% after >5 years of continued treatment with ETV or TDF. NAs can be safely administered in patients with decompensated cirrhosis, HCC, severe exacerbations of chronic hepatitis, or acute liver failure. ETV was reported to be associated with lactic acidosis in one case series of hospitalized patients with decompensated cirrhosis, but other causes such as sepsis may have contributed to lactic acidosis in these acutely sick patients. Mitochondrial toxicity and lactic acidosis are potential adverse effects of all NAs, but documented associations with ETV, TDF, or TAF are extremely rare. TDF has been documented to decrease glomerular filtration rate as well as renal tubular function and to decrease bone mineral density. Although population studies suggest that incidence of adverse events is low,[26] given the need for long durations of NA therapy and increasing age of patients with chronic hepatitis B, safer alternatives are preferred. TAF is a new prodrug of tenofovir. TAF in doses of 25 mg has similar antiviral activity as TDF in doses of 300 mg and is associated with smaller decreases in glomerular filtration rate and bone mineral density.[25] Whether TAF and ETV have similar renal and bone safety profile has not been studied. Lamivudine, telbivudine, and TDF have been shown to be safe for the fetus when used in the first trimester of pregnancy.[27] TDF is preferred because of its high barrier to resistance.

Resistance to NAs

NA treatment may select for viral variants that confer resistance. Resistance to NAs leads to virological breakthrough (>1 $\log_{10}$ increase in HBV DNA from nadir while on treatment) that may be accompanied by biochemical breakthrough, hepatitis flares, and hepatic decompensation. Earlier NAs for hepatitis B were associated with very high rates of antiviral drug resistance—up to 70% after 5 years of lamivudine treatment. The risk of antiviral drug resistance in NA-naive patients is ≤1% after 5 years of continued treatment with ETV and 8 years with TDF, but the risk of resistance to ETV is as high as 50% in patients with lamivudine-resistant HBV.[28,29] For patients with prior NA exposure, TDF or TAF is preferred because mutations conferring resistance to lamivudine or telbivudine (M204V/I) constitute the first step in the 2-hit path to ETV resistance.

Predictors of response

Among HBeAg-positive patients, high ALT or histologic activity and low HBV DNA pretreatment have been consistently shown to be predictive of higher rates of HBeAg loss in response to IFN as well as NA

therapy. HBV genotype is also a strong predictor of IFN-related HBeAg and HBsAg loss.[30] The basis for the genotype effect on IFN-induced HBeAg and HBsAg loss is unclear. Clinical studies showed that patients with genotype A infection do not have lower baseline HBeAg or HBsAg levels. Understanding the biologic differences in HBV genotypes that contribute to more favorable response of genotype A to IFN may help in optimizing the design of new antiviral or immunomodulatory therapies. Among HBeAg-negative patients, there are no consistent baseline predictors of response to IFN or NAs. Because of the low event rate, predictors of HBsAg loss have not been identified for NA therapy while genotype A is the only predictor of HBsAg loss for IFN therapy.

In patients who received IFN therapy, lack of or suboptimal decline in quantitative HBsAg but not HBV DNA has high negative predictive value for response defined as HBeAg loss in HBeAg-positive patients or sustained off-treatment virus suppression in HBeAg-negative patients. This has led to development of stop rules such that patients who are predicted not to have a successful response may discontinue IFN after 12 or 24 weeks, sparing them unnecessary adverse effects.[31] However, these stop rules are genotype-specific and have not been validated in prospective studies.

Choice of antiviral drug and duration of treatment

For patients who are eligible to receive either IFN or NA therapy, the choice depends on patient preference and predicted likelihood of response. IFN has to be administered as injections and is associated with many side effects, but it is given for a finite duration and has a higher rate of HBeAg and HBsAg loss, particularly for patients with genotype A infection. HBeAg-positive patients with genotype A infection, high ALT (>2 times normal), and low HBV DNA (<8 $\log_{10}$ IU/mL) with no cirrhosis and no contraindications to the use of IFN are the best candidates for IFN therapy.

NAs are taken orally and have negligible side effects but need to be administered for many years and, in some patients, for life. Thus, willingness to commit to a long duration of treatment and ability to adhere to the treatment are important deciding factors. ETV, TDF, and TAF are preferred NAs. ETV or TAF is preferred for patients with underlying renal or bone disease. TDF or TAF should be used in patients with prior NA exposure. TDF should be used in pregnant women and women contemplating pregnancy.

De novo combination of NAs has not been shown to be superior to NA monotherapy except for patients with very high baseline HBV DNA (>8 $\log_{10}$ IU/mL) where more rapid decline in viremia is achieved with combination therapy.[32] Similarly, although combination therapy was initially advocated for patients with NA resistance, subsequent studies showed that TDF monotherapy has similar efficacy compared to

combination of TDF plus emtricitabine in patients with lamivudine resistance.[33] Several strategies to combine pegylated IFN and NA have been tried, including *de novo* combination, add-on, and switch-to approaches. Although some studies have shown a benefit compared to monotherapy, the results are not consistent and may not be generalized because many studies only enrolled highly selected patients.[31] A study comparing *de novo* combination of pegylated IFN plus TDF versus pegylated IFN or TDF monotherapy showed that combination therapy was associated with a higher rate of HBsAg loss, but this benefit was mainly observed in patients with genotype A infection.[34] Further studies are needed to identify the subset of patients who are most likely to benefit and to optimize study design of combination therapy with pegylated IFN and NA; however, given the plethora of new antiviral and immunomodulatory therapies in clinical trials, support for these studies might be difficult to garner.

Pegylated IFN is administered for 1 year (48-52 weeks) in both HBeAg-positive and HBeAg-negative patients. A clinical trial comparing 24 versus 48 weeks pegylated IFN in HBeAg-positive patients showed that 24-week duration was inferior.[35] Another trial comparing 48 versus 96 weeks pegylated IFN in HBeAg-negative patients suggests that 96-week duration was superior, but the study included a small number of patients.[36] As discussed earlier, stop rule may be applied at week 12 or 24 to identify patients in whom continued treatment is predicted to be futile.

Guidelines recommend that NA treatment should be continued indefinitely in patients with cirrhosis prior to start of treatment.[12-15] The APASL guidelines indicated NA may be discontinued in selected patients with cirrhosis provided close monitoring is feasible. All guidelines recommend that in HBeAg-positive patients with no cirrhosis NAs should be administered for 12 additional months (consolidation) after HBeAg seroconversion is achieved. Given that only 40% of patients achieve HBeAg seroconversion after 5 years of ETV or TDF,[37,38] most patients will require more than 6 years of treatment. All guidelines recommend that, for HBeAg-negative patients with no cirrhosis, NAs can be stopped when HBsAg is lost, but with <1% of patients achieving this outcome after 5 years of treatment, nearly all patients will require lifelong treatment. All patients must be closely monitored after discontinuation of NA such that treatment can be resumed if there is evidence of clinical relapse (HBV DNA and ALT increasing to levels that meet treatment indications).

Recent studies suggest paradoxically that, for HBeAg-negative patients who had been virally suppressed for >2 to 3 years, withdrawal of NA may be associated with a higher rate of HBsAg loss compared to those who continued NA.[39-41] This has led the APASL and EASL guidelines to include a provision for such an approach as long as patients agree to close monitoring after treatment is discontinued. However,

outcomes, including risks of hepatic decompensation, frequency of monitoring, and criteria for resuming treatment, need to be studied in larger cohorts before this approach is widely adopted.

The reason for a higher rate of HBsAg loss after NA withdrawal is unclear. It has been postulated that viral relapse after a long duration of virus suppression may mimic acute hepatitis B, provoking a rigorous immune response to HBV leading to HBsAg loss. One study found that among patients who experienced clinical relapse those who did not resume treatment had a higher rate of HBsAg loss than those who did suggesting that HBsAg loss is more likely to occur if immune clearance is allowed to proceed and not curbed too soon. However, overly rigorous or prolonged immune lysis of infected hepatocytes can result in liver failure. Moreover, this same study showed that patients who had no viral relapse and those who had viral but not clinical relapse had higher rates of HBsAg loss than those who had clinical relapse indicating that hepatitis flares are not required for HBsAg loss. [40]

The ability to identify which patients will remain in remission, which ones will experience viral relapse only, which ones will experience clinical relapse, and which ones will decompensate when NA is stopped is an important area for research. It is also important to have a standardized definition for viral and clinical relapse such that data from different studies can be compared. Several studies suggested that serum HBsAg, HBV RNA, and hepatitis B core related antigen levels—surrogate markers for cccDNA— prior to stopping treatment may be predictive.[42] Validation of these markers is necessary and, if confirmed, standardized assays must be available for clinical use.

Long-term outcomes

Continued follow-up of patients who completed a course of IFN treatment showed that rates of HBeAg seroconversion increased to 37% after 4 to 5 years and HBsAg loss to 8% after 4 years.[43,44] However, these incremental responses were mainly seen in patients with genotype A infection. Studies have also shown that continued NA treatment is associated with increasing rates of HBeAg seroconversion to 40% after 5 years of ETV or TDF,[37,38] but there is minimal increase in rates of HBsAg loss.

Antiviral treatment has also been shown to improve liver histology. Biopsies performed after 1 year of treatment showed decreased inflammation but not fibrosis, and biopsies performed after 3 to 5 years of continued NA therapy have demonstrated a decrease in both inflammation and fibrosis. A study of 348 patients who had liver biopsies at baseline and after 240 weeks of TDF found that 87% had decreased inflammation and 52% had decreased fibrosis. Importantly, 74% of patients who had cirrhosis at enrollment no longer had cirrhosis.[38] It would be important to know whether patients who have

regression of cirrhosis have lower risk of HCC compared to those without regression. Liver biopsies, particularly serial biopsies, are seldom performed in clinical practice. Studies using biomarker panels or elastography are needed to determine what criteria should be used to assess antiviral-induced fibrosis regression because these noninvasive methods measure not only fibrosis but also inflammation, and initial improvement after treatment may be due to decreased inflammation and not fibrosis.

Antiviral therapy has also been shown to improve clinical outcomes. A landmark randomized controlled trial showed that lamivudine decreased the risk of disease progression and HCC in patients with advanced fibrosis or cirrhosis.[16] Long-term follow-up studies and meta-analyses indicate that IFN and NA therapy decrease the development of cirrhosis, cirrhosis complications, HCC, and mortality.[2,45,46] However, the incidence of HCC is not eliminated, and continued surveillance is required. A recent study showed that the incidence of HCC appeared to level off after 5 years of ETV or TDF in patients with cirrhosis,[47] suggesting that with longer duration of suppressed HBV replication and decreased hepatic inflammation, development of new HCC can be prevented. In this study, all new HCC after year 5 occurred in patients >50 years old. These data support the recommendations to initiate treatment as early as possible. Longer follow-up is needed to determine whether the incidence of HCC will eventually stop in patients with viral suppression on NAs.

Future of HBV treatment

The availability of a simple, safe, and highly effective cure for hepatitis C has reenergized the search for a cure for hepatitis B. However, as described earlier, sterilizing cure with elimination of both cccDNA and integrated HBV DNA may not be possible. Instead, experts agreed that functional cure akin to spontaneous HBsAg loss in patients with chronic HBV infection may be a realistic goal.[3] The endpoint would be HBsAg loss, accompanied by HBeAg loss and undetectable HBV DNA in serum, but HBV DNA may persist in the liver as integrated HBV DNA and as transcriptionally inactive cccDNA. Progression of liver disease would be halted, and over time, fibrosis will regress and incidence of HCC will decrease. Whether seroconversion to hepatitis B surface antibody is necessary to prevent HBsAg seroreversion remains to be determined. This functional cure is currently achievable in a small percent (<10%) of patients after IFN or NA therapy. The vision for the future is to deploy a combination of antiviral drugs directed against new targets and immunomodulatory therapies to restore innate as well as adaptive immune responses with the goal of achieving HBsAg loss in a higher percent (>50%) of patients after a finite course (≥2 years) of treatment. These new strategies may or may not include IFN or NAs.

New antiviral drugs in clinical trials include: entry receptor inhibitors, capsid assembly modifiers, RNA interference, and nucleic acid polymers. [3] To date, capsid assembly modifiers have shown promising results but the effect on HBsAg levels has been modest and there is a concern for antiviral drug resistance. RNA interference has been shown to result in >1 log decrease in HBsAg levels but it has to be administered as injections and there is a concern for off-target effects. Studies of nucleic acid polymers have shown a high rate of HBsAg loss but the design of those studies is complex, the exact mechanism of action of nucleic acid polymers is unclear, and marked ALT flares were observed in a high percent of patients; thus safety needs to be established and efficacy needs to be confirmed. To date, studies of immune modulatory therapies including therapeutic vaccines to stimulate T cell response and toll like receptor agonists to enhance innate immune response have had limited success. Given the recent success of immune therapies in oncology, enthusiasm for immune modulatory therapies including check-point inhibitors and engineered T cells remains high. A major concern of immune modulatory therapies is uncontrolled immune activation leading to fatal hepatitis flares or extra-hepatic organ damage.

Major challenges with developing new drugs for hepatitis B include safety given the excellent safety profile of NAs, ease of administration (NAs are taken as pills once a day), and cost (ETV and TDF are both off patent). However, given the high global burden of hepatitis B, the desire to achieve a "cure" with a finite course of therapy and the need to effectively control HBV replication at an early stage of chronic HBV infection, it is hoped that there will be sufficient interest and commitment from the pharmaceutical industry and the scientific community to work together to make "HBV cure" a reality in the not too distant future.

Figure Legends

FIG. 1. HBV lifecycle and antiviral targets.[3]
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. Small interfering RNA approaches or anti-sense oligonucleotides to block viral replication and viral protein expression.

HBV polymerase. Reverse transcriptase inhibitors include approved nucleos(t)ide analogues. RNAseH 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 HBc (cccDNA regulation and interferon stimulated gene expression).

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

Abbreviations: cccDNA, covalently closed circular DNA; HBe, hepatitis B e protein; HBs, hepatitis B surface antigen; HBx, hepatitis B x protein; MVB, multivesicular body; NTCP, sodium taurocholate co-transporting polypeptide; pgRNA, pregenomic RNA; POL, polymerase; rcDNA, relaxed circular DNA; dslDNA, double stranded linear DNA.

FIG. 2. Phases of chronic HBV infection.[3]

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. Although most patients progress from one phase to the next, not all patients go through each phase, and reversion to an earlier phase can occur.

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 anti-HBe 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.

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV hepatitis B virus.

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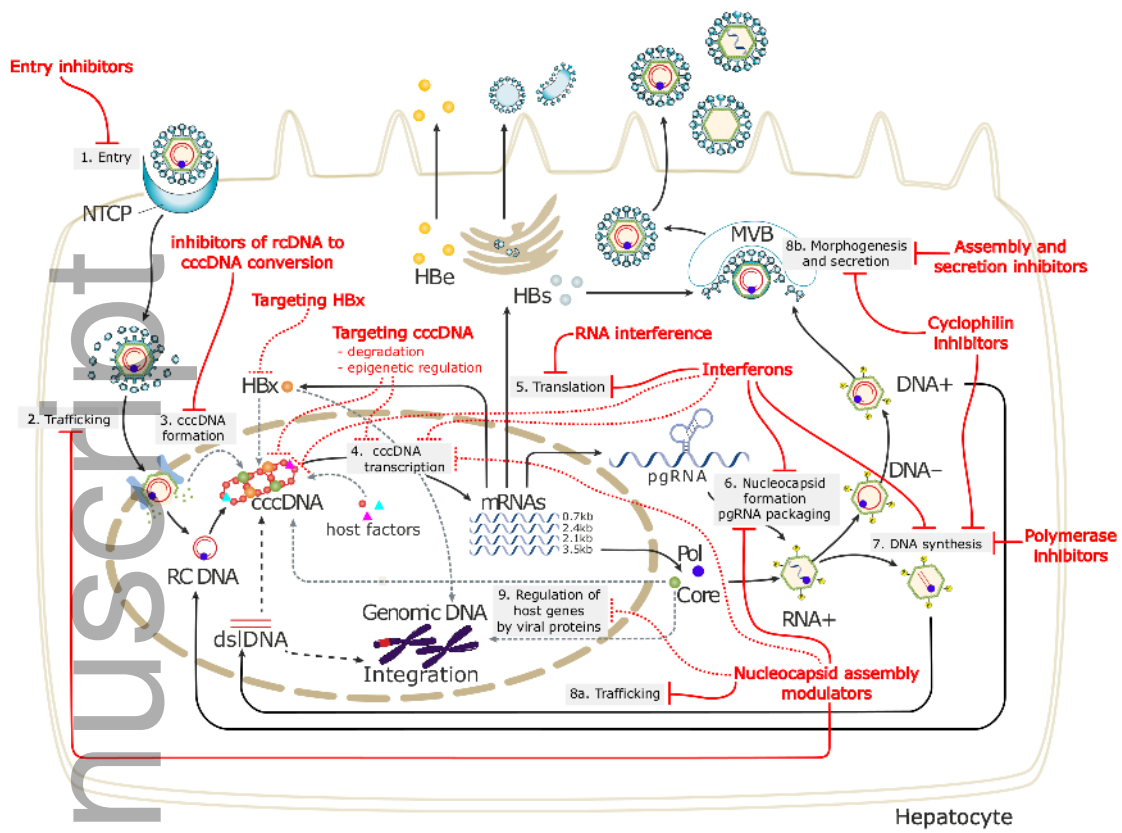Author names in bold designate shared co-first authorship.

TABLE 1. Virological responses to first-line HBV treatments.[13,24]

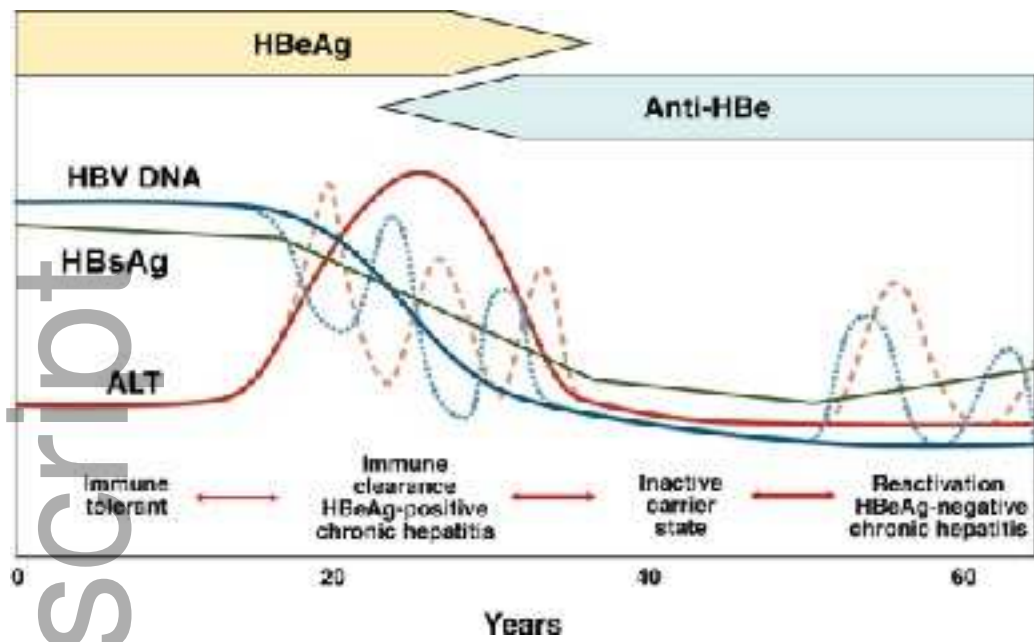|	Entecavir (ETV)	Tenofovir Disoproxil Fumarate (TDF)	Tenofovir Alafenamide (TAF)	Pegylated Interferon
HBeAg-positive patients				
At week 48 or 52				
Undetectable HBV DNA	67	67-76	64	25
HBeAg seroconversion	21	12-21	10	27
HBsAg loss	2	<1-3	1	3
*During extended treatment or follow-up**				
Undetectable HBV DNA	94 (5)	97 (5)	73 (2)	13 (4.5)
HBeAg seroconversion	41 (5)	40 (5)	18 (2)	37 (4.5)
HBsAg loss	5 (5)	10 (5)	1 (2)	8 (4.5)
HBeAg-negative patients				
At week 48 or 52				
Undetectable HBV DNA	90	93	94	63
HBsAg loss	<1	0	0	4
*During extended treatment or follow-up**				
Undetectable HBV DNA	NA	99 (5)	90 (2)	18 (4)
HBsAg loss	NA	0 (5)	<1 (2)	8 (4)

Responses presented as %; NA = not available.

*() Time point in which response was assessed in years while on treatment for ETV, TDF, or TAF and during off-treatment follow-up for pegylated interferon.

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