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Current Best Practice in Hepatitis B Management & Understanding Long-Term Prospects for Cure

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Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; ASO, antisense oligonucleotide; CAR, chimeric antigen receptor; cccDNA, covalently closed circular DNA; cGAS, cyclic GMP-AMP synthetase; CpAMs, core protein allosteric modulators; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; dslDNA, double-stranded linear DNA; EASL, European Association for the Study of the Liver; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBX, HBV X protein; HCC, hepatocellular carcinoma; HDV, hepatitis D virus; ISG, interferon stimulated gene; LAM, lamivudine; MAIT, mucosal-associated invariant T; NAPs, Nucleic acid polymers; NK, natural killer; NTCP, sodium taurocholate co-transporting polypeptide; PD1, programmed cell death protein 1; rcDNA, relaxed circular DNA; RIG-I, retinoid acid inducible gene-I; SC, subcutaneous; siRNA, small interfering RNA; Smc, structural maintenance of chromosomes; STING, stimulator of interferon genes; STOPS, S-

Antigen Transport-inhibiting Oligonucleotide Polymers; TAF, tenofovir alafenamide; TCR, T-cell receptor; TDF, tenofovir disoproxil fumarate; TLR, toll like receptor; ULN, upper limit of normal; WHO, world health organization; WHV, woodchuck hepatitis virus.

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Abstract

The hepatitis B virus (HBV) is a major cause of cirrhosis and hepatocellular carcinoma worldwide. Despite an effective vaccine the prevalence of chronic infection remains high. Current therapy is effective at achieving on-treatment but not off-treatment viral suppression. Loss of hepatitis B surface antigen (HBsAg), the best surrogate marker of off-treatment viral suppression, is associated with improved clinical outcomes. Unfortunately, this endpoint is rarely achieved with current therapy because of their lack of effect on covalently closed circular DNA, the template of viral transcription and genome replication. Major advancements in our understanding of HBV virology along with better understanding of immunopathogenesis have led to the development of a multitude of novel therapeutic approaches with the prospect of achieving functional cure (HBsAg loss) and perhaps complete cure (clearance of cccDNA and integrated HBV DNA). This review will cover current best practice for managing chronic HBV infection and emerging novel therapies for HBV infection and their prospect for cure.

Introduction

The hepatitis B virus (HBV) is a small hepatropic DNA virus that has been infecting humans for millennia. An ancestral strain was likely present in hunter-gatherers during the early Holocene period (~20,000-12,000 years ago).[1]. During human evolution, spread of HBV was likely facilitated by the establishment of agrarian societies in the Neolithic and Bronze Ages.[2] Currently, it is estimated that over 2 billion persons have been exposed to HBV, of whom 296 million (~3.7% of the human population) have chronic infection [3, 4]. Chronic HBV infection is responsible for ~820,000 deaths annually worldwide from complications of cirrhosis and hepatocellular carcinoma (HCC) [5]. Despite the availability of an effective vaccine, ~1.5 million new infections occur annually.

Nevertheless, the HBV vaccine has had a profound impact on the prevalence of chronic HBV infection and complication rate, particularly in high prevalence regions.[6, 7] Given the significant burden on global public health, the World Health Organization (WHO) has set a goal of complete eradication of HBV by 2030, defined as a 65% reduction in mortality and a 90% reduction in incidence compared with the baseline levels obtained in 2015.[8]. Currently, only 12% of countries are on track to meet WHO elimination targets.[9]

HBV lifecycle

The intact virion or Dane particle has an outer lipid envelope that surrounds a viral nucleocapsid containing the viral DNA and polymerase. The genome is partially double-stranded DNA with four

overlapping open reading frames that encode for seven viral proteins: polymerase, core, hepatitis B e antigen (HBeAg), large, middle, and small HBsAg and X protein.[10].

The viral lifecycle is illustrated in **Figure 1**. During replication, double-stranded linear DNA (dslDNA) forms are produced (~5-10%) that may integrate randomly into the host genome by utilizing random sites of host cell DNA breaks.[11] Apart from being a constant source of RNA and viral proteins, integration is also considered to be a contributor to the development of HCC. A substantial proportion of HBsAg may be derived from integrated HBV DNA, particularly among HBeAg negative patients,[12], Figure 1, suggesting that HBsAg loss may ultimately require the elimination of integrated HBV DNA.

Immunopathogenesis of HBV

The immune response contributes to both HBV clearance and liver injury. HBV does not readily activate the intracellular innate defense mechanisms including type I IFN pathway [13, 14]. However, HBV replication is inhibited by pharmacological activation of type I/III IFNs and intracellular antiviral sensors such as the toll like receptors (TLRs) and retinoid acid inducible gene-I (RIG-I) like receptors as well as exogenous IFN therapy [15]. HBV can also induce type III IFN through the interaction between the HBV pregenomic RNA and RIG-I [16] and with a biphasic interferon stimulated gene (ISG) induction in hepatocytes in-vitro [17]. In addition, natural killer (NK) and NKT cells can be activated early in acutely HBV-infected patients [18]. Thus, once HBV replication is established in hepatocytes, type III IFN, and activation of NK and Kupffer cells may help to modulate viral replication and viral spread during the early stages of infection.

As for the adaptive immune response, T-cells play a key role in HBV clearance and liver disease pathogenesis as shown in experimental animal models [19]. CD8 T-cells directly recognize and

kill (or cure) virus-infected hepatocytes that express viral epitopes on class I MHC, whereas CD4 T-cells provide critical T-cell help and orchestrate the overall adaptive immune response. In acutely HBV-infected patients, spontaneous viral clearance and disease resolution is characterized by a broadly specific and durable antiviral CD8 and CD4 T-cell responses as well as HBsAg-specific neutralizing antibody response. Importantly, memory T-cell response to HBV can persist for decades after clinical resolution of acute HBV infection, maintained by trace amounts of virus in-vivo [20] and likely mediating virus control—raising the possibility for their role for sustained virus control of HBV post-therapy. Similarly, a critical role for B-cells in HBV control is suggested by HBV reactivation by immunosuppressive regimens that deplete B cells [21, 22].

Evolution of acute HBV infection to chronic likely involves both host and viral factors [23, 24], although precise mechanisms are not well defined. Established chronic HBV infection is characterized by both HBV-specific and global T- [25, 26] and B-cell dysfunction [27, 28], due to prolonged exposure to viral antigens and inflammatory mediators that leads to immune exhaustion with the induction of regulatory pathways and immune checkpoints including regulatory T-cells, programmed cell death protein 1 (PD1)[29] and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) [26], in addition to altered gammadelta T-cells[30] and metabolic immune dysregulation through myeloid derived suppressor cells and arginase [31] and antiviral T-cell elimination through activated NK cells and death pathways (e.g., Bim) [31, 32]. Lacking adaptive immune control, non-specific inflammatory infiltrates combining innate and adaptive immune cells accumulate in HBV-infected liver and promote hepatocellular injury and fibrosis without virus suppression [33]. Thus, immune-mediated HBV therapy requires a fine balance between immune control of the virus and hepatocellular injury to avoid adverse clinical consequences.

Preventing infection

HBV vaccination has resulted in a significant reduction in both disease prevalence and complications of HBV including HCC [6, 7]. In the U.S., the Advisory Committee on Immunization practices (ACIP)[34] recommends vaccination of all infants, children, adolescents, and adults through 59 years of age as well as adults >60 years with risk factors. Additional information regarding available infant, child, adolescent and adult vaccines, vaccinee schedules and at-risk populations are provided in Supplementary Tables 1 and 2.

Treatment

Goals of treatment

The primary goal of therapy is to prevent cirrhosis, development of HCC and liver-related mortality. However, these endpoints take decades to develop. Therefore, studies evaluating therapies for chronic HBV infection have relied on surrogate endpoints. These include undetectable HBV DNA using a sensitive PCR-based assay, normalization of serum alanine aminotransferase (ALT), loss of HBeAg, loss of HBsAg and histological improvement. HBsAg loss is considered the best endpoint because it is associated with durable suppression of HBV DNA and improvement in clinical outcomes such as hepatic decompensation, hepatocellular carcinoma and liver-related death.[35, 36] However, as reported in two meta-analyses the rate of spontaneous and treatment-related HBsAg loss is low, approximately 1% annually.[37, 38]

Indications for treatment

Chronic hepatitis B (CHB) is a dynamic disease characterized by frequent fluctuations in disease activity. Historically, HBeAg status, HBV DNA and ALT levels are used to assess disease activity. The three major liver societies, the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver ~~Diseases~~ (EASL) and the Asian Pacific Association for the Study of the Liver (APASL) have provided guidance on indications for

treatment, **Table 1**. [39-41] Additionally, the World Health Organization (WHO) has developed a more simplified approach to treatment for low and middle income countries that may lack access to viral load testing.[42] All guidelines strongly agree that patients with decompensated liver disease, cirrhosis and those with active disease (defined as those with elevated HBV DNA and ALT levels) should receive treatment. There are minor regional differences in the choice of HBV DNA level (for example HBV DNA level of 20,000 IU/ml (AASLD and APASL) or 2,000 IU/mL (EASL) in an HBeAg positive patient) and ALT cut-offs (either twice the laboratory upper limit of normal (ULN) (APASL and EASL), >ULN if moderate liver necroinflammation or fibrosis is present (EASL), or gender specific ALT cut-offs -35 U/L for males and 25 U/L for females (AASLD)) to initiate therapy. Similarly, there is general agreement that patients whose disease is inactive (HBeAg negative with low HBV DNA (<2,000 IU/mL) and normal ALT levels can be safely observed without the need for treatment. There is some controversy on how patients with elevated HBV DNA but normal (≤1xULN) or mildly elevated ALT levels (>1-<2xULN) should be managed. In these situations, obtaining additional evidence on disease severity either through a liver biopsy or non-invasive assessment of fibrosis is advised to assist in decision making. Among non-invasive tests, transient elastography (TE)[43] or shear wave elastography (SWE)[44] generally have higher diagnostic accuracy over serum biomarkers such as APRI and FIB-4 and are therefore preferred for assessing fibrosis in the absence of a liver biopsy, **Supplementary Tables 3a and 3b**. Non-invasive tests perform better at excluding than establishing advanced fibrosis/cirrhosis. Additionally, other factors such as age over 40 years, family history of HCC, lengthy disease duration, HBsAg level ≥1,000 IU/mL and a patient's willingness to receive treatment should be considered in the decision to recommend therapy. [39-41, 45, 46]. Other indications for treatment or prophylaxis are listed in **Table 2**.

An alternate and more simplified approach to treatment being put forth by some experts, but not endorsed by any of the major liver society guidelines, is a "treat all" approach in which any HBsAg

positive individual with detectable viremia regardless of ALT level would receive treatment. In the case of HBeAg positive patients with markedly elevated HBV DNA (>10^8 IU/mL) and normal ALT levels (immunetolerant or HBeAg positive chronic infection with no clear evidence of hepatocellular damage), the recommendation to treat is driven by a desire to limit the risk for HBV-specific T-cell depletion, DNA integrations that drive HCC risk, silent fibrosis progression, and risk of transmission. Indeed, this approach is supported by the Risk Evaluation of Viral Load Elevation and Associated Liver Disease (REVEAL) study[47, 48] which showed a relationship between elevated HBV DNA levels and subsequent development of cirrhosis and HCC and a Korean study reporting that untreated immunetolerant patients have higher risk of HCC and death/transplantation than nucleos(t)ide analogue treated immuneactive-phase.[49] However, as data from the REVEAL study was obtained from an older, predominantly male, HBeAg negative cohort, caution is advised in extrapolating to a younger HBeAg positive cohort. Also, in the latter study from Korea, the HCC risk was lowest among patients with highest HBV DNA levels and normal ALT levels (true immunetolerant patients). Notably in that study, the mean age of the immunetolerant patients was 38 years, thus, many or most would have met the three liver association guidelines for treatment. Furthermore, there is currently no evidence that lowering viral load would necessarily reduce HCC incidence in patients with immunetolerant disease. Moreover, spontaneous HBeAg seroconversion occurs in a majority of patients with low rates of progression to HBeAg negative immuneactive disease, cirrhosis or HCC,[50] achieving undetectable HBV DNA is challenging, integration cannot be prevented, and paired liver biopsy studies showed minimal if any fibrosis progression in patients with immunetolerant disease.[51] However, some studies suggest unacceptably high rates of HCC and other clinical outcomes among indeterminate/grey zone patients (HBeAg negative with elevated HBV DNA and mildly elevated ALT levels) or who do not meet criteria for treatment according to APASL, AASLD and EASL and that treatment may be of benefit to these patients.[52-54] Future studies are needed

to address management of these controversial group of patients. Until such results are available, we recommend a “case by case” approach, considering presence of risk factors for disease progression and HCC and patient’s willingness for treatment for cases outside the guideline treatment criteria. Please see supplementary data for more in-depth discussion of management of controversial patients.

Current treatment options

There are seven licensed agents for treatment of chronic HBV infection in the U.S., standard interferon alfa-2b (no longer available in the U.S. and Europe), pegylated interferon alfa-2a, lamivudine (LAM), adefovir, telbivudine, entecavir (ETV), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF). Pegylated interferon is preferred over standard interferon due its more favorable pharmacokinetics and dosing schedule (once weekly versus thrice weekly). Among the nucleos(t)ide analogues, entecavir, TDF and TAF are recommended over LAM, adefovir, telbivudine because of their greater potency and lower rates of antiviral resistance. The sustained, on-treatment viral suppression that can be achieved with these agents is associated with less progression to cirrhosis or even reversal of cirrhosis, prevention of decompensation, reduction but not prevention of HCC and lower mortality.

Two treatment strategies are recommended by liver society guidelines, **Table 3**. One is a finite 48-week treatment course with pegylated interferon alfa-2a and the other is long-term therapy with one of the recommended nucleos(t)ide analogues.

Pegylated interferon alfa-2a

The mechanism of action of pegylated interferon alfa-2a is not fully understood but the drug has both antiviral and immunomodulatory properties. The recommended dose for both HBeAg positive and negative patients is 180 μg once weekly by subcutaneous (SC) injection for 48 weeks.

Pegylated interferon can be discontinued early for futility among HBeAg positive patients, if at week 12 HBsAg levels are ≥20,000 IU/ml for genotypes B and C, or no decline of HBsAg levels are observed for genotypes A and D and at week 24 if HBsAg levels ≥20,000 IU/ml in patients with genotypes A-D. Pegylated interferon can be stopped among HBeAg negative patients with genotype D at week 12 if there is no decrease in HBsAg levels and <2 log10 IU/ml reduction in serum HBV DNA. In addition to the finite dosing schedule, other advantages of pegylated interferon are a relatively high rate of HBeAg loss (>30%) in HBeAg positive patients [55, 56] and the potential to clear HBsAg (2-7% in HBeAg-positive and 4% in HBeAg-negative patients) with a relatively short duration of therapy, especially in patients with HBV genotypes A and B. Moreover, HBeAg loss and HBsAg loss are durable. Disadvantages of pegylated interferon include the need for administration by SC injection as well as the numerous substantial adverse events.[57] Additionally, peginterferon is contraindicated in patients with decompensated cirrhosis and compensated cirrhosis with clinically significant portal hypertension, due to the risk of hepatic decompensation, and during pregnancy. Some studies have shown benefit in extending treatment duration beyond 48 weeks especially among HBeAg negative patients[58, 59] but in practice this is difficult due to poor patient tolerance.

Nucleos(t)ide analogues

Nucleos(t)ide analogues, incorporated into nascent DNA by the HBV reverse transcriptase, inhibit viral replication by functioning as DNA chain terminators.[60] They are more potent inhibitors of viral replication compared to pegylated interferon but recrudescence of viral replication following their withdrawal is common due their lack of activity on covalently closed circular DNA (cccDNA). Recently, it was shown that nucleos(t)ide analogues (tenofovir) may decrease the number of transcriptionally active distinct HBV-host DNA integrations among patients with mild CHB.[61] However, it remains uncertain if nucleos(t)ide analogues can alter clonal expansion of hepatocytes carrying unique viral integrations, the expression level of an integrated sequence or

if reducing integrations would result in a lower incidence of HCC. Efficacy of recommended nucleos(t)ide analogues are shown in **Table 3**. Rates of HBsAg loss after 1 year are 1-3% in HBeAg positive and ≤1% in HBeAg negative patients. In comparison to pegylated interferon, nucleos(t)ide analogues are orally administered, are well tolerated, and have an excellent safety profile. There is a small risk for nephropathy and bone loss, particularly with TDF. They may be used in patients with decompensated cirrhosis and in pregnant women as treatment or for prevention of mother-to-infant transmission. In the absence of co-morbid conditions, selection of one nucleos(t)ide analogue over another is based on patient preference and cost. In patients with renal or bone disease, entecavir or TAF are preferred. For treatment experienced patients or HIV-HBV co-infection, TDF or TAF are the preferred agents due to the high rate of resistance to entecavir in LAM-experienced patients. In pregnant women, TDF is preferred because data on the safety of TAF[62-64] are not yet as extensive as TDF and ETV is contraindicated during pregnancy.

Special populations

Treatment of special populations is beyond the scope of this review and readers are referred to guidelines that cover this topic.[39-41] An abridged overview of management is provided in **Supplementary Table 4**. Patients scheduled to receive immunosuppressive or cytotoxic therapy are at risk for reactivation of HBV infection. Consequently, all patients should be screened for current or past HBV infection using HBsAg and anti-HBc. Those who test positive for HBV markers should be further risk stratified based on the immunosuppressive regimen. Patients at high risk for HBV reactivation (e.g., use of anti-CD20 agent) should receive prophylactic antiviral therapy. Those at moderate risk (e.g., anti-TNF or low dose steroids) could either receive prophylactic antiviral therapy or be monitored closely with HBV DNA and ALT testing every 3 months. If reactivation occurs, patients should immediately start antiviral therapy. Patients at low risk (short term steroids) do not require monitoring for reactivation. The preferred prophylactic treatment of

choice is either TDF, TAF or ETV. Ideally, treatment should be initiated 2-4 weeks before a planned course of immunosuppression and continued for an additional 6 months after immunosuppression is stopped. An exception is for patients receiving B-cell depleting regimens (e.g., anti-CD20) where treatment should continue for 12-18 months after immunosuppression is stopped. [39-41, 65]

Endpoints of treatment

Pegylated interferon is administered for a finite duration of 48 weeks. Among HBeAg positive patients, pegylated interferon-related HBeAg seroconversion is a durable endpoint. Approximately twenty-five percent of patients will have sustained suppression of HBV DNA < 2000 IU/mL in the short-term (6-12 months off-treatment).[66] In contrast, among HBeAg negative patients, only 19% can maintain HBV DNA suppression < 400 copies/mL, off-treatment.[67]

The optimal endpoint during therapy with nucleos(t)ide analogues is HBsAg loss that is confirmed on at least two occasions, with or without development of anti-HBs. Nucleos(t)ide analogues can be discontinued in non-cirrhotic, HBeAg positive patients who achieve HBeAg seroconversion and undetectable HBV DNA and who receive at least 12 months of consolidation therapy. Post-treatment monitoring is advised (every 3 months) for at least a year to detect a return of active disease. For HBeAg negative patients, AASLD recommends indefinite therapy until HBsAg loss occurs. However, APASL and EASL recommend that treatment can be discontinued in selected patients without cirrhosis who achieve undetectable HBV DNA and normal ALT levels for a period of 2-3 years. This recommendation is based on data reporting rates of HBsAg loss of ~20% among Caucasian patients and 2-3% among Asian patients 3 years after withdrawal and 20-30% maintaining a low HBV DNA (<2,000 IU/mL) and normal ALT after withdrawal of nucleos(t)ide analogues.[68] HBsAg cutoffs of <1,000 IU/mL among Caucasians and <100 IU/mL among Asians were associated with the highest rates of HBsAg loss.[69] In practice, virological relapse

is almost universal, withdrawal flares are observed in 10-30% of patients within the first 3 months of stopping treatment and almost half of patients require re-initiation of treatment. Thus, the decision to withdraw treatment requires careful deliberation with the patient and the patient must agree to close monitoring after withdrawal of therapy. Patients with cirrhosis should not stop antiviral therapy due to the risk of hepatic decompensation.

Monitoring untreated patients and Screening

Due to the dynamic nature of chronic HBV infection, untreated patients should be monitored, with serial HBV DNA and ALT levels every 3-6 months, for evidence of disease progression until spontaneous HBsAg loss occurs. Among HBeAg positive patients HBeAg status should be checked every 6-12 months. Among HBeAg negative patients with low HBV DNA levels (<2,000 IU/mL and normal ALT level, (inactive carrier/ HBeAg negative chronic infection)) HBV DNA and ALT levels should be checked every 3 months for one year to confirm inactive disease after which they may be monitored every 6-12 months. Testing for HBsAg loss should be performed annually. If available, monitoring quantitative HBsAg levels annually in HBeAg negative patients with HBV DNA levels <2,000 IU/mL may be helpful to allow HCC risk stratification and determining the monitoring schedule. A non-invasive assessment of liver fibrosis should be considered every 2-3 years.

HCC surveillance is considered cost-effective if the annual risk of HCC is ≥0.2%. Consequently, all patients with cirrhosis should be screened with ultrasound with or without alfa fetoprotein (AFP) testing every 6 months. HBsAg-positive adults without cirrhosis but considered at high risk for HCC including Asian or Black men over 40 years and Asian women over 50 years of age, persons with a first-degree relative with a history of HCC, or persons with hepatitis D virus (HDV) should also undergo screening every 6 months. After HBsAg loss, surveillance for HCC should continue

for patients with cirrhosis, those who have a first-degree relative with HCC, or a long duration of infection (>40 years for males and >50 years for females).

Limitations of current therapy

Although current therapy is associated with less outcomes, it is not curative and does not eliminate HCC risk. This is due to their limited effect on cccDNA and integrated HBV DNA. Additionally, current treatment does not restore the immune dysfunction that is characteristic of chronic HBV infection. Thus, there is an urgent need for short duration regimens that can achieve high rates of HBsAg loss.

Novel treatments

The development of curative therapy for HCV infection and limitations of current therapy for HBV infection has renewed interest in curing chronic HBV infection. A more comprehensive understanding of the HBV lifecycle and immunopathogenesis of persistent infection coupled with innovations in drug development and delivery have led to multiple new approaches to treat chronic HBV infection. These include innovative means to interrupt viral production, **Table 4**, **Figure 1** and/or to restore or boost the exhausted immune response, **Table 4, Figure 2**. Along with the development of novel therapy, newer tools to monitor the virological and immunological response such as HBV RNA, HBcrAg and cytokine panels will be needed. An in-depth review of these markers is beyond the scope of this review, but readers are directed to an excellent review on the topic.[70]

The focus of novel therapy is to achieve HBsAg loss or "*functional cure*". A true cure or "*complete cure*" would require eradication of the non-integrated cccDNA as well as integrated HBV DNA. Currently, this is not technically feasible. An alternate but less desirable goal is sustained off-

treatment inhibition of viral replication or "*partial cure*". Although this is associated with improved clinical outcomes, the response is often not durable.

Direct Antiviral agents in development

Agents Targeting Viral Entry

The premise of targeting viral entry is to block new rounds of hepatocyte infection, thereby preventing cccDNA formation and reducing the cccDNA pool. Bulevirtide (previously Myrcludex B), is a synthetic, N-acylated pre-S1 peptide that irreversibly blocks the sodium taurocholate co-transporting polypeptide (NTCP) receptor, thereby preventing viral entry.[71] Bulevirtide is conditionally approved by the European Medicine Agency (EMA) for the treatment of chronic HDV infection.[72] There is limited clinical data in the HBV mono-infected population. The results of a small unpublished study, indicated that bulevirtide administered at different doses for 12 weeks in patients with HBeAg negative CHB led to a ≥0.5 log IU/mL decline in HBsAg level at week 12 in a minority of patients but none lost HBsAg.[73] Asymptomatic bile acid elevation was noted. These findings are perhaps not surprising given the relatively long half-life of cccDNA. It is predicted that treatment may have to be administered long-term to have any meaningful effect on HBsAg loss. Hepalatide is another NTCP receptor blocker currently being evaluated for CHB treatment in combination with pegylated interferon versus pegylated interferon alone in a double blind, placebo-controlled phase 2 study (NCT04426968).

Rather than targeting the receptor, several monoclonal/polyclonal antibody preparations are being developed that bind to the N-terminal region of pre-S1, the site of viral interaction with the NTCP receptor.[74] In addition to blocking viral entry, monoclonal antibodies can lower viremia and the level of subviral particles and may cross-present viral antigens with stimulation of T-cells leading to HBsAg loss. GC1102 is a recombinant hepatitis B immunoglobulin currently in a phase

2 trial. VIR-3434, is a novel monoclonal antibody in a phase 1 study in virally suppressed patients (NCT04423393). Preliminary results suggest a rapid dose dependent decline in HBsAg levels without significant adverse events.[75] Further studies with longer term follow-up regarding safety and efficacy of entry inhibitors and monoclonal antibodies are eagerly awaited.

Agents Targeting Viral Transcripts

Another approach to inhibit viral production is to target the protein encoding mRNAs via RNA interference and antisense oligonucleotides (ASOs). A therapeutic advantage of this approach is that multiple viral transcripts may be silenced by a single siRNA/ASO because all HBV mRNAs share the same terminal 3′ sequence. Potential advantages of an ASOs are its ability to interact with pre-mRNA, which permits targeting of splicing and increases the amount of target RNA sequence for ASO binding, which can also limit off-target effects and there is no requirement for a carrier vehicle. Conversely, GalNAc conjugation improves accumulation of siRNAs in the target organ and facilitates their cellular uptake

Small interfering RNAs (siRNA) are short nucleic acid duplexes that bind to their target mRNA and induce the cellular RNA-induced silencing complex to degrade the targeted viral RNA. Their delivery and uptake can be enhanced using lipid nanoparticles or conjugation with N-acetylgalactosamine. First generation siRNAs, ARC-520 and ARC-521 demonstrated proof-in-principle, durable knockdown of target genes (HBsAg) in virally suppressed HBeAg positive and negative patients. However, development of these compounds was stopped due to toxicity from the delivery vehicle. Subsequently, studies with other siRNAs AB-729, JNJ-3989, RG6346 and VIR-2218 in viremic and virally suppressed patients on NAs demonstrated mean 1.5 to ~2.0 $\log_{10}$ IU/mL decline in HBsAg levels following monthly administration over 2 to 4 months.[76-79] However, with extended dosing up to one year further decline in HBsAg levels was minimal.[79] Plateau in HBsAg decline suggests that long-term siRNA use is probably not a viable approach.

Rather the siRNA may be administered as induction therapy for a finite period to lower HBsAg levels and then followed by another agent such as an immune modulator e.g. peginterferon or therapeutic vaccine or alternatively as repeated short courses but the benefit of this latter approach is not proven. Suppression of HBsAg with AB-729 was shown to result in an increase in HBV-specific immune response in some but not all patients, providing another benefit of these agents.[80]

ASOs are single-stranded DNA molecules that can bind to viral mRNA and induce their degradation via RNAseH1 or steric hindrance to prevent translation. Two anti-sense molecules, Bepirovirsen, (previously, IONIS-HBVRx or GSK3228836) and IONIS-HBVLRx (GSK33389404) are currently in phase 2a trials among nucleos(t)ide analogue-naïve and treated patients. Administration for four weeks resulted in greater declines among nucleos(t)ide analogue-treated patients 1.99 $\log_{10}$ IU/ml compared to nucleos(t)ide analogue-naïve patients 1.56 $\log_{10}$ IU/ml.[81] Reductions in HBsAg levels were durable in some patients up to six months off treatment. Recently, it was reported that the addition of bepirovirsen 300 mg for 24 weeks to ongoing nucleos(t)ide analogue therapy, resulted in HBsAg below the lower limit of quantitation in 28% (18/64) of patients at end of treatment.[82]

Agents Targeting Core Protein

The HBV core protein has multiple regulatory roles in the viral lifecycle and the host immune response, making it an attractive target for drug development. Its primary role is to serve as the structural protein of the viral nucleocapsid, the site of reverse transcription and replication of the viral genome. The core protein also regulates subcellular trafficking and release of the HBV genome, RNA metabolism, cccDNA transcription and inhibiting the host innate immune response.

Core protein allosteric modulators (CpAMs) are synthetically derived compounds that bind to a small hydrophobic pocket between core protein dimers and augment dimer-dimer interaction to modify nucleocapsid assembly.[83-91] CpAMs inhibit HBV replication through formation of aberrantly assembled nucleocapsids, or morphologically normal capsids devoid of pgRNA, or both. CpAMs are classified based on their mechanism of action. Type 1 CpAMs such as heteroaryldihydropyrimidine (HAP) derivatives lead to the formation of aberrantly assembled nucleocapsids. Type 2 CpAMs for example phenylpropenamides and sulfamoylbenzamides result in the formation of morphologically normal but empty nucleocapsids due to an inability to encapsidate the pre-genomic RNA. Some CpAMs may have additional effects, such as affecting the conversion of rcDNA to cccDNA.

There is great interest in developing CpAMs due to their potent inhibition of viral replication and oral route of administration. At least a dozen CpAMs are in various stages of drug development, **Table 4**. CpAMs as a class are very effective at inhibiting HBV replication across all HBV genotypes. However, they must be combined with one or more antiviral agents of a different class because of rapid development of resistance when used as monotherapy. When combined with NAs in viremic patients, they generally lead to faster and greater inhibition of viral replication compared to NA alone and deeper suppression of HBV DNA in patients already treated with a NA.[83-85, 90] Thus, they may offer the potential to increase on-treatment response particularly in highly viremic patients. However, minimal changes in HBeAg and HBsAg levels with short term administration coupled with a high rate of virological relapse upon withdrawal of the CpAM and nucleos(t)ide analogue,[92] raises questions whether they could achieve functional cure with finite therapy.

Agents Targeting the HBV Polymerase

Drugs targeting the reverse transcriptase function of the HBV polymerase are the most widely used agents to treat chronic HBV infection. Nucleos(t)ide analogues inhibit viral replication but not viral transcription or translation (i.e., viral antigen).[93] The focus of development of next generation nucleos(t)ide analogues are to improve their efficacy and safety, through novel prodrug approaches.

ATI-2173 is a non-competitive, non-chain terminating, clevudine derivative able to inhibit the HBV polymerase via active site distortion.[94] In phase I studies, 28-day dosing resulted in a mean HBV DNA reduction of 2.8 $\log_{10}$IU/mL without any serious adverse events.[95] Expectedly, no changes were seen in HBsAg levels over the short dosing interval. Phase II studies are in progress (NCT04847440). Other new nucleos(t)ide analogue prodrugs, pradefovir, HS-10234 and NCO-48 fumarate, derived from adefovir and tenofovir respectively are designed to increase antiviral potency and reduce metabolite toxicity.[96-98] Preliminary data suggest similar effectiveness to TDF. Agents targeting the RNase H function of the HBV polymerase are in preclinical development.[99]

Agents Targeting HBsAg Release

As HBsAg loss defines functional cure, there is great interest in developing agents to reduce HBsAg levels and limit viral production. In addition, given that HBsAg circulates in vast quantities as subviral particles in chronic HBV infection, it is hoped that therapeutic HBsAg reduction might restore the immune response. Nucleic acid polymers (NAPs) are short, synthetic oligonucleotides able to interact with HBV subviral particles through a poorly understood mechanism.[100] It was proposed that NAPs may interfere with the assembly/release of HBV subviral particles.[101] In a small 40 patient study two NAPs, REP 2139 or REP 2165, were evaluated in combination with tenofovir and pegylated interferon and compared to tenofovir plus pegylated interferon for 48 weeks. At the end of 48 weeks follow-up the addition of NAPs to the regimen was associated with

sustained suppression of HBsAg to below level of detection in 44% all of whom developed anti-HBs compared to the tenofovir and pegylated interferon arm in which 37% achieved sustained suppression of HBsAg, of whom two-thirds developed anti-HBs.[102] Grade 3-4 ALT flares were observed in the majority of NAP treated patients, 90%, compared to 20% among non-NAP treated patients. Controlled studies with larger groups of patients are needed to further evaluate the efficacy and safety of NAPs.

A similar approach to disrupting HBsAg secretion involves the use of S-Antigen Transport-inhibiting Oligonucleotide Polymers (STOPS). Like NAPs, STOPS are single-stranded oligonucleotides that sequester cellular proteins necessary for HBsAg production.[103] STOPS were shown to have greater potency than NAPS *in-vitro*. However, results of a phase 1 study evaluating the STOPS agent ALG-010133 demonstrated no meaningful HBsAg reduction and further development of this compound has been discontinued.[104]

Agents Targeting cccDNA

Elimination of cccDNA within the hepatocyte nucleus is the key to curing chronic HBV infection. Several approaches to eliminate or silence cccDNA are in pre-clinical development. However, the potential for off-target effects is a major safety concern that may limit this exciting approach.

Gene editing technology such as using zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats associated system 9 (CRISP/Cas-9) proteins are being evaluated to inactivate cccDNA by introducing targeted breaks in double stranded DNA that are then repaired by homologous repair creating mutations at the cleavage site. A study utilizing CRISPR/Cas9 systems from *Streptococcus thermophilus* on HBV infected cell lines was successful in eradicating 90% of HBV cccDNA[105]. However, several challenges need to be overcome before this approach can be

used in the clinic, including target specificity, safe and efficient delivery systems to the hepatocyte nucleus, and increasing editing efficiency to eliminate all cccDNA molecules.

In the nucleus, cccDNA is organized into a chromatin-like structure which makes it amenable to epigenetic manipulation.[106] Several compounds have been shown *in-vitro* to silence cccDNA transcription. Interferon-α inhibits transcription of genomic and subgenomic RNAs derived from cccDNA, both in HBV-replicating cells in culture and in HBV-infected chimeric uPA/SCID mice repopulated with primary human hepatocytes.[107] Interestingly, the HBV X protein (HBX) which is essential for viral transcription has been shown to act through degradation of the host structural maintenance of chromosomes (Smc) complex, Smc5/6, which selectively blocks extrachromosomal DNA transcription. HBX destroys the Smc5/6 complex removing the brake on transcription and allowing hepatitis B virus gene expression to occur.[108, 109] Thus, targeting the HBX might be a viable approach to silencing cccDNA. Pevonedistat, a neuronal precursor cell-expressed developmentally down-regulated protein 8- (NEDD-8) activating enzyme inhibitor and dicoumarol, an inhibitor of NAD(P)H:quinone oxidoreductase 1 (NQO1) were shown to reduce HBX expression,[110, 111] restore Smc5/6 levels and suppress viral transcription in cultured hepatocytes [111] and in a humanized mouse model.[110] However, the observation that there is reactivation of cccDNA as soon as HBX becomes available again may be a major limitation to this approach.

Finally, an interesting prospect for targeting cccDNA rests in the enhancement of the apolipoprotein B mRNA editing catalytic subunit 3A and 3B deaminases (APOBEC3A/B). Upregulation of APOBEC3A/B by interferon-α and lymphotoxin-b has been shown to lead to non-cytolytic degradation of cccDNA *in-vitro*, but the degradation of the cccDNA pool is incomplete.[112]

Indirect Antiviral agents

One of the unanswered questions related to therapy is whether cure can be achieved through a purely antiviral approach or if the addition of an immunemodulator will be necessary. Current immunological approaches have been targeting both innate and adaptive immune system to broadly bolster cellular defense and to promote HBV-specific adaptive immune response (**Figure 2**).

Targeting the Innate Immune system

HBV is poorly sensed by the innate immune system and therefore is considered a stealth virus.[13, 14] However, the observation that certain cytokines (IFN-a, IFN-g, TNF-a, and IL-1a), produced by non-parenchymal liver cells, and LTβR-mediated activation of APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide–like) or activation of retinoic acid-inducible gene-I (RIG-I) can suppress or even eradicate HBV from infected hepatocytes through a non-cytolytic mechanism [16, 107, 112, 113] provides a rationale for developing exogenous activators of the innate immune response. Several agonists of pathogen recognition sensors, e.g., TLRs, RIG-I, and stimulator of interferon genes (STING) have been shown to induce production of interferon-stimulated genes (ISGs) and proinflammatory cytokines than can cytopathically or non-cytopathically clear virus.

Agonists of innate immunity

TLRs are expressed in many cell subsets (including immune cells) and play an important role in host defense as sensors of viral and bacterial pathogen-associated molecular patterns (PAMPs). Combination treatment with CpG oligodeoxynucleotides (CpG ODN) and entecavir was shown in

the woodchuck model to suppress woodchuck hepatitis virus (WHV) viral load.[114] Several oral TLR-7/8 agonists are in clinical trials, including GS-9620, RO7020531, RG7795 (ANA773), RG7854, JNJ-4964 (AL-034/TQ-A3334), and GS-9688.

Vesatolimod (GS-9620), a TLR-7 agonist, can activate intrahepatic dendritic cells among others, triggering the production of type I and II interferons and activating intra-hepatic NK and mucosal-associated invariant T (MAIT) cells. In proof-of-principle studies, vesatolimod reduced viral load and HBsAg antigenemia in chimpanzees and woodchucks but not in humans.[115-117] Vesatolimod in untreated viremic and virally suppressed patients on NAs was well-tolerated but did not substantially reduce HBsAg level.[118, 119] Differences in response observed in animal and human studies may relate to use of sub-therapeutic doses in human compared to animal studies**.**

GS-9688 (selgantolimod), a TLR-8 agonist, can activate intrahepatic dendritic cells, NK cells and MAIT cells and induce production of cytokines (IL-12/IL-18). In chronically WHV-infected woodchucks, short duration therapy with GS-9688 induced a sustained antiviral response and reduced WHV surface antigen (WHsAg) levels to below the limit of detection in half of the woodchucks.[120] Among virally suppressed patients, modest declines in HBsAg levels were seen. One patient (5%) achieved HBsAg loss and 16% HBeAg loss.[121] Selgantolimod induced dose dependent cytokine responses that did not correlate with HBsAg decline.

Inarigivir (SB9200), an oral dinucleotide RIG-I and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) agonist in combination with TDF demonstrated dose dependent reduction in HBV DNA level with a maximal reduction of 3.26 $\log_{10}$[122], with serum ALT flares in 10% of patients. Further development of this compound was discontinued due to a patient death possibly related to liver injury.

Cyclic GMP-AMP synthetase (cGAS) can recognize HBV DNA and activate its adaptor protein STING, leading to ISG56 expression thereby inhibiting nucleocapsid formation.[123] Additionally, activation of the cGAS STING pathway by dsDNA or cGAMP was shown to markedly inhibit HBV replication in cell and mouse models.[124] However, a recent study suggesting that human hepatocytes do not express STING raises questions whether such an approach will be clinically effective.[125]

Lymphotoxin-b–mediated activation of APOBEC or activation of RIG-I was reported to suppress HBV replication by cytidine deamination, leading to cccDNA degradation.[112] Lymphotoxin-a (LTa), lymphotoxin-b (LTb), and cluster of differentiation (CD) 258 are the natural ligands of lymphotoxin-b receptor (LTbR). The risk of severe side effects with these cytokines limits their therapeutic use. However, activating the receptor using other ligands, tetravalent bispecific (BS1) and bivalent (CBE11) agonistic anti-LTβR antibodies to non-cytolytically degrade cccDNA has been demonstrated as a proof-in-principle for this approach.[112]

Agents stimulating and restoring adaptive immunity

Several strategies have been explored to re-invigorate the weak adaptive immune responses to HBV with a key consideration being to induce a therapeutic response safely without causing severe hepatocellular damage and clinical decompensation.

Checkpoint Blockade

Given the success of treating certain malignancies with checkpoint inhibitors, there is interest in using this approach for chronic HBV infection. However, use of these agents in the clinic in a non-

malignant setting has been constrained by concerns for widespread hepatocyte death precipitating acute liver failure coupled with the risk of autoimmunity. In-vitro, incubation of T-cells from patients with chronic HBV infection with anti-PD1 antibodies led to proliferation of CD8+ cells with increased production of IL-2 and interferon gamma [126]. In a small pilot study, the PD-1 inhibitor nivolumab was evaluated with and without the therapeutic vaccine GS-4774 in virally suppressed patients on nucleos(t)ide analogue therapy [127]. Minor declines in HBsAg were noted. One patient achieved HBsAg sero-conversion, that was preceded by grade 3 ALT flare. No serious adverse events were observed. A recent phase 2 study evaluated the PD-1 monoclonal antibody envafolimab (ASC22) in nucleos(t)ide analogue experienced patients. A maximum HBsAg reduction of 1.2 Log_{10} IU/mL was seen without significant ALT flares.[128] The treatment was well tolerated. Another approach to targeting PD-1 pathway involves degradation of the PD-1 ligand (PD-L1) mRNA via the ribonuclease H (RNH) pathway.[129] Despite the potential usefulness of checkpoint blockade, concerns regarding safety and unpredictable response may limit their use in chronic HBV infection. Future larger studies are awaited.

Genetically engineered T-cells

Success of immunotherapy in cancer and demonstration of viral clearance in chronically HBV-infected recipients of bone marrow from recovered or vaccinated donors paved the way to develop genetically engineered HBV-specific T-cells[130-132], including ongoing efforts utilizing chimeric antigen receptor (CAR) T-cells [133], T-cell receptor (TCR) gene transfer [134] and TCR activation via immune mobilizing monoclonal T-cell receptors against virus (ImmTAV) molecules[135]. The ImmTAV molecule is a fusion protein consisting of an affinity-enhanced T-Cell receptor with an anti-CD3 T-Cell-activating moiety that can activate and redirect T-cells against HBV infected cells *in-vitro* [135]. Preliminary evidence in an animal model demonstrate reduction of HBsAg and HBV DNA levels without inducing significant liver damage. Proof-of-principle of the safety and efficacy

of using genetically engineered T-cells expressing a HBsAg specific T cell receptor or adoptive transfer of autologous T cells expressing HBV-specific TCR, were demonstrated in patients with HBV-related HCC.[136, 137] Results from clinical studies utilizing these novel approaches in non-HCC patients are necessary to better understand their safety profile and effectiveness.

Novel therapeutic vaccines

The premise behind therapeutic vaccination is to break immune tolerance and augment the HBV-specific T-cell response to mediate functional cure (HBsAg loss). Unfortunately, previous attempts using multiple antigens (pre-S1/S2), peptide-based T-cell vaccines, DNA vaccines with novel adjuvants or nucleos(t)ide analogues were unsuccessful. Updated vaccine approaches are employing unique strategies to enhance vaccine efficacy including: 1) inclusion of multiple antigens to broaden the T-cell response (GS-4774, yeast-based vector with multiple viral antigens including HBsAg, HBcAg and HBX); 2) delivery systems (electroporation e.g. INO-1800 vaccine encoding HBsAg and HBcAg, JNJ-64300535 vaccine encoding HBV core and polymerase proteins); 3) novel adjuvants (INO-1800 vaccine plus INO-9112 (a DNA plasmid for IL-12)); 4) combination with checkpoint inhibitors; 5) use of viral vectors (primed non-replicative human adenovirus, chimpanzee adenovirus (ChAd), modified Vaccinia virus Ankara (MVA) and arenavirus). An advantage of viral vectors is they express antigen intracellularly and induce a robust cytotoxic T lymphocyte (CTL) response. [138] [139] A promising approach to generate high levels of memory T-cells is heterologous prime/boost vaccination. In this strategy, different antigen delivery systems are used to sequentially administer vaccine. In pre-clinical studies, a MVA expressing HBV antigens was used to boost protein-prime (HBsAg and HB core antigen) vaccinations in wildtype and HBV-transgenic (HBVtg) mice. Protein-prime/MVA-boost vaccination was able to overcome HBV-specific tolerance in HBVtg mice with low and medium but not with high antigenemia. Using the same model system, knockdown of viral antigenemia using siRNAs

followed by therapeutic vaccination led to the development of polyfunctional, HBV-specific $CD8^+$ T-cells, and elimination of HBV.[140] This vaccine is being tested in phase I studies.

New approaches to increase immunogenicity of peptide-based vaccines include novel nasal formulation NASVAC [141], the Sci-B-Vac derivative BRII-179[142] and HepTcell which is composed of nine synthetic HBV-derived peptides formulated with IC31®, a TLR9-based adjuvant.

Despite the many approaches and advances, none have been shown to restore immunity and clear infection in patients. Other strategies will therefore be required such as combination with potent antivirals, agents to lower viral antigen burden and other immunological boosters.

Combination Therapy

Given the success of combination therapy in chronic HCV infection and other infectious diseases, a combination approach will likely be needed to achieve durable HBV suppression, likely including both antiviral and immunomodulatory therapy. Almost every possible combination of agents is being evaluated in pre-clinical and clinical studies and results are eagerly awaited. However, a word of caution is in order. A recent study evaluating the combination of siRNA (JNJ-3989) with or without a CpAM (JNJ-6379) plus a NA, reported a surprising result in which the triple arm regimen (siRNA plus CpAM plus NA) had the lowest rate of response (ALT<3XULN, HBV DNA <LLOQ, HBeAg negative and HBsAg <10 IU/mL at end of treatment) compared to the two siRNA plus NA comparator arms.[143] This raises the possibility of an interaction between the CpAM and siRNA and suggests that not all combinations will result on synergy.

Conclusion

Chronic HBV infection results in a chronic hepatitis that carries a lifetime risk for progression to cirrhosis and HCC. Consequently, lifelong monitoring is necessary to detect disease progression and surveillance is recommended for individuals at increased risk for HCC. Persons at risk for cirrhosis and HCC should be offered antiviral treatment. Although current therapy is associated with improved clinical outcome it is not curative because of lack of effect on cccDNA and integrated HBV DNA. Stopping therapy in the absence of HBsAg loss usually leads to relapse to active disease in most patients and thus treatment must be administered long term.

Given the global burden of disease there is an urgent need for more effective therapy. A better understanding of the HBV lifecycle and immunopathogenesis of persistent infection together with innovations in drug development and delivery have led to multiple new approaches to treat chronic HBV infection. A regimen to achieve functional cure will likely require a combination of agents including an antiviral, an agent to reduce viral antigen burden and an immunemodulator to boost the immune response. Complete cure will require the refinement of gene editing therapy. The burden of disease is greatest in low-middle income countries. Therefore, to achieve WHO elimination goals will require development of a safe, effective, finite duration therapy that is affordable. Although many challenges remain, the sheer breadth of therapeutic approaches in development holds great promise for curing and eliminating chronic HBV infection.

References:

1. Kocher A, Papac L, Barquera R, Key FM, Spyrou MA, Hübler R, Rohrlach AB, Aron F, Stahl R, Wissgott A *et al*: **Ten millennia of hepatitis B virus evolution**. *Science* 2021, **374**(6564):182-188.
2. Locarnini SA, Littlejohn M, Yuen LKW: **Origins and Evolution of the Primate Hepatitis B Virus**. *Front Microbiol* 2021, **12**:653684.
3. **Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study**. *Lancet Gastroenterol Hepatol* 2018, **3**(6):383-403.
4. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ: **Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013**. *Lancet* 2015, **386**(10003):1546-1555.
5. **Hepatitis B Fact Sheet** [https://www.who.int/news-room/fact-sheets/detail/hepatitis-b]
6. Chang MH, Shau WY, Chen CJ, Wu TC, Kong MS, Liang DC, Hsu HM, Chen HL, Hsu HY, Chen DS *et al*: **Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls**. *JAMA* 2000, **284**(23):3040-3042.
7. Chang MH, You SL, Chen CJ, Liu CJ, Lai MW, Wu TC, Wu SF, Lee CM, Yang SS, Chu HC *et al*: **Long-term Effects of Hepatitis B Immunization of Infants in Preventing Liver Cancer**. *Gastroenterology* 2016, **151**(3):472-480 e471.
8. Cox AL, El-Sayed MH, Kao JH, Lazarus JV, Lemoine M, Lok AS, Zoulim F: **Progress towards elimination goals for viral hepatitis**. *Nat Rev Gastroenterol Hepatol* 2020, **17**(9):533-542.
9. Analysis. POCfD:https://cdafound.org/polaris-public-faq/#sec13 Last accessed February 14, 2022.
10. Tsukuda S, Watashi K: **Hepatitis B virus biology and life cycle**. *Antiviral Res* 2020, **182**:104925.
11. Pollicino T, Caminiti G: **HBV-Integration Studies in the Clinic: Role in the Natural History of Infection**. *Viruses* 2021, **13**(3).
12. Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, Ferrari C, Given BD, Hamilton J, Kanner SB *et al*: **RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg**. *Sci Transl Med* 2017, **9**(409).
13. Wieland S, Thimme R, Purcell RH, Chisari FV: **Genomic analysis of the host response to hepatitis B virus infection**. *Proc Natl Acad Sci U S A* 2004, **101**(17):6669-6674.
14. Wieland SF, Chisari FV: **Stealth and cunning: hepatitis B and hepatitis C viruses**. *J Virol* 2005, **79**(15):9369-9380.
15. Chang J, Guo JT: **Treatment of chronic hepatitis B with pattern recognition receptor agonists: Current status and potential for a cure**. *Antiviral Res* 2015, **121**:152-159.
16. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, Watanabe T, Iijima S, Sakurai Y, Watashi K *et al*: **The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus**. *Immunity* 2015, **42**(1):123-132.
17. Shlomai A, Schwartz RE, Ramanan V, Bhatta A, de Jong YP, Bhatia SN, Rice CM: **Modeling host interactions with hepatitis B virus using primary and induced pluripotent stem**

cell-derived hepatocellular systems. *Proceedings of the National Academy of Sciences of the United States of America* 2014, **111**(33):12193-12198.

18. Fisicaro P, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C: **Early kinetics of innate and adaptive immune responses during hepatitis B virus infection**. *Gut* 2009, **58**(7):974-982.
19. Guidotti LG, Chisari FV: **Immunobiology and pathogenesis of viral hepatitis**. *Annu Rev Pathol* 2006, **1**:23-61.
20. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV: **The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response**. *Nat Med* 1996, **2**(10):1104-1108.
21. Hwang JP, Barbo AG, Perrillo RP: **Hepatitis B reactivation during cancer chemotherapy: an international survey of the membership of the American Association for the Study of Liver Diseases**. *Journal of viral hepatitis* 2015, **22**(3):346-352.
22. Di Bisceglie AM, Lok AS, Martin P, Terrault N, Perrillo RP, Hoofnagle JH: **Recent US Food and Drug Administration warnings on hepatitis B reactivation with immune-suppressing and anticancer drugs: just the tip of the iceberg?** *Hepatology* 2015, **61**(2):703-711.
23. Milich D, Liang TJ: **Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection**. *Hepatology* 2003, **38**(5):1075-1086.
24. Asabe S, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, Purcell RH, Chisari FV: **The size of the viral inoculum contributes to the outcome of hepatitis B virus infection**. *Journal of virology* 2009, **83**(19):9652-9662.
25. Ferrari C, Boni C, Rossi M, Vecchi A, Barili V, Laccabue D, Fisicaro P, Missale G: **T cell regulation in HBV-related chronic liver disease**. *Journal of hepatology* 2017, **66**(5):1096-1098.
26. Park JJ, Wong DK, Wahed AS, Lee WM, Feld JJ, Terrault N, Khalili M, Sterling RK, Kowdley KV, Bzowej N *et al*: **Hepatitis B Virus-Specific and Global T-Cell Dysfunction in Chronic Hepatitis B**. *Gastroenterology* 2016, **150**(3):684-695 e685.
27. Salimzadeh L, Le Bert N, Dutertre CA, Gill US, Newell EW, Frey C, Hung M, Novikov N, Fletcher S, Kennedy PT *et al*: **PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection**. *J Clin Invest* 2018, **128**(10):4573-4587.
28. Burton AR, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, Swadling L, Alberts E, Davidson BR, Kennedy PT, Gill US *et al*: **Circulating and intrahepatic antiviral B cells are defective in hepatitis B**. *J Clin Invest* 2018, **128**(10):4588-4603.
29. Bengsch B, Martin B, Thimme R: **Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation**. *J Hepatol* 2014, **61**(6):1212-1219.
30. Chang KM, Traum D, Park JJ, Ho S, Ojiro K, Wong DK, Wahed AS, Terrault NA, Khalili M, Sterling RK *et al*: **Distinct phenotype and function of circulating Vdelta1+ and Vdelta2+ gammadeltaT-cells in acute and chronic hepatitis B**. *PLoS Pathog* 2019, **15**(4):e1007715.
31. Fisicaro P, Barili V, Rossi M, Montali I, Vecchi A, Acerbi G, Laccabue D, Zecca A, Penna A, Missale G *et al*: **Pathogenetic Mechanisms of T Cell Dysfunction in Chronic HBV Infection and Related Therapeutic Approaches**. *Front Immunol* 2020, **11**:849.

32. Lopes AR, Kellam P, Das A, Dunn C, Kwan A, Turner J, Peppa D, Gilson RJ, Gehring A, Bertoletti A *et al*: **Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection**. *J Clin Invest* 2008, **118**(5):1835-1845.
33. Traum D, Wang YJ, Schwarz KB, Schug J, Wong DK, Janssen H, Terrault NA, Khalili M, Wahed AS, Murray KF *et al*: **Highly multiplexed 2-dimensional imaging mass cytometry analysis of HBV-infected liver**. *JCI Insight* 2021, **6**(7).
34. **Hepatitis B Vaccination: Information for Healthcare Providers** [https://www.cdc.gov/vaccines/vpd/hepb/hcp/index.html]
35. Lok AS, Zoulim F, Dusheiko G, Ghany MG: **Hepatitis B cure: From discovery to regulatory approval**. *J Hepatol* 2017, **67**(4):847-861.
36. Cornberg M, Lok AS, Terrault NA, Zoulim F: **Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference**. *Hepatology* 2019.
37. Yeo YH, Ho HJ, Yang HI, Tseng TC, Hosaka T, Trinh HN, Kwak MS, Park YM, Fung JYY, Buti M *et al*: **Factors Associated With Rates of HBsAg Seroclearance in Adults With Chronic HBV Infection: A Systematic Review and Meta-analysis**. *Gastroenterology* 2019, **156**(3):635-646.e639.
38. Zhou K, Contag C, Whitaker E, Terrault N: **Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses**. *Lancet Gastroenterol Hepatol* 2019, **4**(3):227-238.
39. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS, Jr., Bzowej NH, Wong JB: **Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance**. *Hepatology* 2018, **67**(4):1560-1599.
40. **EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection**. *J Hepatol* 2017, **67**(2):370-398.
41. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN *et al*: **Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update**. *Hepatol Int* 2016, **10**(1):1-98.
42. WHO: **http://apps.who.int/iris/bitstream/handle/10665/154590/9789241549059_eng.pdf**. *Last accessed 3/10/22* 2015.
43. Singh S, Muir AJ, Dieterich DT, Falck-Ytter YT: **American Gastroenterological Association Institute Technical Review on the Role of Elastography in Chronic Liver Diseases**. *Gastroenterology* 2017, **152**(6):1544-1577.
44. Herrmann E, de Ledinghen V, Cassinotto C, Chu WC, Leung VY, Ferraioli G, Filice C, Castera L, Vilgrain V, Ronot M *et al*: **Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: An individual patient data-based meta-analysis**. *Hepatology* 2018, **67**(1):260-272.
45. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH: **Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level**. *Jama* 2006, **295**(1):65-73.
46. Chen CF, Lee WC, Yang HI, Chang HC, Jen CL, Iloeje UH, Su J, Hsiao CK, Wang LY, You SL *et al*: **Changes in serum levels of HBV DNA and alanine aminotransferase determine**

risk for hepatocellular carcinoma. *Gastroenterology* 2011, **141**(4):1240-1248, 1248.e1241-1242.

47. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH, Group R-HS: **Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level**. *JAMA* 2006, **295**(1):65-73.
48. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ, Risk Evaluation of Viral Load E, Associated Liver Disease/Cancer-In HBVSG: **Predicting cirrhosis risk based on the level of circulating hepatitis B viral load**. *Gastroenterology* 2006, **130**(3):678-686.
49. Kim GA, Lim YS, Han S, Choi J, Shim JH, Kim KM, Lee HC, Lee YS: **High risk of hepatocellular carcinoma and death in patients with immune-tolerant-phase chronic hepatitis B**. *Gut* 2018, **67**(5):945-952.
50. Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF: **Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels**. *Am J Med* 2004, **116**(12):829-834.
51. Hui CK, Leung N, Yuen ST, Zhang HY, Leung KW, Lu L, Cheung SK, Wong WM, Lau GK, Hong Kong Liver Fibrosis Study G: **Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase**. *Hepatology* 2007, **46**(2):395-401.
52. Sinn DH, Kim SE, Kim BK, Kim JH, Choi MS: **The risk of hepatocellular carcinoma among chronic hepatitis B virus-infected patients outside current treatment criteria**. *J Viral Hepat* 2019, **26**(12):1465-1472.
53. Choi GH, Kim GA, Choi J, Han S, Lim YS: **High risk of clinical events in untreated HBeAg-negative chronic hepatitis B patients with high viral load and no significant ALT elevation**. *Alimentary Pharmacology and Therapeutics* 2019, **50**(2):215-226.
54. Hsu YC, Chen CY, Chang IW, Chang CY, Wu CY, Lee TY, Wu MS, Bair MJ, Chen JJ, Chen CC *et al*: **Once-daily tenofovir disoproxil fumarate in treatment-naive Taiwanese patients with chronic hepatitis B and minimally raised alanine aminotransferase (TORCH-B): a multicentre, double-blind, placebo-controlled, parallel-group, randomised trial**. *Lancet Infect Dis* 2021, **21**(6):823-833.
55. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, Feinman SV, Mach T, Akarca US *et al*: **Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b**. *Gastroenterology* 2008, **135**(2):459-467.
56. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA *et al*: **Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial**. *Lancet* 2005, **365**(9454):123-129.
57. Konerman MA, Lok AS: **Interferon Treatment for Hepatitis B**. *Clin Liver Dis* 2016, **20**(4):645-665.
58. Zhu YY, Wu YL, Chen J, Zheng Q, Dong J, Jiang JJ: **[Prolonged duration of the routine pegylated-interferon alfa-2a therapy produces superior virological response in HBeAg-positive chronic hepatitis B patients: a single-center cohort study]**. *Zhonghua Gan Zang Bing Za Zhi* 2012, **20**(10):737-741.

59. Cao ZH, Ma LN, Zhang HW, Liu YL, Chen XY: **Extended treatment with peginterferon α-2a in combination with lamivudine or adefovir for 96 weeks yields high rates of HBeAg and HBsAg seroconversion**. *J Dig Dis* 2013, **14**(8):446-450.
60. Menendez-Arias L, Alvarez M, Pacheco B: **Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance**. *Curr Opin Virol* 2014, **8**:1-9.
61. Hsu YC, Suri V, Nguyen MH, Huang YT, Chen CY, Chang IW, Tseng CH, Wu CY, Lin JT, Pan DZ *et al*: **Inhibition of Viral Replication Reduces Transcriptionally Active Distinct Hepatitis B Virus Integrations with Implications on Host Gene Dysregulation**. *Gastroenterology* 2022.
62. Li B, Liu Z, Liu X, Liu D, Duan M, Gu Y, Liu Q, Ma Q, Wei Y, Wang Y: **Efficacy and safety of tenofovir disoproxil fumarate and tenofovir alafenamide fumarate in preventing HBV vertical transmission of high maternal viral load**. *Hepatol Int* 2021, **15**(5):1103-1108.
63. Zeng QL, Yu ZJ, Ji F, Li GM, Zhang GF, Xu JH, Chen ZM, Cui GL, Li W, Zhang DW *et al*: **Tenofovir Alafenamide to Prevent Perinatal Hepatitis B Transmission: A Multicenter, Prospective, Observational Study**. *Clin Infect Dis* 2021, **73**(9):e3324-e3332.
64. Ding Y, Cao L, Zhu L, Huang Y, Lin C, Wang Y, Liu Y, Sheng Q, Wang S, Fan J *et al*: **Efficacy and safety of tenofovir alafenamide fumarate for preventing mother-to-child transmission of hepatitis B virus: a national cohort study**. *Aliment Pharmacol Ther* 2020, **52**(8):1377-1386.
65. Loomba R, Liang TJ: **Hepatitis B Reactivation Associated With Immune Suppressive and Biological Modifier Therapies: Current Concepts, Management Strategies, and Future Directions**. *Gastroenterology* 2017, **152**(6):1297-1309.
66. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW *et al*: **Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B**. *N Engl J Med* 2005, **352**(26):2682-2695.
67. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C *et al*: **Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B**. *N Engl J Med* 2004, **351**(12):1206-1217.
68. Tout I, Lampertico P, Berg T, Asselah T: **Perspectives on stopping nucleos(t)ide analogues therapy in patients with chronic hepatitis B**. *Antiviral Res* 2021, **185**:104992.
69. Hirode G, Choi HSJ, Chen CH, Su TH, Seto WK, Van Hees S, Papatheodoridi M, Lens S, Wong G, Brakenhoff SM *et al*: **Off-Therapy Response After Nucleos(t)ide Analogue Withdrawal in Patients With Chronic Hepatitis B: An International, Multicenter, Multiethnic Cohort (RETRACT-B Study)**. *Gastroenterology* 2021.
70. Coffin CS, Zhou K, Terrault NA: **New and Old Biomarkers for Diagnosis and Management of Chronic Hepatitis B Virus Infection**. *Gastroenterology* 2019, **156**(2):355-368.e353.
71. Blank A, Markert C, Hohmann N, Carls A, Mikus G, Lehr T, Alexandrov A, Haag M, Schwab M, Urban S *et al*: **First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B**. *J Hepatol* 2016, **65**(3):483-489.
72. Kang C, Syed YY: **Bulevirtide: First Approval**. *Drugs* 2020, **80**(15):1601-1605.

73. **A Phase 1b/2a Randomized, Open-label Clinical Trial of Daily Myrcludex B vs Entecavir in Patients With HBeAg Negative Chronic Hepatitis B** [https://clinicaltrials.gov/ct2/show/study/NCT02881008]
74. Tu T, Urban S: **Virus entry and its inhibition to prevent and treat hepatitis B and hepatitis D virus infections**. *Curr Opin Virol* 2018, **30**:68-79.
75. Agarwal K YM, Wedemeyer H, Arizpe A, Shen L, Cloutier D, Tay C, Gupta S, Fanget M, Dholakiya S et al: **Rapid HBsAg reduction in chronic hepatitis B virus infection: preliminary re-sults from a phase 1 study evaluating a single dose of VIR-3434, a novel neutralizing, vaccinal monoclonal antibody.** *Hepatology* 2021, **74**:514A, Abstract 839.
76. Yuen MF LT KW, Tongkijvonich P, Yoon JH, Sievert W et. al.: **HBV RNAi INHIBITOR RG6346 IN PHASE 1b-2a TRIAL WAS SAFE, WELL-TOLERATED, AND RESULTED IN SUBSTANTIAL AND DURABLE REDUCTIONS IN SERUM HBsAg LEVELS.** . *Hepatology* 2020, **72**:LO9.
77. Gane E LY, Cloutier D, et: **Safety and antiviral activity of VIR-2218, an X-targeting RNAi therapeutic, in participants with chronic hepatitis B infection: week 48 follow-up results.** . *Journal of Hepatolgy* 2021, **75**:S287:OS244;.
78. Gane E LS, Lim TH, Strasser S, Sievert W, Cheng W, et .al.: **Short interfering RNA JNJ-3989 combination therapy in chronic hepatitis B shows potent reduction of all viral markers but no correlate was identified for HBsAg reduction and baseline factors**. *Journal of Hepatology* 2021, **75**:S289:(Abstract OS-1430).
79. Yuen MF BE, Kim YJ, Holmes JA, Lim Y-S, Strasser SI et. al.: **Safety and pharmacodynamics of the GalNAc-siRNA AB-729 in subjects with chronic hepatitis B infection.** *Hepatology* 2020, **72**:62A:Abstract 83.
80. Paratala B PJ-J, Ganchua SC, Gane E, Yuen M-F et al: **Inhibition of HBsAg in patients with CHB by RNA interference therapeutic AB-729 is accompanied by upregulation of HBVspecific T cell activation markers**. *J Hepatology* 2021, **75**(2):S294–S803.
81. Yuen MF, Heo J, Jang JW, Yoon JH, Kweon YO, Park SJ, Tami Y, You S, Yates P, Tao Y *et al*: **Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: a phase 2 randomized controlled trial**. *Nat Med* 2021, **27**(10):1725-1734.
82. Yuen M-F PR, Lim SG, Tsuji K, Diaconescu G, Gadano A, et al: **Efficacy and safety of bepirovirsen in patients with chronic hepatitis B virus infection on stable nucleos (t)ide analogue therapy: interim results from the randomised phase 2b B-Clear study**. *Journal of Hepatology* 2022, **77(S1);S13:LB004B**.
83. Yuen MF, Gane EJ, Kim DJ, Weilert F, Yuen Chan HL, Lalezari J, Hwang SG, Nguyen T, Flores O, Hartman G *et al*: **Antiviral Activity, Safety, and Pharmacokinetics of Capsid Assembly Modulator NVR 3-778 in Patients with Chronic HBV Infection**. *Gastroenterology* 2019, **156**(5):1392-1403.e1397.
84. Yuen MF, Agarwal K, Gane EJ, Schwabe C, Ahn SH, Kim DJ, Lim YS, Cheng W, Sievert W, Visvanathan K *et al*: **Safety, pharmacokinetics, and antiviral effects of ABI-H0731, a hepatitis B virus core inhibitor: a randomised, placebo-controlled phase 1 trial**. *Lancet Gastroenterol Hepatol* 2020, **5**(2):152-166.
85. Zoulim F, Lenz O, Vandenbossche JJ, Talloen W, Verbinnen T, Moscalu I, Streinu-Cercel A, Bourgeois S, Buti M, Crespo J *et al*: **JNJ-56136379, an HBV Capsid Assembly**

Modulator, Is Well-Tolerated and Has Antiviral Activity in a Phase 1 Study of Patients With Chronic Infection. *Gastroenterology* 2020, **159**(2):521-533.e529.

86. Jacobson IM MX, Nguyen T, Schiff ER, Yuen MF, Hann H-WL et. al.: **Analysis of the longer-term safety profile of the hepatitis B virus core inhibitor ABI-H0731 in an open-label extension study.** . *Hepatology* 2020, **72**:501A:Abstract 820.
87. Gane EJ YM, Jucov A, Le K, Westland C, Schwabe C, Agarwal K et. al.: **Safety, Pharmacokinetics (PK), and Antiviral Activity of the Capsid Assembly Modulator (CAM) ALG-000184 in Subjects with Chronic Hepatitis B (CHB).** *Heptology* 2021, **74**.
88. Yuen M-F CW-L, Peng C-Y, Jeng W-J, Su W-W, Chang T-T, et. al.: **EDP-514, a Novel Pangenotypic Class II Hepatitis B Virus Core Inhibitor Demonstrates Significant HBV DNA and HBV RNA Reductions in a Phase 1b Study in Viremic, Chronic Hepatitis B Infected Patients** *Hepatology* 2021, **74**:505A:Abstract 823.
89. Feld JJ LE, Nguyen T, Lalezari JP, Hassanein T, Martin P, et. al.: **EDP-514, A NOVEL PANGENOTYPIC CLASS II HEPATITIS B VIRUS CORE INHIBITOR: PRELIMINARY RESULTS OF A 28-DAY PHASE 1b STUDY IN NUC-SUPPRESSED CHB PATIENTS**. *Hepatology* 2021, **74**:504A:Abstract 822.
90. Yuen MF, Zhou X, Gane E, Schwabe C, Tanwandee T, Feng S, Jin Y, Triyatni M, Lemenuel-Diot A, Cosson V *et al*: **Safety, pharmacokinetics, and antiviral activity of RO7049389, a core protein allosteric modulator, in patients with chronic hepatitis B virus infection: a multicentre, randomised, placebo-controlled, phase 1 trial**. *Lancet Gastroenterol Hepatol* 2021, **6**(9):723-732.
91. https://investor.arbutusbio.com/news-releases/news-release-details/preliminary-data-shows-arbutus-capsid-inhibitor-ab-836-generally. Last accessed January 4th.
92. Yuen MF MX, Hassanein TI, Kwo PY, Ma J, Li L, et al: **HBV PGRNA AND DNA BOTH REBOUND IMMEDIATELY FOLLOWING DISCONTINUATION OF THE CORE INHIBITOR VEBICORVIR DESPITE CONTINUED NRTI TREATMENT IN PATIENTS WITH HBEAG POSITIVE CHRONIC HEPATITIS B VIRUS INFECTION: FINDINGS FROM A PHASE 2 OPEN LABEL STUDY**. *Hepatology* 2021, **74**:65A Abstract 96.
93. Marcellin P, Wong DK, Sievert W, Buggisch P, Petersen J, Flisiak R, Manns M, Kaita K, Krastev Z, Lee SS *et al*: **Ten-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B virus infection**. *Liver Int* 2019, **39**(10):1868-1875.
94. Squires KE, Mayers DL, Bluemling GR, Kolykhalov AA, Guthrie DB, Reddy P, Mitchell DG, Saindane MT, Sticher ZM, Edpuganti V *et al*: **ATI-2173, a Novel Liver-Targeted Non-Chain-Terminating Nucleotide for Hepatitis B Virus Cure Regimens**. *Antimicrob Agents Chemother* 2020, **64**(9).
95. Mayers D JA, Anastasiy I, Ghicavii N, Ogilvie L, Squires K et. al.: **Phase 1 Results for ATI-2173, a novel active site polymerase inhibitor nucleotide (ASPIN), in HBV- infected subjects.** . *Journal of Hepatology* 2021, **75(2)**:S743:Abstract PO- 1251.
96. Gao Y, Kong F, Song X, Shang J, Yao L, Xia J, Peng Y, Liu W, Gong H, Mu M *et al*: **Pradefovir Treatment in Patients with Chronic Hepatitis B: Week 24 Results from a Multicenter, Double-blind, Randomized, Noninferiority, Phase 2 Trial**. *Clin Infect Dis* 2021.
97. Zhang H, Hu Y, Wu M, Liu J, Zhu X, Li X, Chen H, Li C, Liu C, Niu J *et al*: **Randomised clinical trial: safety, efficacy and pharmacokinetics of HS-10234 versus tenofovir for**

the treatment of chronic hepatitis B infection. *Aliment Pharmacol Ther* 2021, **53**(2):243-252.

98. Zhi L VE, Vrishabhendra L, Hu D, Lu T-S and Marschke KB: **Single-dose Pharmacokinetics and Safety of NCO-48 Fumarate, a Novel Liver-Targeted Prodrug (LTP) of Tenofovir (TFV)**. *Hepatology* 2021, **74**:519A: Abstract 847.
99. Pierra Rouviere C, Dousson CB, Tavis JE: **HBV replication inhibitors**. *Antiviral Res* 2020, **179**:104815.
100. Al-Mahtab M, Bazinet M, Vaillant A: **Safety and Efficacy of Nucleic Acid Polymers in Monotherapy and Combined with Immunotherapy in Treatment-Naive Bangladeshi Patients with HBeAg+ Chronic Hepatitis B Infection**. *PLoS One* 2016, **11**(6):e0156667.
101. Vaillant A: **Nucleic acid polymers: Broad spectrum antiviral activity, antiviral mechanisms and optimization for the treatment of hepatitis B and hepatitis D infection**. *Antiviral Res* 2016, **133**:32-40.
102. Bazinet M, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, Jimbei P, Iarovoi L, Smesnoi V, Musteata T *et al*: **Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy**. *Gastroenterology* 2020, **158**(8):2180-2194.
103. Kao CC, Nie Y, Ren S, De Costa N, Pandey RK, Hong J, Smith DB, Symons JA, Beigelman L, Blatt LM: **Mechanism of action of hepatitis B virus S antigen transport-inhibiting oligonucleotide polymer, STOPS, molecules**. *Mol Ther Nucleic Acids* 2022, **27**:335-348.
104. Aligos: **Aligos Halting Further Development of STOPS™ Drug Candidate, ALG-010133**. In.; 2022.
105. Kostyushev D, Brezgin S, Kostyusheva A, Zarifyan D, Goptar I, Chulanov V: **Orthologous CRISPR/Cas9 systems for specific and efficient degradation of covalently closed circular DNA of hepatitis B virus**. *Cell Mol Life Sci* 2019, **76**(9):1779-1794.
106. Tropberger P, Mercier A, Robinson M, Zhong W, Ganem DE, Holdorf M: **Mapping of histone modifications in episomal HBV cccDNA uncovers an unusual chromatin organization amenable to epigenetic manipulation**. *Proc Natl Acad Sci U S A* 2015, **112**(42):E5715-5724.
107. Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, Petersen J, Raimondo G, Dandri M, Levrero M: **IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome**. *J Clin Invest* 2012, **122**(2):529-537.
108. Xu Z, Yen TS, Wu L, Madden CR, Tan W, Slagle BL, Ou JH: **Enhancement of hepatitis B virus replication by its X protein in transgenic mice**. *J Virol* 2002, **76**(5):2579-2584.
109. Decorsière A, Mueller H, van Breugel PC, Abdul F, Gerossier L, Beran RK, Livingston CM, Niu C, Fletcher SP, Hantz O *et al*: **Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor**. *Nature* 2016, **531**(7594):386-389.
110. Cheng ST, Hu JL, Ren JH, Yu HB, Zhong S, Wai Wong VK, Kwan Law BY, Chen WX, Xu HM, Zhang ZZ *et al*: **Dicoumarol, an NQO1 inhibitor, blocks cccDNA transcription by promoting degradation of HBx**. *J Hepatol* 2021, **74**(3):522-534.
111. Sekiba K, Otsuka M, Ohno M, Yamagami M, Kishikawa T, Seimiya T, Suzuki T, Tanaka E, Ishibashi R, Funato K *et al*: **Pevonedistat, a Neuronal Precursor Cell-Expressed**

Developmentally Down-Regulated Protein 8-Activating Enzyme Inhibitor, Is a Potent Inhibitor of Hepatitis B Virus. *Hepatology* 2019, **69**(5):1903-1915.

112. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T *et al*: **Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA**. *Science* 2014, **343**(6176):1221-1228.
113. Maini MK, Gehring AJ: **The role of innate immunity in the immunopathology and treatment of HBV infection**. *J Hepatol* 2016, **64**(1 Suppl):S60-S70.
114. Meng Z, Zhang X, Pei R, Zhang E, Kemper T, Vollmer J, Davis HL, Glebe D, Gerlich W, Roggendorf M *et al*: **Combination therapy including CpG oligodeoxynucleotides and entecavir induces early viral response and enhanced inhibition of viral replication in a woodchuck model of chronic hepadnaviral infection**. *Antiviral Res* 2016, **125**:14-24.
115. Lanford RE, Guerra B, Chavez D, Giavedoni L, Hodara VL, Brasky KM, Fosdick A, Frey CR, Zheng J, Wolfgang G *et al*: **GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees**. *Gastroenterology* 2013, **144**(7):1508-1517, 1517 e1501-1510.
116. Menne S, Tumas DB, Liu KH, Thampi L, AlDeghaither D, Baldwin BH, Bellezza CA, Cote PJ, Zheng J, Halcomb R *et al*: **Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B**. *J Hepatol* 2015, **62**(6):1237-1245.
117. Gane EJ, Lim YS, Gordon SC, Visvanathan K, Sicard E, Fedorak RN, Roberts S, Massetto B, Ye Z, Pflanz S *et al*: **The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection**. *J Hepatol* 2015, **63**(2):320-328.
118. Janssen HLA, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen AH, Joshi A, Woo J, Lau AH, Gaggar A *et al*: **Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B**. *J Hepatol* 2018, **68**(3):431-440.
119. Agarwal K, Ahn SH, Elkhashab M, Lau AH, Gaggar A, Bulusu A, Tian X, Cathcart AL, Woo J, Subramanian GM *et al*: **Safety and efficacy of vesatolimod (GS-9620) in patients with chronic hepatitis B who are not currently on antiviral treatment**. *J Viral Hepat* 2018, **25**(11):1331-1340.
120. Daffis S, Balsitis S, Chamberlain J, Zheng J, Santos R, Rowe W, Ramakrishnan D, Pattabiraman D, Spurlock S, Chu R *et al*: **Toll-Like Receptor 8 Agonist GS-9688 Induces Sustained Efficacy in the Woodchuck Model of Chronic Hepatitis B**. *Hepatology* 2021, **73**(1):53-67.
121. Gane E DP BA, Zhao Y, Tan SK et al.: **Efficacy and Safety of 24 Weeks Treatment with Oral TLR8 Agonist Selgantolimod (GS-9688, SLGN) in Virally Suppressed Adult Patients with Chronic Hepatitis B: A Phase 2 Study**. *Journal of Hepatology* 2020, **73** S52:Abstract ASO71.
122. Yuen MC CC, Liu CY et al.: **Ascending dose cohort study of inarigivir-A novel RIG I agonist in chronic HBV patients: Final results of the ACHIEVE trial**. *J Hepatology* 2019, **70**:e45-79.
123. Dansako H, Ueda Y, Okumura N, Satoh S, Sugiyama M, Mizokami M, Ikeda M, Kato N: **The cyclic GMP-AMP synthetase-STING signaling pathway is required for both the innate immune response against HBV and the suppression of HBV assembly**. *FEBS J* 2016, **283**(1):144-156.

124. He J, Hao R, Liu D, Liu X, Wu S, Guo S, Wang Y, Tien P, Guo D: **Inhibition of hepatitis B virus replication by activation of the cGAS-STING pathway**. *J Gen Virol* 2016, **97**(12):3368-3378.
125. Thomsen MK, Nandakumar R, Stadler D, Malo A, Valls RM, Wang F, Reinert LS, Dagnaes-Hansen F, Hollensen AK, Mikkelsen JG *et al*: **Lack of immunological DNA sensing in hepatocytes facilitates hepatitis B virus infection**. *Hepatology* 2016, **64**(3):746-759.
126. Fisicaro P, Valdatta C, Massari M, Loggi E, Biasini E, Sacchelli L, Cavallo MC, Silini EM, Andreone P, Missale G *et al*: **Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B**. *Gastroenterology* 2010, **138**(2):682-693, 693.e681-684.
127. Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, Schwabe C, Dunbar PR: **Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study**. *J Hepatol* 2019, **71**(5):900-907.
128. Wang G QJ, Cui Y, Yan Y, He H, Wu J et. al.: **A Phase IIa trial of Subcutaneously Administered PD-L1 Antibody ASC22 (Envafolimab) in Patients with Chronic Hepatitis B.** *Hepatology* 2021, **74**:19: Late Breaking Abstract LO12; .
129. Geretti AM GE, Lacombe K, Balabanska R, Attley G, Teixeira P, Luangsay S, Triyatni M, Lu. S, Lin S et al:: **LIVER DIRECTED TARGETING OF PD-L1 WITH R07191863, A LOCKED NUCLEIC ACID, IN CHRONIC HEPATITIS B: FIRST REPORT OF PHASE 1 TOLERABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS.** *Hepatology* 2021, **74**:54: Late breaking abstract LP29.
130. Tan E, Gakhar N, Kirtane K: **TCR gene-engineered cell therapy for solid tumors**. *Best Pract Res Clin Haematol* 2021, **34**(3):101285.
131. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L: **'Off-the-shelf' allogeneic CAR T cells: development and challenges**. *Nat Rev Drug Discov* 2020, **19**(3):185-199.
132. Lau GK, Lok AS, Liang RH, Lai CL, Chiu EK, Lau YL, Lam SK: **Clearance of hepatitis B surface antigen after bone marrow transplantation: role of adoptive immunity transfer**. *Hepatology* 1997, **25**(6):1497-1501.
133. Kruse RL, Shum T, Tashiro H, Barzi M, Yi Z, Whitten-Bauer C, Legras X, Bissig-Choisat B, Garaigorta U, Gottschalk S *et al*: **HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice**. *Cytotherapy* 2018, **20**(5):697-705.
134. Gehring AJ, Xue SA, Ho ZZ, Teoh D, Ruedl C, Chia A, Koh S, Lim SG, Maini MK, Stauss H *et al*: **Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines**. *J Hepatol* 2011, **55**(1):103-110.
135. Fergusson JR, Wallace Z, Connolly MM, Woon AP, Suckling RJ, Hine DW, Barber C, Bunjobpol W, Choi BS, Crespillo S *et al*: **Immune-Mobilizing Monoclonal T Cell Receptors Mediate Specific and Rapid Elimination of Hepatitis B-Infected Cells**. *Hepatology* 2020, **72**(5):1528-1540.
136. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, Khakpoor A, Zhan H, Ciccorossi P, Gilmour K, Cavallone D *et al*: **Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient**. *J Hepatol* 2015, **62**(2):486-491.

137. Meng F, Zhao J, Tan AT, Hu W, Wang SY, Jin J, Wu J, Li Y, Shi L, Fu JL *et al*: **Immunotherapy of HBV-related advanced hepatocellular carcinoma with short-term HBV-specific TCR expressed T cells: results of dose escalation, phase I trial**. *Hepatol Int* 2021, **15**(6):1402-1412.
138. Zoulim F, Fournier C, Habersetzer F, Sprinzl M, Pol S, Coffin CS, Leroy V, Ma M, Wedemeyer H, Lohse AW *et al*: **Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial**. *Hum Vaccin Immunother* 2020, **16**(2):388-399.
139. Chinnakannan SK, Cargill TN, Donnison TA, Ansari MA, Sebastian S, Lee LN, Hutchings C, Klenerman P, Maini MK, Evans T *et al*: **The Design and Development of a Multi-HBV Antigen Encoded in Chimpanzee Adenoviral and Modified Vaccinia Ankara Viral Vectors; A Novel Therapeutic Vaccine Strategy against HBV**. *Vaccines (Basel)* 2020, **8**(2).
140. Michler T, Kosinska AD, Festag J, Bunse T, Su J, Ringelhan M, Imhof H, Grimm D, Steiger K, Mogler C *et al*: **Knockdown of Virus Antigen Expression Increases Therapeutic Vaccine Efficacy in High-Titer Hepatitis B Virus Carrier Mice**. *Gastroenterology* 2020, **158**(6):1762-1775 e1769.
141. Akbar SMF, Al Mahtab M, Aguilar JC, Yoshida O, Khan S, Penton E, Gerardo GN, Hiasa Y: **The Safety and Efficacy of a Therapeutic Vaccine for Chronic Hepatitis B: A Follow-Up Study of Phase III Clinical Trial**. *Vaccines* 2022, **10**(1).
142. Ma H, Lim TH, Leerapun A, Weltman M, Jia J, Lim YS, Tangkijvanich P, Sukeepaisarnjaroen W, Ji Y, Le Bert N *et al*: **Therapeutic vaccine BRII-179 restores HBV-specific immune responses in patients with chronic HBV in a phase Ib/IIa study**. *JHEP Rep* 2021, **3**(6):100361.
143. Yuen MF AT JI, Brunetto M, Janssen HLA et al: **Efficacy and Safety of the siRNA JNJ-3989 and/or the Capsid Assembly Modulator JNJ-6379 for the Treatment of Chronic Hepatitis B Virus Infection : Results From the Phase 2b REEF-1 Study** *Hepatology* 2021, **74**:17: Late Breaking Abstract LO10.
144. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, Poordad F, Halota W, Horsmans Y, Tsai N *et al*: **Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B**. *Hepatology* 2010, **51**(2):422-430.
145. Schulze A, Gripon P, Urban S: **Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans**. *Hepatology* 2007, **46**(6):1759-1768.
146. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H *et al*: **Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus**. *Elife* 2012, **1**:e00049.

Table 1: Indications for Treatment by Liver Society Guidelines and World Health Organization.

Indication	AASLD	EASL	APASL	WHO
Cirrhosis (any detectable HBV DNA)	Treat	Treat	Treat	Treat
HBeAg positive CHB	Treat if: ^ALT≥ 2 X ULN and HBV DNA> 20,000 IU/mL	Treat if: HBV DNA> 2,000 IU/mL, #ALT>ULN and/or at least moderate liver necroinflammation or fibrosis*	Treat if: a. HBV DNA> 20,000 IU/mL and #ALT >2 X ULN (if no concern of hepatic decompensation, observe) b. HBV DNA >20,000 IU/mL and ALT<2 ULN, treat if moderate to severe inflammation or fibrosis*. **c.** HBV DNA <20,000 and any ALT treat if moderate to severe inflammation or fibrosis*.	Treat all adults above the age of 30 if: a. HBV DNA> 20,000 IU/mL and #ALT >ULN (tested 3 times during a 6–12-month period) b. ALT >ULN and other causes of ALT elevation have been excluded (if HBV DNA testing unavailable)
HBeAg negative CHB	Treat if: ALT≥ 2 X ULN and HBV DNA> 2,000 IU/mL	Treat if: HBV DNA> 2,000 IU/mL, ALT>ULN and/or at least moderate liver necroinflammation or fibrosis*	Treat if: a. HBV DNA> 2,000 IU/mL and ALT >2 X ULN (if no concern of hepatic decompensation, observe) b. HBV DNA >2,000 IU/mL and ALT<2 X ULN, treat if moderate to severe inflammation or fibrosis*. HBV DNA <2,000 IU/mL treat if moderate	

			to severe inflammation or fibrosis*.	
CHB reactivation	Treat	Treat	Treat	Treat
Pregnant women with HBV DNA >200,000 IU/mL on 3rd trimester	Treat	Treat	Treat**	Decision to treat should be based on regular treatment indications. No specific recommendation regarding prevention of vertical transmission

^ Normal ALT defined as ≤35 and ≤25 U/L for males and females, respectively.
# Normal ALT defined as ≤ laboratory upper limit of normal (~40 U/L)

*Based on histologic assessment of liver biopsy including moderate to severe inflammation by either Ishak activity score >3 or METAVIR activity score above A2. Fibrosis by Ishak score > 3 or METAVIR >2, Elastography (Fibroscan©) > 8kPa.

**If HBV DNA is above 6-7 log IU/mL

ALT, alanine aminotransferase, AASLD, American Association for the Study of Liver Diseases; EASL, European Association for the Study of the Liver; APASL, Asian-Pacific Association for the Study of Liver Diseases; WHO, World Health Organization.

Table 2. Indications for treatment and prophylaxis

Indications for treatment	**Indications for prophylaxis (Prevention of HBV transmission/re-activation)**
Decompensated cirrhosis	Post-liver transplantation
Compensated cirrhosis regardless of HBV DNA and ALT levels	Post-liver transplantation from anti-HBc positive donor to HBsAg negative recipient
HBV presenting with acute liver failure	HBsAg positive mother during the third trimester with HBV DNA >200,000 IU/mL
HBeAg positive immune active (HBV DNA >20,000 IU/mL and ALT >2XULN)	HBsAg positive patients receiving immunosuppression/chemotherapy
HBeAg negative immune active (HBV DNA >2,000 IU/mL and ALT >2XULN)	HBsAg negative, anti-HBc positive patients receiving immunosuppression / chemotherapy and at high risk for reactivation
HBV/HDV Co-infection with HBV DNA >2,000 IU/mL	
HBV / HIV Co-infection	
HBV presenting with extrahepatic manifestations	
HBsAg positive healthcare worker with HBV DNA>2,000 IU/mL	

Table 3. Efficacy of Currently Approved Agents for Therapy of Chronic Hepatitis B

HBeAg positive	**PegIFN** (180mcg/week SC)	**Entecavir** (0.5mg/day PO)	**Tenofovir disoproxil fumarate** (245-300mg/day PO)	**Tenofovir alafenamide** (25mg/day PO)
Anti-HBeAg seroconversion	32%[1]	21%[2] 23%[3]	21%[2] 27%[4]	10%[2]
HBV DNA < 60-80 IU/mL	14%[1]	67%[2] 94%[3]	76%[2] 98%[4]	64%[2]
ALT normalization	41%[1]	68%[2] 80%[3]	68%[2] 78%[4]	72%[2]
HBsAg loss	3-7%[1]	2%[2] 1.4%[3]	3%[2] 5%[4]	1%[2]
HBeAg negative	**PegIFN** (180mcg/week)	**Entecavir** (0.5mg/day)	**Tenofovir disoproxil fumarate** (245-300mg/day)	**Tenofovir alafenamide** (25mg/day)
HBV DNA < 60-80 IU/mL	19%[1]	90%[2]	93%[2] 100%[4]	94%[2]
ALT normalization	59%[1]	78%[2]	76%[2] 83%[4]	83%[2]
HBsAg loss	4%[1]	0%[2]	0%[2] 3%[4]	0%[2]

Table adapted from EASL Clinical practice guidelines J Hepatol 2017; 67:370-398 and Terrault N et al Hepatology 2018; 67:1560-1599.

pegIFN, pegylated interferon alfa-2a; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B s antigen; ALT, alanine aminotransferase.

ALT normalization defined by laboratory upper limit of normal

[1]Evaluated 6 months following 48-52 weeks of treatment
[2]Evaluated at 48-96 weeks of continuous therapy
[3]The entecavir long-term cohort consisted of 183 HBeAg positive patients who received ≥1 year of entecavir 0.5 mg in the registration trial (ETV-022) and then entered long-term treatment (ETV-901) with a treatment gap ≤35 days. In ETV-901 the entecavir dose was increased to 1.0 mg daily.[144]
[4] Results based on a sub-set of patients 203/641 (32%) HBeAg-positive (n=80) and HBeAg-negative (n=118) patients who were initially randomized and treated and who were followed for 10 years.[93]

Table 4. Direct and Indirect Antiviral Agents Currently in Development

Target	Mechanism of Action	Agent in Development	Current Stage of Development
Direct			
Viral Entry	Blockage of the NTCP receptor	Bulevirtide	Phase 3*
		Hepalatide	Phase 2
	Monoclonal antibody against the pre-S1 domain	VIR-3434	Phase 1
Viral Transcription	mRNA disruption by siRNA	JNJ-3989	Phase 2
		AB-729	Phase 2
		RG6346	Phase 2
		VIR-2218	Phase 2
		ALG-125755	Phase 1
		BB-103	Pre-Clinical
	mRNA disruption by ASO	Bepirovirsen	Phase 2
		IONIS-HBVLRx	Phase 2
Core Protein	Capsid Inhibitor	EDP-514	Phase 2
		Morphothiadin	Phase 2
		RG7907	Phase 2
		Vebicorvir	Phase 2
		JNJ 56136379	Phase 2
		ABI-H3733	Phase 1
		AB-836	Phase 1
		ALG-000184	Phase 1
		QL-007	Phase 1
		VNRX-9945	Phase 1
		ZM-H1505R	Phase 1
		GLP-26	Pre-Clinical
		ABI-4334	Pre-Clinical
cccDNA	Reducing HBX expression	Pevonedistat	Pre-Clinical
		Dicoumarol	Pre-Clinical
HBV polymerase	Prodrugs of nucleotide analogues	Pradefovir	Phase 3
		HS-10234	Phase 3
		NCO-48 fumarate	Phase 1
	Non-chain terminating nucleotide analogue	AT-2173	Phase 2
HBsAg release	Nucleic Acid Polymers (NAPs)	REP 2139/2165	Phase 2
	S-Antigen Transport-inhibiting Oligonucleotide Polymer (STOPS)	ALG-010133	Discontinued
Indirect			
Innate immunity	TLR 7 agonist	Vesatolimod	Phase 2
		RG7854	Phase 1
	TLR 8 agonist	GS-9688	Phase 2

		SBT 8230	Preclinical
Adaptive immunity	Checkpoint inhibitor	Nivolumab	Phase 2
		Envafolimbab (ASC22)	Phase 2
	Immune Mobilizing Monoclonal T-cell Receptors Against Virus (ImmTAV)	IMC-I109V	Phase 1/2
Therapeutic vaccines	DNA vaccines	GS-4774	Phase 2
		HB-110	Phase 1
		INO-1800/9112	Phase 1
		JNJ-64300535	Phase 1
		MVA-HBV (VTP-300)	Phase 1
		TG1050	Phase 1
		VRON-0200	Preclinical
	T-cell or B-cell epitope vaccine	εPA-44	Phase 3
		FP-02.2 (HepTcell)	Phase 2
	HBV envelope antigen vaccines	NASVAC	Phase 4
		BRII-179	Phase 2
		VVX001	Phase 2

NTCP, sodium taurocholate co-transporting polypeptide; HBX, HBV X protein; HBsAg, HBV surface antigen; cccDNA, covalently closed circular DNA; siRNA, small interfering RNAs; ASO, antisense oligonucleotides.

*For HBV/HDV co-infection not HBV monotherapy.

Figure Legends

Figure 1: HBV lifecycle and targets of drug development

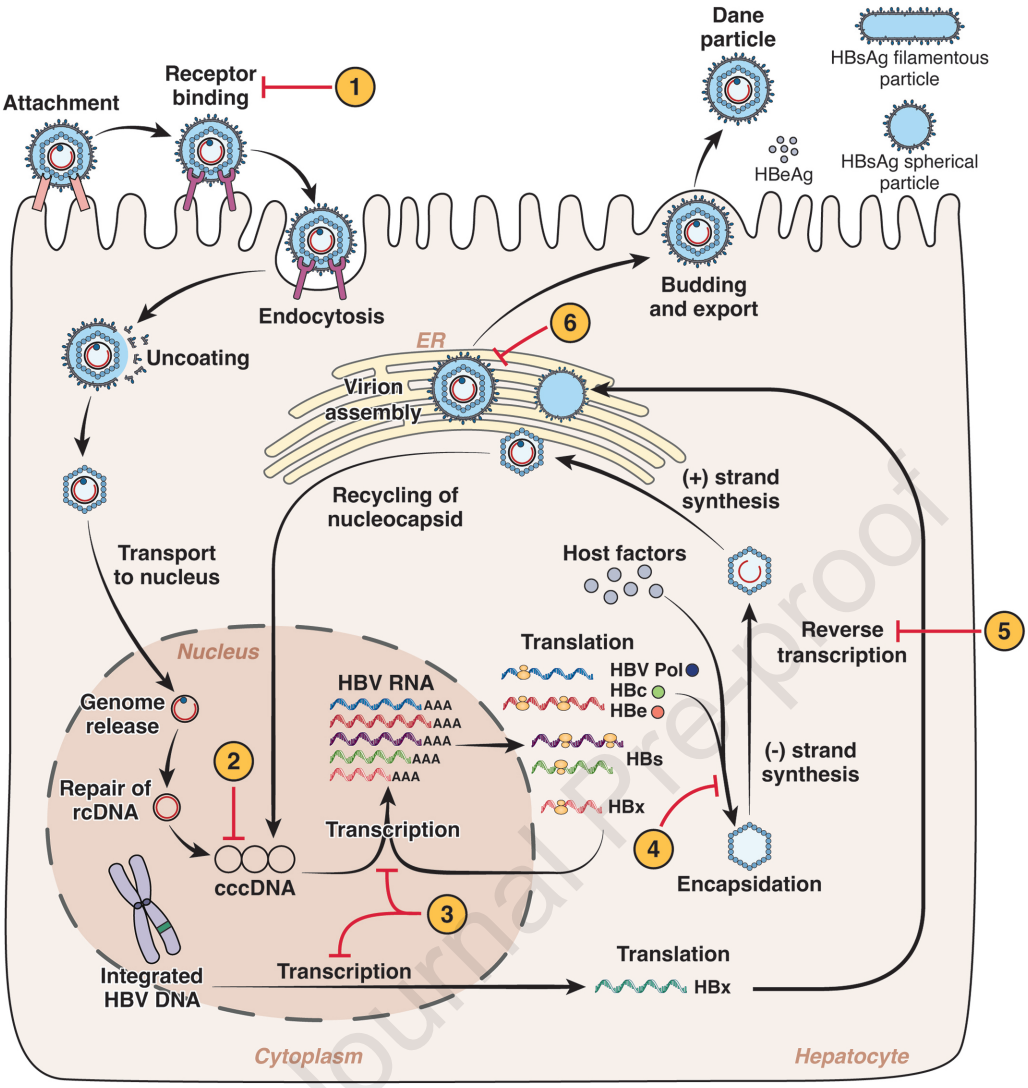
Viral entry is a multi-step process beginning with viral attachment to the hepatocyte surface via a loose interaction with heparan sulfate proteoglycans[145]. This is followed by stronger interaction between the pre-S1 domain and the hepatocyte bile salt transporter, the sodium taurocholate co-transporting polypeptide (NTCP) which facilitates entry[146]. The NTCP receptor confers species specificity to HBV. Viral entry is thought to occur via endocytosis. Following entry, there is uncoating and release of the partially double-stranded, relaxed circular DNA genome (rcDNA), which is transported to the hepatocyte nucleus where host cellular enzymes repair the rcDNA to form the covalently closed circular DNA (cccDNA). cccDNA serves as the transcriptional template for all mRNAs including the pre-genomic RNA, which also serves as the template for genome replication. Viral transcription and translation are under the control of viral promoters and enhancers. Four viral transcripts, polymerase, core, surface, and X are transported to the cytoplasm where they are translated into 7 viral proteins. In the cytoplasm, core proteins self-assemble and through an encapsidation reaction, the pgRNA and viral polymerase are packaged to form the nucleocapsid. Viral replication occurs within the nucleocapsid through a reverse transcription step. The mature viral capsids containing rcDNA are then enveloped with the small, medium, and large (S, M, L) surface proteins in the endoplasmic reticulum and secreted from the infected cell as intact virions, (Dane particle), or transported back to the nucleus to replenish the cccDNA pool. Several sub-viral filamentous and spherical particles that are devoid of viral DNA are also produced in vast excess of the Dane particle.

1)Targeting viral entry; 2) Targeting covalently closed circular DNA (cccDNA) via elimination or silencing; 3) Targeting viral transcription 4) Targeting the HBV core protein; 5) Targeting the HBV polymerase; and 6) Targeting hepatitis B surface antigen (HBsAg) secretion.

Figure 2: Immune subsets involved in HBV pathogenesis and approaches for immune-modulatory therapy.

Multiple immune subsets participate in virus control and disease pathogenesis in chronic hepatitis B. Adaptive immune modulatory approaches in exploration include augmentation of antiviral T and B cells by therapeutic vaccination, checkpoint inhibition (e.g. blockade of PD1/PDL1 or CTLA4/CD28 interactions) as well as supplementation by providing engineered T-cells or antibodies. Innate immune modulatory include IFN alpha (already in clinical use with pleiotropic antiviral and immune modulatory effects) in addition to evolving clinical and pre-clinical evaluations for cellular antiviral pathways including agonists for toll like receptors (e.g TLR7/8), RIG-I,* STING agonists and lymphotoxins.

*No longer in clinical development



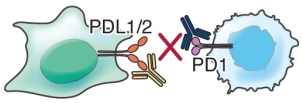
Adaptive immune modulation

Anti-HBs

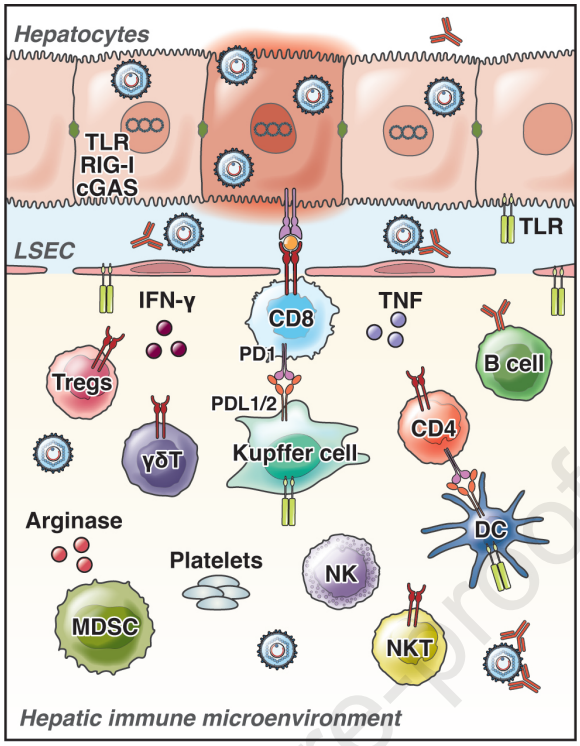
Therapeutic vaccine

Checkpoint inhibition

Anti-PD1/PDL1

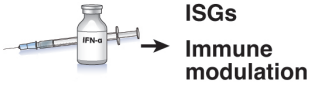


Engineered T-cells



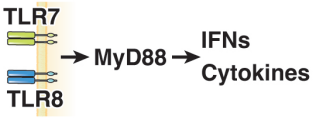
Innate immune modulation

IFN alpha



Toll-like receptor (TLR)

TLR7/8 agonists



Other intracellular pathways:

- Retinoic acid inducible gene-I (RIG-I)
- Stimulator of IFN gene (STING) agonists
- Lymphotoxin

Combinations

Nuc, immune modulation, vaccine

Supplementary Table 1. Available HBV vaccines for adults, dosing schedules and target populations for vaccination.

Vaccine	Dose	Schedule	Target Population
*Recombivax HB	Adults: 10 mcg HBsAg (1.0 mL) Predialysis and Dialysis Patient: 40 mcg (1.0 mL)	3 doses at 0, 1, 4-6 months	1. Sex partners of hepatitis B-positive persons 2. Sexually active persons who are not in a long-term, mutually monogamous relationship (e.g., persons with more than one sex partner during the previous 6 months) 3. Persons seeking evaluation or treatment for a sexually-transmitted disease 4. Men who have sex with men 5. Persons who inject drugs 6. Household contacts of hepatitis B-positive persons 7. Persons born in countries where hepatitis B infection is endemic should be tested and vaccinated if susceptible 8. International travelers to regions with high or intermediate rates of endemic hepatitis B infection 9. Health care and public safety workers that may be exposed to blood or blood-contaminated body fluids 10. Residents and staff of facilities for developmentally disabled persons, corrections facilities, and other facilities that serve adults at risk for hepatitis B infection 11. Persons with end-stage renal disease, including pre-dialysis, hemodialysis,
*Engerix-B	20 mcg HBsAg (1.0 mL)	3 doses at 0, 1, 4-6 months	
Heplisav-B	20 mcg of HBsAg and 3000 mcg of CpG 1018 adjuvant (0.5 mL)	2 doses at 0 and 1 month.	
PreHevbrio	10 mcg HBsAg (S, pre-S1 and pre-S2) (1.0 mL)	3 doses at 0, 1, 6 months	
Twinrix (Hepatitis B vaccine combined with Hepatitis A vaccine	Hep A as Havrix 720 El.U, Hep B as Engerix-B 20 mcg	3 or 4 doses 0, 1, 6 months or 0, day 7, day 21-30, 12 months	

	peritoneal dialysis, and home dialysis patients 12. Persons with chronic liver disease 13. Persons to age 60 years with diabetes 14. Persons with HIV infection 15. All other persons seeking protection from hepatitis B infection.

*There should be at least 4 weeks between doses 1 and 2, and at least 8 weeks between doses 2 and 3.

*The minimum interval for the overall series from dose 1 to final dose is 4 months (16 weeks).

Notes
Testing for immune response should be done 1-2 months after dose completion.

In the absence of an immune response to the vaccine doses, a repeat series is advised.

Hemodialysis patients are at risk for loss of immunity and should be tested annually for anti-HBS. In cases where anti-HBs levels fall <10 mIU/mL a booster dose of vaccine should be given.

Immunocompromised patients should receive a double vaccine dose

Supplementary Table 2. Available HBV vaccines for infants, children and adolescents and dosing schedules.

Vaccine	Dose	Schedule
Infants/children/adolescents		
*Engerix-B #Birth through 19 years	10 mcg HBsAg (0.5 mL)	3 doses at 0, 1, and 6 months
*Recombivax HB #Birth through 19 years Adolescents 11 through 15 years	5 mcg (0.5 mL) 10 mcg (1.0mL)	3 doses at 0, 1, and 6 months 2 doses at 0- and 4-6-months
Pediarix (Hepatitis B Vaccine combined with Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, and Inactivated Poliovirus Vaccine)	10 mcg HBsAg (0.5 mL)	3 doses at 2, 4, and 6 months of age
Vaxelis (Hepatitis B Vaccine combined with Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Hemophilus b Conjugate)	10 mcg HBsAg (0.5mL)	3 doses at 2, 4, and 6 months of age

*Engerix-B is approved for use in individuals of all ages.

*Recombivax HB is approved for use in individuals of all ages.

*Pediarix may be given as early as 6 weeks of age through 6 years of age (prior to the 7th birthday)

*Vaxelis is approved for use as a 3-dose series in children from 6 weeks through 4 years of age (prior to the 5th birthday)

#Birth dose should be administered within 24 hours of birth

HBsAg Hepatitis B surface antigen

Supplementary Table 3a. Performance characteristics of non-invasive tests for evaluation of advanced fibrosis* in CHB.

	Cut-off for detection of advanced fibrosis	AUROC	Sensitivity	Specificity	Positive predictive value	Negative predictive value
APRI[1, 2]	>0.5	0.68-0.87	70-84%	50-69%	52-64%	53-84%
FIB-4[2, 3]	>1.5	0.77	65-70.4%	70.2-73.6%	76.2%	43-81.4%
Transient Elastography[4]	8.8 kPa		59%	85%	58%	82%
Shear Wave Elastography[5, 6]	8.1kPa		94.9%	73.1%	77.0%[c]	97.4%[c]

*Advanced fibrosis defined as ≥F2 Metavir or ≥F3 Ishak fibrosis stage

[a] Overall estimation in a population with assumed 15.9% severe fibrosis and 16% cirrhosis due to either HBV, HCV and NAFLD.

Non-invasive tests perform better at excluding than establishing advanced fibrosis/cirrhosis.

Supplementary Table 3b. Performance characteristics of non-invasive tests for evaluation of cirrhosis in CHB.

	Cut-off for detection of advanced fibrosis	AUROC	Sensitivity	Specificity	Positive predictive value	Negative predictive value
APRI[1, 2]	>2.0	0.75	28-73%	70-87%	18-36%	82-97%
FIB-4[2, 7]	>3.25	0.75	16.2-17.4%	95.2-97.1%	44-62.2 %	46.8%
Transient Elastography[5, 6]	11 kPa		81%	83%	20%[a], 67%[b]	99%[a], 91%[b]
Shear Wave Elastography[5, 6]	11.5kPa		79.9%	93.3%	48.3%[c]	95.4%[c]

*Cirrhosis defined as F4 Metavir or F5-6 Ishak fibrosis stage

[a] In a population with assumed low prevalence (5%) of cirrhosis.

[b] In a population with assumed high prevalence (30%) of cirrhosis

[c] Overall estimation in a population with assumed 15.9% severe fibrosis and 16% cirrhosis due to either HBV, HCV and NAFLD.

Supplementary Table 4. Management of CHB in Special Populations

	Indication for treatment	Recommended treatment	Screening for HCC
Hepatitis C Virus (HCV)	Treat if HBsAg positive or if evidence of HBV reactivation Standard treatment indications for HBsAg positive persons apply	TDF, TAF or ETV	*Screening by US recommended every 6 months
Hepatitis D Virus (HDV) co-infection	Treat if cirrhotic, if active HBV replication and prior to treatment initiation for HDV	TDF, TAF or ETV	*Screening by US recommended every 6 months
Human immunodeficiency virus (HIV)	Treatment recommended for all patients using a regimen that includes 2 drugs active against HBV	TDF or TAF plus lamivudine or emtricitabine.	*Screening by US recommended every 6 months
Immunosuppressed patient	Treatment indicated for patients with moderate-high risk for reactivation (see text) or if evidence of reactivation present	TDF, TAF or ETV. Start 2-4 weeks prior to immunosuppression and continue until immune reconstitution occurs (moderate risk) or 12-18 months after stopping immunosuppression (high risk)	Consider screening based on individual risk
Decompensated Cirrhosis	Treatment indicated. Patients with MELD score ≥15 should also be referred for transplant evaluation	TDF, TAF or ETV. pegIFN contraindicated	*Screening by US recommended every 6 months
Acute HBV infection	Treatment indicated if features of acute liver failure manifest including INR > 1.5, total bilirubin > 3 mg/dL, encephalopathy, ascites	TDF, TAF or ETV. pegIFN contraindicated	No indication for screening among individuals who recover from acute infection
Post-liver transplant	Lifelong prophylactic treatment indicated for those	TDF, TAF or ETV. HBIG use should be	Consider screening based on individual

	transplanted for HBV-liver disease.	considered on individual basis	risk e.g. Cirrhosis in allograft
Post-non-liver solid organ transplant	Lifelong treatment indicated in patients who are HBsAg positive. Patients with evidence of past infection (HBsAg negative, anti-HBc positive) should be closely monitored and prophylactically treated during intense immunosuppression	TDF, TAF or ETV.	Consider screening based on individual risk
Children (2 to <18 years of age)	Treatment indications same as adults. Treatment indicated among HBeAg positive patients with elevated ALT and detectable HBV DNA with the goal of HBeAg seroconversion	PegIFN, TDF or ETV.	Consider screening based on individual risk (presence of advanced fibrosis and family history of HCC)

TDF, Tenofovir disoproxil fumarate; TAF, Tenofovir alafenamide; ETV, entecavir, pegIFN; pegylated interferon alfa-2a, HBIG, Hepatitis B Immune Globulin; HCC; Hepatocellular carcinoma; US, ultrasound, MELD, Model for End-stage Liver Disease.

*The AASLD guideline for HCC recommends US surveillance every 6 months for persons at high risk of HCC. There is insufficient evidence for or against the addition of AFP every 6 months to screening algorithms. AFP alone is not recommended except in those circumstances where US is unavailable or cost is an issue.

HBV Prevention

Vaccination of individuals at high risk for exposure including infants born to chronic carriers, and those at high risk for chronicity such as immunocompromised patients, is a critical component of infection control. Administration of birth dose vaccine which is critical for interrupting mother-to-infant transmission and for success of HBV elimination programs remains low. Globally less than 38% of babies born worldwide received the birth dose vaccine within 24 hours after birth.

Management of Controversial Patients (Immune tolerant, Inactive Carriers and Grey Zone/Indeterminant)

An alternate and more simplified approach to treatment being put forth by some experts, but not endorsed by any of the major liver society guidelines, is a “treat all” approach in which any HBsAg positive individual with detectable viremia regardless of ALT level would receive treatment. In the case of HBeAg positive patients with markedly elevated HBV DNA (>10^8 IU/mL) and normal ALT levels (immunetolerant or HBeAg positive chronic infection with no clear evidence of hepatocellular damage), the recommendation to treat is driven by a desire to limit the risk for HBV-specific T-cell depletion, DNA integrations that drive HCC risk, silent fibrosis progression, and risk of transmission. Indeed, this approach is supported by the Risk Evaluation of Viral Load Elevation and Associated Liver Disease (REVEAL) study[8, 9] which showed a relationship between elevated HBV DNA levels and subsequent development of cirrhosis and HCC and a Korean study that reported untreated immunetolerant patients had a 2-fold higher incidence of HCC, 1.05 vs 0.51 per 100 patient-years, and death/liver transplantation, 0.76 vs 0.32 per 100 patient-years, than nucleos(t)ide analogue treated patients in the immuneactive-phase.[10] In addition, a small retrospective study of untreated HBeAg positive patients with elevated HBV DNA and normal ALT levels who underwent liver biopsy noted significant histology, defined as ≥stage 2 fibrosis or stage 1 fibrosis plus ≥grade 2 inflammation, among older patients - 22% among 36-50 years and 45% among >50 years compared to none ≤35 years.[11] However, as data from the REVEAL study was obtained from an older, predominantly male, HBeAg negative cohort, caution is advised in extrapolating to a younger HBeAg positive cohort. Also, in the study from Korea, the HCC risk was lowest among patients with highest HBV DNA levels and normal ALT levels (true immunetolerant patients). Furthermore, there is currently no evidence that lowering viral load would necessarily reduce HCC incidence in patients with immunetolerant disease. Moreover, spontaneous HBeAg seroconversion occurs in a majority of patients with low rates of progression

to HBeAg negative immuneactive disease, cirrhosis or HCC.[12] Additionally, several studies demonstrate that achieving undetectable HBV DNA is challenging in this population. In one randomized study comparing TDF/placebo to TDF/emtricitabine for 192 weeks among 126 immunetolerant patients only 55% and 76%, respectively, were able to achieve HBV DNA <69 IU/ml and 5% HBeAg seroconversion.[13] In another trial of pegylated interferon plus ETV for 48 weeks, no patient achieved the primary endpoint of HBeAg loss and HBV DNA ≤ 1,000 IU/mL 48 weeks after end of treatment.[14] Finally integration cannot be prevented, and paired liver biopsy studies showed minimal if any fibrosis progression in patients with immunetolerant disease.[15] On balance, the data support current recommendations that true immunetolerant patients (HBV DNA $>10^8$ with normal ALT) have a good prognosis and do not require treatment, at least in the short term. Older patients ≥35 years should be evaluated for treatment on an individualized basis.

The argument for treating inactive carriers is less convincing and is based on small retrospective studies suggesting significant histological disease in a proportion of inactive carriers. In one study, among 97 patients with three normal ALT levels and HBV DNA <2,000 IU/ml, 19% were found to have ≥F2 stage fibrosis.[16] However, the patient's disease status before classification as an inactive carrier was unknown and it is possible that fatty liver disease may have contributed to the observed fibrosis as more than three-quarters of patients were overweight or obese. Inactive carriers may achieve high rates of HBsAg loss which favors treatment of this population. A non-randomized study of pegylated interferon with or without adefovir compared to no treatment among inactive carriers reported HBsAg loss after 96 weeks of therapy in 45% who received pegylated interferon, 38% in those who received combination therapy compared to 2% among untreated patients.[17] Despite these data the majority of studies report favorable clinical outcomes among inactive carriers. For example, among 3,673 HBeAg negative carriers with a normal ALT level, those who maintained a normal ALT level and who did not have hepatic steatosis, n=1,476, rates of cirrhosis, HCC and liver-related death were 0.9%, 0.1% and 0% after 13.4 years of follow-

up. Similarly, among 1,932 inactive carriers followed for a mean of 13.1 years in the REVEAL cohort, the annual incidence rates of HCC and liver-related death were 0.06% and 0.04%, respectively.[18] Whether, patients who developed outcomes had underlying cirrhosis or remained as inactive carriers during follow-up could not be determined. Given the low rate of complications, many patients who likely would not benefit from treatment, would have to be treated to prevent complications from developing. Therefore, we do not support therapy of inactive carriers unless there are other factors associated with poor outcome present such as elevated HBsAg levels. Inactive carriers do need continual monitoring as previously discussed.

There is more controversy whether indeterminate/grey zone patients should receive treatment. Some studies suggest high rates of HCC and other clinical outcomes among HBeAg negative patients with elevated HBV DNA and mildly elevated ALT levels or who do not meet criteria for treatment according to APASL, AASLD and EASL guidelines and that such patients may benefit from treatment.[19-21] A retrospective analysis of 3,366 predominantly Asian patients of whom 1,303 were in the indeterminate phase and followed for a mean of 12.5 years reported a significantly higher 10-year cumulative incidence of HCC among those who remained in the indeterminate phase compared to those who remained as inactive carriers, 4.6% versus 0.5%.[22] The HCC rate was notably higher in those older than 45 years. One concern with the results was the infrequent monitoring of patients. Over a mean follow-up of 12.5 years, the mean number of HBV DNA tests was 3.4 and ALT tests 8.1 with a mean time between ALT tests of 16 months. This long duration between monitoring meant that there could have been patients who transitioned to the active phase and should have been treated, who might have been missed. However, other studies from Europe and the U.S. report very low rates of HCC 0 to <1% over 4-8 years of follow-up. Interestingly, the TORCH study, comparing TDF to observation for patients with mild disease defined as HBV DNA >2,000 IU/mL and minimally raised serum ALT levels >1 to <2 x ULN reported that treated patients had a significantly lower rate of fibrosis progression (defined as an

increase in Ishak Fibrosis by >1 point) compared to no treatment, 26% versus 47%. However, this study included both HBeAg positive and negative patients and the role of treatment in preventing clinical outcomes such as HCC was not evaluated. Future studies are needed to address management of indeterminate patients. Until such results are available, we recommend a “case by case” approach, considering presence of risk factors for disease progression and HCC and patient’s willingness for treatment for cases outside the guideline treatment criteria.

References:

1. Jin W, Lin Z, Xin Y, et al. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis B-related fibrosis: a leading meta-analysis. BMC Gastroenterol 2012;12:14.
2. Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. Hepatology 2015;61:292-302.
3. Dong B, Lyu G, Chen Y, et al. Comparison of two-dimensional shear wave elastography, magnetic resonance elastography, and three serum markers for diagnosing fibrosis in patients with chronic hepatitis B: a meta-analysis. Expert Rev Gastroenterol Hepatol 2021;15:1077-1089.
4. Sporea I, Sirli R, Deleanu A, et al. Liver stiffness measurements in patients with HBV vs HCV chronic hepatitis: a comparative study. World J Gastroenterol 2010;16:4832-7.
5. Singh S, Muir AJ, Dieterich DT, et al. American Gastroenterological Association Institute Technical Review on the Role of Elastography in Chronic Liver Diseases. Gastroenterology 2017;152:1544-1577.
6. Herrmann E, de Ledinghen V, Cassinotto C, et al. Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: An individual patient data-based meta-analysis. Hepatology 2018;67:260-272.
7. Sonneveld MJ, Brouwer WP, Chan HL, et al. Optimisation of the use of APRI and FIB-4 to rule out cirrhosis in patients with chronic hepatitis B: results from the SONIC-B study. Lancet Gastroenterol Hepatol 2019;4:538-544.
8. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
9. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678-86.
10. Kim GA, Lim YS, Han S, et al. High risk of hepatocellular carcinoma and death in patients with immune-tolerant-phase chronic hepatitis B. Gut 2018;67:945-952.
11. Nguyen MH, Garcia RT, Trinh HN, et al. Histological disease in Asian-Americans with chronic hepatitis B, high hepatitis B virus DNA, and normal alanine aminotransferase levels. Am J Gastroenterol 2009;104:2206-13.
12. Chu CM, Hung SJ, Lin J, et al. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. Am J Med 2004;116:829-34.
13. Chan HL, Chan CK, Hui AJ, et al. Effects of tenofovir disoproxil fumarate in hepatitis B e antigen-positive patients with normal levels of alanine aminotransferase and high levels of hepatitis B virus DNA. Gastroenterology 2014;146:1240-8.
14. Feld JJ, Terrault NA, Lin HS, et al. Entecavir and Peginterferon Alfa-2a in Adults With Hepatitis B e Antigen-Positive Immune-Tolerant Chronic Hepatitis B Virus Infection. Hepatology 2019;69:2338-2348.
15. Hui CK, Leung N, Yuen ST, et al. Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. Hepatology 2007;46:395-401.
16. Abdo AA, Bzeizi KI, Babatin MA, et al. Predictors of significant fibrosis in chronic hepatitis B patients with low viremia. J Clin Gastroenterol 2014;48:e50-6.

17. Cao Z, Liu Y, Ma L, et al. A potent hepatitis B surface antigen response in subjects with inactive hepatitis B surface antigen carrier treated with pegylated-interferon alpha. Hepatology 2017;66:1058-1066.
18. Chen JD, Yang HI, Iloeje UH, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology 2010;138:1747-54.
19. Sinn DH, Kim SE, Kim BK, et al. The risk of hepatocellular carcinoma among chronic hepatitis B virus-infected patients outside current treatment criteria. J Viral Hepat 2019;26:1465-1472.
20. Choi GH, Kim GA, Choi J, et al. High risk of clinical events in untreated HBeAg-negative chronic hepatitis B patients with high viral load and no significant ALT elevation. Alimentary Pharmacology and Therapeutics 2019;50:215-226.
21. Hsu YC, Chen CY, Chang IW, et al. Once-daily tenofovir disoproxil fumarate in treatment-naive Taiwanese patients with chronic hepatitis B and minimally raised alanine aminotransferase (TORCH-B): a multicentre, double-blind, placebo-controlled, parallel-group, randomised trial. Lancet Infect Dis 2021;21:823-833.
22. Huang DQ, Li X, Le MH, et al. Natural History and Hepatocellular Carcinoma Risk in Untreated Chronic Hepatitis B Patients With Indeterminate Phase. Clin Gastroenterol Hepatol 2022;20:1803-1812 e5.