Dan L. Longo, M.D., *Editor*

New Approaches to Chronic Hepatitis B

Geoffrey Dusheiko, M.D., Kosh Agarwal, M.D., and Mala K. Maini, M.D., Ph.D.

From University College London (G.D.), Kings College Hospital London (G.D., K.A.), Kings College London (K.A.), and the Institute of Immunity and Transplantation, University College London (M.K.M.) — all in London. Dr. Dusheiko can be contacted at g.dusheiko@ucl.ac.uk or at Kings College Hospital, Denmark Hill, London SE5 NRS, United Kingdom.

This article was updated on January 5, 2023, at NEJM.org.

CHRONIC HEPATITIS B IS CAUSED BY THE HEPATITIS B VIRUS (HBV), A HEPA-totropic DNA virus that can replicate at high levels and cause minimal disease or severe liver injury. The clinical spectrum of chronic hepatitis B ranges from no symptoms to progressive hepatic fibrosis, advanced cirrhosis, and hepatocellular carcinoma. An estimated 296 million people have chronic hepatitis B, of whom 221 million live in low- and middle-income countries.[1] Without intervention, deaths from HBV are expected to peak at 1.14 million by 2035.[2]

VIROLOGIC FEATURES OF HBV

HBV is a partially double-stranded DNA virus. The life cycle and therapeutic targets are shown in Figures 1 and 2. The spherical 42-nm virion comprises an envelope that is outside a 30-nm nucleocapsid that encloses a circular 3.2-kb DNA genome. The lipid bilayer of the virion envelope incorporates the large (42-kD) hepatitis B virus surface protein (LHBs), the medium (33-kD) surface protein (MHBs), and the small (26-kD) surface protein (SHBs). The minus-strand HBV DNA genome is synthesized by reverse transcription of the 3.5-kb pregenomic RNA (pgRNA), after copackaging of the pgRNA and HBV polymerase inside the icosahedral nucleocapsids.

The RNA primer can remain bound to the direct repeat 1 region to prime linear double-stranded DNA synthesis.[3] Linear double-stranded integrants of the HBV DNA genome are integrated into the hepatocyte genome at sites of DNA breaks. The linear genomes do not circularize, and integrated HBV DNA is therefore unable to produce progeny virus. However, the randomly dispersed integrated HBV genomes act as a source of subgenomic RNA transcripts encoding LHBs, MHBs, SHBs, and possibly HBx. Integration could favor viral persistence, since the hepatitis B surface antigen (HBsAg) burden transcribed from integrated genomes may contribute to T-cell and B-cell exhaustion. Insertion into hepatocellular DNA may contribute to the pathogenesis of hepatocellular carcinoma by invoking a *cis*-mediated oncogenic mechanism, driving downstream cellular transcription, or through *trans*-mediated expression of viral proteins, particularly HBx.[4,5]

NATURAL HISTORY AND IMMUNOPATHOGENESIS OF HEPATITIS B

HBV generally causes a self-limiting illness in adults, with viral control mediated primarily by the adaptive immune response.[6] In contrast, more than 90% of cases of perinatal infection develop into chronic infection. The hierarchical contribution of causal pathways of chronicity and immunopathogenesis is poorly understood.[7] Persistent viral replication cannot be contained by the depleted and defective HBV-specific CD4 and CD8 T-cell response that results from the combination of high antigen-dose exhaustion and liver-tolerizing pathways, including inhibitory checkpoints (e.g., programmed death 1 [PD-1]), regulatory cells, and metabolic dysfunction.[8]
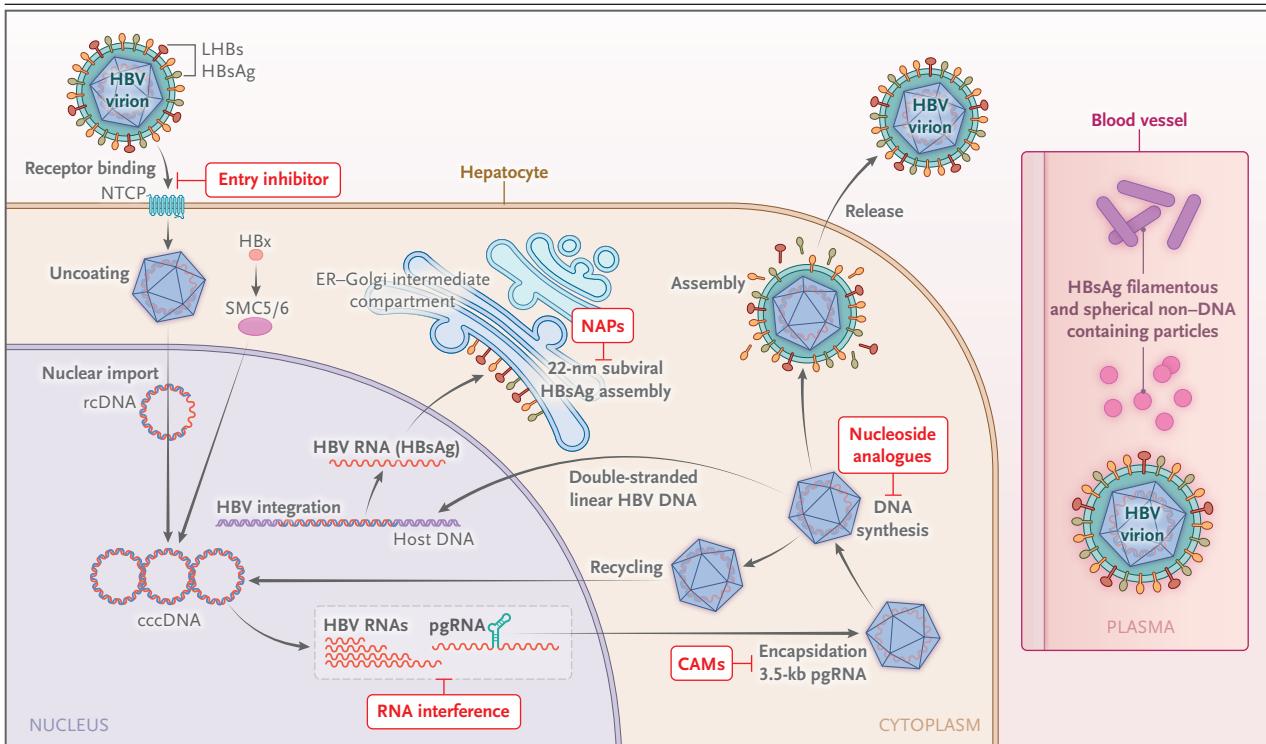
55

Figure 1. Hepatitis B Virus (HBV) Life Cycle.

The life cycle of HBV and the site of action of entry inhibitors, nucleoside analogues, RNA interference (RNAi) therapeutic agents, capsid assembly modulators (CAMs), and nucleic acid polymers (NAPs) are shown. The infectious virion attaches by binding of the pre-S1 domain of the large HBV surface protein (LHBs) to the hepatocyte entry receptor (NTCP [sodium–taurocholate cotransporting polypeptide]). The nucleocapsid is disassembled in the nucleus, releasing relaxed circular DNA (rcDNA). The rcDNA genome is repaired to form a covalently closed circular DNA (cccDNA) minichromosome. The pregenomic RNA (pgRNA) is packaged within the nucleocapsid and reversely transcribed to minus-strand HBV DNA to begin replication. The pgRNA is also enveloped and secreted. The viral capsids are enveloped with hepatitis B surface antigen (HBsAg) and released as virions. Capsids containing rcDNA shuttle back to the nucleus and replenish the cccDNA reservoir. Transcription from the cccDNA minichromosome is subject to epigenetic and HBx protein regulation. Double-stranded linear DNA HBV genomes can integrate into the hepatocyte genome at sites of DNA breaks. ER denotes endoplasmic reticulum, and SMC5/6 structural maintenance of chromosomes 5 and 6.

B-cell–depleting therapy with rituximab highlights the contribution of memory B cells to HBV control; antibodies against HBsAg are inadequately produced by dysfunctional HBsAg-specific B cells.[9-11]

Several disease phases are recognized on the basis of serum aminotransferase levels, hepatitis B e antigen (HBeAg) status, HBV DNA levels, and histologic characterization or noninvasive methods (Table 1). None of the current phase terminologies are fully consistent with the virologic or immunologic stages of the disease, but these definitions serve as indicators of clinical phenotypes and treatment.[12]

HBeAg-positive infection occurs predominantly in young persons. Near-normal aminotransfer-

ase levels despite high levels of viremia (>7 $\log_{10}$ IU per milliliter) reflect the noncytopathic nature of HBV. The absence of inflammation in this phase has been considered indicative of immunologic tolerance.[13] The observation that adolescents have some functional HBV-specific T cells provides evidence against central tolerance.[6,14] Discernible HBV integrants and clonal expansion of hepatocytes suggest progressive disease.[15]

HBeAg-positive disease is characterized by elevated HBV DNA levels (typically 5 to 7 $\log_{10}$ IU per milliliter) and abnormal aminotransferase levels, with exacerbations leading to necroinflammation and hepatic fibrosis. Spontaneous HBeAg seroconversion occurs in approximately 15% of cases per annum. Depending on the tim-

ing, the transition to HBeAg-negative, inactive infection reduces the risk of progression, but the disease necessitates longitudinal monitoring.[16] Conversely, HBeAg seroconversion can augur a change to HBeAg-negative disease, with mutations in the precore or basal core promoter down-regulating HBeAg, despite continued HBV replication.[17] HBsAg levels can remain high (>1000 IU per milliliter), which reflects expression predominantly from integrated HBV genomes.[18] Spontaneous seroclearance of HBsAg has been associated with improved clinical outcomes but is infrequent (accounting for an estimated 1 to 2% of cases per year).[19]

The annual incidence of hepatocellular carcinoma is low among patients without cirrhosis but is increased, to up to 10%, among those with cirrhosis.[20] Although there is a linear relationship between HBV DNA levels and the risks of cirrhosis and hepatocellular carcinoma among HBeAg-negative patients,[21] among HBeAg-positive patients, the immediate risk is lower for those with HBV DNA levels exceeding 8 $\log_{10}$ IU per milliliter than for those with HBV DNA levels of 5 to 7 $\log_{10}$ IU per milliliter.[22] In HBeAg-positive patients with active disease, the infection is likely to be transitioning from a low inflammatory–high replicative state to a different immunologic phenotype and phase. The transition is characterized by a decrease in HBV DNA levels and an increase in necroinflammatory damage, which lead to an increase in hepatocyte turnover (and to the gradual selection of HBeAg-negative variants).

DIAGNOSTIC MARKERS

HBsAg, HBeAg, anti-HBc (antibodies against hepatitis B core antigen [HBcAg]), and hepatitis B DNA are established markers of chronic hepatitis B (Table 1). Serologic assays for HBsAg detect virion and subviral particles in blood. Non–DNA-containing subviral HBsAg particles greatly outnumber virions.[23] SHBs antigen is the predominant component of 22-nm spherical particles; virion particles are enriched in LHBs antigen. The isoforms cannot be identified by current immunoassays, which target the common antigenic epitope within the "a" determinant of HBsAg protein. Neither can current HBsAg assays distinguish between HBsAg origi-

nating from covalently closed circular DNA (cccDNA) and HBsAg derived from integrated viral genomes.[18] HBV DNA levels are an indicator of HBV replication, used to identify the phase of chronic hepatitis B infection, determine indications for treatment, and assess the efficacy of antiviral therapy.

HBcAg induces both B-cell and T-cell responses; anti-HBc indicates exposure to hepatitis B. HBeAg is a secreted HBV protein, derived from the HBcAg reading frame with the use of the first of the two start codons, but is not required to maintain infection (Fig. 3). HBeAg in serum signals high levels of replication and infectivity, but since HBeAg expression is affected at the transcriptional, translational, and post-translational levels, it may not necessarily correlate with HBV DNA levels.

HBV has 10 listed genotypes and at least 24 subtypes. The evolution and distribution of HBV genotypes and subtypes can be traced to complex introductions into host populations, as well as to the movement of people in recent years.[24-26] HBV genotypes and subtypes influence clinical outcomes, including HBeAg and HBsAg seroconversion rates, mutational patterns in the precore and core promoter regions, the risks of cirrhosis and hepatocellular carcinoma, and the treatment response.[27]

QUANTITATIVE HBSAG

Low HBV DNA levels (<2000 IU per milliliter), plus low HBsAg levels (<1000 IU per milliliter) and normal serum aminotransferase levels, signify an improved outcome. Long-read sequences indicate that integrated HBV DNA becomes the predominant source of HBsAg in HBeAg-negative infection.[16] Higher HBsAg levels increase the risk of hepatocellular carcinoma among patients with low HBV DNA levels,[28] a finding that could reflect active transcription from accumulated HBV integrants and potentially influence carcinogenesis.

NEW MARKERS OF CCCDNA TRANSCRIPTION

Although several technologies for quantifying cccDNA in liver tissue are in development, no accepted quantitative standard is available.[29] Instead, two new serum markers, HBV RNA and HBV core-related antigen (HBcrAg), can be used to estimate transcription from cccDNA.[30]
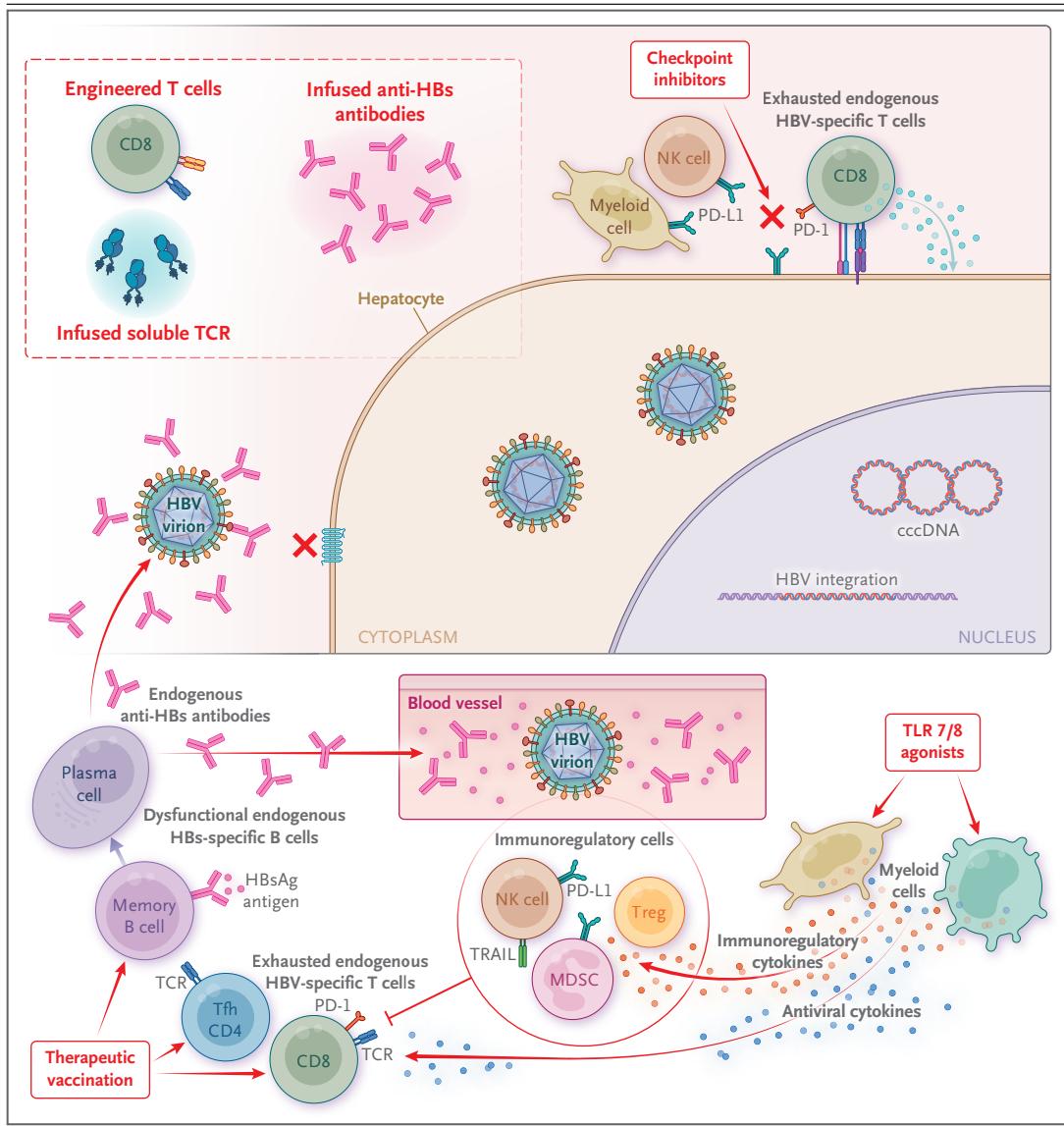
HBV RNA

During HBV replication, pgRNA is reversely transcribed to minus-strand DNA. However, a minority of pgRNA transcripts are not transcribed but are encapsidated, releasing HBV RNA–containing capsids into serum. Several methods are available for quantitating pgRNA[31]; two higher-throughput assays have been developed.[32,33]

Increased levels of HBV RNA are present in HBeAg-positive infection. HBV RNA correlates poorly with HBsAg levels in HBeAg-negative persons because of HBsAg derived from integrated genomes.[34] Since nucleoside analogues

have a minimal effect on cccDNA transcription to pgRNA, HBV RNA remains detectable for years during treatment.[35]

HBCRAG

HBcrAg measures a composite of HBeAg, HBcAg, and p22cr protein (a 22-kD truncated core protein). The components of HBcrAg thus originate exclusively from HBV cccDNA (Fig. 3).[36] Serum HBcrAg is measured by means of a chemiluminescent enzyme immunoassay (Lumipulse G system, Fujirebio) with an analytic range between 3.0 and 6.8 $\log_{10}$ U per milliliter. A more sensi-

Figure 2 (facing page). Potential Molecular and Immuno-therapeutic Targets.

Immunotherapies aim to revive endogenous, exhausted HBV-specific CD4 and CD8 T cells and dysfunctional HBsAg-specific memory B cells or replace them with exogenous supplies of specific effectors (dashed red box). Therapeutic vaccination should stimulate HBV-specific CD8 T cells (for cytolytic and cytokine-mediated HBV control), CD4 responses (including follicular helper T [Tfh] cells to support humoral immunity), and HBsAg-specific memory B cells to differentiate into plasma cells that are able to produce anti–hepatitis B surface (anti-HBs) antibodies. Anti-HBs antibodies can neutral-ize virions to block their entry but can also be absorbed by large quantities of circulating subviral particles of HBsAg. Endogenous HBV-specific T cells are constrained by checkpoints such as programmed death 1 (PD-1); the PD-1 ligand (PD-L1) is expressed by multiple cell types in the liver (including myeloid cells, liver-resident natural killer [NK] cells, and hepatocytes). Checkpoint inhibitors can release the breaks on endogenous immu-nity in combination with vaccines or toll-like receptor (TLR) agonists. Oral TLR7/8 agonists activate myeloid cells to produce immunomodulatory cytokines, which have antiviral potential (directly and through T cells and NK cells) but can also expand immunoregulatory cells (e.g., TRAIL [tumor necrosis factor–related apoptosis-inducing ligand] plus NK cells, PD-L1 plus myeloid-derived suppressor cells [MDSC], and regulatory T cells [Treg]) that constrain effectors. Infused soluble, affinity-matured, bispecific T-cell receptors (TCRs) harness local T cells to target infected hepatocytes. Adoptively trans-ferred TCR-engineered T cells are being tested for HBV-related hepatocellular carcinoma. Broadly neutralizing anti-HBs antibodies can be infused for virion and HBsAg neutralization and antibody-dependent cellular phago-cytosis and can be engineered to enhance dendritic-cell cross-presentation for a T-cell "vaccinal" effect.

tive assay, with a limit of detection of 2.1 $\log_{10}$ U per milliliter, has been developed.[37]

Although their clinical relevance is still be-ing defined, HBV RNA and HBcrAg identify active cccDNA transcription and have the po-tential both to identify patients at increased risk for progressive disease and to predict re-lapse after cessation of nucleoside analogue therapy.[35,38] These markers overcome the limita-tions of quantitative HBsAg (an inaccurate in-dicator of cccDNA in HBeAg-negative patients), HBV DNA, and HBeAg (a marker subject to genomic change). Both HBcrAg and HBV RNA could potentially be used to separate HBeAg-negative disease from less active infection, re-fining indications for treatment.

CURRENT ANTIVIRAL TREATMENT

INDICATIONS FOR TREATMENT

The major guidelines base treatment on serum aminotransferase and HBV DNA levels and on the severity of liver disease.[39-41] Consistency among guidelines is confounded by their complexity and differences in set points. Simplified guide-lines are required for the expansion of program-matic access to testing and treatment. Although several factors affect the outcome, prospective and retrospective studies have shown that an HBV DNA level of more than 2000 IU per milliliter is a strong predictor of cirrhosis and hepatocellu-lar carcinoma.[42] Patients with HBV DNA levels under 2000 IU per milliliter and normal amino-transferase levels are at lower risk for these conditions.[21] All guidelines recommend treat-ment for patients with cirrhosis and detectable HBV DNA.

The guidelines have not recommended treat-ment for "immunotolerant" HBeAg-positive pa-tients, because the immediate risk is low. Accu-mulating evidence suggests that new norms apply, since a higher risk of hepatocellular carci-noma has been reported among untreated im-munotolerant patients than among matched treated cohorts.[22] Treatment for all patients with HBV DNA levels exceeding 2000 IU per milliliter confers several benefits, including circumvent-ing the transition to active disease, reducing transcriptionally active integrations, and induc-ing a slow decline in the cccDNA pool[43] and hepatic clone size to prevent hepatocellular car-cinoma.[44] Treatment of young adults would also decrease sexual transmission, as well as vertical transmission from a mother to her infant. The benefit may be smaller for those at lower risk, but any negative effect of prolonged antiviral therapy could in the future be mitigated by fi-nite, curative treatment regimens.

NUCLEOSIDE ANALOGUES

Tenofovir, tenofovir alafenamide, and entecavir compete with the endogenous nucleotide sub-strate to bind the active site of the HBV poly-merase, disrupting the 5′ to 3′ phosphodiester linkage and terminating DNA chain elongation. Tenofovir alafenamide is a phosphoramidite pro-drug of tenofovir. Liver targeting of the conge-ner reduces systemic exposure.

Table 1. Phases of Hepatitis B Virus (HBV) Infection, Nomenclature, and Biomarkers.*

Variable	Phase					
	HBeAg-Positive Chronic HBV Infection	HBeAg-Positive Chronic Hepatitis B	HBeAg-Negative Chronic HBV Infection	HBeAg-Negative Chronic Hepatitis B	"Gray Zone"	Occult Hepatitis B
Other phase names	Immune tolerant	Immune (re)active	Inactive carrier state	HBeAg-negative disease	Indeterminate	None
Serologic testing						
HBsAg	Positive	Positive	Positive	Positive	Positive	Negative
Quantitative HBsAg ($\log_{10}$ IU/ml)†	3.5–4.5	3.5–4.5	2.5–3.5	2–3	2–3	Negative
HBeAg	Positive	Positive	Negative	Negative	Negative	Negative
HBe antibodies	Negative	Negative	Positive	Positive	Positive	May be positive
HBV DNA (IU/ml)	Typically >10^7	Typically >10^5 to 10^7	<10^3	Typically >10^3 to ≤10^5	2×10^3 ($3.3 \log_{10}$) to 2×10^4 ($4.3 \log_{10}$)	Low, at detection limit
Alanine aminotransferase	Near ULN	Elevated	Near ULN	Elevated	Fluctuates near ULN	Near ULN
Histologic features on liver biopsy	Minimal necroinflammation or fibrosis	Moderate-to-severe necroinflammation and varying degrees of fibrosis	Minimal necroinflammation and fibrosis	Moderate-to-severe necroinflammation or fibrosis	Minimal or low necroinflammation	Usually minimal or low necroinflammation; fibrosis can be present
cccDNA (assumed copy no./cell)‡	Relatively high	Relatively high	Relatively low, or transcriptional activity	Relatively low, or transcriptional activity	Relatively low, or transcriptional activity	Data uncertain
Integrated HBV DNA§	Present	Present	Present and accounts for majority of HBsAg	Present and accounts for majority of HBsAg	Present	Present
HBcrAg level	High	High	Low or undetected	Lower than HBeAg-positive states	May be detected	Data not available
HBV RNA level	High	High	Low or undetected	Lower than HBeAg-positive states	May be detected	Data not available

* The abbreviation cccDNA denotes covalently closed circular DNA, HBcrAg HBV core-related antigen, HBeAg hepatitis B e antigen, HBsAg hepatitis B surface antigen, and ULN upper limit of the normal range.
† Quantitative HBsAg levels are derived from baseline data in clinical trials of small interfering RNA. Ranges can vary significantly in HBeAg-negative patients, depending on the HBV genotype and HBsAg expression.
‡ The low number of infected cells in some HBeAg-negative patients, the low cccDNA copy number (1 to 10 per infected cell), and the lack of standards for quantitation allow only a qualitative assumption of cccDNA copy number.
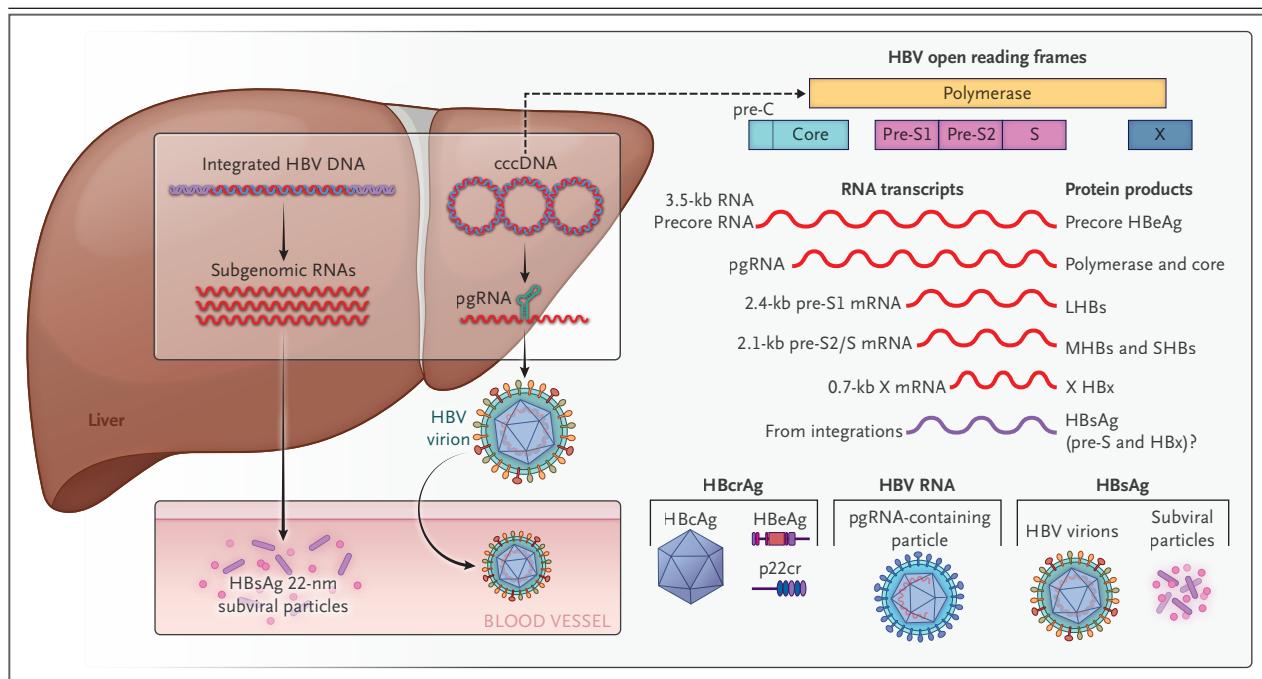§ The presence of integrated HBV DNA is usually assumed.

Figure 3. HBV Genome Open Reading Frames, RNA Transcripts, Protein Products, and Biomarkers.

The cccDNA serves as the template for transcription of pgRNA and subgenomic messenger RNAs (mRNAs) and encodes the large, medium, and small surface proteins, precore hepatitis B e antigen (HBeAg), and core, polymerase, and X proteins. The linear delineation of the four HBV open reading frames is shown (colored boxes), as are the dual sources of HBsAg from integrated HBV DNA and cccDNA. The mRNA transcripts derived from cccDNA and integrated viral genomes are also shown. The protein products are listed. Biomarkers in serum, including HBsAg, HBeAg, HBV RNA, and HBV core-related antigen (HBcrAg), are shown at the bottom of the figure. (Untranscribed pgRNA is also secreted.) The polymerase open reading frame encodes the large polymerase and core protein. Hepatitis B core antigen (HBcAg) and HBeAg are two translation products of the precore–core open reading frame that share homologous amino acid sequences but have different properties. The core proteins are assembled in the nucleocapsid. Translation from the upstream initiation codon and processing from the precore protein result in HBeAg, a soluble, dimeric protein that is secreted into serum. The first 19 aa of the extra 29 aa encoded by the precore region serve as the signal peptide directing the precore–core protein to the secretory pathway. A 2.4-kb pre-S1 mRNA transcript encodes the LHBs protein, a 2.1-kb pre-S2/S mRNA encodes the medium surface HBV (MHBs) protein and the small surface (SHBs) protein, and a 0.7-kb X mRNA encodes the X protein. The transcripts from cccDNA are shown with a common polyadenylation site. The S transcripts from HBV integrations indicate transcription from chromosomal DNA.

Nucleoside analogues are safe and effective.[45] Decreases in renal function and bone mineral density may occur with tenofovir, effects that are lessened in trials of tenofovir alafenamide.[46] Current guidelines suggest a change to entecavir or tenofovir alafenamide for patients who are older than 60 years of age or have renal dysfunction. Cholesterol and low-density lipoprotein levels increase after a switch to tenofovir alafenamide.[47] Weight gain has been reported.

The goals of treatment are to reduce HBV DNA to undetectable levels, normalize serum aminotransferase levels, and reduce inflammation and fibrosis. In addition, tenofovir is used to reduce viremia in pregnant women in order to limit mother-to-child transmission of HBV. Nucleoside analogues are used to treat fulminant hepatitis B and decompensated cirrhosis. They can be prescribed at appropriate doses in patients with renal failure and in children and adolescents. Other indications include prevention of HBV reactivation during immunosuppressive treatment. Prevention of a recurrence after liver transplantation by means of preemptive nucleoside analogue treatment is now the rule. Treatment complements HBV vaccination and safe sexual practices to reduce the risk of sexually transmitted HBV infection.

DISADVANTAGES AND LIMITATIONS OF NUCLEOSIDE ANALOGUES

Nucleoside analogues target a late stage in the viral life cycle. They act on the reverse-transcription step of formation of progeny virus but exert

no direct effect on pgRNA transcription and do not directly affect HBsAg expression from integrated genomes.[48] Thus, HBeAg loss is uncommon (occurring in 20 to 30% of patients after 1 to 2 years), and HBsAg loss is rare. The reported average incidence of HBsAg seroclearance is 0.22% per year, with a 10-year cumulative incidence of 2.11%,[49] but HBsAg loss, if it occurs, improves outcomes.[50]

Nucleoside analogues do not eliminate the risk of hepatocellular carcinoma. Several reports have suggested that the reduction in the risk of new or recurrent hepatocellular carcinoma is greater with tenofovir than with entecavir,[51,52] but these data may have been confounded by methodologic pitfalls.[53] Resistance to entecavir develops after 5 years in only 1% of previously untreated patients. Three substitutions must occur for phenotypic resistance to entecavir to develop: rtL180M (where rt denotes reverse transcriptase), T184L, and M204V. Although case reports have identified a quadruple mutation conferring tenofovir resistance,[54] computer analysis of full-length HBV sequences suggests that this mutation is rare.[55]

NUCLEOSIDE ANALOGUE WITHDRAWAL

Several guidelines suggest that nucleoside analogues may be discontinued in HBeAg-negative patients to maintain a low replicative state off treatment and to trigger HBsAg loss. However, the guidelines of the American Association for the Study of Liver Diseases suggest that HBsAg loss is the only acceptable end point for stopping nucleoside analogue treatment. Cirrhosis contraindicates discontinuation of treatment. Withdrawal of nucleoside analogues results in relapse in most patients; the relapse occurs earlier with tenofovir withdrawal than with entecavir withdrawal.[56] At cessation of treatment, HBsAg levels associated with HBsAg seroclearance are less than 100 IU per milliliter in Asian patients and less than 1000 IU per milliliter in White patients. Off-treatment HBsAg seroclearance occurs in 4 to 19% of HBeAg-negative patients.[57] Since discontinuation carries the risk of hepatic decompensation, the benefits and the disadvantages should be carefully weighed. Treatment should be reinstated before any rise in serum aminotransferase levels if a rapid increase in the HBV DNA level (to 3 to 4 $\log_{10}$ IU per milliliter) is detected.

HBV REACTIVATION

An array of treatments may induce HBV reactivation, including cancer chemotherapy, checkpoint inhibitor therapy, immunosuppressive therapy, bone marrow and stem-cell treatment, anti–tumor necrosis factor therapy, treatment with newer classes of immunobiologic agents (including tyrosine kinase inhibitors), chimeric antigen receptor T-cell treatment, and treatment of coexisting hepatitis C.[58] Rituximab treatment is particularly hazardous. Although the composite risk can be difficult to assess, preemptive use of an antiviral agent is recommended when the risk is considered to be high.

PEGYLATED INTERFERON ALFA

Interferon alfa, a pleotropic cytokine, inhibits transcription of pgRNA and subgenomic RNA from cccDNA. Activation of the lymphotoxin beta receptor by interferon alfa causes degradation of cccDNA by inducing cytidine deamination through up-regulation of APOBEC3 (apolipoprotein B messenger RNA–editing catalytic polypeptide-like 3) deaminases.[59] Interferon alfa is also an important immunoregulatory modulator: aminotransferase levels frequently increase during treatment in patients who have a response to the agent. A controlled trial of tenofovir plus pegylated interferon alfa-2a resulted in loss of HBsAg in 9% of patients — a higher percentage than with monotherapy.[60]
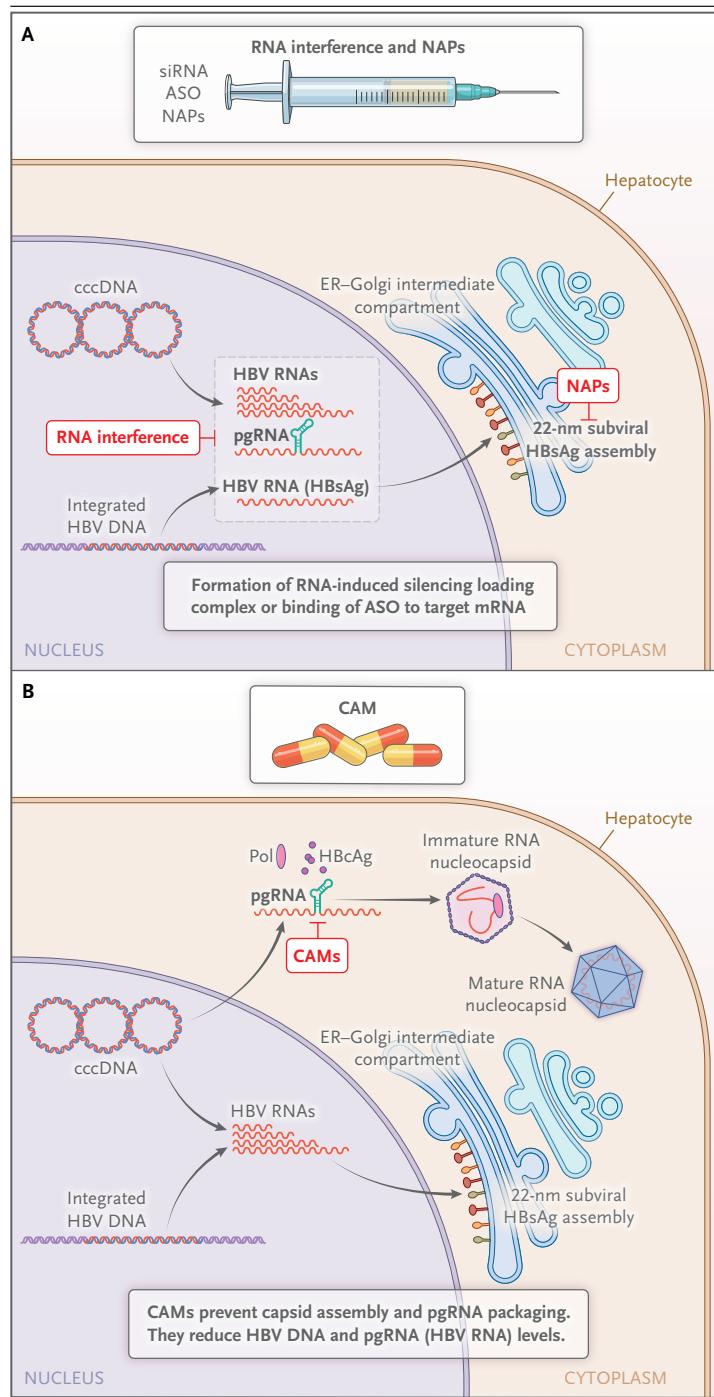
NEW INVESTIGATIONAL THERAPIES IN DEVELOPMENT

To reduce the need for lifelong treatment, a functional cure, defined as a sustained loss of HBsAg (undetectable at a level of <0.05 IU per milliliter) and undetectable HBV DNA after cessation of treatment, is sought. True cure of HBV infection requires eradication, degradation, or silencing of cccDNA; silencing of integrated viral genomes; and correction of antigen-specific immune dysfunction. The most advanced investigational treatments include entry inhibitors, RNA interference agents (small interfering RNA [siRNA]) and HBsAg assembly agents, capsid (core) assembly modulators (CAMs), and immunomodulatory approaches (Figs. 1, 2, and 4, Table 2, and Table S3).

Figure 4. Mechanisms of Action of RNA Interference Therapeutics, CAMs, and NAPs.

RNAi therapeutics (small interfering RNA [siRNA] and antisense oligonucleotides [ASOs]) suppress HBsAg from both integrated HBV DNA and cccDNA, without a direct effect on cccDNA or integrated viral DNA (Panel A). These agents target S and X and thus target transcripts of the HBV genome and subgenomic integrants, which leads to simultaneous inhibition of multiple viral genes due to overlapping mRNA transcripts and knockdown of all viral proteins and pgRNA expressed from cccDNA. Dose-dependent declines in HBsAg are observed, proving that RNAi agents target a hitherto inaccessible source of HBsAg. Liver levels and delivery have been established with newer conjugates (GalNAc). The siRNA has a longer duration of action than ASOs and requires less frequent dosing. Current compounds have minimal off-target effects. Serum aminotransferase elevations are more common with ASOs than with siRNA. Both agents show evidence of immune activation. NAPs bind to a putative chaperone to inhibit assembly of 22-nm subviral particles derived from integrated HBV DNA genomes within the ER–Golgi intermediate compartment and may induce a cytolytic response, resulting in frequent serum alanine aminotransferase elevations. CAMs impair encapsidation of pgRNA and at higher doses may inhibit the formation of new cccDNA. CAM-A results in aberrant capsid formation. CAM-E results in normal but empty capsids. An additive effect with nucleoside analogues causes substantial reductions in HBV DNA and pgRNA (HBV RNA) levels. The most clinically significant effect is seen in HBeAg-positive patients who have not received nucleoside analogues. A smaller reduction in HBV RNA has been observed in patients whose pgRNA levels are low at baseline; these patients include HBeAg-positive patients who were treated with nucleoside analogues and HBeAg-negative patients who either received or did not receive nucleoside analogue treatment. Maintenance treatment with CAMs requires combined treatment with nucleoside analogues, since amino acid substitutions in the binding pocket confer reduced sensitivity. Rebound replication occurs after cessation of treatment. Interference with efficacy has been observed in studies of treatment combined with an siRNA. Other potential toxic effects and drug–drug interactions have been observed.

ENTRY INHIBITORS

Bulevirtide (Hepcludex, Gilead), a synthetic pre-S1 lipopeptide, binds to and inactivates the sodium taurocholate cotransporting polypeptide, inhibiting entry of HBV (and hepatitis D virus). A reduction in HBsAg has not been observed with monotherapy, and the role of bulevirtide in the treatment of chronic hepatitis B is undefined. Anti-HBV pre-S1 monoclonal antibodies neutralize HBV infection by blocking attachment of HBV to hepatocytes, as well as binding subviral particles to reduce circulating HBsAg. The VIR-3434 antibody also has an Fc region engineered to induce a vaccinal effect[61] (Table S3 in

Table 2. New Anti-HBV Compounds in Clinical Development.

Type and Compound	Originator	Phase*
Capsid assembly modulators		
Vebicorvir (ABI-H0731)	Assembly Biosciences	Phase 2 terminated
ABI-H3733		Phase 1
ABI-4334		Preclinical studies
Morphothiadin (GLS4)	HEC Pharma	Phase 2
JNJ-6379	Janssen	Phase 2b
EDP-514	Enanta	Phase 2
RG7907	Roche	Phase 2
QL-007	Qilu	Phase 1
Canocapavir	Zhimeng Biopharma	Not available
ALG-000184	Aligos Therapeutics	Phase 1
AB-836	Arbutus	Phase 1 and 2
VNRX-9945	Venatorx	Phase 1 terminated
O7049839	Roche	Phase 1
siRNA agents†		
RG6346 (RO7445482)	Roche/Dicerna	Phase 2
JNJ-3989	Janssen	Phase 2b
AB7-29–001	Arbutus	Phase 2
VIR-2218	Vir Biotechnology	Phase 2
ALG-125755	Aligos Therapeutics	Phase 1
Antisense oligonucleotides		
Bepirovirsen (GSK3228836)	GSK	Phase 2
RO7062931	Roche	Phase 1
ALG-020572–401	Aligos Therapeutics	Phase 1
Nucleic acid polymers: REP 2139, REP 2165	Replicor	Phase 2
Active-site polymerase inhibitor: ATI-2173	Antios	Phase 2
Entry inhibitor: bulevirtide	Gilead	Phase 3 (hepatitis D)
Transcriptional inhibitor: nitazoxanide	Romark Laboratories/Lupin	Phase 2

* The information on study status is from ClinicalTrials.gov, accessed December 4, 2022.
† The abbreviation siRNA denotes small interfering RNA.

the Supplementary Appendix, available with the full text of this article at NEJM.org).

NUCLEIC ACID POLYMERS

Nucleic acid polymers are amphipathic phosphorothioate oligonucleotides (Figs. 1 and 4). The target for REP 2139 (Replicor) has been puta-tively identified as the HSP40 (heat-shock protein, 40 kD) chaperone DNAJB12 and thus inhibits trafficking of 22-nm HBsAg subviral particles. The efficacy of REP 2139 has been tested in combination with tenofovir and pegylated interferon. After 48 weeks of follow-up, HBsAg was undetectable in 14 of 40 study participants. It remains to be proved that alanine aminotransferase flares during treatment are indicative of immune reconstitution.[62]

CAPSID ASSEMBLY MODULATORS

Several classes of oral, small-molecule CAMs have been developed that interfere with capsid assembly through allosteric binding to the core dimer and thereby misdirect capsid formation and disrupt encapsidation of pgRNA[63] (Figs. 1 and 2 and Table 2). These agents result in decreases in serum HBV DNA levels (up to 4 $\log_{10}$ IU per milliliter) and HBV RNA levels (up to 3 $\log_{10}$ copies per milliliter) within 28 days. Declines in HBsAg levels are negligible, since CAMs do not reduce established cccDNA levels or transcription from cccDNA and have no direct effect on HBsAg derived from integrated HBV genomes. HBV RNA and HBcrAg rebound after discontinuation of treatment, underscoring the inability of CAMs to eradicate the pool of cccDNA. The place of CAMs in curative strategies remains uncertain.

RNA INTERFERENCE

RNA-interfering agents, including siRNA and antisense oligonucleotides (ASOs) exploit a complementary sequence of the target RNA to trigger specific RNA degradation and perturb translation (Figs. 1 and 4 and Table 2). Current RNA-interfering agents, targeted to X or S RNA, are designed to silence all HBV transcripts derived from cccDNA or integrated genomes.[64]

SIRNA

The siRNA sense strands bind to the complementary target messenger RNA (mRNA); the duplexes load to the RNA-induced silencing complex and lead to mRNA cleavage. Lipid nanoparticle delivery and conjugation to N-acetylgalactosamine facilitate hepatocyte uptake. The siRNAs have a longer duration of action than ASOs. In phase 1 and 2 trials, 60 to 75% of patients treated with siRNA had a reduction in HBsAg of more than 2.0 $\log_{10}$ IU per milliliter and HBsAg

levels of less than 100 IU per milliliter within 24 to 48 weeks, with a relatively slow rebound after treatment.[65]

The results of the first large phase 2b clinical trials of the combination of an siRNA and CAM have recently been reported. The six-group REEF-1 study, involving 470 patients, investigated the efficacy of JNJ-3989 (an siRNA), injected every 4 weeks, plus the CAM JNJ-6379, plus a nucleoside, given for 48 weeks. In the group treated with 200 mg of JNJ-3989 alone, 74% of patients had an HBsAg level of less than 100 IU per milliliter, but no patients had a functional cure at week 24 of follow-up. An unexplained antagonistic effect of the CAM combined with the siRNA occurred.[66]

REEF-2 assessed the efficacy of 48 weeks of treatment with the combination of JNJ-3989 (200 mg every 4 weeks), JNJ-6379 (250 mg daily), and a nucleoside analogue, as compared with a nucleoside analogue alone, in 140 HBeAg-negative patients. Although 71% of the patients receiving combination therapy had an HBsAg level of less than 100 IU per milliliter at week 48 of treatment (which was reduced to 46% at 48 weeks after cessation of treatment), no patients had HBsAg seroclearance at week 24 of follow-up without restarting nucleoside analogue treatment.[67] A participant in the control group required liver transplantation for hepatic decompensation after withdrawal of the nucleoside analogue, underscoring the need for stringent criteria to enable cessation of nucleoside analogue treatment.[68]

ASOS

ASOs are synthetic, single-stranded oligonucleotides with various biochemical characteristics. ASOs bind to complementary HBV RNA transcripts to form a hybrid ASO-RNA complex, resulting in cleavage by ribonuclease H. Serum aminotransferase levels can increase after the HBsAg level has reached a nadir.[69] In the recently reported phase 2b B-Clear study of bepirovirsen involving participants with chronic HBV infection, 9% of participants who received 300 mg of bepirovirsen subcutaneously once weekly for 24 weeks plus a nucleoside analogue and 10% who received bepirovirsen alone reached the primary end point of an undetectable HBsAg level (<0.05 IU per milliliter) and an HBV DNA level below 20 IU per milliliter 24 weeks after discontinuing bepirovirsen. Among participants with a low baseline HBsAg level of no more than 3 $\log_{10}$ IU per milliliter who did not receive nucleoside analogue treatment, 25% reached the primary end point, as compared with 16% of those who received nucleoside analogue treatment. HBsAg levels declined to less than 100 IU per milliliter at the end of treatment in 63% of those who received 300 mg of bepirovirsen plus a nucleoside analogue and in 59% of those who did not receive a nucleoside analogue.[70]

The dose-dependent decline in HBsAg during treatment and the prolonged effect with siRNA and ASOs are encouraging findings, and even though the end points for a functional cure have not been met to date, these data indicate that an HBsAg reduction to less than 100 IU per milliliter can be achieved in the majority of patients. Given these results, a partial functional cure is being posited, defined as a decline in HBsAg to less than 100 IU per milliliter 6 months after finite treatment, resulting in a lower replicative state in HBsAg-positive patients and possibly immune activation or restoration. A summary of the efficacy of current trials is presented in Table S1.

PRECLINICAL RESEARCH APPROACHES

There are several preclinical research approaches. These include X gene targeting, cccDNA or RNA destabilization or cccDNA reduction, host targets or targeting by CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and associated Cas9 homing endonucleases) to base edit cccDNA.

IMMUNOMODULATORY THERAPY

Early data suggest that new direct antiviral agents alone are insufficient to restore effective immunologic control. Therefore, immunomodulatory treatments to restore and replenish exhausted, sparse, or dysfunctional HBV-specific T-cell and B-cell responses by activating or replacing endogenous immunity are being researched (Fig. 2 and Table S3).

Pegylated interferon alfa is being added to siRNA and nucleic acid polymers in current clinical trials. Several oral selective toll-like receptor agonists are being tested, but promising results in animal models have not been confirmed in clinical studies to date.[71]

Antibody or small-molecule checkpoint inhibitors against the PD-1 axis (used in cancer treatment to overcome T-cell exhaustion) have had limited initial effects, which may be augmented after HBsAg reduction or in combination with more immunogenic therapeutic vaccines to prime multispecific T-cell and B-cell responses. Several new therapeutic vaccines are in development (Table S3) for testing in combination after siRNA knockdown of HBsAg.[72]

Replacement of failed immunity with HBV-specific mediators such as bispecific soluble T-cell receptors or Fc-engineered monoclonal antibodies is also being tested in clinical trials and adoptive cell therapy (in HBV-related hepatocellular carcinoma).[61] The progressive immunologic impairment, with prolonged antigen exposure to high HBsAg titers and aging, supports the introduction of immunotherapy for chronic hepatitis B at an earlier stage of disease.[14,73] Immunomodulatory strategies carry the risk of on-target and off-target toxic effects, including immune-mediated liver injury.

The combinations of new antiviral agents and immunomodulatory strategies that will achieve a functional cure may differ for different phases of hepatitis B. Further empirical evidence from clinical trials will be required to identify such combinations.

PREVENTION AND CONTROL

PROPHYLACTIC VACCINATION

The inclusion of HBV vaccine in the expanded program of immunization has decreased the prevalence of hepatitis B in children under the age of 5 years and has reduced incident cases of chronic hepatitis B and hepatocellular carcinoma.[74] Despite advocacy by the World Health Organization (WHO), however, global birth-dose vaccination (i.e., vaccination within 24 hours after birth) coverage is unsatisfactory. Also, vaccine protection may fail in more than 10% of infants born to mothers with a high viremic load ($>2 \times 10^5$ IU of HBV DNA per milliliter). Current HBV A2 (serotype adw) vaccines may not completely prevent transmission from mothers infected with, for example, genotype E (serotype ayw4) in West Africa, which may explain residual mother-to-child transmission despite birth-dose vaccination.[75] The rationale for nucleoside

analogue prophylaxis in the third trimester to prevent peripartum infection is recognized, but this approach necessitates testing during pregnancy for HBsAg and HBV DNA.

SCREENING AND PRIMARY PREVENTION

To decrease incident acute hepatitis B, the U.S. Advisory Committee on Immunization Practices has recommended universal hepatitis B vaccination for adults between the ages of 19 and 59 years and has liberalized the recommendation for vaccination of adults who are 60 years of age or older. The committee has proposed universal one-time hepatitis B screening for all adults (≥18 years).

The WHO recommends focused testing for most affected populations (people who inject drugs, men who have sex with men, sex workers, people living with human immunodeficiency virus [HIV], health care workers, migrants from regions where hepatitis B is endemic, and children of HBV-positive mothers), for persons in whom there is a clinical suspicion of chronic viral hepatitis, and for family members or sexual partners of affected persons. General population testing is recommended in areas where the prevalence of HBsAg exceeds 2%. Unfortunately, these programmatic policies have not been implemented in many regions where hepatitis B is endemic and where current interventions will be insufficient to reach 2030 elimination goals.

CONCLUSIONS

Prophylactic vaccination alone will not alleviate the burden of hepatitis B. HBV continues to be a widespread cause of death because of inadequate screening, testing, and treatment, as well as late presentation — a major global public health failing. It is critical for agencies to advance affordable diagnostics in order to identify pregnant women and other persons who qualify for treatment. The cost of generic antiviral agents is not a barrier. Lack of community access and referral presents a greater obstacle than the low cost of drugs.

Innovative curative strategies could reshape the treatment of chronic hepatitis B. Maintaining momentum in developing such strategies will require continued investment and patient involvement, as well as a candid realization that

the search for a cure for chronic hepatitis B presents myriad challenges.

Current levels of diagnosis and treatment of hepatitis B are inadequate. Approximately 10% of people living with hepatitis B knew their status in 2019. Of this group, only about 6.6 million people with a diagnosis of hepatitis B received treatment in 2019. The WHO African region leads the way in HIV testing and treatment, but by the end of 2019, hepatitis B had been diagnosed in only 2% of the 82 million persons living with hepatitis B in this region and only 0.1% of the currently infected persons had received treatment. While we look beyond current treatments, we should not lose sight of the cardinal responsibility to improve awareness and access to treatment of hepatitis B with existing antiviral therapies.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES

1. Sheena BS, Hiebert L, Han H, et al. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Gastroenterol Hepatol 2022; 7:796-829.

2. Nayagam S, Thursz M, Sicuri E, et al. Requirements for global elimination of hepatitis B: a modelling study. Lancet Infect Dis 2016;16:1399-408.

3. Tu T, Zhang H. Viral integrations in chronic hepatitis B infection: purposeless passenger or problematic promoter of persistence? Hepatology 2022;76:15-7.

4. Tu T, Zhang H, Urban S. Hepatitis B virus DNA integration: in vitro models for investigating viral pathogenesis and persistence. Viruses 2021;13:180.

5. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7:6.

6. Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. Nat Rev Immunol 2022; 22:19-32.

7. Heim K, Neumann-Haefelin C, Thimme R, Hofmann M. Heterogeneity of HBV-specific CD8+ T-cell failure: implications for immunotherapy. Front Immunol 2019;10:2240.

8. Barili V, Vecchi A, Rossi M, et al. Unraveling the multifaceted nature of CD8 T cell exhaustion provides the molecular basis for therapeutic T cell reconstitution in chronic hepatitis B and C. Cells 2021; 10:2563.

9. Burton AR, Pallett LJ, McCoy LE, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. J Clin Invest 2018;128:4588-603.

10. Salimzadeh L, Le Bert N, Dutertre C-A, et al. PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. J Clin Invest 2018;128:4573-87.

11. Neumann-Haefelin C, Thimme R. Entering the spotlight: hepatitis B surface antigen-specific B cells. J Clin Invest 2018; 128:4257-9.

12. Cornberg M, Lok AS, Terrault NA, Zoulim F, 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B — report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference. J Hepatol 2020;72:539-57.

13. Milich DR. The concept of immune tolerance in chronic hepatitis B virus infection is alive and well. Gastroenterology 2016;151:801-4.

14. Kennedy PTF, Sandalova E, Jo J, et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143: 637-45.

15. Mason WS, Gill US, Litwin S, et al. HBV DNA integration and clonal hepatocyte expansion in chronic hepatitis B patients considered immune tolerant. Gastroenterology 2016;151(5):986-998.e4.

16. Suslov A, Meier M-A, Ketterer S, Wang X, Wieland S, Heim MH. Transition to HBeAg-negative chronic hepatitis B virus infection is associated with reduced cccDNA transcriptional activity. J Hepatol 2021;74:794-800.

17. Podlaha O, Gane E, Brunetto M, et al. Large-scale viral genome analysis identifies novel clinical associations between hepatitis B virus and chronically infected patients. Sci Rep 2019;9:10529.

18. Meier M-A, Calabrese D, Suslov A, Terracciano LM, Heim MH, Wieland S. Ubiquitous expression of HBsAg from integrated HBV DNA in patients with low viral load. J Hepatol 2021;75:840-7.

19. Yeo YH, Ho HJ, Yang HI, 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.e9.

20. Papatheodoridis GV, Sypsa V, Dalekos GN, et al. Hepatocellular carcinoma prediction beyond year 5 of oral therapy in a large cohort of Caucasian patients with chronic hepatitis B. J Hepatol 2020;72: 1088-96.

21. 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.

22. Choi GH, Kim G-A, Choi J, Han S, Lim Y-S. High risk of clinical events in untreated HBeAg-negative chronic hepatitis B patients with high viral load and no significant ALT elevation. Aliment Pharmacol Ther 2019;50:215-26.

23. Vaillant A. HBsAg, subviral particles, and their clearance in establishing a functional cure of chronic hepatitis B virus infection. ACS Infect Dis 2021;7:1351-68.

24. Lago BV, Mello FC, Kramvis A, Niel C, Gomes SA. Hepatitis B virus subgenotype A1: evolutionary relationships between Brazilian, African and Asian isolates. PLoS One 2014;9(8):e105317.

25. Kramvis A, Paraskevis D. Subgenotype A1 of HBV–tracing human migrations in and out of Africa. Antivir Ther 2013;18:513-21.

26. Glebe D, Goldmann N, Lauber C, Seitz S. HBV evolution and genetic variability: impact on prevention, treatment and development of antivirals. Antiviral Res 2021;186:104973.

27. Revill PA, Tu T, Netter HJ, Yuen LKW, Locarnini SA, Littlejohn M. The evolution and clinical impact of hepatitis B virus genome diversity. Nat Rev Gastroenterol Hepatol 2020;17:618-34.

28. Tseng T-C, Liu C-J, Yang H-C, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. Hepatology 2013;57:441-50.

29. Zhang H, Tu T. Approaches to quantifying hepatitis B virus covalently closed circular DNA. Clin Mol Hepatol 2022;28: 135-49.

30. Ghany MG, King WC, Lisker-Melman M, et al. Comparison of novel biomarkers with conventional HBV markers among untreated adults with chronic hepatitis B in North America. Hepatology 2021;74: 2395-409.

31. Kramvis A, Chang KM, Dandri M, et al. A roadmap for serum biomarkers for hepatitis B virus: current status and future outlook. Nat Rev Gastroenterol Hepatol 2022;19:727-45.

32. Butler EK, Gersch J, McNamara A, et al. Hepatitis B virus serum DNA and RNA

67

levels in nucleos(t)ide analog-treated or untreated patients during chronic and acute infection. Hepatology 2018;68:2106-17.

33. Scholtès C, Hamilton AT, Plissonnier M-L, et al. Performance of the cobas HBV RNA automated investigational assay for the detection and quantification of circulating HBV RNA in chronic HBV patients. J Clin Virol 2022;150-151:105150.

34. Giersch K, Allweiss L, Volz T, Dandri M, Lütgehetmann M. Serum HBV pgRNA as a clinical marker for cccDNA activity. J Hepatol 2017;66:460-2.

35. Carey I, Gersch J, Wang B, et al. Pregenomic HBV RNA and hepatitis B core-related antigen predict outcomes in hepatitis B e antigen-negative chronic hepatitis B patients suppressed on nucleos(t)ide analogue therapy. Hepatology 2020;72:42-57.

36. Mak L-Y, Wong DK-H, Cheung K-S, Seto W-K, Lai C-L, Yuen M-F. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. Aliment Pharmacol Ther 2018;47:43-54.

37. Inoue T, Kusumoto S, Iio E, et al. Clinical efficacy of a novel, high-sensitivity HBcrAg assay in the management of chronic hepatitis B and HBV reactivation. J Hepatol 2021;75:302-10.

38. Lok J, Dusheiko G, Carey I, Agarwal K. Review article: novel biomarkers in hepatitis B infection. Aliment Pharmacol Ther 2022;56:760-76.

39. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560-99.

40. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1-98.

41. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370-98.

42. Lee MH, Yang HI, Liu J, et al. Prediction models of long-term cirrhosis and hepatocellular carcinoma risk in chronic hepatitis B patients: risk scores integrating host and virus profiles. Hepatology 2013;58:546-54.

43. Chow N, Wong D, Lai C-L, et al. Effect of antiviral treatment on hepatitis B virus integration and hepatocyte clonal expansion. Clin Infect Dis 2022 May 20 (Epub ahead of print).

44. Hsu YC, Suri V, Nguyen MH, et al. Inhibition of viral replication reduces transcriptionally active distinct hepatitis B virus integrations with implications on host gene dysregulation. Gastroenterology 2022; 162(4):1160-1170.e1.

45. Wong GL-H, Tse Y-K, Wong VW-S, Yip TC-F, Tsoi KK-F, Chan HL-Y. Long-term

safety of oral nucleos(t)ide analogs for patients with chronic hepatitis B: a cohort study of 53,500 subjects. Hepatology 2015; 62:684-93.

46. Agarwal K, Brunetto M, Seto WK, et al. 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. J Hepatol 2018;68:672-81.

47. Suzuki K, Suda G, Yamamoto Y, et al. Effect of switching from tenofovir disoproxil fumarate to tenofovir alafenamide on lipid profiles in patients with hepatitis B. PLoS One 2022;17(1):e0261760.

48. Dandri M, Petersen J. cccDNA maintenance in chronic hepatitis B — targeting the matrix of viral replication. Infect Drug Resist 2020;13:3873-86.

49. Hsu Y-C, Yeh M-L, Wong GL-H, et al. Incidences and determinants of functional cure during entecavir or tenofovir disoproxil fumarate for chronic hepatitis B. J Infect Dis 2021;224:1890-9.

50. Yip TC, Wong GL, Chan HL, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol 2019;70:361-70.

51. Yuan B-H, Li R-H, Huo RR, Li M-J, Papatheodoridis G, Zhong J-H. Lower risk of hepatocellular carcinoma with tenofovir than entecavir treatment in subsets of chronic hepatitis B patients: an updated meta-analysis. J Gastroenterol Hepatol 2022;37:782-94.

52. Kim WR, Telep LE, Jump B, et al. Risk of hepatocellular carcinoma in treatment-naïve chronic hepatitis B patients receiving tenofovir disoproxil fumarate versus entecavir in the United States. Aliment Pharmacol Ther 2022;55:828-35.

53. Choi W-M, Yip TC-F, Lim Y-S, Wong GL-H, Kim WR. Methodological challenges of performing meta-analyses to compare the risk of hepatocellular carcinoma between chronic hepatitis B treatments. J Hepatol 2022;76:186-94.

54. Park E-S, Lee AR, Kim DH, et al. Identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients. J Hepatol 2019;70:1093-102.

55. Velkov S, Protzer U, Michler T. Global occurrence of clinically relevant hepatitis B virus variants as found by analysis of publicly available sequencing data. Viruses 2020;12:1344.

56. Sonneveld MJ, Chiu S-M, Park JY, et al. Probability of HBsAg loss after nucleo(s) tide analogue withdrawal depends on HBV genotype and viral antigen levels. J Hepatol 2022;76:1042-50.

57. Berg T, Simon K-G, Mauss S, et al. Long-term response after stopping tenofovir disoproxil fumarate in non-cirrhotic HBeAg-negative patients — FINITE study. J Hepatol 2017;67:918-24.

58. Papatheodoridis GV, Lekakis V, Voulgaris T, et al. Hepatitis B virus reactivation

associated with new classes of immunosuppressants and immunomodulators: a systematic review, meta-analysis, and expert opinion. J Hepatol 2022;77:1670-89.

59. Lucifora J, Xia Y, Reisinger F, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 2014;343:1221-8.

60. Marcellin P, Ahn SH, Ma X, et al. Combination of tenofovir disoproxil fumarate and peginterferon α-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B. Gastroenterology 2016;150(1):134-144.e10.

61. Maini MK, Burton AR. Restoring, releasing or replacing adaptive immunity in chronic hepatitis B. Nat Rev Gastroenterol Hepatol 2019;16:662-75.

62. Bazinet M, Pântea V, Placinta G, et al. Safety ad 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; 158:2180-94.

63. Taverniti V, Ligat G, Debing Y, Kum DB, Baumert TF, Verrier ER. Capsid assembly modulators as antiviral agents against HBV: molecular mechanisms and clinical perspectives. J Clin Med 2022;11: 1349.

64. Hui RW-H, Mak L-Y, Seto W-K, Yuen M-F. RNA interference as a novel treatment strategy for chronic hepatitis B infection. Clin Mol Hepatol 2022;28:408-24.

65. Yuen M-F, Schiefke I, Yoon J-H, et al. RNA interference therapy with ARC-520 results in prolonged hepatitis B surface antigen response in patients with chronic hepatitis B infection. Hepatology 2020;72: 19-31.

66. Yuen M-F, Asselah T, Jacobson IM, et al. Effects of the siRNA JNJ-3989 and/or the capsid assembly modulator JNJ-6379 on viral markers of chronic hepatitis B: results from the REEF-1 study. In: Proceedings and Abstracts of the 2022 International Liver Congress, June 22–26, 2022. London: European Association for the Study of the Liver, 2022.

67. Agarwal K, Buti M, Van Bommel F, et al. Efficacy and safety of finite 48-week treatment with the siRNA JNJ-3989 and the capsid assembly modulator (CAM-N) JNJ-6379 in HBeAg negative virologically suppressed (VS) chronic hepatitis B (CHB) patients: results from the REEF-2 study. In: Proceedings and Abstracts of the 2022 AASLD Liver Meeting, November 4–6, 2022. Washington, DC: American Association for the Study of Liver Disease, 2022.

68. Agarwal K, Lok J, Carey I, et al. A case of HBV-induced liver failure in the REEF-2 phase II trial: implications for finite treatment strategies in HBV 'cure.' J Hepatol 2022;77:245-8.

69. Yuen M-F, Heo J, Jang J-W, 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;27:1725-34.

70. Yuen MF, Lim SG, Plesniak R, et al. Efficacy and safety of bepirovirsen in chronic hepatitis B infection. N Engl J Med 2022;387:1957-68.

71. Gehring AJ, Protzer U. Targeting innate and adaptive immune responses to cure chronic HBV infection. Gastroenterology 2019;156:325-37.

72. Michler T, Kosinska AD, Festag J, 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.e9.

73. Aliabadi E, Urbanek-Quaing M, Maasoumy B, et al. Impact of HBsAg and HBcrAg levels on phenotype and function of HBV-specific T cells in patients with chronic hepatitis B virus infection. Gut 2022;71:2300-12.

74. Wong GL-H, Hui VW-K, Yip TC-F, et al. Universal HBV vaccination dramatically reduces the prevalence of HBV infection and incidence of hepatocellular carcinoma. Aliment Pharmacol Ther 2022;56: 869-77.

75. Shimakawa Y, Veillon P, Birguel J, et al. Residual risk of mother-to-child transmission of hepatitis B virus infection despite timely birth-dose vaccination in Cameroon (ANRS 12303): a single-centre, longitudinal observational study. Lancet Glob Health 2022;10(4):e521-e529.
Copyright © 2023 Massachusetts Medical Society.

IMAGES IN CLINICAL MEDICINE

The *Journal* welcomes consideration of new submissions for Images in Clinical Medicine. Instructions for authors and procedures for submissions can be found on the *Journal*'s website at NEJM.org. At the discretion of the editor, images that are accepted for publication may appear in the print version of the *Journal*, the electronic version, or both.