

Evolving New Strategies for the Medical Management of Chronic Hepatitis B Virus Infection

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Abstract: Is a cure for chronic hepatitis B virus (HBV) infection possible? Hepatitis C virus infection is now routinely cured medically. There is a growing expectation that new drugs for the management of chronic HBV infection should provide substantial benefit over and above that of current chronic HBV medications, if not be curative. Although the definition of medically induced cure for chronic HBV infection varies, most include sustained off-drug absence of viremia and negativity for other virologic markers. There are currently more than 29 drugs in the pipeline being tested for the management of chronic HBV infection. This article discusses the potential drugs with respect to their possible contributions to achieving medically induced cure.

The current management of chronic hepatitis C virus (HCV) infection almost routinely leads to sustained virologic suppression, or functional cure, and there is excitement and even expectation that a similar cure should be possible for chronic hepatitis B virus (HBV) infection.^{1,2} The current medical management of chronic HBV infection requires either parenteral injections of interferons or indefinite use of a nucleos(t)ide (NUC) HBV polymerase inhibitor.^{3,4} However, neither approach regularly achieves sustained off-drug virologic suppression. The state of chronic HBV management could be said to be where HCV was a decade ago, despite HBV being discovered 25 years before HCV.

Thus, with HCV infection being regularly cured by direct-acting antiviral (DAA) agents, there seems to be another race to develop new HBV therapeutics. It is often reasoned that medical management of chronic HBV infection is likely more difficult than that of HCV because, as outlined in Table 1, HBV infection involves covalently closed circular DNA (cccDNA), which is the nuclear form of the viral genome and is the template for all viral gene products. HBV cccDNA persists stably within the infected cell and can reactivate even after decades of inactive disease.⁵⁻⁹ In some cases, the reactivation can be attributed to immunosuppression.^{8,9} Perhaps for that and other reasons, HBV infection also appears to be less responsive than HCV infection to interferon therapy.¹⁰⁻¹³ Moreover, because people with chronic HBV infection do not usually have detectable

Table 1. Comparison of Chronic Hepatitis B Virus and Hepatitis C Virus

	Hepatitis B Virus	Hepatitis C Virus
Genome	DNA	RNA
Chronic Infection	Yes	Yes
Chronic (Inflammatory) Liver Disease	Yes	Yes
Hepatocellular Carcinoma	Yes	Yes
Hepatocellular Carcinoma in the Background of Cirrhosis	Not always	Almost always
Immunology of Chronic Infection	<ul style="list-style-type: none"> • No detectable anti-HBs • Detectable HBcAb • T-cell dysfunction • Unclear if there is virus-mediated repression of the immune system 	<ul style="list-style-type: none"> • Hepatitis C antibody–positive • Virus-mediated repression of the immune system
Interferon Responsiveness	Rare	More common
Nuclear, Stable Genome	Yes, cccDNA and integration	No

anti-HBs, antibody to hepatitis B surface antigen; cccDNA, covalently closed circular DNA; HBcAb, hepatitis B core antibody.

circulating antibodies to hepatitis B surface antigen (anti-HBs) and have only very weak circulating HBV T-cell responses,^{5,14,15} there is a school of thought that a sustained virologic response, off medication, for chronic HBV will require both antiviral suppression and immunorestitution.¹⁵ This article considers the varying therapeutic goals and definitions of cure for HBV infection, different strategies to achieve these goals, and specific drugs in the development pipeline.

The Goal of Therapy

The caregiving and advocacy communities are setting expectations for new medications very high.² The goal is cure. However, the definition of cure has been more ambiguous. Perhaps the most meaningful definition of cure is clinical cure, in which an individual's age-adjusted risk of death, or loss of quality of life, due to liver disease returns to that of an individual without HBV infection (or at least to that of an individual with a resolved infection).¹⁶ However, for practical reasons, cures are defined descriptively as functional or virologic, using endpoints that can be practically evaluated (Table 2). That being said, most current definitions call for sustained off-drug suppression of viremia with loss of circulating hepatitis B surface antigen (HBsAg) and normalization of serum alanine aminotransferase (ALT) levels (Table 2).^{2,17} It should be noted, however, that ALT normalization may be rare if fatty liver disease and alcohol use are present.

The most ambitious definitions (complete virologic cure^{2,18} and complete cure¹⁸) call for elimination or permanent silencing of all viral cccDNA. Removal and/or silencing of integrated HBV DNA is also important.

Table 2. Definitions of Cure for Chronic Hepatitis B Virus Infection

	Definition	Reference(s)
Clinical Cure	A chronically infected individual's risk of death or morbidity due to liver disease returns, off drug, to that of an individual of the same age who has never been infected with hepatitis B.	Block et al ¹⁶
Functional Cure	A patient with off-drug suppression of viremia and antigenemia, as well as normalization of alanine aminotransferase levels and other markers of liver damage.	Liang et al ¹⁷
Complete Virologic Cure	Virologic cure plus elimination of all virologic markers (ie, cccDNA)	Revill et al ² and Zeisel et al ¹⁸

cccDNA, covalently closed circular DNA.

None of the currently approved medications reliably achieve these goals, and intracellular HBV cccDNA and circulating HBsAg levels persist, even long after suppression of viremia with the currently used NUCs.^{19,20} Indeed, even after 10 years of virus suppression with NUCs, the number of deaths due to liver disease is only reduced by 50% to 80%.^{17,21,22} Although these reductions are impressive, there is still significant mortality even after a decade

of intervention, making clear the need for new therapeutics and perhaps earlier times of intervention.

Current Medical Options for Chronic Hepatitis B Virus Infection

There are currently 7 therapeutic drugs approved by US and European regulatory agencies for use in the management of chronic HBV infection.^{3,4,23} These consist of interferons (biologic therapies) and polymerase inhibitors (NUCs). The interferons are cellular cytokines that induce hundreds of different cellular genes, many associated with enhancing a cellular state of refractoriness to viral infections, as well as enhancing cellular immunologic activity.^{5,24} Interferon therapy is recommended for use in a subset of patients with chronic HBV infection and is associated with adverse reactions that may result in the patient prematurely discontinuing use.^{3,4,17} The NUCs are competitive inhibitors of the viral reverse transcriptase enzyme and are orally bioavailable and effective at repressing viremia in most patients.⁴ It should be noted that in some patients, clearing of HBV infection is accompanied by a flare of ALT, indicating an immune response and improved viral control. For both therapeutic approaches, the achievement of functional cure, as defined in Table 2, occurs in less than 12% of hepatitis B e antigen (HBeAg, or envelope antigen)-positive patients and in even smaller percentages of HBeAg-negative patients.^{2,3,7,25}

In addition, NUC therapy is not indicated for HBsAg-positive individuals with low viral DNA levels who do not have cirrhosis. In this category are a significant number of patients with elevated ALT levels who may be at an increased risk of significant liver disease. Because of this elevated risk, these individuals would benefit from effective therapy, but there are currently no medications that can be used in this subpopulation (as neither NUCs nor interferons are recommended). Thus, there continues to be a need for new medications that can lead not just to functional cure but to full virologic or sterilizing cure, in which all viral DNA and gene products are eliminated.

Another area of unmet, or under-met, need is management of hepatitis delta virus (HDV) infection. HDV requires the HBV envelope proteins to produce infectious virus; thus, prior or coinfection of HBV is necessary for HDV to establish infection. Worldwide, there are estimated to be at least 15 to 20 million people with HDV/HBV coinfection, and their risk of death from liver disease is such that beneficial intervention is indicated.²⁶ However, medical management of HDV infection has been particularly challenging. HDV/HBV-coinfecting individuals usually experience a much more aggressive course of liver disease than those with chronic HBV infection alone.^{26,27} Even the limited benefit observed

in some HBV-infected patients with interferon therapy has not been reliably seen in HDV infection.^{26,28} It had been hoped that the effective viremia suppression of HBV achieved by HBV polymerase inhibitors (NUCs) would have a secondary HDV-suppressive effect and, therefore, benefit because HDV depends upon HBV gene products for its infectivity. However, even in individuals in whom HBV viremia has been repressed by many orders of magnitude, HDV-mediated disease and viremia are largely unaffected, at least over the period of time studied.^{29,30} This is presumably because even in individuals in whom HBV viremia is repressed by NUCs by several orders of magnitude, the numbers of HBV-infected cells and the production of HBV gene products (via nuclear HBV DNA and incomplete replication suppression) remain sufficient to provide the HBV envelope proteins necessary for HDV infection. Thus, new medications are needed to manage HDV infection.

Strategies for the Medical Management of Chronic Hepatitis B Virus Infection

Medical interventions for the management of chronic HBV infection can generally be divided into 2 categories: those that target the virus, such as NUCs (DAA agents), and those that target the host, such as interferons (host-targeting, indirect-acting antiviral agents). There are now more than 29 experimental drugs for chronic HBV infection at various stages of development (from preclinical to clinical phases). As Figure 1 and Tables 3 and 4 show, lonafarnib is exclusively indicated for HDV infection³¹ and 6 other medications are expected to have dual activity against HDV and HBV. Figure 1 organizes experimental drugs by stage of development and indicates whether or not they are direct- or indirect-acting antiviral agents. Tables 3 and 4 list the name and target/drug substance of each experimental drug, as well as the commercial sponsor or discovery organization.

The most advanced compounds in the pipeline are the prodrugs of tenofovir, with tenofovir alafenamide (Gilead) already approved for the treatment of HIV. Not many HBV drugs have reached the phase 2 human subject trial designation, and it is interesting that at least 2 of these drugs are from an entirely new class: Toll-like receptor-targeting compounds (GS-9620, Gilead and RG 7795, Roche).

Another phase 2 drug is the capsid inhibitor NVR-1221 (Janssen, formerly Novira). With twice-daily dosing, it demonstrated a clear and safe proof of concept in achieving 1- to 2-log reductions of viremia, even as monotherapy.³² There has also been phase 2 research on REP 2139-Ca (Replicor), a nucleic acid oligomer reported to inhibit HBsAg formation, secretion, or stability. Although

the number of patients in the study was small and their complete profiles were not clear, the results were interesting, with several patients no longer having detectable HBV DNA viremia, 9 of 12 achieving 2-log or greater reductions in HBsAg, and several becoming anti-HBs-positive.³³ Assuming that these individuals were chronically infected with HBV at the time of treatment and that they remain HBsAg-negative, HBV DNA-negative, and anti-HBs-positive, as well as that these milestones can be attributed to treatment and remain off treatment, functional cure has been achieved.

New molecular delivery systems such as nanoparticles and glycoconjugates are enabling oligonucleotide RNA interference (RNAi) mechanism-based drugs to become more efficient in cell penetration and, hence, be more practical.³⁴ At least 4 of these drugs are being tested for HBV in human trials. The RNAi agent ARC-520 (Arrowhead) was shown to be safe in phase 1 study. However, although the design of the drug, now in phase 2 study, was intended to selectively target cccDNA-derived transcripts, this selectivity may actually cause the agent to miss repressing transcripts derived from integrated host genomes.^{35,36} The developers of the agent plan to return to the clinic to develop a construct that targets cccDNA-derived as well as integrated HBV DNA-derived transcripts, but the original construct may have revealed a truth about chronic HBV infection. Briefly, it has been known for decades that HBV DNA integrates into the host chromosomes.^{6,16,37} Although there is uncertainty about the extent to which these integrations (integrants) increase the risk of liver cancers, there has been a general agreement that these integrants rarely, if ever, lead to productive infectious virus.³⁸ Moreover, although integrated HBV genomes can produce viral transcripts and, hence, viral proteins, there has been little consideration of how much antigen load is really contributed by transcripts generated by integrated genomes. The results from the work on ARC-520 imply that the amount of circulating HBsAg derived from integrated DNA, at least in HBeAg-negative patients, may be very significant.³⁶ This is a paradigm shift in conventional wisdom and will have a profound implication for treatment design, as it may mean that the elimination of HBsAg may not be possible by only targeting HBV cccDNA.

Another drug in phase 2 development is Myrcludex B (MYR GmbH), which is a mimetic of the HBV attachment binding region of the large envelope protein.³⁹ The mimetic interferes with HBV envelope protein-mediated attachment to the virus receptor, the sodium taurocholate cotransporting polypeptide protein, on hepatocytes.⁴⁰ The agent has demonstrated proof of concept in animals and is now being examined in human subjects. It should be noted that the drug is also being tested in patients for

activity against HDV and that RNAi agents and REP 2139-Ca should also be effective against HDV. This may help patients infected with HDV because interferons and NUCs have not been useful.⁴¹

Birinapant (TetraLogic) is a second mitochondrial activator of caspase (SMAC) agonist, an activator of caspases that could control cell death of HBV-infected cells.⁴² The idea is that HBV-infected cells are more sensitive to the cell killing mediated by this compound than uninfected cells. The concept of selective sensitivity and elimination of HBV-infected cells by what has been called chemical T cells¹ is intriguing. However, problems such as the occurrence of Bells palsy as a possible drug-related event in some patients treated for cancers and commercial problems have placed development of this drug in question.

As Figure 1 and Tables 3 and 4 indicate, there are a number of drugs in preclinical phases, ranging from immunotherapies to agents targeting steps in the virus life cycle that have not been previously attempted. The drugs listed in the figure and tables are very diverse. However, there is a lack of drugs that directly target the HBV X (HBx) protein and/or cccDNA, which are considered to be difficult targets. Although recent work suggests that X influences cccDNA transcription by accelerating the decay of cellular DNA-binding proteins,^{43,44} there is still no clear biochemical function of X to assay against. Selective targeting of cccDNA is also challenging because cccDNA persists in the nucleus and is thought to be indistinguishable from host DNA. The HBx protein is necessary for efficient *in vivo* viral replication and was recently reported to be involved in the regulating of viral transcription (from the cccDNA).²⁰ Thus, HBx could provide a cccDNA surrogate target for DAA agents.

Another option for direct cccDNA-targeting agents involves HBV-specific clustered regularly interspaced short palindromic repeats-associated (CRISPR-Cas) systems, which would aim to selectively cleave or mutate the cccDNA.^{6,45} If the problem of delivery to the nucleus can be solved for CRISPR-Cas systems, which are a complex of protein and RNA, selective destruction of HBV cccDNA becomes a possibility. Otherwise, repressing cccDNA will rely upon host-acting drugs. There are also several strategies that target cccDNA epigenetics, but these are in very early stages (data not published).

What Is Critical for Achieving Functional Cure?

The currently used medical interventions (NUCs and interferons) were approved for use largely based upon their ability to reduce viremia and improve the histologic appearance of infected livers (under biopsy), with normalizations of ALT levels, after less than 1 year (usually 48

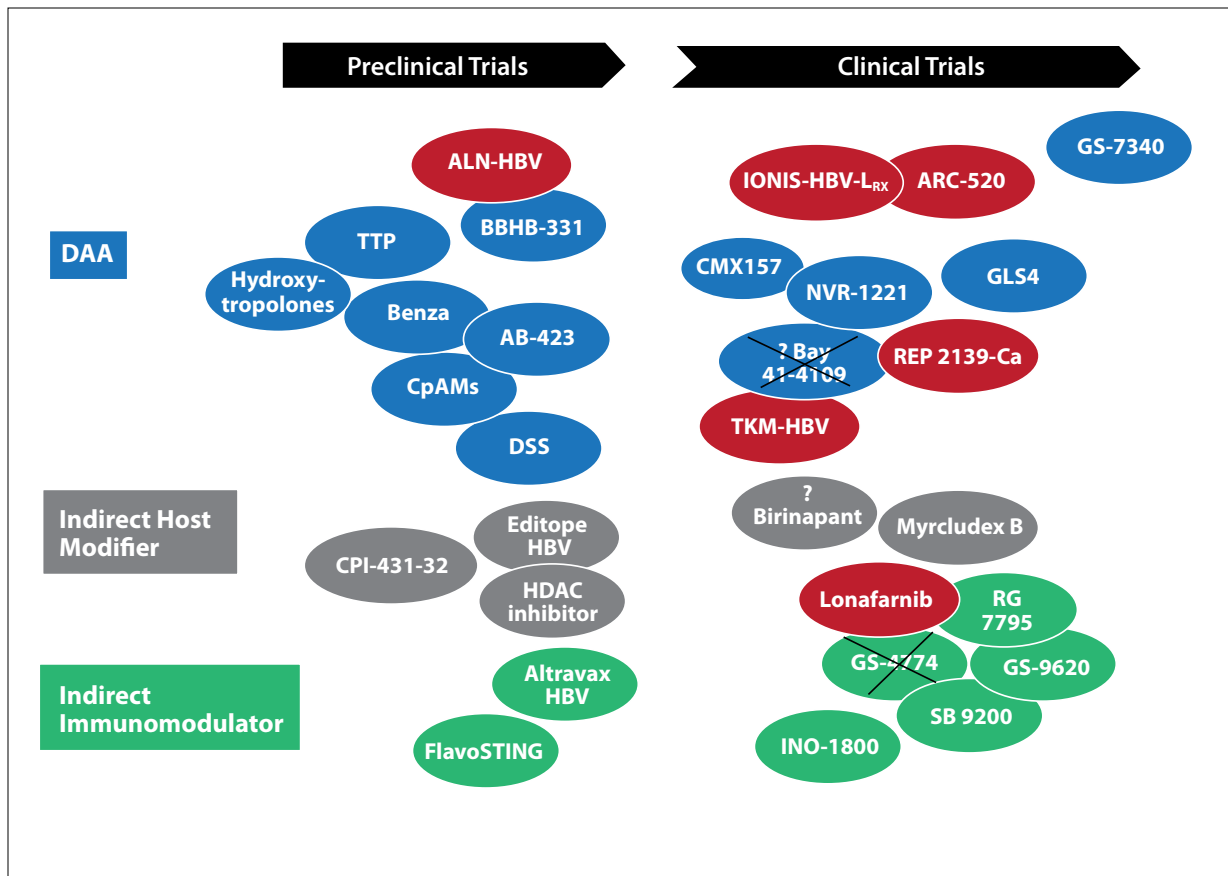


Figure 1. An overview of the HBV therapeutic pipeline, as of March 2016. Most of the compounds are categorized as either a DAA agent, indirect host modifier, or indirect immunomodulator; however, a few compounds, such as REP 2139-Ca, are in between categories. Compounds are also classified according to their relative stage of development: preclinical (nonhuman) or clinical (human) trials. Drugs thought to be suspended or removed from development are crossed out. The color of each oval indicates its category (blue=DAA agent, gray=indirect host modifier, and green=indirect immunomodulator), with red signifying an HDV active (antiviral) drug, regardless of category. A question mark indicates that it is unclear whether the drug is still in clinical trial. More detail, including HBV vaccines and immunotherapies, is available in Tables 3 and 4 and in the text.

DAA, direct-acting antiviral; DSS, disubstituted sulfonamides; HBV, hepatitis B virus; TTP, triazol pyrimidines.

weeks) of therapy.^{13,25,46} Although it is difficult to precisely associate these endpoints with clinical improvements, high viral loads are associated with greater cancer risk,^{47,48} and patients are now routinely observed to experience reversals in fibrotic liver disease (if present at the beginning of therapy) and reductions in the likelihood of death from liver cancer over 10 years of continuous therapy.^{21,23}

More recent suggestions of what the outcome and endpoints of therapy should be usually involve achievement of all the previous endpoints plus sustained off-drug suppression of viremia, and perhaps HBsAg antigenemia, satisfying the definition of functional cure and virologic cure (Table 2). Although these more demanding endpoints, such as the elimination of HBsAg, are rational and supported by circumstantial evidence,²³ the degree

to which medical suppression of antigenemia is beneficial has not been fully determined.

Are Direct-Acting Antiviral Agents Enough?

Complete viral polymerase suppression of HBV replication should, in theory, reduce viremia and intrahepatic levels of replicative forms of HBV DNA to 0, and even HBV cccDNA should be eliminated, as the infected cells are eventually replaced with uninfected cells.¹ In this model, as illustrated in Figure 2, people with HBV could be cured with DAA agents alone. Although the length of time for this replacement would likely (and has been estimated to) take several years of treatment, virologic cure has not occurred in most treated individuals. Indeed,

Table 3. Experimental Direct-Acting Antiviral Drugs in Development for the Management of Chronic Hepatitis B Virus Infection

Compound Name	Target/Drug Substance	Development Phase	Commercial Sponsor or Discovery Organization
GS-7340	Pol/prodrug of tenofovir [nucleos(t)ide]	Phase 3	Gilead
ARC-520	RNA/RNAi	Phase 2	Arrowhead
NVR-1221	Capsid/small molecule	Phase 1/2	Janssen (formerly Novira)
REP 2139-Ca	HBsAg/oligomeric nucleic acid	Phase 2	Replicor
TKM-HBV	RNA/RNAi	Phase 1b/2	Arbutus
CMX157	Pol/prodrug of tenofovir [nucleos(t)ide]	Phase 1	ContraVir
IONIS-HBV-L _{RX}	RNA/antisense RNA	Phase 1	Ionis
BBHB-331	RNA/DNA-directed shRNA	Preclinical	Benitec
AB-423 and benza	Capsid/small molecule	Animal	Arbutus
GLS4	Capsid/small molecule	Phase 2	HEC Pharma
CpAMs	Capsid/small molecule	Animal	Assembly
Disubstituted sulfonamides	cccDNA/small molecule	Preclinical	Arbutus
Triazol pyrimidines	HBsAg	Animal	Arbutus
ALN-HBV	RNA/RNAi	Preclinical	Alnylam
Hydroxytropolones	RNase H inhibitors	Preclinical	Arbutus

cccDNA, covalently closed circular DNA; CpAMs, core protein allosteric modifiers; HBsAg, hepatitis B surface antigen; pol, polymerase; RNAi, RNA interference; RNase H, ribonuclease H; shRNA, short hairpin RNA.

HBsAg levels decline modestly even after 1 to 2 years of multi-log suppression of viremia.

Perhaps this goal has not been routinely achieved in the past because the suppression of HBV gene expression (from HBV cccDNA and integrated forms) as well as replication may have been inadequate. Certainly, there is evidence for persistence of substantial functionally active HBV cccDNA, as well as replicative DNA, and even reactivation of the virus long after NUC therapy.^{7,19,20} Thus, the remaining intrahepatic HBV DNA levels, even after over a year of more than 5-log reductions of viremia, suggest that the current polymerase inhibitors are not actually inhibiting HBV polymerase sufficiently to clear HBsAg and/or cccDNA. Even reducing viremia by 99%, although impressive, is still very incomplete. For example, a 2-log reduction of intrahepatic viral DNA, assuming at least 1 genome per infected cell and a total of 10⁹ to 10¹⁰ infected cells, leaves an enormous viral load in the liver. Principles of enzyme inhibition dictate that every additional 10-fold suppression of the polymerase will require significantly greater amounts of the polymerase inhibitor. These are amounts and serum concentrations of the currently used polymerase inhibitors that may not be safely tolerated in people. It should be acknowledged that the current polymerase inhibitors were optimized, and are now dosed, mostly to reduce viremia, not intrahepatic viral DNA. Perhaps if there were more potent polymerase inhibitors or alternative means of viral replication inhibi-

tion, it would be possible to cure more people of HBV with DAA agents in the same way that HCV is now being cured.

The routine curing of HCV infection has required more than a single medical intervention to achieve sustained off-drug virus suppression. Combinations of drugs targeting different steps in the HCV life cycle were needed. Whether or not combinations of DAA agents targeting different viral steps in the HBV life cycle will be sufficient is unknown at this time, but seems likely.

Is cccDNA Repression/Elimination Necessary?

As previously mentioned, cccDNA is the stable, circular (episome-like) nuclear form of the HBV genome, and is the natural source of all progeny viral RNA and hence infectious virus.⁶ As also discussed, suppression of the viral polymerase will prevent synthesis of new virus, but does not directly affect the viral cccDNA that has already been established within the infected cell. Therefore, even complete suppression of viral polymerase would not directly stop cccDNA from being transcribed into RNA and the production of viral proteins. Eventually, as previously discussed, as the infected cells die, cccDNA levels and their gene products would diminish. Nevertheless, it has become accepted that the silencing or elimination of cccDNA will be important, if not necessary, to achieve

Table 4. Host- and Immune-Targeting Drugs in Development for the Management of Chronic HBV Infection

Compound Name (If Available)	Target/Drug Substance	Development Phase	Commercial Sponsor or Discovery Organization
Entecavir, IFN- α , IL-2, and HBV vaccine	Antiviral immunoenhancer combination	Phase 4	Tongji Hospital, China
Entecavir, IFN- α , and HBV vaccine	Antiviral immunoenhancer combination	Phase 4	Seoul National University, Korea
GS-9620	Toll-like receptor-7 agonist	Phase 2	Gilead
GS-4774	Therapeutic vaccine/viral antigens	Phase 2/3	Gilead
RG 7795 (ANA 773)	Toll-like receptor-7 agonist	Phase 2	Roche
Lonafarnib	HDV, prenylation/small molecule	Phase 2	Eiger
ANRS HB102	Therapeutic vaccine/viral antigens	Phase 1/2	French Recherche Nord et Sud Sida HIV Hepatitis
Myrcludex B	Blocks NTCP entry/lipopeptide	Phase 2	MYR GmbH
HBIG, HBV vaccine, and GM-CSF	Vaccine, immunoenhancer	Phase 2	Beijing 302 Hospital, China
SB 9200	RIG I/small molecule	Phase 1/2	Spring Bank Pharmaceuticals
Nivolumab	PD-1/antibody	Phase 1 ^a	Bristol-Myers Squibb
TG1050	Adenovirus vector HBV antigens	Phase 1/1b	Transgene
Birinapant	SMAC agonist/small molecule	Phase 1	TetraLogic
Hepilisav 601	Therapeutic vaccine	Phase 1	Dynavax
INO-1800	Therapeutic vaccine	Phase 1	Inovio
FlavoSTING	STING/small molecule	Animal	Arbutus/Blumberg Institute
Altravax HBV	Therapeutic HBV vaccine	Animal	Altravax
Editope HBV	ER glucosidase/small molecule	Animal	Blumberg Institute
HDAC inhibitor	HDAC	Preclinical	Arbutus/Blumberg Institute
CPI-431-32	Cyclophilins/small molecule	Animal	ContraVir

^aIn clinical trials for cancer, not HBV.

ER, endoplasmic reticulum; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBIG, hepatitis B immune globulin; HBV, hepatitis B virus; HDV, hepatitis delta virus; IFN- α , interferon-alpha; IL-2, interleukin-2; NTCP, sodium taurocholate cotransporting polypeptide; PD-1, programmed death receptor-1; RIG I, retinoic acid inducible gene I; SMAC, second mitochondrial activator of caspase; STING, stimulator of interferon genes.

the goals of cure, as outlined in Table 2. It does seem likely, however, that preventing cccDNA expression is an important part of a cure strategy. How this is achieved (by a DAA agent that targets cccDNA, or indirectly by awaiting cell turnover), however, is a matter of practicality, and the different strategies needed is a matter of debate.

Is Eliminating Hepatitis B Surface Antigen or Hepatitis B e Antigen Meaningful?

In the circulation of patients with chronic HBV infection, HBsAg exists in amounts 100 to 1000 times in excess of the infectious virus, making it a significant serum protein, often present in 1 to 50 $\mu\text{g/mL}$.⁴⁹ It has been tempting to assume that HBsAg has a role in immunosuppression or

at least in complexing with or neutralizing anti-HBs. In addition, there is certainly evidence that declining levels of HBsAg, on interferon therapy, correlate with favorable outcomes.⁴⁹ Also, studies with REP 2139-Ca are consistent with the value of suppressing HBsAg.³⁵ However, a direct role for HBsAg as an immunosuppressive agent has not been fully proven. The experimental evidence for a tolerizing role for HBeAg is a bit more compelling. Tian and colleagues have shown, for example, that perinatal passage of HBeAg in murine systems from mother to newborn is sufficient and necessary for the establishment of persistent infection in the newborn, and this is mediated by an HBeAg effect upon Kupffer cells.⁵⁰ A role for HBeAg in maintaining chronic infection was not observed.

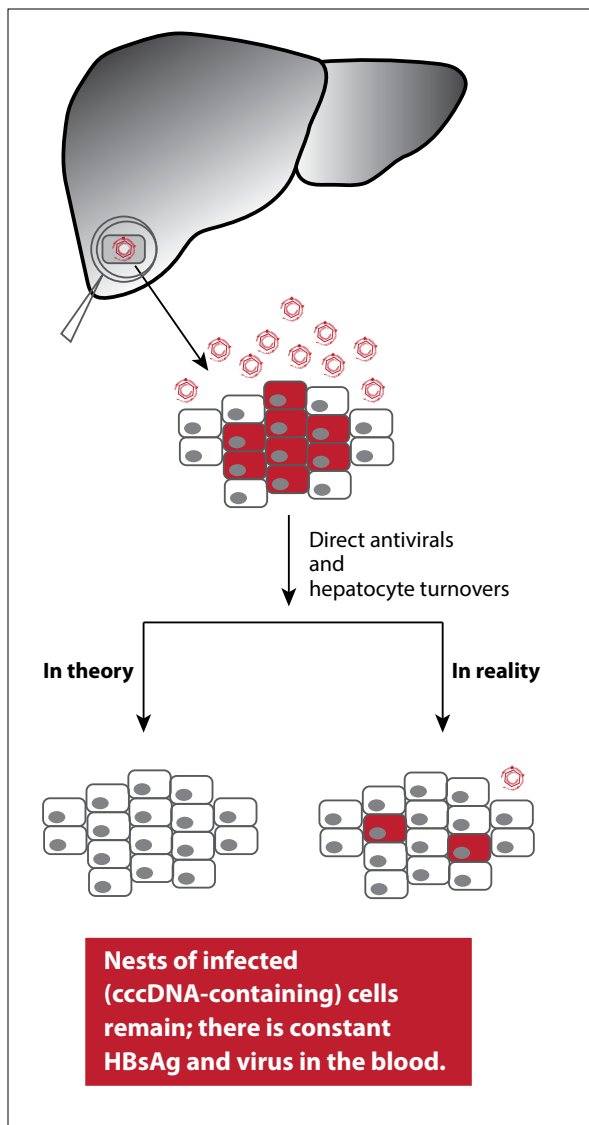


Figure 2. The fate of HBV-infected cells in the liver following nucleos(t)ide therapy. HBV-infected and virus-producing cells are indicated in red, with a region of the liver magnified. The virus progeny produced from the infected cells are indicated as reddish spheres with white centers. Two possible outcomes following nucleos(t)ide treatment for more than 10 hepatocyte half-lives are shown. In theory, if complete viral suppression occurs, there should be few or no infected cells. In reality, infected cell nests remain.

cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Is Inhibiting Hepatitis B Virus X Protein Meaningful?

The mechanism of action of HBx remains uncertain, although it is apparently necessary for productive infec-

tions *in vivo*, at least in the woodchuck. Also, HBx is necessary for efficient cccDNA transcription, as demonstrated with the human virus in tissue culture systems.^{6,43} Thus, it is another attractive direct antiviral target, and it may provide a means of repressing cccDNA without directly targeting a host function. It is therefore thought that selective and safe X-acting drugs would be a valuable addition to the armamentarium of HBV therapeutics. Likely because of current assay difficulties, other than RNAi (which targets X gene transcripts), there are no X-active agents in the pipeline of which we are aware.

Is Activation of the Innate or Circulating Adaptive Immune Response Necessary?

Resolved HBV infection, even after a period of chronic disease, is characterized by undetectable levels of viremia, antigenemia, robust HBV-specific T-cell response, and detectable levels of circulating anti-HBs.^{5,14,51,52} Therefore, there is a school of thought that a stable off-drug response (ie, functional cure) will need to be accompanied by the appearance of neutralizing anti-HBs as a marker of B-cell response and also a T-cell response to denote a robust innate host defense. HBV can reactivate from seemingly indolent disease from occult or “hidden” viral cccDNA genomes even after years of little, if any, productive infection.^{8,9} Therefore, it is easy to appreciate how virus reactivation from small numbers of de-repressed genomes, years after virus suppression via drugs has stopped, could lead to a rebound of infection (if the infections are unopposed by antibodies and/or a cellular response). This situation is different from that seen with HCV, in which DAA agents appear to be sufficient to eliminate productive infection with the help of cellular immune response, and nests of indolent viral genomes with reactivation potential do not occur. Of course, it is possible that if viral gene product amounts can be pushed below some threshold, innate host defenses, if not adaptive responses, could gain the upper hand. This is achievable with DAA agents alone in HCV, but there is a compelling argument that immunorestitution with enhancing agents will be needed for HBV.

When and Who to Treat

It is now clear that chronic HBV is a heterogeneous disease that varies in terms of physiology, immunology, virology, and length of time of infection.⁵³ This could affect which drugs are most appropriate to use and when to use them. It is already accepted practice that interferons are not to be used in patients with advanced fibrosis or cirrhosis.^{4,54,55} Moreover, as Figure 3 shows, patients with high viremia, elevated ALT levels, and decades of infection may require and benefit from drugs that are different than

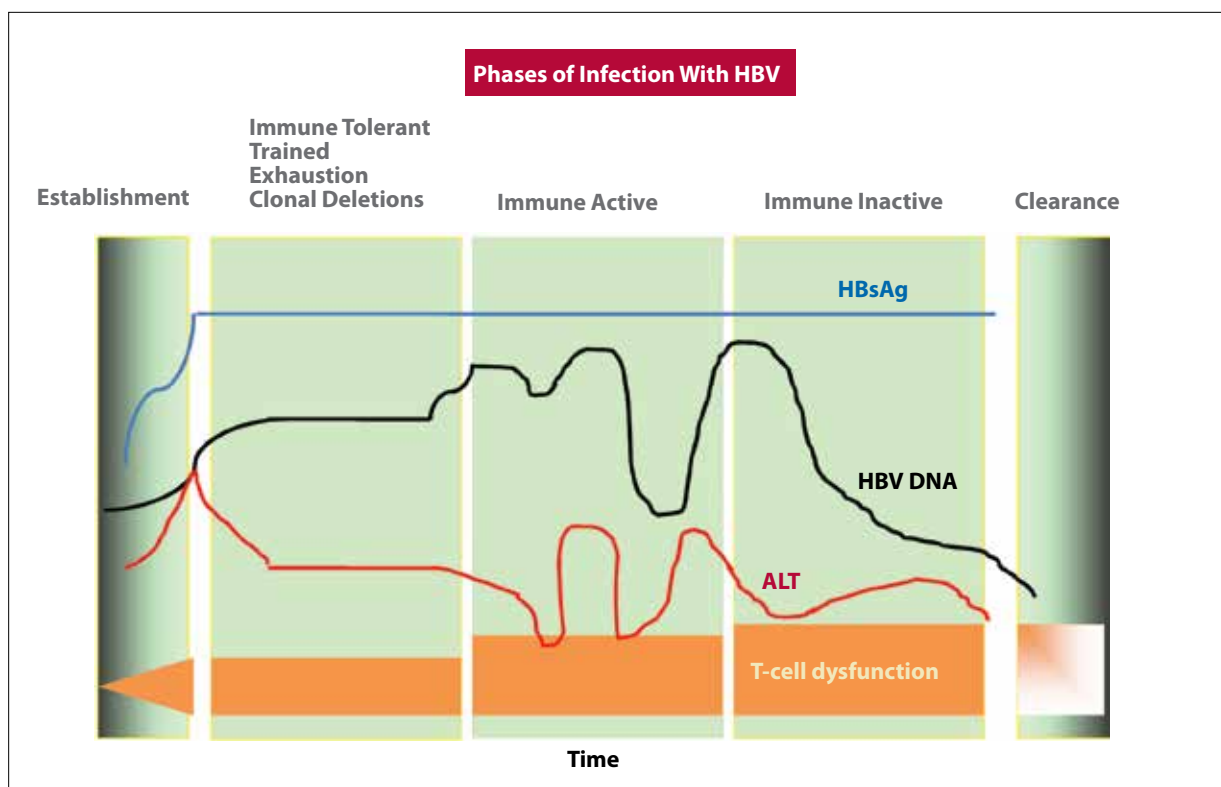


Figure 3. The various phases of chronic HBV infection. Although not necessarily progressive, chronic HBV infection has been divided into specific phases, based largely upon the levels of viremia and serum ALT.^{4,25} The different phases of infection may influence the patient's needs and type of medical intervention.

ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

those used in patients with low to no viremia and elevated ALT levels. There is also evidence that the host immune system experiences T-cell clonal deletion or becomes progressively exhausted or retrained, and this T-cell fatigue/exhaustion or training with respect to HBV antigens may increase with time (years) of exposure.¹⁴ This could mean that patients with chronic HBV infection would benefit from immunostimulatory interventions early in the course of chronic infection, before their T-cell responsiveness has been irreversibly exhausted. Thus, new drugs targeting the immune systems or mechanisms of antiviral action other than the polymerase may have indications and limitations specific to their mechanisms.

Endpoints for Treatment and Drug Development

The currently used and approved medications for HBV infection were evaluated largely based upon their ability to suppress viremia and normalize serum ALT levels.^{7,13,56,57} New therapeutics are likely to be used as add-on therapies. Therefore, the current endpoints will be inadequate to detect benefits contributed by these new medications.

Moreover, because it is expected that the new therapeutics should achieve goals approximating cures, as outlined in Table 2, endpoints that correlate with sustained responses will be needed. The new endpoints will likely involve biologic and host markers, such as assessments of viral antigenemia, intracellular viral DNA loads, and immunorestitution (as outlined in Table 5). This is an area of active investigation, but as of yet, we are not aware of the validation of markers other than those in current use.

Conclusion

The advent of DAA agents, as well as the success they have had in routinely curing HCV patients, has given hope that a similar approach can be applied to discovering a cure for chronic HBV infection. Although parallels exist between HCV and HBV, significant differences between the viruses create a unique set of challenges for a chronic HBV cure. These obstacles include the heterogeneity of HBV-infected patients, establishment of more demanding treatment endpoints, persistence of HBV cccDNA, restoration of host immunity, and HBV/HDV coinfection and superinfection. Despite substantially reducing

Table 5. New Endpoint Markers That Are Needed to Evaluate Emerging Therapeutics

Likelihood of Sustained Off-Drug Response	Evidence of Beneficial Immune Reawakening
<ul style="list-style-type: none"> • Infected cell number • cccDNA amount and transcription 	<ul style="list-style-type: none"> • Innate host activity • T-cell mobilization • Humoral activity

cccDNA, covalently closed circular DNA.

mortality, the current treatment regimens of interferons and NUC reverse transcriptase inhibitors are beneficial only to a subset of patients with chronic HBV infection and fail to adequately cure patients in sustained off-drug suppression of viremia and other viral markers.

The current experimental therapeutics for chronic HBV infection vary widely in terms of stage of development, patient scope, and mechanism of action. These drugs include extensions of the current gold standard NUCs, HBV capsid inhibitors, agents that target HBsAg, RNAi mechanism-based therapeutics, peptide mimetics that interfere with HBV envelope proteins, SMAC agonists, and HBV-specific CRISPR-Cas systems. Furthermore, a potentially promising therapeutic target that has yet to be fully explored is HBx. Ideally, several of these drugs will prove to be effective at curing chronic HBV infection. It is likely that a combination of drugs with distinct targets in the viral cycle will be required to establish an effective therapy for chronic HBV infection.

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has a speakers contract to do promotional talks for Alexion, Bayer, Bristol-Myers Squibb, Gilead, Salix/Valeant, AbbVie, and Merck; is a minor stock shareholder (liver space noted only) of Kinex, Synageva, RiboSciences, and CoCrystal; has stock options for Arrowhead; and has served as consultant and promotional speaker for Quest.

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