New Therapeutic Targets and Drugs for the Treatment of Chronic Hepatitis B

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Abstract

Chronic hepatitis B virus (CHB) is managed effectively with either nucleoside/nucleotide-based or interferon-based therapies. However, most patients receiving these therapies do not establish long-term, durable control of infection after treatment withdrawal. In particular, rates of hepatitis B surface-antigen loss and seroconversion to antisurface-antigen antibody are very low. Thus, novel therapies and treatment modalities are necessary to achieve either elimination of the virus from the liver or durable immune control of hepatitis B virus (HBV) infection in the absence of chronic therapy ("functional cure"). Here the authors review new targets and approaches for treating CHB. These approaches can be divided into two broad categories—those targeting the virus or host factors required by the virus and those targeting the innate or adaptive immune systems. Unfortunately, although a variety of promising strategies have been identified and several new approaches have achieved preclinical validation, relatively few novel drug candidates are in active clinical studies to treat CHB. Thus, functional cure of CHB infection remains an important therapeutic challenge.

Keywords

► hepatitis B virus (HBV)
► antiviral therapy
► immunotherapy
► chronic hepatitis B
► treatment

Chronic hepatitis B (CHB) remains a significant unmet medical need. An estimated 350 million people are chronically infected with hepatitis B virus (HBV) worldwide and are therefore at significantly increased risk of advanced liver disease.[1] Substantial progress in the treatment of CHB has been made in the past two decades with the approval of interferon- (IFN-) based and nucleoside/nucleotide-based therapies. Although these two classes of agents have revolutionized the management of CHB, each has limitations.[2,3] Pegylated interferon-alpha (PEG-IFN-α) represents a finite treatment course, but is poorly tolerated and only elicits a sustained clinical response in a subset of patients. In contrast, the current nucleoside/nucleotides of choice (tenofovir disoproxil fumarate [tenofovir DF] and entecavir) represent safe, well-tolerated, highly-effective and durable on-treatment therapies. However, although nucleoside/nucleotide therapies significantly improve clinical end points, they generally require chronic administration to maintain viral suppression and benefit.

Overall, the ability to "cure" HBV infection (with cure being defined as the loss of both HBV DNA and HBV surface antigen [HBsAg] plus seroconversion to anti-HBsAg antibody-positive, and maintenance of these end points after the cessation of therapy) presents a formidable challenge that is not adequately met by current therapies. A long-term study of tenofovir DF in HBV e antigen- (HBeAg-) positive CHB patients indicated that 5 years of therapy led to HBsAg loss in 10% of patients and full HBsAg seroconversion in 8% of patients.[4] Similarly, HBsAg loss was observed in 8% of HBeAg-positive patients treated with PEG-IFN-α for 1 year and followed for 3 additional years (HBsAg loss in 8% of patients 4 years after treatment initiation).[5]

This review will focus on new approaches for the treatment of CHB. New therapeutic targets and approaches can be divided into two main categories: (1) therapies that target the virus either directly or by targeting host factors required by the virus, and (2) therapies targeting the host innate or adaptive immune response. Before discussing new targets, it is helpful to review the replication cycle of HBV and the challenges it presents.
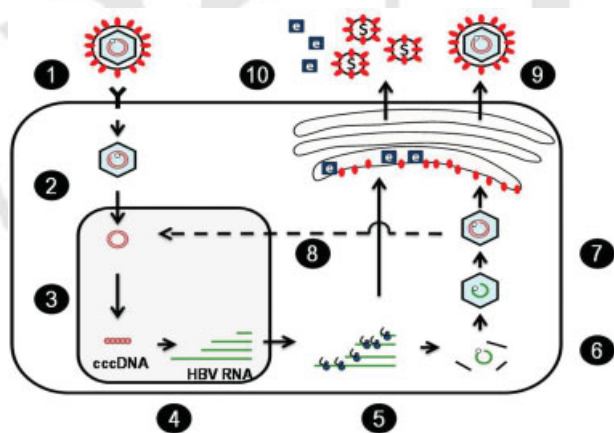
The HBV Viral Lifecycle

The viral lifecycle is summarized in ►**Fig. 1** and the reader is referred to reviews of HBV molecular virology for further details.[6,7] Several aspects of the viral lifecycle have profound implications for antiviral therapy and bear emphasis. First, HBV is a small virus (3.2 kilobases) that encodes few proteins and is highly dependent on host machinery for replication (e. g., transcription, translation, virus secretion). HBV therefore presents few targets for selective antiviral intervention. Second, covalently closed circular DNA (cccDNA) is a highly stable episomal form of the viral genome that persists during potent nucleoside/nucleotide therapy.[8] This is because nucleoside/nucleotides act downstream of cccDNA at the reverse transcription step (►**Fig. 1**, step 7). Although nucleoside/nucleotides can block the formation of new cccDNA (►**Fig. 1**, step 8), they do not directly impact existing copies of cccDNA. Third, it is important to note that nucleoside/nucleotide therapy blocks production of new viral genomes (►**Fig. 1**, step 7), but does not inhibit transcription of viral messenger RNA (mRNA) (►**Fig 1**, step 4), translation of viral antigens (►**Fig. 1**, step 5), or secretion of viral antigens (step 10). In other words, antigenemia can occur independently of viremia (or suppression of viremia).

Fig. 1 The hepatitis B virus (HBV) viral lifecycle. Infection of the host hepatocyte starts with (1) attachment of the virus to a host receptor and entry into the cytoplasm. (2) Once inside the cell, the virus disassembles and delivers its genome to the nucleus. (3) During this process, the relaxed circular genome of HBV is repaired and super-coiled and chromatinized to form a covalently closed circular DNA genome (cccDNA). (4) Host ribonucleic acid (RNA) polymerases transcribe cccDNA to yield four HBV messenger RNAs, which are exported to the cytoplasm and (5) transcribed by host ribosomes to form viral proteins. (6) Viral core protein can polymerize around a "pregenomic" viral RNA and the viral polymerase protein to form immature nucleocapsids. (7) Within the nucleocapsids, viral reverse transcription occurs and converts the pregenomic mRNA into a partially double-stranded DNA genome. Mature DNA containing nucleocapsids can either (8) cycle back to the nucleus to amplify the nuclear pool of cccDNA or (9) acquire an HBsAg envelope and then be secreted from the cell. (10) In parallel, but independently from virion secretion, hepatitis B surface antigen (HBsAg) particles and viral e antigen (HBeAg) protein are also secreted from the cell.

Approaches Targeting the Viral Lifecycle

New approaches targeting the virus directly or through host factors required by the virus are reviewed below and are presented sequentially according to the processes outlined in ►**Fig. 1**.

Viral Entry

The HBV entry process has historically been poorly understood due to the lack of robust in vitro infection systems. To date, limited HBV infection has only been supported in primary human or primary tupaia (tree shrew) hepatocytes and in the well-differentiated human hepatic cell line HepaRG. Using these systems, a series of peptide inhibitors derived from the large surface antigen of HBV were identified to inhibit HBV infection with nanomolar potency.[9] Not only can these peptides prevent in vitro infection of both HBV and HDV, but they are also effective in small animal models of infection after subcutaneous administration.[10,11] A therapeutic candidate designated Myrcludex B was selected for development and consists of a myristylated peptide containing amino acids 2 to 48 of the HBV large surface antigen. Mechanistically, Myrcludex B (or close analogs) appears to work through binding the host receptor for HBV infection (recently identified to be the sodium taurocholate cotransporting polypeptide [NTCP]) and blocking attachment of the virus.[12] Myrcludex B recently completed a phase 1 safety and pharmacokinetic study in healthy volunteers.[13] Future Myrcludex B studies including a phase 2 multiple-dose therapeutic study in CHB patients are eagerly awaited.

cccDNA

As indicated above, cccDNA represents a central obstacle to curative therapy. The formation of new cccDNA (amplification) in already infected cells can be blocked by replication inhibitors (e.g., nucleoside and nucleotide analogs). However, the existing pool of cccDNA in infected cells is refractory to treatment with nucleoside/nucleotides. Conceptually, an approach directly targeting and eliminating cccDNA could be curative. However, despite the high value of targeting cccDNA, efforts in this area are at early stages. Recently, two structurally related sulfonamide compounds were reported that block conversion of relaxed circular HBV DNA into cccDNA at micromolar concentrations.[14] The compounds were identified through a cell-based high throughput screen and neither the mechanism nor the target for these compounds is currently known.

A distinct approach to directly target cccDNA is the use of zinc-finger nucleases (ZFNs). ZFNs combine a nonspecific restriction enzyme domain with zinc-finger motifs that introduce sequence specificity. Although this approach is at an early stage of investigation, in vitro data indicate that ZFNs are able to cleave HBV DNA in hepatoma cells.[15] In addition, although cleaved target DNA can be repaired, this process often introduces mutations or deletions; mutation of HBV DNA was also observed during in vitro analyses. Because the HBV genome is compact and encodes multiple overlapping reading frames, it may be highly susceptible to inactivation by mutation. However, although the concept of specifically

inactivating HBV DNA by cleavage and/or mutation is attractive, this approach will face the clinical challenges of preferentially delivering ZFNs to the liver; it also has the potential for off-target cleavage. Currently, adeno-associated viral vectors are being explored for ZFN delivery.[16]

Viral Transcription and mRNA Stability

Although it would be ideal to eradicate cccDNA from infected cells, another approach is to functionally inactivate cccDNA by either preventing transcription or degrading viral mRNA. cccDNA transcription can be blocked using zinc-finger proteins (ZFPs), in this case devoid of restriction nuclease domains. ZFPs can selectively recognize specific HBV target sequences with nanomolar affinity, and after binding, block the binding of transcription factors to cccDNA and/or hinder RNA polymerase read-through transcription. Proof of concept has been established in vitro using duck HBV (DHBV) and in cell lines with stable integrations of the X gene.[17,18]

Another approach to prevent transcription of cccDNA is to functionally "silence" it epigenetically. Recent results suggest that inactivation of cccDNA through epigenetic modifications represents an important mechanism of action for IFN-α.[19] Specifically, experiments in both cell culture and HBV-infected uPA-SCID mice transplanted with human hepatocytes indicated that HBV mRNA levels are markedly reduced by IFN-α at time points when cccDNA is not substantially affected. Furthermore, reductions in viral mRNA correlated with reductions in acetylated histones bound to cccDNA. Epigenetic regulation of cccDNA therefore represents a novel opportunity for modulation of cccDNA function. Although promising, a conceptual challenge for this approach is selectivity, as inhibition or activation of host epigenetic regulators may also modulate host gene transcription.

As an alternative to interfering with transcription from cccDNA, viral mRNA can be targeted directly by antisense oligonucleotides, ribozymes, or RNA interference (RNAi). HBV presents an ideal target for these technologies because all viral transcripts overlap in the 3′ region and are coterminal.[6] By targeting the 3′ region of HBV mRNAs, the production of all HBV proteins could conceivably be blocked with a single RNA therapeutic molecule. There is in vitro proof of concept that hammerhead and hairpin ribozymes, as well as antisense oligonucleotides reduce HBV transcript levels in cell culture models.[20–22] However, few recent reports in ribozyme and antisense areas exist. In contrast, more progress has been made using RNAi. Multiple investigators have demonstrated that viral mRNA can be potently knocked down by RNAi, not only in cell culture, but also in small animal models of HBV.[23–25] However, although the potential to knock down viral proteins using RNA targeting approaches is tantalizing scientifically, significant challenges remain in the area of drug delivery. Nevertheless, multiple RNAi-based therapeutics have moved into the clinic for other indications; therefore, this approach could be considered for HBV.[26]

Viral Assembly

As indicated above, HBV genome replication takes place exclusively within nucleocapsids; therefore, dysregulation of capsid assembly will effectively block viral replication. Multiple classes of compounds that interfere with HBV capsid formation have been described. These include a series of heteroarylpyrimidines (HAPs),[27,28] as well as a series of phenylpropenamides.[29–31] This class of inhibitors can have very potent antiviral activity (nM EC_{50} values) and is fully active against nucleoside-resistant strains of HBV.[32] Mechanistically, both phenylpropenamides and HAPs increase the kinetics of capsid assembly, which disrupts the formation of replication-competent nucleocapsids.[33,34] HAPs have been shown to have antiviral effects in small animal models of HBV replication.[35,36] However, there is no reported clinical experience with any compounds from this class to date.

Reverse Transcription

There are five widely approved nucleoside/nucleotide inhibitors for the treatment of CHB (tenofovir DF, entecavir, lamivudine, adefovir dipivoxil, telbivudine). Clinical results and the utility of these compounds have been well described elsewhere.[2,3] Although new nucleoside/nucleotides with anti-HBV activity have been reported, both tenofovir DF and entecavir are highly potent and have high barriers to resistance. It is currently unclear whether new drugs in this class would translate into further improvements in HBsAg seroconversion.

Antigen and Virion Secretion

Current therapies are largely ineffective in preventing viral antigen secretion; therefore, this represents a potential new approach for therapy. Secreted viral antigens have been reported to cause immune defects; hence, blocking antigen secretion could foster improved host responses to chronic infection.[37,38] Functional inhibition of cccDNA transcription (either by targeting cccDNA, viral transcription, or viral mRNA stability as described above) will result in downstream inhibition of antigen production. In addition, compounds with more direct effects on antigen secretion have been reported.

An exploratory amphipathic oligonucleotide therapeutic termed REP 9AC′ is currently in clinical development for the treatment of CHB.[39] REP 9AC′ blocks secretion of HBsAg subviral particles and causes HBsAg to accumulate inside cells. Interestingly, REP 9AC′ does not block the secretion of virions, consistent with studies that have suggested the pathways for virion and subviral particles are distinct.[40] In pilot clinical studies, weekly intravenous infusions of REP 9AC′ resulted in the clearance of HBsAg from the serum of HBeAg-positive CHB patients. Some patients who cleared HBsAg became seropositive for anti-HBsAg antibody, and the titers of anti-HBsAg were further enhanced by add-on therapy with either PEG-IFN-α or thymosin-α, a peptide with immunomodulatory activity.

Small molecule inhibitors of HBsAg secretion have also been described. A class of imino sugars termed "glucovirs" (e.g., N-nonyl-deoxynojirimycin) reduced secreted HBsAg and HBV virions in vitro, and also had antiviral activity in woodchucks infected with woodchuck hepatitis virus (WHV).[41,42] Glucovirs are believed to act by mimicking

glucose residues on glycosylated proteins (including HBsAg) and inhibiting host α-glucosidases. More recently, a class of triazolopyrimidines that inhibit HBsAg secretion was identified through a high throughput screening effort.[43,44] The prototype "hit," HBF-0259, inhibited the accumulation of all three forms of HBsAg (large, middle, and small) in the media of HBV-expressing cell lines with an EC_{50} of 1.5 μM. The mechanism of action remains to be determined, but appears to be distinct from that of the glucovirs.

Immunotherapeutic Strategies

Viruses that establish chronic infection employ a wide variety of strategies to avoid sterilizing immunity. With regard to HBV, evasion of innate immunity may contribute to the development of persistence, while a profoundly dysfunctional T-cell response is likely a key factor in the maintenance of chronicity.[45] Accordingly, over the past 20 years multiple immunotherapeutic strategies have been evaluated in CHB patients with the goal of reconstituting effective antiviral control. Initial proof-of-concept was demonstrated by resolution of CHB in the recipient of a bone marrow transplant from a donor with immunity to HBV.[46] In line with studies linking HBV persistence to a defect in adaptive immunity,[45] these early transplantation studies suggested that restoration of HBV-specific T-cell immunity can clear persistent infection.[47] Unfortunately, therapeutic translation of this knowledge has proven difficult. Indeed, a variety of therapeutic vaccination trials have yielded disappointing results and efforts to boost antiviral immunity with recombinant cytokines have also been largely unsuccessful. Accordingly, an urgent need remains to develop new immunotherapeutics to improve the rate of functional cure. Fortunately, recent advances in our understanding of the nature of immune dysfunction in chronic viral infections are leading to novel immune-based strategies to treat CHB. These approaches are briefly described below.

IFN Therapy

Currently, the only approved widely immunotherapeutic agent for the treatment of CHB patients is IFN-α (pegylated or nonpegylated). Despite more than 20 years of clinical use, the mechanism(s) by which IFN-α controls HBV replication are not completely understood. This remains an important goal because mechanistic understanding of IFN-α activity could drive rational design of novel immunotherapeutic strategies. It is therefore noteworthy that recent studies have shed new light on PEG-IFN-α treatment response. Most importantly, it was determined that PEG-IFN-α does not improve peripheral HBV-specific T-cell responses, and that virologic response may instead be related to activation of an NK cell subset.[48,49] In addition, as discussed above, IFN-α inhibition of cccDNA transcription may also be an important mechanism of viral control.[19]

The importance of the direct antiviral response to IFN-α is in line with the therapeutic activity of IFN-λ which, by virtue of a restricted receptor expression pattern, presumably lacks many of the immunomodulatory functions (and associated toxicities) of IFN-α.[50,51] An interim analysis of a phase 2 clinical study with PEG-IFN-λ1a (BMS-914143) revealed that this agent displayed at least comparable efficacy to PEG-IFN-α through 24 weeks of treatment, with a superior safety profile in most respects.[52] Because these data indicate that direct activation of hepatocytes by IFNs can induce a robust virologic response, targeted delivery of IFN-α to the liver may also be a useful therapeutic strategy.[53]

Cytokine Therapeutics

Elegant studies in the LCMV mouse model have highlighted the potential of interleukin-7 (IL-7) and IL-21 for the treatment of chronic viral diseases.[54–58] Furthermore, IL-21 has been shown to play a key role in the age-dependent response to HBV in a transgenic mouse model,[59] although defective IL-21-producing T-cells have not been observed in young CHB patients.[60] Both rIL-7 and rIL-21 are in clinical development. Although rIL-21 (BMS-982470) has so far only been tested in cancer patients,[61] rIL-7 (CYT107) is currently being evaluated in a phase 1/2a trial in CHB patients in combination with nucleoside/nucleotides (tenofovir DF or entecavir) and vaccination (GenHevac).[62] Encouragingly, rIL-7 has been well tolerated in early-phase clinical studies in cancer and human immunodeficiency virus (HIV) patients;[62] therefore, data from CHB patients are eagerly awaited.

Toll-Like Receptor (TLR) Agonists

Toll-like receptors are pattern-recognition receptors (PRRs) that recognize a variety of broadly conserved pathogen-associated molecular patterns (PAMPs). Pharmaceutical activation of TLRs is an attractive approach for the treatment of CHB because agonism of these receptors triggers innate immune responses and also stimulates adaptive immunity. This approach is supported by the demonstration that TLR activation can suppress hepadnavirus replication in vitro and in animal models.[63] Most notably, the small molecule oral TLR7 agonist GS-9620 demonstrated S-antigen loss and seroconversion in the woodchuck model of CHB[64] and sustained reduction of viremia and antigenemia in chronically infected chimpanzees.[65] GS-9620 is currently in phase 1b studies in treatment-naïve patients as well as in patients virologically suppressed with tenofovir DF.

Restoring HBV-Specific T-Cell Immunity

It is broadly accepted that clearance of acute HBV infection requires a vigorous, multispecific CD8^{+} cytotoxic T-cell response, whereas persistent infection is associated with a limited and dysfunctional T-cell response.[66] Although the failure of nucleoside/nucleotides to typically achieve durable HBV suppression is likely related to the lack of sustained recovery of virus-specific T-cell function,[67] a recent study demonstrated that the HBV-specific T-cell response can in fact be restored (at least after in vitro expansion) in those select patients that do achieve HBsAg loss and seroconversion upon therapy.[68] This suggests that T-cell dysfunction during CHB is reversible; therefore, therapeutic strategies to restore effective antiviral T-cell response should be considered.

Therapeutic Vaccination

Over the past 10 years, therapeutic vaccination has been the most frequently tested strategy to improve HBV-specific T-cell function. Unfortunately, although vaccination with anti-HBs/HBsAg complexes demonstrated modest virologic response in a phase 2b study,[69] the therapeutic efficacy of classical vaccine approaches has been disappointing.[70] However, the recent approval of the autologous dendritic cell vaccine Provenge (Sipuleucel-T) for the treatment of metastatic prostate cancer has demonstrated that therapeutic vaccination can be a viable immunotherapeutic approach. Although the complexity and high cost of autologous dendritic cell vaccination may preclude its use for the treatment of CHB, promising alternative therapeutic vaccine strategies are currently under development. These include adenovirus-based (TG1050) and yeast-based (GS-4774) approaches. The latter is of particular interest as this Tarmogen (targeted molecular immunogen) incorporates multiple viral antigens (HBx, HBsAg, and HBcAg), can induce effector CD4$^+$ and CD8$^+$ T-cells following ex vivo stimulation of healthy and chronic HBV donor samples and displayed significant activity in an HBV-antigen-positive tumor-protection study in mice.[71,72]

Targeting T-Cell Inhibitory Receptors

Another potential strategy to boost antiviral T-cell responses is to inhibit coinhibitory signals mediated by receptors such as PD-1 and CTLA-4.[73] Blocking PD-1 (or its ligand, PD-L1) is a particularly attractive approach because it sits atop the hierarchy of inhibitory receptor expression by intrahepatic HBV-specific CD8$^+$ T-cells[74]; the function of virus-specific T-cells from CHB patients can be improved by blocking PD-1 ex vivo.[75] In addition, treatment of chronically infected woodchucks with an anti-PD-L1 antibody together with entecavir and therapeutic vaccination led to prolonged control of viremia and antigenemia (M. Roggendorf, personal communication). An anti-PD-1 antibody (BMS-936558) and an anti-PD-L1 antibody (BMS-936559) have demonstrated striking responses in patients with advanced cancer, although treatment was associated with a relatively high frequency of grade 3 and 4 toxicities.[76,77] Similarly, blockade of CTLA-4 can also be associated with serious side effects.[78] Despite a compelling biological rationale, safety concerns likely explain the lack of reported development for antibodies to PD-1 or other inhibitory T-cell receptors for CHB.

Blocking Suppressive Cytokines and Regulatory T-Cells in the Liver

In addition to reducing the profound antigen load in HBV patients (discussed above), successful boosting of antiviral T-cell immunity may also benefit from modulation of suppressive cytokines in the liver microenvironment. Both IL-10 and TGF-β can contribute to the tolerogenic properties of the liver and blocking these cytokines can improve antiviral T and/or NK cell function.[79–82] Various inhibitors of TGF-β are currently in preclinical and clinical development.[83] Although somewhat controversial, several studies have indicated that regulatory T-cells (Tregs) may also play a role in the inhibition of HBV-specific T-cell function.[84] Pharmaceutically, Tregs could be depleted and reprogrammed with a CD25-blocking monoclonal antibody such as daclizumab[85] or inhibited by TLR activation.[86] Therapeutic inhibition of TGF-β or Tregs has not been clinically validated in CHB patients, nor did these approaches induce a virologic response in chronically infected woodchucks when combined with IL-12 treatment.[87]

Conclusions and Perspectives for New Therapies

Approved therapies, particularly tenofovir DF and entecavir are able to manage disease in the majority of CHB patients, although the rate of functional cure (HBsAg loss and seroconversion) with these agents is low. Thus, there is an urgent need to develop novel therapeutics to achieve a functional cure, ideally with finite courses of therapy.

Strategically, any new approaches based on targeting the virus, either directly or by inhibiting a host factor required for infection, will need to establish that target inhibition ultimately translates into improved HBsAg seroconversion. For example, simply blocking viral replication, even by a mechanism other than nucleoside/nucleotides, is unlikely to cure infection unless it impacts the cccDNA reservoir or fosters an enhanced immune response capable of controlling viral replication. Directly targeting cccDNA is an attractive strategy, although as a nucleic acid, cccDNA represents a challenge for conventional drug discovery. Approaches such as zinc-finger nucleases and modulation of epigenetic regulators face a challenge to establish selectivity. Instead, approaches such as blocking antigen secretion or directly targeting viral RNA are more likely to be technically feasible in the near term. It is also hoped that the recent discovery of the HBV entry receptor will open new avenues to drug discovery by enabling more relevant in vitro models for virus replication and host response.[12]

The natural resolution of CHB in a small percentage of patients annually highlights the potential of host immunity to affect sustained viral control. Although immunotherapeutic approaches have been largely unsuccessful to date, our growing understanding of the immune defects in CHB are enabling the development of new strategies. PEG-IFN-λ1a and the TLR7 agonist GS-9620 represent promising immunotherapeutic approaches; hopefully, additional immune-based agents will soon follow these into the clinic. Although there is cause for optimism with new immune-based approaches, it is important to recognize the challenges of safely activating the immune system. The high safety bar for new CHB therapies may impede the development of immunotherapeutic approaches (e.g., anti-PD-1 antibodies) that have demonstrated great promise in oncology.

Targeting viral proteins that have been reported to subvert host immunity is an attractive approach because it alleviates concerns related to "on target" toxicities. Unfortunately, these host–virus interactions have yet to be comprehensively validated in physiologically relevant systems. This illustrates the current challenge for HBV research and development: although many potential new approaches to treat CHB have been identified, therapeutic translation has been challenging

and relatively few drug candidates have emerged and entered clinical studies. In the coming years, it is hoped that this trend can be reversed and that major strides can be made in alleviating the global health care burden of HBV.

Acknowledgments

References

1 Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11(2):97–107

2 Kwon H, Lok AS. Hepatitis B therapy. Nat Rev Gastroenterol Hepatol 2011;8(5):275–284

3 Papatheodoridis G, et al; European Association for the Study of the Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012;57(1):167–185

4 Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381(9865): 468–475

5 Buster EH, Flink HJ, Cakaloglu Y, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology 2008;135(2):459–467

6 Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev 2000;64(1):51–68

7 Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Knipe DM, Howley PM, eds. *Fields Virology* (Vol. 2). Philadelphia, PA: Lippincott Williams & Wilkins; 2007:2977–3030

8 Werle-Lapostolle B, Bowden S, Locarnini S, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology 2004;126(7):1750–1758

9 Gripon P, Cannie I, Urban S. Efficient inhibition of hepatitis B virus infection by acylated peptides derived from the large viral surface protein. J Virol 2005;79(3):1613–1622

10 Petersen J, Dandri M, Mier W, et al. Prevention of hepatitis B virus infection in vivo by entry inhibitors derived from the large envelope protein. Nat Biotechnol 2008;26(3):335–341

11 Volz T, Allweiss L, M Barek MB, et al. The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. J Hepatol 2012

12 Yan H, Zhong G, Xu G, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human. hepatitis B and D virus. eLife 2012;1:e00049

13 Haefeli WE, Blank A, Mikus G, Mier W, Alexandrov A, Urban S. Successful first administration of Myrcludex B, a first-in-class hepatitis B and D virus entry inhibitor, in humans. Hepatology 2012;56:372A

14 Cai D, Mills C, Yu W, et al. Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. Antimicrob Agents Chemother 2012;56(8):4277–4288

15 Cradick TJ, Keck K, Bradshaw S, Jamieson AC, McCaffrey AP. Zinc-finger nucleases as a novel therapeutic strategy for targeting hepatitis B virus DNAs. Mol Ther 2010;18(5):947–954

16 Händel EM, Gellhaus K, Khan K, et al. Versatile and efficient genome editing in human cells by combining zinc-finger nucleases with adeno-associated viral vectors. Hum Gene Ther 2012;23 (3):321–329

17 Zimmerman KA, Fischer KP, Joyce MA, Tyrrell DL. Zinc finger proteins designed to specifically target duck hepatitis B virus covalently closed circular DNA inhibit viral transcription in tissue culture. J Virol 2008;82(16):8013–8021

18 Zhao X, Zhao Z, Guo J, et al. Creation of a six-fingered artificial transcription factor that represses the hepatitis B virus HBx gene integrated into a human hepatocellular carcinoma cell line. J Biomol Screen 2013;18(4):378–387

19 Belloni L, Allweiss L, Guerrieri F, et al. IFN-α inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest 2012;122(2):529–537

20 Nash KL, Alexander GJ, Lever AM. Inhibition of hepatitis B virus by lentiviral vector delivered antisense RNA and hammerhead ribozymes. J Viral Hepat 2005;12(4):346–356

21 Welch PJ, Tritz R, Yei S, Barber J, Yu M. Intracellular application of hairpin ribozyme genes against hepatitis B virus. Gene Ther 1997;4(7):736–743

22 zu Putlitz J, Wieland S, Blum HE, Wands JR. Antisense RNA complementary to hepatitis B virus specifically inhibits viral replication. Gastroenterology 1998;115(3):702–713

23 McCaffrey AP, Nakai H, Pandey K, et al. Inhibition of hepatitis B virus in mice by RNA interference. Nat Biotechnol 2003;21 (6):639–644

24 Morrissey DV, Lockridge JA, Shaw L, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nat Biotechnol 2005;23(8):1002–1007

25 Klein C, Bock CT, Wedemeyer H, et al. Inhibition of hepatitis B virus replication in vivo by nucleoside analogues and siRNA. Gastroenterology 2003;125(1):9–18

26 Keaney J, Campbell M, Humphries P. From RNA interference technology to effective therapy: how far have we come and how far to go? Ther Deliv 2011;2(11):1395–1406

27 Deres K, Schröder CH, Paessens A, et al. Inhibition of hepatitis B virus replication by drug-induced depletion of nucleocapsids. Science 2003;299(5608):893–896

28 Wang XY, Wei ZM, Wu GY, et al. In vitro inhibition of HBV replication by a novel compound, GLS4, and its efficacy against adefovir-dipivoxil-resistant HBV mutations. Antivir Ther 2012;17 (5):793–803

29 Delaney WE IV, Edwards R, Colledge D, et al. Phenylpropenamide derivatives AT-61 and AT-130 inhibit replication of wild-type and lamivudine-resistant strains of hepatitis B virus in vitro. Antimicrob Agents Chemother 2002;46(9):3057–3060

30 Feld JJ, Colledge D, Sozzi V, Edwards R, Littlejohn M, Locarnini SA. The phenylpropenamide derivative AT-130 blocks HBV replication at the level of viral RNA packaging. Antiviral Res 2007;76(2): 168–177

31 Wang P, Naduthambi D, Mosley RT, et al. Phenylpropenamide derivatives: anti-hepatitis B virus activity of the Z isomer, SAR and the search for novel analogs. Bioorg Med Chem Lett 2011;21 (15):4642–4647

32 Billioud G, Pichoud C, Puerstinger G, Neyts J, Zoulim F. The main hepatitis B virus (HBV) mutants resistant to nucleoside analogs are susceptible in vitro to non-nucleoside inhibitors of HBV replication. Antiviral Res 2011;92(2):271–276

33 Katen SP, Chirapu SR, Finn MG, Zlotnick A. Trapping of hepatitis B virus capsid assembly intermediates by phenylpropenamide assembly accelerators. ACS Chem Biol 2010;5(12):1125–1136

34 Stray SJ, Zlotnick A. BAY 41-4109 has multiple effects on Hepatitis B virus capsid assembly. J Mol Recognit 2006;19(6):542–548

35 Weber O, Schlemmer KH, Hartmann E, et al. Inhibition of human hepatitis B virus (HBV) by a novel non-nucleosidic compound in a transgenic mouse model. Antiviral Res 2002;54(2):69–78

36 Brezillon N, Brunelle MN, Massinet H, et al. Antiviral activity of Bay 41-4109 on hepatitis B virus in humanized Alb-uPA/SCID mice. PLoS ONE 2011;6(12):e25096

37 Xu Y, Hu Y, Shi B, et al. HBsAg inhibits TLR9-mediated activation and IFN-alpha production in plasmacytoid dendritic cells. Mol Immunol 2009;46(13):2640–2646

38 Woltman AM, Op den Brouw ML, Biesta PJ, Shi CC, Janssen HL. Hepatitis B virus lacks immune activating capacity, but actively inhibits plasmacytoid dendritic cell function. PLoS ONE 2011;6(1): e15324

39 Mahtab MA, Bazinet M, Vaillant A. REP 9AC′: A second generation HBsAg release inhibitor with improved tolerability. Hepatology 2012;56:401A–402A

40 Patient R, Hourioux C, Roingeard P. Morphogenesis of hepatitis B virus and its subviral envelope particles. Cell Microbiol 2009;11 (11):1561–1570

41 Block TM, Lu X, Platt FM, et al. Secretion of human hepatitis B virus is inhibited by the imino sugar N-butyldeoxynojirimycin. Proc Natl Acad Sci U S A 1994;91(6):2235–2239

42 Block TM, Lu X, Mehta AS, et al. Treatment of chronic hepadnavirus infection in a woodchuck animal model with an inhibitor of protein folding and trafficking. Nat Med 1998;4(5):610–614

43 Dougherty AM, Guo H, Westby G, et al. A substituted tetrahydro-tetrazolo-pyrimidine is a specific and novel inhibitor of hepatitis B virus surface antigen secretion. Antimicrob Agents Chemother 2007;51(12):4427–4437

44 Yu W, Goddard C, Clearfield E, et al. Design, synthesis, and biological evaluation of triazolo-pyrimidine derivatives as novel inhibitors of hepatitis B virus surface antigen (HBsAg) secretion. J Med Chem 2011;54(16):5660–5670

45 Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012;61(12):1754–1764

46 Ilan Y, Nagler A, Adler R, Tur-Kaspa R, Slavin S, Shouval D. Ablation of persistent hepatitis B by bone marrow transplantation from a hepatitis B-immune donor. Gastroenterology 1993;104(6): 1818–1821

47 Lau GK, Suri D, Liang R, et al. Resolution of chronic hepatitis B and anti-HBs seroconversion in humans by adoptive transfer of immunity to hepatitis B core antigen. Gastroenterology 2002;122 (3):614–624

48 Penna A, Laccabue D, Libri I, et al. Peginterferon-α does not improve early peripheral blood HBV-specific T-cell responses in HBeAg-negative chronic hepatitis. J Hepatol 2012;56(6): 1239–1246

49 Micco L, Peppa D, Loggi E, et al. Differential boosting of innate and adaptive antiviral responses during pegylated-interferon-alpha therapy of chronic hepatitis B. J Hepatol 2013;58(2):225–233

50 Byrnes-Blake KA, Pederson S, Klucher KM, et al. Pharmacokinetics and pharmacodynamics of pegylated interferon lambda-1 in cynomolgus monkeys. J Interferon Cytokine Res 2012;32(5):198–206

51 Witte K, Gruetz G, Volk HD, et al. Despite IFN-lambda receptor expression, blood immune cells, but not keratinocytes or melanocytes, have an impaired response to type III interferons: implications for therapeutic applications of these cytokines. Genes Immun 2009;10(8):702–714

52 Chan H, et al. Peginterferon lambda, a new potential therapeutic option for the treatment of chronic hepatitis B: a phase 2B comparison with peginterferon alfa in patients with HBeAg-positive disease. Hepatology 2012;56:1525A–1526A

53 Ji C, Sastry KS, Tiefenthaler G, et al. Targeted delivery of interferon-α to hepatitis B virus-infected cells using T-cell receptor-like antibodies. Hepatology 2012;56(6):2027–2038

54 Elsaesser H, Sauer K, Brooks DG. IL-21 is required to control chronic viral infection. Science 2009;324(5934):1569–1572

55 Fröhlich A, Kisielow J, Schmitz I, et al. IL-21R on T-cells is critical for sustained functionality and control of chronic viral infection. Science 2009;324(5934):1576–1580

56 Yi JS, Du M, Zajac AJ. A vital role for interleukin-21 in the control of a chronic viral infection. Science 2009;324(5934):1572–1576

57 Pellegrini M, Calzascia T, Toe JG, et al. IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. Cell 2011;144(4):601–613

58 Nanjappa SG, Kim EH, Suresh M. Immunotherapeutic effects of IL-7 during a chronic viral infection in mice. Blood 2011;117 (19):5123–5132

59 Publicover J, Goodsell A, Nishimura S, et al. IL-21 is pivotal in determining age-dependent effectiveness of immune responses in a mouse model of human hepatitis B. J Clin Invest 2011;121 (3):1154–1162

60 Kennedy PT, Sandalova E, Jo J, et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143(3):637–645

61 Hashmi MH, Van Veldhuizen PJ. Interleukin-21: updated review of phase I and II clinical trials in metastatic renal cell carcinoma, metastatic melanoma and relapsed/refractory indolent non-Hodgkin's lymphoma. Expert Opin Biol Ther 2010;10(5): 807–817

62 Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol 2011;11(5):330–342

63 Zhang X, Kraft A, Broering R, Schlaak JF, Dittmer U, Lu M. Preclinical development of TLR ligands as drugs for the treatment of chronic viral infections. Expert Opin Drug Discov 2012;7(7): 597–611

64 Menne S, et al. Anti-viral efficacy and induction of an antibody response against surface antigen with the TLR7 agonist GS-9620 in the woodchuck model of chronic HBV infection. J Hepatol 2011;54: S441

65 Lanford RE, Guerra B, Chavez D, et al. GS-9620, an oral agonist of toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanze. Gastroenterology 2013 Feb 13 [Epub ahead of print]

66 Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005;5(3):215–229

67 Boni C, Penna A, Bertoletti A, et al. Transient restoration of antiviral T-cell responses induced by lamivudine therapy in chronic hepatitis B. J Hepatol 2003;39(4):595–605

68 Boni C, Laccabue D, Lampertico P, et al. Restored function of HBV-specific T-cells after long-term effective therapy with nucleos(t) ide analogues. Gastroenterology 2012;143(4):963–973

69 Xu DZ, Zhao K, Guo LM, et al. A randomized controlled phase IIb trial of antigen-antibody immunogenic complex therapeutic vaccine in chronic hepatitis B patients. PLoS ONE 2008;3(7): e2565

70 Bertoletti A, Gehring A. Therapeutic vaccination and novel strategies to treat chronic HBV infection. Expert Rev Gastroenterol Hepatol 2009;3(5):561–569

71 Kemmler CB, et al. Recombinant yeast therapeutic vaccines expressing hepatitis B virus (HBV) X, S, and core antigens generate antigen specific T-cell responses in immune samples from healthy and chronic HBV donors. Hepatology 2012;56: 373A–374A

72 Guo Z, et al. Recombinant yeast therapeutic vaccines expressing hepatitis B virus (HBV) X, S and core antigens generate antigen specific T-cell responses and tumor protection in mice. Hepatology 2012;56:375A–376A

73 Crawford A, Wherry EJ. The diversity of costimulatory and inhibitory receptor pathways and the regulation of antiviral T cell responses. Curr Opin Immunol 2009;21(2):179–186

74 Fisicaro P, Valdatta C, Massari M, et al. Combined blockade of programmed death-1 and activation of CD137 increase responses of human liver T-cells against HBV, but not HCV. Gastroenterology 2012;143(6):1576–1585, e4

75 Fisicaro P, Valdatta C, Massari M, et al. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. Gastroenterology 2010;138(2): 682–693, e1–e4

76 Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366 (26):2443–2454

77 Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366(26):2455–2465

78 Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363(8):711–723

79 Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 2006;12(11):1301–1309

80 Ejrnaes M, Filippi CM, Martinic MM, et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 2006;203(11):2461–2472

81 Tinoco R, Alcalde V, Yang Y, Sauer K, Zuniga EI. Cell-intrinsic transforming growth factor-beta signaling mediates virus-specific CD8+ T-cell deletion and viral persistence in vivo. Immunity 2009;31(1):145–157

82 Peppa D, Micco L, Javaid A, et al. Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathog 2010;6(12):e1001227

83 Akhurst RJ, Hata A. Targeting the TGFβ signalling pathway in disease. Nat Rev Drug Discov 2012;11(10):790–811

84 Manigold T, Racanelli V. T-cell regulation by CD4 regulatory T-cells during hepatitis B and C virus infections: facts and controversies. Lancet Infect Dis 2007;7(12):804–813

85 Rech AJ, Mick R, Martin S, et al. CD25 blockade depletes and selectively reprograms regulatory T-cells in concert with immunotherapy in cancer patients. Sci Transl Med 2012;4(134): 34ra62

86 Sutmuller RP, Morgan ME, Netea MG, Grauer O, Adema GJ. Toll-like receptors on regulatory T-cells: expanding immune regulation. Trends Immunol 2006;27(8):387–393

87 Otano I, Suarez L, Dotor J, et al. Modulation of regulatory T-cell activity in combination with interleukin-12 increases hepatic tolerogenicity in woodchucks with chronic hepatitis B. Hepatology 2012;56(2):474–483