Review

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New antiviral targets for innovative treatment concepts for hepatitis B virus and hepatitis delta virus

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Summary

Current therapies of chronic hepatitis B (CHB) remain limited to pegylated-interferon-alpha (PegIFN-α) or any of the five approved nucleos(t)ide analogues (NUC) treatments. While viral suppression can be achieved in the majority of patients with the high-barrier-to-resistance new-generation of NUC, i.e. entecavir and tenofovir, HBsAg loss is achieved by PegIFN-α and/or NUC in only 10% of patients, after a 5-year follow-up. Attempts to improve the response by administering two different NUC or a combination of NUC and PegIFN-α have not provided a dramatic increase in the rate of *functional cure*. Because of this and the need of long-term NUC administration, there is a renewed interest regarding the understanding of various steps of the HBV replication cycle, as well as specific virus-host cell interactions, in order to define new targets and develop new antiviral drugs. This includes a direct inhibition of viral replication with entry inhibitors, drugs targeting cccDNA, siRNA targeting viral transcripts, capsid assembly modulators, and approaches targeting the secretion of viral envelope proteins. Restoration of immune responses is a complementary approach. The restoration of innate immunity against HBV can be achieved, with TLR agonists or specific antiviral cytokine delivery. Restoration of adaptive immunity may be achieved with inhibitors of negative checkpoint regulators, therapeutic vaccines, or autologous transfer of engineered HBV-specific T cells. Novel targets and compounds will readily be evaluated using both relevant and novel *in vitro* and *in vivo* models of HBV infection. The addition of one or several new drugs to current therapies should offer the prospect of a markedly improved response to treatments and an increased rate of *functional cure*. This should lead to a reduced risk of antiviral drug resistance, and to a decreased incidence of cirrhosis and hepatocellular carcinoma (HCC).

Keywords: Hepatitis B virus; Viral targets; Direct acting antivirals (DAA); Host-targeting antivirals (HTA); Immunotherapy.

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Abbreviations: CHB, chronic hepatitis B; NUC, nucleos(t)ide analogues; PegIFN-α, pegylated-interferon-alpha; cccDNA, covalently-closed-circular DNA; siRNA or RNAi, small interfering RNA; HBV, hepatitis B virus; TLR, toll-like receptor, HCC, hepatocellular carcinoma; WHO, world health organization; IFN, interferon; HBsAg, small envelope protein antigen; pgRNA, pregenomic RNA; HDV, hepatitis delta virus; CHD, chronic hepatitis D; DAA, direct acting antiviral; HTA, host-targeting antiviral; HBpol, HBV polymerase; TP, terminal protein domain; RT domain, reverse transcription domain; RNAse H, ribonuclease H; rcDNA, relaxed-circular DNA; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; HBc or Cp, core proteins; CpAM, core protein allosteric modulator; ETV, entecavir; cIAP, cellular inhibitors of apoptosis proteins; NK, natural killer; NKT, natural killer T cell; SVR, sustained virologic response; pDC, plasmacytoid dendritic cell; PRR, pathogen recognition receptor; RLR, RIG-like receptor; NLR, NOD-like receptor; IFN, interferon; TAM, tumor-associated macrophages; TCR, T cell receptor; CAR, chimeric antigen receptor; WHV, Woodchuck hepatitis virus.

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Introduction

Chronic hepatitis B virus (CHB) infections remain a major public health problem worldwide. Despite the availability of an efficient vaccine, the coverage rate remains unsatisfactory in highly endemic areas [1]. There are currently 250 million chronic carriers of the virus (World Health Organisation (WHO); fact-sheet n° 204), who are at a high risk of developing hepatocellular carcinoma (HCC) [2,3]. Indeed, CHB infections are the first cause of HCC worldwide, and HCC ranks 3rd in terms of cancer mortality. Presently, there are two main classes of antiviral drugs approved for CHB treatment. Pegylated interferon-alpha (PegIFN-α), administered subcutaneously for 48 weeks, can induce viral suppression in approximately 25% of patients, but it is associated with side effects [4,5]. The administration of Nucleos(t)ide analogues (NUC) achieves a stronger viral suppression in the majority of patients, when using drugs with both high antiviral potency and barrier to resistance, i.e. tenofovir or entecavir [4,5]. Antiviral drug resistance remains an issue in many highly endemic countries, because of the former or current use of less expensive drugs, which have a low barrier to resistance and have generated the selection of resistant strains. The requirement for long-term NUC administration is also a problem for healthcare management in these countries. The safety profile of NUC is generally excellent, thus allowing long-term therapy and thereby preventing relapse of viral replication

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as well as re-occurrence of liver damage. Several cohort studies have shown that interferon (IFN) or NUC based antiviral therapy is associated with a decreased risk of HCC, but not with its elimination. Because of the cohort heterogeneity in these studies, in terms of infection duration, disease severity, and duration of treatment, it is not yet clear which patient population benefit the most from these treatments [6]. The major clinical feature of a favorable outcome is the loss of serum small envelope antigen (HBsAg), which allows the interruption of therapy, and is associated with a decreased risk of developing HCC, especially when it occurs at a young age. Unfortunately, based on the long-term follow-up of patients, current treatments achieve HBsAg clearance in a mere 10% of patients [4,5].

In order to foster the management of this deadly infection, it is critical to develop new antiviral strategies achieving more than viral suppression, i.e. *functional cure* of the infection. This would facilitate the implementation of antiviral treatments over a finite period of time, potentially reducing their cost, increase drug accessibility to populations living in highly endemic areas, and impact HCC development.

Key point

Chronicity of HBV infection is mainly due to the persistence of viral cccDNA and to defective immune responses.

HBV is a non-cytopathic DNA virus, which belongs to the *Hepadnaviridae* family [7]. Viral persistence relies on a covalently-closed-circular DNA (cccDNA) located within the nucleus of infected hepatocytes. cccDNA binds to histones to form a viral mini-chromosome, and is the template for all viral RNA transcriptions, including the viral pregenomic RNA (pgRNA) [8]. pgRNA is packed and reverse-transcribed within the nucleocapsids, which are subsequently used for the formation of virions or recycling to the nucleus for cccDNA maintenance [7,8]. The pathobiology of the infection mainly involves host immune responses required to control viral replication. HBV has evolved mechanisms to evade both innate and adaptive immune responses in order to establish persistent infections [9]. This has implications regarding the development of a more efficient treatment, which would impair immune evasion.

Key point

Novel antiviral treatments are necessary to increase the rate of functional cure of the infection, thereby allowing treatments with a finite duration and an expected benefit in terms of prevention of liver disease complications.

Based on current knowledge, the following definitions for an "HBV cure" have been summarized by the scientific community [10] (Fig. 1):

i) *Functional cure* (equivalent to resolved acute infection): HBsAg loss with or without anti-HBs seroconversion, with undetectable serum DNA, but persistence of cccDNA, which is not transcriptionally active, allowing treatment cessation. This implies that residually infected cells are controlled by host antiviral immunity. From a pragmatic point of view, the achievement of higher rates of HBsAg loss has become a major aim in on-going and future clinical trials.

ii) *Complete cure:* as for functional cure, but with the physical elimination of cccDNA.

It is worth noting, that even with the achievement of functional or complete cure, the persistence of integrated viral sequences in the host genome and molecular damage in the infected hepatocytes may represent an issue for a more complete prevention of HBV-induced HCC, which will need to be evaluated by future translational studies.

Major advances have been made in the last few years to improve our understanding of several key steps of the viral cycle (Fig. 2) and viral interplay with the immune system, most of which are reviewed in the present issue of the *Journal*. These include the identification of cellular receptor for viral entry, the determination of key nuclear enzymes involved in cccDNA formation, the discovery of the partial cccDNA degradation induced by IFN or NF-κB signaling pathways, as well as a better understanding of the mechanism involved in HBV-specific T cell exhaustion. Improved experimental models have also been established to study HBV replication and pathobiology in appropriate *in vitro* hepatocyte culture systems and animal models.

It is noteworthy that an estimated 15–20 million of HBV positive patients are co-infected with the hepatitis delta virus (HDV) worldwide [11]. Chronic HDV infection (CHD), as one of the most severe form of viral hepatitis, is known to accelerate the progression of chronic hepatitis B towards cirrhosis and its ensuing complications. Nevertheless, HDV infection is still considered as an orphan disease. The only available drug effective against chronic HDV infection is PegIFN-α, efficient in only 20–35% of patients [12]. Therefore, specific drug discovery efforts are needed to improve treatment of chronic HDV [13,14]. Research efforts aimed at curing HBV might also lead to improving the response to the treatment of CHD.

In this manuscript, we review investigational and early clinical efforts regarding the identification and characterization of antiviral targets that are being evaluated for the development of innovative treatment concepts for chronic HBV and HDV infections.

Direct acting antivirals

Key point

Direct acting antivirals targeting different steps of the viral life cycle, i.e. viral entry, cccDNA, viral transcripts, HBx, virus packaging and egress are under investigation.

Drug developers are in general more inclined to design direct acting antivirals (DAAs), inhibiting viral enzymatic activities or viral protein functions (Fig. 2), since DAA are less prone to adverse effects. Conversely, host-targeting agents (HTA), which are meant to inhibit a host cell function involved in the virus life cycle, may potentially lead to more undesirable effects. Regarding HBV, the only success in drug development

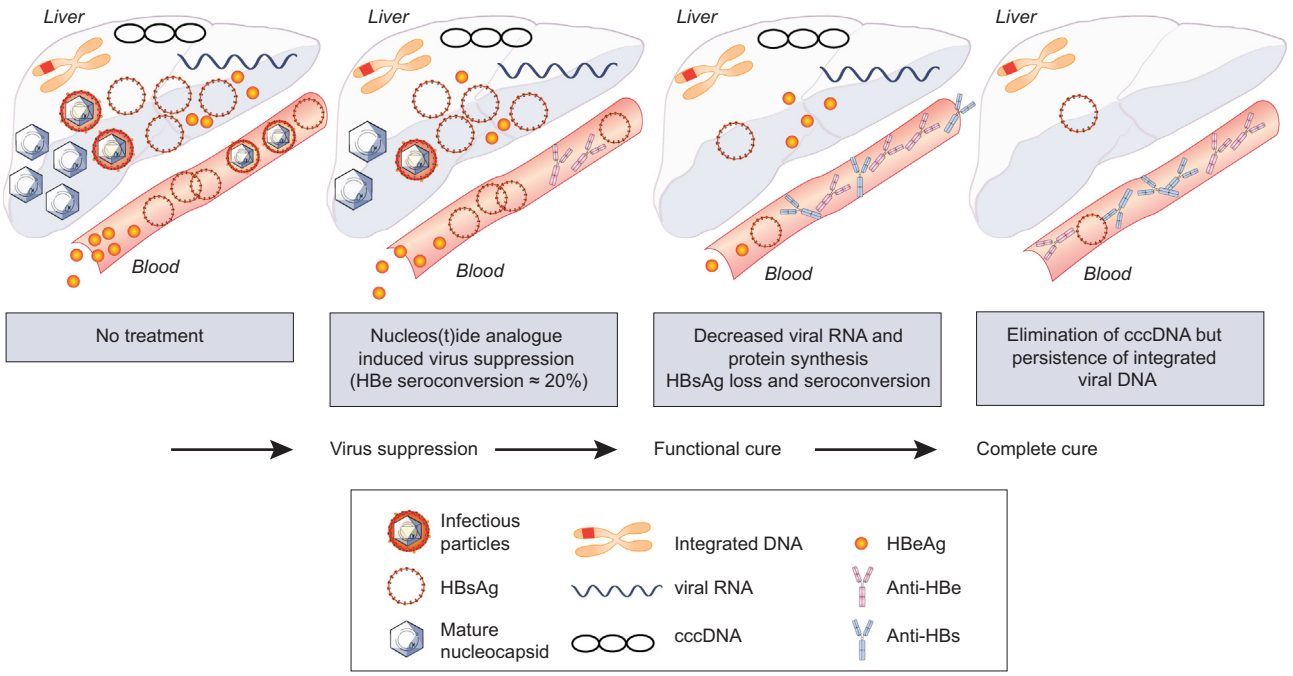

Fig. 1. Schematical representation of various types of "cure". When no treatment is applied in HBe+ patients, all virologic parameters are detectable in both liver and blood compartment (top left panel). When a NUC treatment is applied, all DNA containing particles are almost completely eliminated from bloodstream, and are strongly reduced inside the liver; this corresponds to virus-suppression. In this case sero-conversion from HBeAg to anti-HBeAb is a rare event (top right panel). A reduction of HBsAg levels by specific mean or reduction of intracellular viral RNA and protein synthesis could lead to functional cure (bottom left and middle panels). A complete cure would be obtained when eradication of cccDNA would be obtained (bottom right panel).

targeted the HBV polymerase (HBpol). HBpol is a multi-domain protein featuring terminal protein (TP), spacer, reverse transcriptase (RT), and ribonuclease H (RNAseH) subdomains [7]. So far, only drugs targeting the RT activities (i.e. priming and polymerization) have been developed [15]. One particular feature of the synthesis of genomic HBV DNA (relaxed circular (rc)DNA) from pgRNA is its tight link with the encapsidation process [7]. Inhibition of capsid formation could, therefore, complement polymerase inhibitors. Alternatively, RNAseH inhibitors could also be used to develop combinatory approaches targeting this step of the viral life cycle [16].

Targeting reverse transcription within the viral nucleocapsid

Prodrugs of HBV polymerase and other polymerase inhibitors

Tenofovir alafenamide fumarate (TAF) is a nucleotide RT inhibitor and a novel prodrug of tenofovir (TDF) [17]. It is under development for use in the treatment of chronic HIV and HBV infections. TAF was shown to be highly bioavailable, stable in plasma and suitable for the efficient delivery of its active form (TDF-diphosphate) to hepatocytes and lymphoid tissues, allowing lower doses of TDF to be used and reducing systemic exposures of TDF. In phase-1b clinical studies, no differences in viral load were observed after the administration of 8–120 mg of TAF, and the level of viral suppression over 4 weeks was similar to TDF (refer to NCT01671787 at clinicaltrials.gov). Yet, this drug seems to have a better safety profile in the long-term, in particular with respect to nephrotoxicity, and is currently evaluated in phase-3 clinical trials (NCT01940471 and NCT01940341). Other prodrugs are currently under clinical development (Table 1).

Despite the availability of such potent NUC, drug development could be improved by focusing polymerase inhibitors that target not only RNA- and DNA-dependent DNA synthesis, but also the priming of reverse transcription in order to maximize the effect on the replenishment of the cccDNA pool [15].

Is there a place for RNAseH inhibitors?

HBV ribonuclease H (RNaseH) activity is essential for viral replication, but it has so far not been exploited as a drug target [16]. Recent low-throughput screening of compound classes with anti-HIV RNaseH activity led to the identification of HBV RNaseH inhibitors from three different chemical families blocking HBV replication [18]. These inhibitors are, thus, interesting candidates

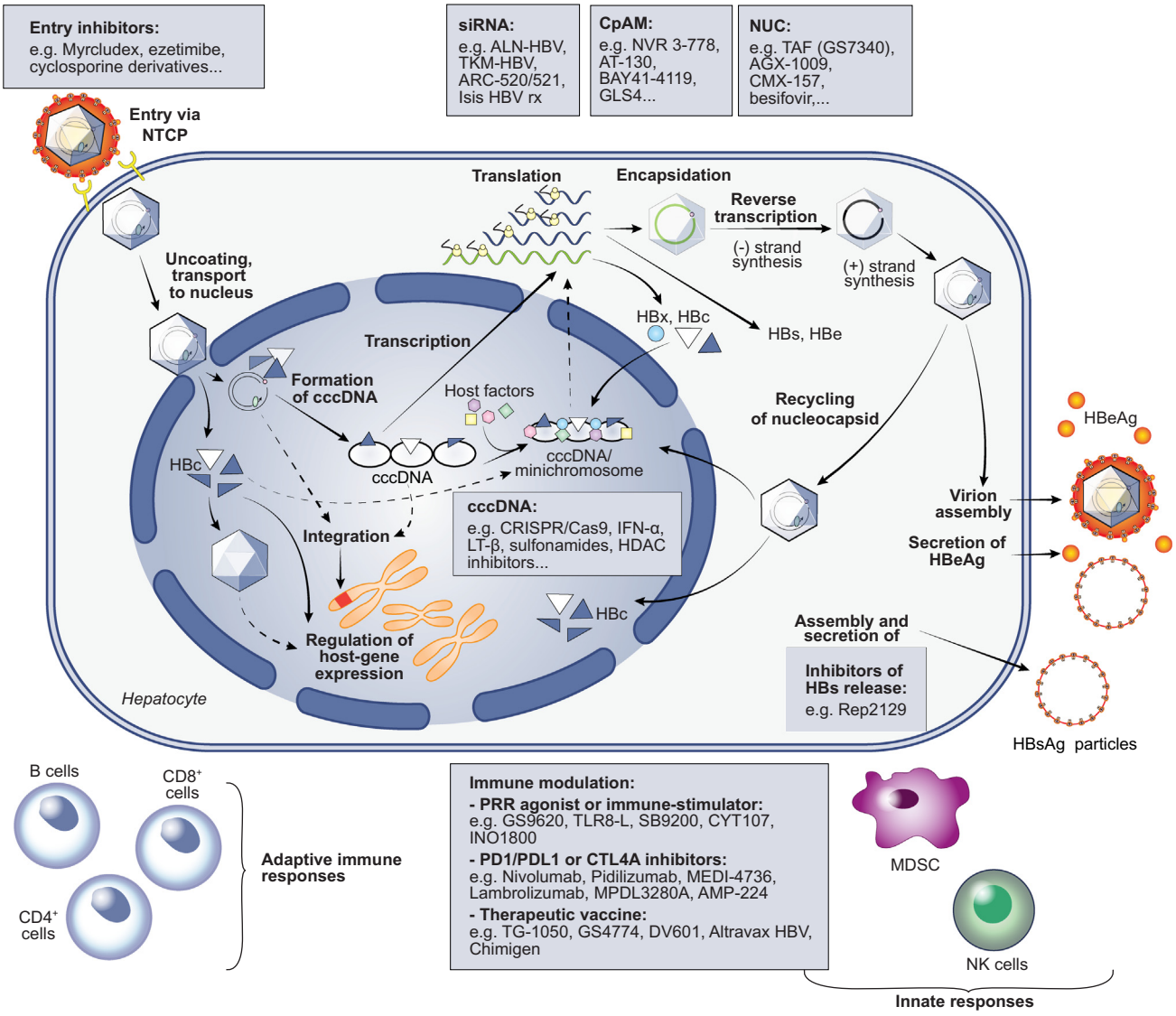

Fig. 2. HBV life cycle and main classes of antivirals in development.

for developing new anti-HBV drugs, and could be used in combination with existing anti-HBV drugs and/or with other novel inhibitors under development, to improve treatment efficacy. The safety profile of these RNAseH inhibitors will need to be carefully evaluated to continue their development.

Core allosteric modulators (CpAM)

The core/HBc/Cp protein of HBV has recently re-emerged as a promising direct antiviral target. Indeed, this multifunctional HBV protein plays a role in the cytoplasmic "encapsidation" process [7], as well as in the nucleus of infected cells for the regulation of cccDNA and host-gene expression [19–21]. Previous findings established HBc could be localized in the nucleus of infected hepatocytes in liver samples from chronically infected patients (see for instance [22]). These observations were confirmed *in vitro*, since it was shown that HBc could bind to cccDNA [20,23] and to host-genes, including those involved in the innate immune response [19]. Once bound, it was shown to modulate gene expression by recruiting histone methyltransferases (e.g. EZH2) responsible for the modulation of repressive epigenetic marks (e.g. H3K27me3) [19]. Therefore, capsid inhibitors or the HBc allosteric modulator (CpAM) may have several functions, beside the "canonical" inhibition of nucleocapsid assembly in the cytoplasm [23].

Thanks to the knowledge of the 3-dimensional structure of HBc [24], several classes

Table 1. A summary of clinical trials and their strategies for HBV treatment.

	Targets	Compounds	Developer	Stage of development	ClinicalTrials.gov identifier
DAA	HBpol	GS-7340; Tenofovir Alafenamide (prodrug of tenofovir)	Gilead	Phase 3	NCT01940471 and NCT01940341
	HBpol	AGX-1009 (prodrug)	Agenix	Phase 3 (?)	No identifier found
	HBpol	Besifovir	IlDong Pharmaceutical	Phase 3	NCT01937806
	HBpol	CMX-157 (lipid acyclic nucleoside phosphonate)	Contravir	Phase 1	NCT02585440
	HBc	GLS-4 (Morphothiadine mesilate)	HEC Pharm/SUnshine	Phase 2	China-CFDA
	HBc	NVR 3-778	Novira Pharmaceuticals	Phase 1	NCT02112799 & NCT02401737
	HBs	REP-2139 (nucleic acid polymers)	Replicor	Phase 2 for both HBV and HDV	NCT02565719 and NCT02233075
	Viral RNAs	siRNA: ARC-520/ARC-521	Arrowhead	Phase 2	NCT02604212 and NCT02604199
	Viral RNAs	siRNA: ISIS-HBVRx	Ionis pharmaceuticals	Phase 1 or 2 (?)	No identifier found
HTA	NTCP	Myrcludex	Hepatera and MYR GmbH	Phase 2 for both HBV and HDV	Development in Russian Federation
	Promotion of apoptosis in infected cells	Birinapant	Tetralogic	Phase 1	NCT02288208
	Prenylation/farnesylation	Lonafarnib	Eiger BioPharmaceuticals	Phase 2 for HDV	NCT02430181, NCT02430194, NCT02511431
	Immune stimulation	Thymosin alpha	Seoul National University Hospital	Phase 4	NCT00291616
	pDC stimulation	GS-9620 (TLR7 agonist)	Gilead	Phase 2	NCT02166047 & NCT02579382
	Immune stimulation	INO-1800	Inovio Pharmaceuticals	Phase 1	NCT02431312
	Immune stimulation	Cyt-107 (IL-7)	Cythesis	Phase 1/2 (discontinued)	NCT01027065
	Immune stimulation	IFN-lambda	BMS	Phase 2 (discontinued)	NCT01204762
	Adaptive responses	ABX-203	Abivax	Phase 2/3	NCT02249988
	Adaptive responses	GS-4774 (therapeutic vaccine)	Gilead	Phase 2	NCT01943799 & NCT02174276
	Adaptive responses	TG-1050 (therapeutic vaccine)	Transgene	Phase 1	NCT02428400
	Adaptive responses	DV-601 (therapeutic vaccine)	Dynavax	Phase 1	NCT01023230
	Adaptive response	HB-110	Genexine	Phase 1	NCT01641536
	Adaptive responses	Nivolumab (Anti-PD1 mAb)	Ono Pharmaceuticals/ BMS	Phase 1/2 for HCC	NCT01658878

of non-nucleosidic small molecules, amongst which phenylpropenamides (e.g. AT-130; [25]) and heteroaryldihydropyrimidines (HAP; e.g. BAY41-4109; [26]) derivatives have been developed. These molecules inhibit either pgRNA encapsidation or capsid formation, leading to the arrest in the neo-synthesis of viral rcDNA, since reverse transcription of pgRNA only occurs in the capsids [27]. Interestingly, these CpAM can inhibit the replication of HBV mutants resistant to NUC [28–30], and are less likely to foster the development of specific resistance, due to the natural pressure linked with capsid assembly.

To date three CpAMs are under early clinical development: BAY41-4109 (Bayer; development on hold), NVR 3-778 (NCT02112799; Novira Therapeutics), and GLS-4 (trial with China-CFDA; Morphothiadine Mesilate, HEC Pharm) (Table 1), while several others are the focus of pre-clinical evaluations and should enter clinical trials soon [27].

Is the complete suppression of viral DNA synthesis important?

cccDNA is initially formed in the hepatocytes from rcDNA contained in viruses either upon entry or when a persistent infection is established. Replenishment of cccDNA occurs through the recycling of nucleocapsids to the nucleus of infected cells.

While it was shown that NUC administration was successful in reducing viral load, it did not prevent the formation of cccDNA from incoming virions. Unfortunately, the absence of complete viral suppression may lead to residual viremia,

with levels below the detection threshold of current commercial PCR assays, which in turn can cause the infection of new hepatocytes and the formation of a new pool of cccDNA. Incomplete suppression of viral DNA synthesis may also account for the continuing replenishment of established cccDNA within a single infected cell.

Several recent clinical studies have shown the persistence of 1) detectable HBV DNA in serum using sensitive PCR assay despite being non-quantifiable [31] and 2) of intrahepatic HBV DNA synthesis after long-term TDF administration (Boyd *et al.* J. Hepatol., *in revision*).

Overall, these observations substantiate the need for improving the development of treatments inhibiting the replication of viral genome within infected hepatocytes. This could be achieved either by enhancing the antiviral activity of NUC, or by combining NUC with other viral replication inhibitors, such as RNAseH inhibitors, CpAMs, or other DAAs.

Targeting viral protein expression and/or function

An issue regarding the design of drugs targeting viral replication *per se*, is that, in the absence of an effect on cccDNA, HBV proteins continue to be produced and exert their pathogenic and/or immunopathologic effects. This includes the secretion of HBV antigens (e.g. HBsAg and HBeAg) into the bloodstream, which seem to play a crucial role in the immune evasion [9], or the expression of HBx, which was shown to be required for the initiation of infection [32] and to contribute to oncogenic events [33]. The complete and concomitant inhibition of all viral protein synthesis could represent a powerful treatment strategy. This could be achieved by focusing on the development of therapies targeting the shared 3' end of HBV transcripts, possibly through RNA interference.

RNA interference (RNAi)

The use of RNAi to inhibit the replication of HBV has been extensively conducted *in vitro*, was validated in animal models, and is the subject of a recent thorough review [34]. The *in vivo* delivery of HBV-specific small interfering RNA (siRNA) to infected hepatocytes was the main challenge raised by these studies. Indeed siRNA are small, double-stranded oligoribonluceotides, which are hydrophilic and negatively charged, and are, therefore, difficult to deliver to the cytosol where the RNA-induced silencing complex resides. Various methodologies are currently used *in vivo* to deliver siRNA to hepatocytes [34], including i) the use of cationic/neutral-lipid nucleic acid nanoparticles, ii) conjugation to a chemical moiety capable of interacting with a given hepatocyte receptor (i.e. N-acetyl galactosamine as a ligand of asialoglycoprotein receptor), and iii) the use of dynamic polyconjugates (DPC), where siRNA is conjugated to cholesterol and co-injected with an hepatotropic cell penetrating peptide [34]. Based on these technologies, three siRNA molecules are (or will soon be) under clinical trial, including ARC-520 (phase-2: NCT02604212 and NCT02604199; Arrowhead Research Corporation), TKM-HBV (Tekmira/Arbutus Biopharma), and ALN-HBV (Alnylam) (Table 1).

ARC 520 was evaluated in several chimpanzee studies. In one such study, NUC was initially administered leading to a decrease in the total amount of liver HBV DNA by 1.1–2.5 $\log_{10}$, and in cccDNA by 0.7 ± 0.6 $\log_{10}$ in HBeAg+ chimpanzees. The effect was negligible in HBeAg- chimpanzees. Following the addition of ARC-520 in HBeAg+ individuals, the amount of total liver DNA and cccDNA decreased compared to the baseline by 1.5–2.9 $\log_{10}$ and 1.4 ± 0.7 $\log_{10;}$ respectively. The reduction was largely correlated with the duration of the treatment. Neither total HBV DNA nor cccDNA levels changed remarkably in HBeAg– animals during the study [35]. This raised the issue of integrated HBV DNA with viral transcripts harboring an altered 3' end, therefore not targeted by the siRNA that could trigger HBsAg production, and led to the readjustment of the sequence targeted by siRNA via arrowhead. ARC-520 has also been evaluated in phase-2 clinical trials in entecavir naïve or exposed CHB patients treated with a single dose. An intravenous administration of up to 4 mg/kg did not result in any significant adverse effects, and led to a maximum reduction of HBsAg by 1.5 $\log_{10}$ in HBeAg+ patients *vs.* 0.5 $\log_{10}$ in HBeAg- patients [36]. ARC-520 produced deep and durable knockdown of viral antigens and DNA in a phase 2 study in patients with chronic hepatitis B. This is the first proof-of-concept regarding the use of siRNAs as therapeutic molecules in CHB patients.

Three increasing doses (amount injected reaching 0.5 mg/kg) of ALN-HBV siRNA were administered, at days 0, 21 and 42, to 4 chimpanzees, and resulted in an average 2-log10 reduction in the level of HBsAg (at day-60) (unpublished data; c.f. available proprietary data disclosure http://www.alnylam.com/web/assets/ALN-HBV_RNAi_Roundtable_072815.pdf). Furthermore, recently disclosed results showed that the administration of 3 weekly doses of 3 mg/kg of ALN-HBV into AAV-HBV-transduced mice, reduced the average level of HBsAg by 2.9 log10, and that HBsAg knockdown persisted more than 100 days following the administration of the last dose [35]. Injections (n = 6) of 0.3 mg/kg of TKM-HBV over 28 days, also led to a reduction in the level of HBsAg (a 90% decrease) and,

interestingly, to a concomitant 50% reduction in cccDNA in these animals (unpublished data).

The difference between these siRNAs and their formulations in terms of antiviral efficacy across clinical situations, routes of administration, and side effects will be an important issue to address in the near future. Antisense oligonucleotides have also been tested for efficacy *in vivo* and *in vitro* using HBV transgenic and hydrodynamic transfection mouse and cell culture HBV infection models, respectively [37].

Strategies aimed at reducing the level of HBsAg

Owing to the high amount of HBsAg circulating in CHB patients, the design of therapeutic monoclonal antibodies aimed at neutralizing and/or "titrating" HBsAg from the bloodstream may prove to be difficult or inefficient. Alternatively, a therapeutic approach could be to specifically prevent the secretion of HBsAg, however, this may also be unsuccessful since HBsAg uses canonical host cell secretory pathways. Nevertheless, Rep2139 (Replicor), a nucleic acid polymer (NAP), was shown to inhibit the secretion of HBsAg, through a yet unknown mechanism (Table 1). During clinical trials (NTC02233075), this drug administered in combination with PegIFN-α induced a significant decline in viremia and in the levels of HBsAg, resulting in a high rate of anti-HBsAb seroconversion in favorable HBsAg responders [35,38]. Further data are required to confirm these preliminary results and novel clinical trials have been registered (NCT02565719).

Targeting HBx functions

HBx is a multifunctional protein, which is essential for the initiation and maintenance of HBV infection *in vivo* and *in vitro* [39,32], mainly through mechanisms involved in the regulation of cccDNA transcriptional activity, inhibition of effectors of innate immunity, and degradation of host factors with antiviral properties [7]. Targeting HBx remains a difficult task, since it has no enzymatic activity. The only option is to target interactions at the interface with host partners. This was recently the subject of a study that reported that HBx was capable of interacting with and inducing the degradation of Smc5/6, a protein that restricts the transcription of cccDNA [40]. The HBx degradation observed resulted from the interaction between HBx and the DDB1-CUL4 ubiquitin machinery. It would be interesting to verify whether a drug interfering with either HBx-DDB1 or HBx-host factor interactions can be developed.

Elimination of cccDNA

The initial formation of cccDNA from rcDNA following nuclear delivery, and its maintenance by nucleocapsid recycling, represent important antiviral targets. The cellular and biochemical events required for this process involve (a) the transport of nucleocapsids to the nucleus and the transformation of rcDNA into cccDNA via the removal of the viral polymerase covalently linked to the viral antisense DNA strand, (b) the removal of the short RNA primer (used for the sense DNA strand synthesis), (c) the completion of sense DNA strands, and (e) the removal of the viral antisense DNA strand redundancy [8]. These steps include several host nuclear enzymes, for which it will be difficult to appoint a specific function in the viral life cycle [8]. Interestingly, it was recently reported that small molecules might specifically target cccDNA formation. Two structurally related disubstituted-sulfonamide compounds were identified and may potentially serve as proof-of-concept drug candidates to eliminate cccDNA from chronic HBV infection by preventing the initial formation and/or maintenance of cccDNA by nucleocapsid recycling, but not by degrading already formed cccDNA [41].

Therefore, one of the remaining major issues will be to determine if the established pool of cccDNA in chronically infected cells can be degraded. It was recently shown that IFN-α and lymphotoxin-β receptor agonists could, via their interaction with their respective receptors, up-regulate either APOBEC3A or APOBEC3B (two cytidine deaminases), which in turn induced non-hepatotoxic degradation of cccDNA [42]. Interestingly, it was proposed that HBV core proteins could mediate APOBEC3A/B recruitment onto cccDNA, resulting in cytidine deamination, apurinic/apyrimidinic site formation, and finally to a "supposedly specific" partial degradation of cccDNA without affecting host genome. However, this concept remains to be validated. Experimentally, it was recently shown that IFN-γ and TNF-α may degrade cccDNA in a non-cytolytic and APOBEC3-dependent manner [43], unveiling novel perspectives to achieve cccDNA degradation. The use of cccDNA specific meganuclease (or related sequence-specific homing endonucleases) delivered to infected cells by gene therapy could also be an interesting approach to degrade cccDNA [44,45]. An interesting proof-of-concept was made recently with the use of the CRISPR/Cas9 system, where it was shown that inhibition of replication was due to mutations and deletions in cccDNA similar to those observed with chromosomal DNA cleaved by Cas9 and repaired by nonhomologous end joining [44,46]. This demonstrated that Cas9 could be recruited to cccDNA, highlighting the possibility of developing future antiviral strategies aimed at targeting cccDNA via endonucleolytic cleavage. The risk of off-target effect will need to be assessed carefully in pre-clinical models, although recent advances in genetic diseases suggest that this approach

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might be applied to the clinic in a near future [47,48].

Host-targeting antivirals (except immune modulation)

Entry inhibitors

The discovery of a cellular receptor for the entry of HBV, namely hNTCP (human sodium taurocholate cotransporting polypeptide; also known as SLC10A1) [49], provided extremely valuable information for the development of entry inhibitors [50]. Prior to this discovery, it was shown that myristoylated preS peptide (Myrcludex B), a lipopeptide derived from the preS1 domain of the HBV envelope, could prevent HBV infection in hepatocyte cultures, as well as *in vivo* in liver humanized uPA/SCID mice [51]. Using the same mouse model it was reported that treatment with this molecule efficiently inhibited the establishment of HDV infection, which requires HBV envelopes for its infectivity. Retrospectively, it was interesting to see that the inhibition of viral entry by the preS peptide was due to its interaction with hNTCP. Furthermore, drugs that inhibit the function of hNTCP, such as cyclosporine**,** also decrease viral infectivity in cell culture models [50] (Table 1). Since the turnover and reinfection cycles of hepatocyte might be needed to maintain a persistent infection, this could make a reasonable case for the evaluation of such an entry inhibitor in the context of chronic infections. The efficacy of entry inhibitors in the treatment of CHB is currently being evaluated in clinical trials (clinical trials are run in the Russian Federation).

Silencing of cccDNA

Interfering with cccDNA-associated chromatin proteins is another exciting approach. Indeed, the acetylation and/or methylation status of the histones bound to cccDNA affect its transcriptional activity. It was shown, in cell culture and in humanized mice, that the administration of IFN induces cccDNA-bound histone hypoacetylation, as well as active recruitment onto the cccDNA of transcriptional co-repressors [52]. IFN-α treatment also reduced binding of the STAT1 and STAT2 transcription factors to active cccDNA. This may represent a molecular mechanism in which IFN-α mediates the epigenetic repression of cccDNA transcriptional activity, which may assist in the development of novel therapeutic strategies. A potential issue associated with the use of epigenome modifiers in oncology is their lack of specificity for viral genome sequences, which may lead to serious side effects. The identification of viral mechanisms involved in epigenetic regulation of cccDNA is, therefore, crucial. The role of HBx in this respect has been clearly established [32,40], whereas the role of HBc remains to be elucidated. Targeting these proteins as previously discussed could thus represent a more specific approach for cccDNA silencing.

Re-induction of cell apoptosis in HBV-infected cells?

In recent studies it was shown that blocking the activity of cellular inhibitors of apoptosis proteins (cIAP), which naturally antagonize the pro-apoptotic effect of TNF-α, could promote cell death and contribute to HBV clearance in hydrodynamically infected mice [53]. Interestingly, birinapant, a molecule that inhibits cIAP, was shown to favor the clearance of HBV in these mice by an apoptosis mediated mechanism associated with TNF-α production and activation of a HBV-specific CD4 T cell response [54]. This yet unexplored strategy is currently being studied in a phase1/2 clinical trial. It is worth noting that birinapant is in the phase-3 of its development for various cancers (e.g. ovarian, colorectal, lymphoma…); therefore, *in vivo* safety data are already available for this drug [55]. It has recently entered phase-1 trial in CHB patients (NCT02288208; TetraLogic Pharmaceuticals) (Table 1).

Immune restoration

It is obvious that restoration of HBV targeting immune responses will be an important component of the novel approaches aiming at "curing" the infection. Whether this may be achieved as a result of novel mechanisms of inhibition of viral replication or will require specific immunotherapeutic advances remains to be determined. A better understanding of the interplay between HBV and the immune system is, therefore, critical, since many studies have so far been conducted in non-relevant conditions (non-human models, transformed hepatocytes, or immune cells derived from blood and not from the liver). The findings derived from these studies show that clearance of HBV during an acute infection requires the infected individual to mount a broad (several specific antigens) and strong T cell response in a timely and orchestrated manner [56,57]. In contrast, in a CHB infection, there are anergic and/or exhausted phenotypes of specific T cells, due to i) a strong and long-term exposition to secreted HBV antigens (i.e. APC-induced T cell suppression), ii) an overall lack of activation of innate immune cells and a lack of relevant antigen presentation by

infected cells, and iii) a highly tolerogenic environment within the infected liver due to the over representation of immune-modulatory cells (e.g. T-reg, "M2-like" or suppressive myeloid cells, PD-L1-expressing cells etc.) and secretion of tolerogenic molecules (e.g. IL-10, TGF-β, k, adenosine etc.) [56,57]. Moreover, HBV-specific T cells could be anergised/exhausted by negative signals transmitted by PD-1/PD-L1, CTLA-4/B7-1, or Tim3/Gal9 interactions. Beside a poor cytotoxic activity and impaired cytokine production of effector T cells, it has been also shown that other cell types including natural killer (NK)/NKT dendritic cells were impaired in their functions and cross talks, thus contributing to the overall lack of immune response against the virus in a chronic situation [58,59]. Some practical and theoretical aspects regarding immunotherapy of CHB and the need to consider the immunological microenvironment of the liver are addressed below.

A better use of IFN-α based either on the identification of better pre-treatment predictive biomarkers of response and/or on improved combinations of PegIFN-α NUCs is currently evaluated [60,61]. As CHB patients treated with PegIFN-α or with NUC showed partial restoration of NK/NKT or T cell functions, respectively [62], a combined therapy could be beneficial in a subset of patients, highlighting the need for biomarkers of immune restoration to identify the right patients and the right timing to ensure the success of combination therapies

Restoration of innate immune functions

Other IFNs and cytokines

Other IFNs, including modified IFN-α, IFN-β, IFN-γ, type-III IFN, as well as other cytokines/chemokynes (e.g. TNFα, IL-1β, IL-6, IL-2, IL-12, IL-18 etc.) were shown to have a direct or indirect effect on HBV replication in *in vitro* and in animal models (see [63] for a recent review). However, none of these molecules have been successfully tested or have shown a higher efficacy (cases of IFN-β and IFN-λ) than IFN-α in clinical trials.

PRR agonists and other immune-stimulatory molecules

The therapeutic approach of injecting high amounts of recombinant cytokines has been largely disappointing since severe side effects due to systemic immune activation frequently occur. The local and targeted restoration of the endogenous production of antiviral cytokines could, on the other hand, represent an interesting approach [63]. This could lead, in the case of IFNs, to the production of a wider variety of factors (i.e. 14 types of IFN-α) and to a higher biologically active mixture of cytokines at the site of viral replication. Two types of cells are specialized in the productions of IFN-α and IFN-λ (type-III IFNs; λ1 to λ3) upon either TLR7/TLR9 or TLR3 agonisation, namely pDC and mDC-BDCA3+/CLEC9A+ cells [64,65]. When activated these cells can in turn activate other innate immune cells and *in fine* adaptive cells. Hence, agonist-induced activation of PRR in these cells could represent a novel approach for the treatment of CHB [66,67].

Effect of TLR7 agonisation via an orally delivered ligand. GS-9620, an oral agonist of TLR-7, was found to induce a strong anti-HBV effect, with a 2-log10 reduction in viremia in chimpanzees [68]. Surprisingly, in one animal, administration of GS-9620 led to an off-drug, long-term suppression of serum and liver HBV DNA, thus suggesting that cccDNA could be targeted. This was further confirmed in infected woodchucks [69] where it was shown that, using a larger test group and increasing injection doses, cccDNA could be reduced, leading to a sharp reduction in the levels of HBsAg and seroconversion to anti-HBs in many animals treated at the highest concentrations. A phase II trial is currently underway in patients where the combination of tenofovir and GS-9620 is compared to the effect of a tenofovir monotherapy (NCT02166047 & NCT02579382; Gilead), following the completion of several phase 1B studies (NCT01590641 & NCT01590654) [70] (Table 1).

A place for others PRR agonists? TLR7 agonists mainly act via plasmacytoid dendritic cell (pDC) activation and a type-I IFN mediation, since their receptors are scarcely, or not at all, expressed in other cell types, including hepatocytes bearing HBV replication. If such an agonist could have an interesting therapeutic effect, one could expect that an agonist targeting both specialized immune cells and infected hepatocytes, because they both express its receptor, could be a better choice. Indeed, it was shown that hepatocytes expressed functional TLR2 or TLR3, as well as other RIG-like, NOD-like, and many DNA sensors, thus unveiling the possibility of using their respective agonists [71,72]. A study dating back to 2005 revealed that in transgenic mice, many PRR agonists are able to induce an antiviral effect [73]. However, the model was not optimal, since cccDNA was not expressed. Recent data have suggested that HBV replication was sensitive to direct TLR1/2, TLR2/6, TLR3, TLR4, RIGI/MDA5, and STING agonisation in hepatocytes [74,75], thus revealing novel PRR agonists that could be tested in relevant animal models. Two aspects have to be taken into consideration regarding such an approach, namely i) the toxicity resulting

Key point

Strategies to induce restoration of innate immunity or adpative immune responses are under evaluation.

from a "cytokine storm" that may be provoked by a systemic injection, and ii) the ability of the virus to downregulate a given innate sensor, which could lead to a decreased efficiency. With respect to the latter, it was shown that, in HBe-positive CHB patients, the level of expression of TLR2 decreased in hepatocytes and myeloid cells [76], which could theoretically interfere with the function of TLR2-L. However, in the woodchuck model, long-term therapy with NUC was associated with the restoration of TLR2 expression [77]. Therefore, a therapy combining NUC and the TLR2 agonist could be envisaged to restore innate immune functions in the liver microenvironment [78].

Depletion/inhibition of immune-modulatory innate cells: the case of "M2" macrophages or MDSCs

It is well established that different innate immune cells can enhance the immune tolerance of cancer cells by tumor infiltration. The concept of tumor-associated-macrophages (TAM) has led to the development of strategies aimed at eliminating or re-differentiating these cells to break tolerance to cancer cells. Hence anti-CSF-1R antibodies have been successfully tested in tumors displaying massive TAM infiltration [79,80]. Such concepts could also be true in the case of chronic infections.

A recent ground breaking publication demonstrated that the number of myeloid-derived suppressive cells augmented in the liver of CHB patients, in particular those in the immune tolerant phase [81]. The increase in the number of suppressive cells was associated with a dampening of HBV-specific and bystander T cell responses, via a metabolic regulation involving the depletion of arginine by MDSC-secreted arginase. The elimination or re-differentiation (into antiviral myeloid cells) of such cells could represent an interesting approach to restore the function of T cells.

Restoration of adaptive immune functions

Many strategies have been undertaken to break tolerance to HBV in CHB patients by attempting to restore the functions of adaptive immune cells. The latest results obtained in this area of research are non-exhaustively summarized hereafter.

Therapeutic vaccination

The aim of a therapeutic vaccination is to restore/induce a specific T cell response by improving the quality/quantity of antigen presentation by professional antigen presenting cells, in a context where there are i) a massive production of antigens thought to be responsible for a systemic and local T cell exhaustion phenotype, and ii) a likely inadequate presentation of antigens by the HBV-replicating hepatocytes [82].

The initial strategies involving the use of recombinant HBV proteins to stimulate the production by B cells of virus-neutralizing antibodies, remained unsuccessful. The direct increase in HBV-specific CD8 T cells was attempted by using various formulations of recombinant HBV proteins, the injection of plasmid DNA encoding viral epitopes, and/or the transduction with viral vectors (e.g. vaccinia, adenovirus etc.). Prime-boost strategies also were undertaken to improve the response to therapeutic vaccination, but without real success in clinical trials (for an overview see [83,84]). The latest results in this domain published by Fontaine and colleagues, conducted in a phase-1/2 clinical trial, showed that an HBV envelope-expressing DNA vaccine administered in association with NUC could not decrease the risk of relapse after NUC cessation or the rate of virological breakthrough in HBV-treated patients, and did not restore an anti-HBV immune response despite the effective viral suppression by NUC [85]. It is probable that therapeutic vaccines would have to be used in combination with adjuvants/PRR agonists (to boost innate responses and T cell proliferation/differentiation) and/or checkpoint inhibitors. This has been undertaken in pre-clinical trials using the woodchuck model, and was recently summarized in a review [84].

Currently, several companies are developing therapeutic vaccines in phase 1 or 2 trials, including Transgene (TG1050 product; results in mice presented at 2015 EASL meeting; NCT02428400), Gilead Sciences (GS4774; heat killed vaccine vector expressing a fusion protein with HBsAg sequences from 4 genotypes; NCT01943799 & NCT02174276; see [86]), and Dynavax (DV601; HBsAg and core antigens; NCT01023230; see [87]) (Table 1).

T cell therapy

In order to increase the number of HBV-specific T cells, an adoptive transfer of autologous cells could be envisaged, either through the reinfusion of T cells expressing HBV-specific chimeric-antigen-receptors (CAR), which would enable HLA-independent recognition of infected hepatocytes, or through the transfer of engineered T cells overexpressing HLA-restricted HBV-specific T cell receptors (TCRs) [84]. The overexpression of either CAR or HBV-specific TCRs, was until recently accomplished using retroviral transduction, which may pose safety issues in patients. Therefore, recent developments have focused on the use of electroporation/nucleoporation for delivering RNAs transiently encoding recombinant TCRs into resting T cells. Such an approach has been used efficiently in a patient bearing a

HBsAg-positive liver disease and a metastatic tumor [88,89]. An alternative promising new immune-therapeutic approach involves retargeting immune effector cells towards HBV-infected hepatocytes using T cell receptor-like antibodies (TCR-L) recognizing HBV core and S epitopes genetically linked to cytokines (e.g. TCR-L/IFN-α fusion proteins) or bi-specific antibody constructs harboring two immunoglobulin domains (one targeting HBsAg/the other containing effector specificities for T cells) [84].

Depletion/inhibition of immune-modulatory adaptive cells

Checkpoint inhibitors. The HBV-specific T cell exhausted phenotype is particularly associated with the overexpression of co-inhibitory receptors, including programmed cell death (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4 or CD152), lymphocyte activation gene 3 (Lag-3), T cell immunoglobulin domain and mucin domain 3 (TIM-3), and CD244 (2B4) [56,57,90,91]. This exhausted phenotype is maintained by the presence in the microenvironment of immunosuppressive cytokines/chemokines, including IL-10 and TGF-β, produced by tolerogenic-prone innate immune cells and T-reg cells that are enriched in the liver of CHB patients [56,57,90,91]. Recent studies in the field of cancer therapy have highlighted the clinical relevance of blocking these co-inhibitory receptors via antibodies. In advanced melanoma, the combined use of nivolumab (anti-PD-1) and ipilimimab (anti-CTLA-4) was shown to be associated with a significant increase in survival [92]. Since chronic HBV infection and tumor immunology share similar characteristics in terms of immune subversion, blocking co-inhibitory receptors may be an attractive concept for HBV therapy. In a mouse model engineered to mimic HBV persistence, it was recently shown that an anti-PD-1 antibody could reverse immune dysfunctions and help clear the HBV infection to some extent (60% negativity for HBsAg compared to 20% in control animals) [93]. Recent studies performed in animals chronically infected with the woodchuck hepatitis virus (WHV) tested the effect of a combined ETV, anti-PD-L1 MAb, and WHV DNA vaccine. Inhibition of PD-L1 was shown to function synergistically with ETV and the therapeutic vaccination, to control viral replication and restore WHV-specific T cell responses [94]. Moreover, in *ex vivo* experiments conducted on $CD8^+$ T cells isolated from 98 CHB patients aimed at comparing the efficacy of inhibitory receptor blockade strategies targeting PD-1, 2B4, Tim-3, CTLA-4, and BTLA, it was shown that the anti-PD-1 molecule led to the strongest restoration of function. This suggested the importance of blocking PD-1/PD-L1 in the context of CHB [95]. Further studies are required to investigate the use of strategies aimed either at blocking the receptor and/or its ligand, since several aspects linked to their clinical evaluation remain unsolved: i) what will be the risk of uncontrolled T cell mediated hepatocyte lysis in the context of a chronically damaged liver? ii) what will be the risk of off-target immune restoration, i.e. autoimmunity? Studies targeting CHB patients with HCC and receiving checkpoint inhibitors for their cancer should help in obtaining the first results necessary to decide whether or not these programs should be continued for the treatment of CHB alone.

Inhibitors of CD39/CD73. Extracellular adenosine, generated in the microenvironment through ATP hydrolysis, acts as an immune-regulatory signal that modulates the function of several cellular components of the adaptive and innate immune responses [96]. Indeed, extracellular adenosine was shown to prevent activation, proliferation, cytokine production and cytotoxicity of T cells via the stimulation of the purinergic receptors (e.g. P2X and P2Y), and, therefore, contributed to the anergisation of these cells. CD39 and CD73 are the two ectonucleotidases involved in the generation of extracellular adenosine. Within immune-suppressive microenvironments, such as in tumors or in chronically infected tissues, the levels of expression of CD39 and CD73 increased significantly [96]. T-regs, which massively infiltrate the liver of CHB patients, express a high amount of CD39 [97], and are thus thought to participate in the metabolic regulation of T cells via adenosine production. Hence, the development of therapeutic strategies targeting ectonucleotidases could help restoring immune functions by depletion of adenosine, as well as antibodies targeting either CD39 or CD73.

Perspectives regarding immune restoration

The implementation of immunotherapeutic approaches will not be straightforward, based on the previous unsuccessful efforts. A tentative road map would be to sequentially combine in patients displaying NUC induced virus-suppression i) a strategy to lower HBV antigen secretion, followed either by ii) blocking the inhibitory pathways (e.g. antibodies directed against checkpoint molecules or/and depletion of inhibitory cells (e.g. T-reg, MDSC. . .)) and/or a stimulating the innate immune functions (e.g. PRR agonist), and finally iii) by stimulating T cells (by therapeutic vaccine or T cell adoption), before envisaging to interrupt treatments completely.

Key point

Novel antiviral strategies to combat HBV/HDV co-infections are under evaluation.

Key point

Combinations of direct antivirals and restoration of immune responses may be needed for the sustainability of the functional cure.

Novel targets for inhibiting HDV

HDV is a satellite virus of HBV and cannot propagate without its help. Therefore, it is expected that a *functional cure* for HBV would concomitantly lead to the clearance of HDV. At present, patients co-infected by HBV and HDV have to be treated with both a NUC and PegIFN-α. But the success rate of these treatments is rather low, with only around 25% of patients displaying undetectable levels of HDV RNA in the bloodstream, 24 weeks after treatment cessation (result from HIDIT-1 and 2 studies) [98–101]. Moreover, late relapses of HDV have been observed [102], thus decreasing the long-term virologic response rate and warranting the discovery of novel approaches.

There is currently no specific antiviral treatment for HDV. This is mainly due to the fact that the virus does not encode enzymatic activities. A better understanding of the biology of HDV is required to identify virus-modified host cell functions that could be targeted [13,14].

Since HDV uses the same host receptors as HBV, i.e. hNTCP, it is quite possible that HBV entry inhibitors would also be beneficial against this virus [50], and this was, indeed, reported *in vitro* and in animal models [103]. Results of a phase 2A clinical trial (run in the Russian Federation) with Myrcludex B ± PegIFN-α have also been disclosed and showed an encouraging anti-HDV effect, with most patients experiencing a drop in the level of HDV RNA (>1-log) after being treated for 24 weeks, and a few patients even displayed a negative viremia (in both experimental arms) [104].

NAPs (Rep2139), which inhibit the secretion of HBsAg, could also have a positive effect on HDV. Preliminary results of a phase-2 clinical study (NCT02233075), showed a significant reduction in the level of HDV RNA secreted in the blood of 12 Caucasian patients co-infected with HBV and HDV [35], though further results are needed to draw final conclusions about the potential benefit of NAPs for CHD patients.

Since HDV assembly depends on the prenylation/farnesylation of the last four amino acids of the large HDV protein (protein necessary for assembly), inhibitors of farnesyl-tranferase, which were previously used as anticancer drugs (e.g. Oncogenic Ras is farnesylated) [105], have been tested and were shown to be efficient *in vitro* and in mice against HDV. Hence, lonafarnib was integrated in clinical trials, and preliminary results of phase 2A studies (several NCT number; c.f. Table 1) revealed that treatment with lonafarnib was associated with a dose-dependent, yet modest (−1.54 log IU/ml) decrease in HDV viremia. However, no effect on the activity of transaminases and a universal off-drug rebound of viremia was observed, suggesting that further drug optimization is needed, especially since its administration was also associated with gastrointestinal side effects [106].

Prospect for future therapies of CHB

What kind of "cure" can we envisage in the future?

This seems to be the right moment to direct research efforts from fundamental research to applied translation and clinical research. In parallel, industrial efforts are required to discover new drugs and address remaining technical challenges in order to assess the efficacy of the new medications being developed. Although a complete cure for HBV infection, including the clearance of cccDNA and the removal of integrated viral sequences is desirable, this outcome is very unlikely in patients who spontaneously recover from acute infections, thus rendering this treatment goal difficult to achieve in chronically infected individuals. It will require major research efforts and a long-term commitment of the medical and scientific community. The achievement of a *functional cure* is more likely, since it is currently observed in resolved acute infections, as well as in a minority of treated patients who lose HBsAg from the serum. Given the current drug discovery research activity and the rapid progression of several compounds to phase 1B and phase 2 clinical trials, it is conceivable that new treatment options, increasing the rate of success of functional cures, will become available in a near future.

Prospects for drug development strategies

Given the complexity of the HBV life cycle and its immune-pathogenesis, several issues will have to be overcome. Overall, the path towards an increased rate of HBV *functional cure* will be challenging. Since the currently used NUCs have long-term favorable safety data and provide significant clinical benefit, the newly developed antivirals will need to have an extremely good safety profile. Phase 2 clinical trials will need to rely on new treatment endpoints, probably including the quantification of HBsAg because of the lack of accessibility to the liver compartment for virologic and immunologic studies. An important aspect will lie in the determination of the HBsAg threshold upon which a drug will continue to be administered and evaluated alone, or will be given in combination with other compounds. Other virologic and immunologic biomarkers will also need to be evaluated. This raises the issue of identifying clinical correlates of functional cures. In near future, it is most likely that combination therapies relying on direct acting antivirals and drugs to restore

innate and/or adaptive immune responses will be needed to increase the rate of successful functional cure. However, one can also imagine that strategies that would silence cccDNA efficiently and/or significantly decrease viral antigen could restore robust antiviral immunity. This highlights the need to accompany early clinical development programs with strong translational research programs to provide a strong scientific rationale for the design of clinical studies based on these novel drugs. It will be also important in clinical trials to address all of the existing conditions, such as patients with NUC induced viral suppression (mainly in western or developed countries), treatment-naïve patients (mainly from highly endemic areas), the classic chronic active hepatitis patients *vs.* the so-called immune tolerant patients, patients infected with different viral genotypes or mutants, adult *vs.* young patients. The design of these trials will be different according to the type of antivirals or immune-therapeutics used in monotherapy and in combination therapy. The regulatory pathway for the development of drugs will also need to evolve, as this was the case for HIV and HCV, to allow a faster evaluation of new compounds and their combination, as well as an efficient translation to clinical applications in order to find a cure for HBV infection.

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