Review paper

New directions in hepatitis B therapy research

Marta Lewandowska, Anna Piekarska

Department of Infectious Diseases and Hepatology, Medical University in Lodz, Lodz, Poland

Abstract

Chronic hepatitis B treatment is available for a long period, allowing disease control and infection suppression, but it is rarely responsible for HBsAg clearance. None of the drugs available aim at cccDNA, the obstacle in HBV infection eradication. Complications related to CHB, such as liver insufficiency, cirrhosis, and hepatocellular carcinoma are reduced in conditions of good viremia suppression, but still exist even after HBsAg seroclearance, what makes a need for urgent forthcoming of new therapeutics. Recent years brought promising and interesting results of experimental approaches, which are directed against different phases of HBV life cycle, target ccc DNA, or boost, and restore host immune response. Unfortunately, encouraging results in vitro and on animal models are not always reflected in human. Nevertheless, the multiplicity of novel antivirals allows to expect that at least some of them will enter clinical practice and relieve patients from chronic hepatitis B, fatal and devastating disease.

Key words: HBV life cycle, cccDNA silencing, novel HBV antivirals, immunotherapeutics.

Address for correspondence
Marta Lewandowska, Department of Infectious Diseases and Hepatology, Pasieczna 8/12 m. 137, 91-362 Łódź, Poland, e-mail: marta.strycharz@outlook.com

Introduction

According to WHO estimation, 240 million people are chronically infected with hepatitis B (defined as hepatitis B surface antigen positive for at least 6 months). Hepatitis B virus (HBV) infection affects 1 in 50 people in Europe. More than 686,000 people die every year due to complications of hepatitis B, including cirrhosis and liver cancer [1].

It has been over 24 years since interferon, the first indirect (host) acting drug against HBV was registered. Currently, in the United States and Europe, there are seven drugs approved and available, of which 5 are direct acting oral agents targeting the same viral gene product, polymerase. These drugs are effective in viremia suppression, decrease disease progression, and the eventual risk of hepatocellular carcinoma. Entecavir and tenofovir are highly potent antiviral agents with the high genetic barrier to drug resistance what results in a sustained virological response (SVR).

That makes hepatitis B a treatable disease, but it still remains incurable. The pending goal at present is to develop new therapeutic strategies that would allow HBV eradication, following clinical cure. Clinical cure is a return an individual to the risk of death and illness due to liver disease to that of an age and gender adjusted uninfected individual [2]. Nowadays, in practice, only functional sustained cure, off drug virological response (loss of HBV viremia with desired HBsAg antigen) is achievable, but we lack curative response.

Achieving HBsAg seroclearance, the ultimate treatment endpoint of chronic HBV infection depends on administered drugs, and range from HBsAg loss after 12 months of nucleoside therapy 0-1% on lamivudine, 2-3% on tenofovir and entecavir consecutively, to 3-7% in HBsAg positive patients at 6 months, following 12 months of pegylated interferon (PEG-INF) treatment [3].

Regrettably, even in conditions of HBsAg seroclearance, there is a threat of liver-related complications and HBV reactivation due to persistence of intrahepatic co-
valently closed circular DNA (cccDNA), the transcriptional template of HBV [4]. Immunosuppressed patients, HIV-positive, dialyzed, during organ transplantation procedure, on chemotherapy, or immunosuppressive therapy due to rheumatological or gastrological disorders are especially at risk of HBV reactivation.

Therefore, a need for new treatment approaches that eliminate the HBV cccDNA to achieve virological cure is crucial. There are different categories of new medicines that are expected in the next few years. They either target different phases of HBV replication cycle – entry inhibition, mRNA transcription, capsid assembly, viral protein secretion, silence, and eliminate cccDNA, or modulate immune response (Table 1).

**Chronic hepatitis infection as an immunological disorder**

Hepatitis B is a viral infection that strongly affects immune system, leading to disorders in innate and adaptive response, which makes it invisible for a host in the early phase of infection and extremely weaken hosts potential to fight infection in chronic phase. Unsuccessful activation of CD4+ T cells, induce functionally impaired CD8+ cells response that results in infection persistence [5]. What is more, it is known that despite a small percentage of patients who achieve satisfactory virological response during 48-week course of PEG-INF-α treatment, this number increases in the following years after finishing therapy [6]. It demands exploring and allows promising results in field of therapeutic immune modulatory therapies of chronic hepatitis B (CHB). There are several new approaches on the horizon, including TLR agonists, lymphotoxin-β receptor agonists, PD-1/PD-L1 inhibitors, and therapeutic vaccines.

**Therapeutic vaccines**

Viral-based vaccines presents an excellent safety profile and are used as a toll against infectious diseases and cancers [7]. Vaccine technology used to stimulate immune system is one of the new approaches in future HBV treatment. Currently, six therapeutic vaccines designed by different concerns are investigated.

TG 1050 is a novel immunotherapeutic vaccine, based on non-replicative adenovirus serotype 5, which guarantee an induction of optimal CD8 response, essential in HBV clearance. TG 1050 is composed of truncated HBV core, modified HBV polymerase, 2 envelope domains, and reveals high antigenic complexity. It displays cytolytic activity in HBV infected mouse models with significant reduction of circulating viral parameters [8].

### Table 1. New drug candidates in chronic hepatitis B

| Drug class                  | Representative molecule                          | Clinical advantage                                                                 |
|-----------------------------|--------------------------------------------------|------------------------------------------------------------------------------------|
| Therapeutic vaccines        | GS 4774                                          | Stimulate immune system elicit hepatitis B virus (HBV)-specific T-cell responses    |
|                             | HepTcell                                         | Initiate powerful T cell immune responses                                          |
| Capsid inhibitors           | AT-61; AT-130                                     | Nonnucleoside analogue inhibitors of hepatitis B virus (HBV) replication           |
|                             |                                                  | Decrease the amount of RNA-containing cytoplasmic HBV core particles               |
|                             | NVR 3-778                                        | Interferes with the viral DNA protein shield                                      |
| HBsAg inhibitors            | REP 2139; REP 2165                                | Prevent the release of subviral particles                                          |
|                             |                                                  | Rapid reduction and elimination of hepatitis surface antigen                       |
|                             |                                                  | The restoration of the immune system                                              |
| Entry inhibitors            | Myrcludex B (synthetic 47-amino-acid              | Highly stable binding and inactivation of HBV receptors NTCP, misdirecting HBV to |
|                             | N-myristoylated lipopeptide, derived from the preS region of hepatitis B virus | an unproductive pathway and preventing an infection of the cell                    |
| TLR 7 agonist               | GS96 20                                          | Innate and adaptive immune stimulation                                            |
| New polymerase inhibitors   | TAF tenofovir alafenamide                        | Advantageous safety renal profile                                                  |
|                             |                                                  | Reduced plasma tenofovir exposures                                                |
|                             |                                                  | Blood stability                                                                    |
|                             | Besifovir (LB80380)                              | Proven antiviral activity against wild type and drug resistant mutation virus      |
| Targeting HBV RNA           | ARC-520; ARC-521                                 | Silence all HBV gene products                                                     |
|                             |                                                  | Constrict HBV replication                                                         |
|                             |                                                  | HBsAg and HBsAg suppression                                                       |
|                             |                                                  | Inducing INF response                                                             |
Preclinical studies on animal models (HBV replicating transgenic mice, HBV infected chimpanzees, woodchuck) have clearly show benefit of DNA vaccine-based combination therapies for CHB treatment; however, some experts indicate that the results of clinical trials conducted with HBV patients have not proved its efficacy and are disappointing, especially with regards to promising results on animals [9].

GS 4774 is a recombinant, heat killed, yeast-based T-cell vaccine designed to elicit HBV-specific T-cell responses. In phase II clinical study conducted by Lock et al., patients with CHB were treated for 1 year with oral antivirals, and then either continued the therapy or maintained it with several doses of GS 4774. The vaccine was well tolerated, but there were no clinical benefits regarding HBsAg decline in serum [10].

One of the promising immunotherapy product is HepTcell, completely synthetic peptide-based T-cell vaccine, encoding a high density of CD4 and CD8, focused against multiple HBV genotypes, being in phase I studies.

Cytokines and their receptors agonists, in this case INF-α and lymphotxin (LT) β receptor agonists (BS1 or CB11), have an ability to deaminate and degrade cccDNA. Up-regulation of located in nucleus APOBECs (apolipoproteins B mRNA editing enzyme catalytic polypeptide 3A and cytidine deaminases) deaminates cccDNA. Under treatment with this agents, HBV cccDNA degradation and HBV replication inhibition was observed [11]. Nonetheless, till now, they remain in phase in vitro trials and animal studies. Programmed cell death-1 (PD-1) are hyperexpressed on CD8+ cells, relying on previous studies interfering in PD-1/PD-1 ligand improves antiviral activity of T cells [12], as an example on mice model with viral infection blockage of PD-1/PD-L1 inhibitory pathway restored ability of CD8 T cells to undergo proliferation, secrete cytokines, kill infected cells, and decrease viral load [13]. Other research confirm enhanced percentage of responding T cells and production of IFN-γ and cytokine production [14]. Benefits of mentioned above blockage was also observed in patients with HIV/HBV coinfection, where survival and cytokine secretion of HBV CD8+ T cells increased; the same authors indicate that HBV DNA suppression due to adefovir therapy reduced PD-1 expression [15].

Pattern recognition receptors (PRRs) recognize conserved microbial structures called PAMPs (pathogen associated molecular patterns); they are the basis of innate immune response. Toll-like receptors belong to group of PRRs and are located on numerous cells of immune response, macrophages, dendritic cells, mast cells, eosinophils, B lymphocytes, neutrophils, and epithelial cells. TLRs activated by microbial products trigger innate immune response, and up-regulate cytokines secretion and dendritic cell maturation, what permits more effective antigen presentation (toll-like). TLR-7, expressed in lysosomal/endosomal compartments of plasmacytoid dendritic cells (pDCs), and B lymphocytes, recognize viral single stranded RNAs, what turns on a cascade of INF and other cytokines production, stimulating NK cells and T cytotoxic lymphocytes [16]. PDCs are the primary interferon-producing cells in the blood in response to viral infections [17].

GS96 20 is an oral agonist of toll-like receptor-7, currently being in II phase of clinical trials. One of the studies in chimpanzees with chronic HBV infection, proved rapid reduction of HBV DNA, reduction of HBsAG and HBeAg level, decreased number of infected hepatocytes, and prolonged suppression of HBV viremia. The same authors report dose dependent increase in INF-β production and overall good toleration of therapy [18]. In the study aimed to prove safety and good tolerance of GS 9620, 75 healthy volunteers were randomized to receive a single dose of GS-9620 starting from 0,3 mg to 1, 2, 4, 6, 8, and 12 mg or placebo. Adverse effects were minimal, flu-like, resolved within 72 hours, occurred at doses 8 and 12 mg, and were connected with serum interferon detection. Interestingly, the doses equal to or over 2 mg asseverated chemokines, cytokines, and INF-stimulated genes induction [19].

Strategies targeting cccDNA

Zinc finger proteins

Zinc finger proteins (ZNFs) are huge and diverse family of proteins, serving various biological functions. These small, functional domains require at least one zinc ion to stabilize the integration of protein itself. Term “finger” refers to their secondary structures (α-helix and β-sheet), that are held by zinc ion. ZNFs typically bind DNA, RNA, proteins, serving as interactor [20]. ZNFs beside TALEN’s and CRISP are in the group of three most commonly used engineered DNA-binding proteins used to target cccDNA.

In one of the studies, ZNFs were specially designed to bind/block DNA sequences in duck HBV enhancer region, which is a model of human HBV. They give an unique and direct possibility to target cccDNA. Authors proved that in presence of ZFNs, viral RNA and protein levels were significantly reduced, which resulted in decreased intracellular viral particle production [21]. Another research, conducted within cultured cells, proved effective cleavage of viral DNA by HB-specific ZFNs. What is more, it showed misreper-
ation of cleaved fragments in a way that could potentially inactivate HBV. Frameshift mutations leading to truncations of the viral core protein were observed, demonstrated the possibility of targeting episomal cccDNA [22].

Transcription activator-like effector nucleases

Chen et al. worked on another DNA-binding proteins, transcription activator-like effector nucleases (TALENs), which are the transcription activator-like (TAL) effector nucleases, enzymes that present ability to cleave sequence-specific DNA targets. The effect of TALENs was examined in cells transfected with linear full-length HBV DNA and in a hydrodynamic injection-based mouse model. TALENs were designed to target highly conserved regions, assuring suitability for multiple HBV genotypes. The results suggest a therapeutic potential of TALENs as it showed a reduction in cccDNA level. Moreover, a synergistic effect of TALENs and IFN-α on inhibiting HBV transcription was observed, since pgRNA reduction in cells under INF-α-TALENs cotreatment was twice as high as in cells treated with INF-α alone. Authors indicate that the inhibitory effect of TALENs is largely dependent on the FokI endonuclease activity, based on data that TALENs with no endonuclease activity barely inhibited the HBeAg and HBcAg expression [23]. According to Bloom et al., TALENs was responsible for 31% disruption of cccDNA molecules and its correlation with HBsAg secretion [24].

Clustered regulatory interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) protein endonucleases

The CRISPR-Cas systems mediate immunity to invade genetic elements via three-stage process – adaptation, expression, and interference, to finally target foreign DNA or RNA, and cleaving it within the proto-spacer sequence [25]. CRISPR/Cas9 system, as a new developed toll, is now a subject of research to examine whether this system can cleave HBV genomes. Data form Su-Ru Lin et al. suggest that the CRISPR/Cas9 system could disrupt the HBV expressing templates both in vitro and in vivo, indicating its potential in eradicating persistent HBV infection [26].

Capsid assembly inhibitors

In this group, three types of new compounds can be distinguished: phenylpropenamide derivatives (AT-61, AT-130), sulfamoylbenzamide (SBA) derivatives, and heteroaryldihydropyrimidine (HAP). Phenylpropenamides derivatives are assembly accelerators, which transgress the assembly by speeding it up. It leads to decreased level of RNA-containing cores [27]. The influence of AT-61 on HBV replication cycle is seen before pgRNA, and is packed into immature core particles [28]. AT-130 and AT-60 inhibit, the packaging steps in HBV life cycle.

Sulfamoylbenzamide (SBA) derivatives are the inhibitors of pregenomic RNA (pgRNA) containing nucleocapsids of HBV formation. Similarly to AT-61, SBAs accelerate the decay of pgRNA-containing nucleocapsids. It results in a stoppage of subsequent HBV DNA replication. The effect is dose depended. In viral replication cycle, they act in the step prior to viral DNA synthesis, but do not affect viral DNA synthesis directly. That explains the fact that all these compounds influence NA-resistant and wild type HBV [29].

Heteroaryldihydropyrimidine (HAP) affects HBV capsid assembly and preformed HBV capsids. In vitro, it accelerate and misdirect capsid assembly. Inhibition of viral replication by HAP occurs by inappropriate assembly induction and capsid destabilization [30].

NVR 3-778 is a capsid assembly modulator, which initiates defective capsid assembly, inhibits RNA encapsidation, and DNA replication, resulting in HBV RNA and quantitative HBeAg reductions. In phase Ib clinical trial, the first of NVR 3-778 in HBV-infected patients showed efficacy to increase long lasting treatment response rates of this drug alone or in combination with PEG-INF, as well as satisfactory safety profile.

Inhibiting HBV entry receptor

Discovery of the human sodium taurocholate co-transporting polypeptide (hNTCP) as a liver specific receptor of HBV and HDV, revealed new targets for drugs against chronic hepatitis B and D. NTCP is a sodium dependent uptake transporter located on the basolateral membrane (blood-side) of hepatocytes; is not detected in any other tissue of human body. Its primary function is the enterohepatic circulation of bile acids; it is also one of the key transporters in it and has restricted specificity for drug transport. Yet, this receptor was unidentified. Three kinds of envelope proteins form outer coats of HBV and HDV are called the pre-S1 domain, one of them plays a main role in the HBV and HDV virus-receptor interaction [31].

Myrcludex B (synthetic 47-amino-acid N-myristoylated lipopeptide, derived from the preS region of hepatitis B virus) is a new drug, the first-in-class entry inhibitor for treatment of chronic hepatitis B (HBV) and chronic hepatitis delta (HDV) infection. The myristoylated N-terminal preS1 domain of the L protein is specifi-
cally bound to the sodium taurocholate co-transporting polypeptide NTCP, what determines the susceptibility of human liver to HBV and HDV; NTCP-expressing cell lines can be efficiently infected with these viruses [31]. Myrcludex blocks in vitro and in vivo receptor functions of NTCP by competing for the binding with HBsAg.

In phase I clinical trial to 36 healthy volunteers, the first in humans administration of Myrcludex B, the drug was well tolerated, no serious or relevant adverse effects were observed, applied doses were ascending up to 20 mg intravenously or 10 mg subcutaneously. It showed 2-compartment target mediated drug disposition model, and had 85% bioavailability of the subcutaneous application [32].

According to Volz, in mice reconstituted with human hepatocytes 6 weeks after Myrcludex B treatment, no increase in HBV viremia, in antigen levels, and amount of HBCAg-positive human hepatocytes was observed. Authors indicate that Myrcludex B may also delay amplification of the cccDNA pool in initially infected hepatocytes [33].

HDV is using HBV envelope and may be targeted by Myrcludex B. Myrcludex B inhibits the essential HBV receptor. Until now, clinical trials phase 2a has been completed, showing clinical efficacy and good safety profile of Myrcludex B. Hepatitis D infection is possible only in state of previous HBV infection or coinfection HBV/HDV. HDV superinfection may rev up cirrhosis progression, leading to liver insufficiency, and is the risk factor of HCC. HDV is a RNA virus, which does not code majority enzymes necessary for its replication; instead, it exploits its host enzymatic activity. There is no HDV specific polymerase. No target point in HDV life cycle preclude direct antiviral therapy. On PEG-INF treatment, 25% of patients achieve SVR; however, this effect if further diminished by relapses [34].

Myrcludex will be a new therapeutic option in HDV infection, where no approved treatment options exist. First results of a phase Ib/Ia study confirm previous observations, showing synergistic antiviral effects on HDV RNA and HBV DNA under Myrcludex-IFN combined therapy, and a strong effect on HDV RNA level, inducing ALT normalization during monotherapy simultaneously [35]. Bogomolov et al. divided patients to three cohorts, consecutively receiving: first Myrcludex in monotherapy, second Myrcludex and PEG-INF, third PEG-INF in monotherapy, for 24 weeks; after that, either continuing PEG-INF for the next 24 weeks in second and third group, or starting 48 weeks PEG-INF treatment in the first cohort. However, the results of this research were mystifying, as decline in HDV RNA level was not accompanied by decrease in serum HBsAg [35].

Targeting HBV RNA

RNA interference is an important antiviral defense mechanism in organisms. That gives possibilities for another HBV inhibition strategy, which was widely analyzed by Ebert et al. [36]. All HBV RNAs share common 3’ sequences, which may be a target for small interfering (si)RNAs. SiRNAs are responsible for inducing INF response and for controlling not only HBV replication, but antigen secretion as well. SiRNA reduces expression levels of HBV envelope protein L and capsid protein core, what is known to be gene silencing effect [36]. In research conducted on mice, siRNA was responsible for decreasing the level of viral transcripts, viral antigens, and viral DNA detected in the livers and sera [37]. The same authors reported that viral RNA production inhibition was accompanied by a > 80% decline in the secretion of viral HBsAg and HBeAg.

RIG-I-like receptor dsRNA helicase enzyme that is encoded (in humans) by the DDX58 gene. RIG-I is part of the RIG-I-like receptor family, and is a pattern recognition receptor that is a sensor for some viruses. It is expressed in HBV infected hepatocytes. SiRNA induced type I INF response is dependent on RIG-1 [36].

ARC-520 is a new drug, currently under clinical trials. It silence all HBV gene products and intervene upstream of the reverse transcription process. ARC-520 constrict HBV replication by delivering siRNA and caused HBeAg and HBsAg suppression. The drug is composed of 2 cholesterol-conjugated siRNA sand, a hepatocyte-targeted membrane-lytic-peptide (NAG-MLP). It is designed to be given intravenously. Cholesterol siRNA is taken up by hepatocytes and released from endosomes by the action of NAG-MLP, then in the cytoplasm, the siRNAs engage the RNAi machinery [38].

Clinical trials on ARC-520 and ARC-521 has been recently abandoned due to substantial advances in field of new subcutaneous (subQ) and extra-hepatic delivery systems. Although previously ARC-520, ARC-521 in human clinical trials appeared to show good toleration, currently discontinuation of clinical trials is also related with investigation of the cause of deaths in a non-clinical toxicology study in non-human primates connected with drug EX1 delivery vehicle utilized by these drug candidates.

New polymerase inhibitors

Tenofovir disoproxil fumarate (TDF) is an antiretroviral drug, successfully used in HIV and HBV infection therapy for a long period of time. However, it is riddled with a risk of developing clinically significant nephrotoxicity or bone density loss. TAF tenofovir
alafenamide is a new prodrug of tenofovir, devoided of these adverse effects. TAF presents much better safety renal profile and reduces plasma tenofovir exposures by 90%, what decreases off-target side effects and has been shown to be significantly more stable in blood and plasma. It does not interact with renal transporters OAT-1 and OAT-3 (organic anion transporters), exhibit no OAT-dependent toxicity, and is unlikely to accumulate in renal proximal tubes. TAF is converted intracellularly to TFV, shows higher intracellular levels of TFV-DP, lower circulating levels of TFV than TDF, and accumulates in liver, lymphoid tissue, and peripheral blood mononuclear lymphocytes. CatA as the major hydrolase is responsible for the intracellular activation of TAF [39].

The metabolism of TAF in primary human hepatocytes in vitro and in dogs in vivo was evaluated, and presented high levels of pharmacologically active metabolite tenofovir diphosphate (TFV-DP), with half-life over 24 hours [40].

As known from the latest data derived from HIV-positive patients, there was a significant improvement in hip and spine bone density and glomerular filtration in patients on tenofovir alafenamide, comparing to those receiving tenofovir disoproxil fumarate. In the regard of viral suppression treatment, shifting to those receiving tenofovir disoproxil fumarate in patients on tenofovir alafenamide, comparison in hip and spine bone density and glomerular filtration in patients on tenofovir alafenamide, compared to entecavir 0.5 mg in 48 weeks, in order to confirm non-inferiority of besifovir and entecavir treatment in CHB naïve patients. HBV DNA reduction was seen in besifovir group of more than 5 and 4.5 logs HBeAg-positive and negative hepatitis B patients, respectively [46]. Up to 48-week only, one patient had a virological breakthrough, with no specific mutations identified; none of the patients developed besifovir resistance till the end of treatment. Five patients in each besifovir group, compared to seven in entecavir, passed through ALT breakthrough (41-372 IU/ml), which was transient. There were no serious adverse events. Dose dependent L- carnitine depletion was observed, and patients required L-carnitine supplementation. Pivalic moiety presence in compounds is responsible for L-carnitine reduction, as the similar effect was observed in HIV positive patients during ADV treatment, which contains the pivalic moiety as well [47]. Hypophosphatemia (1.9 mg/dl and 1.6 mg/dl) with no clinical signs and returning to normal in following 4 weeks, occurred in 2 patients receiving besifovir (n = 75).

Besiﬁvir (LB80380) is an oral nucleotide prodrug, chemically similar to adefovir dipivoxil and tenofovir disoproxil fumarate. The parent drug LB80331 is formed by the removal of the two pivaloyl groups (deacetylation) in the liver and intestine, then is further metabolized to LB80317 (by oxidation of the nucleoside base at the 6 position). The process is mediated through enzyme oxidases (aldehyde oxidase and xanthine oxidase). The active metabolite with the antiviral effect is LB80317. The drug is excreted via the urinary system (approximately 80% of the drug excretion) [43]. Besiﬁvir (LB80380) has proven antiviral activity against wild type and drug resistant mutation virus, demonstrated in phase II trials [44].

Besiﬁvir in doses 90 mg or 150 mg, compared to etecavir 0.5 mg in 96 weeks of treatment, showed the same antiviral property, respectively: undetectable HBV DNA in 80.7%, 78.6%, and 80%, ALT normalization in 90.3%, 78.6%, and 93.3%; and HBsAg loss in 20%, 21.4%, and 22.2%. Virological breakthrough due to drug non-compliance was noted in one patient receiving besiﬁvir in dose 90 mg, but none of the patients developed drug resistance. Drug was well tolerated and had a good clinical safety proﬁle; the most common side effect related to besiﬁvir was carnitine depletion. No patient had increased creatinine level > 0.5 mg/dl from baseline [45].

In another study, besiﬁvir in doses 90 mg and 150 mg was compared to etecavir 0.5 mg in 48 weeks, in order to conﬁrm non-inferiority of besiﬁvir and entecavir treatment in CHB naïve patients. HBV DNA reduction was seen in besiﬁvir group of more than 5 and 4.5 logs HBeAg-positive and negative hepatitis B patients, respectively [46]. Up to 48-week only, one patient had a virological breakthrough, with no specific mutations identified; none of the patients developed besiﬁvir resistance till the end of treatment. Five patients in each besiﬁvir group, compared to seven in entecavir, passed through ALT breakthrough (41-372 IU/ml), which was transient. There were no serious adverse events. Dose dependent L- carnitine depletion was observed, and patients required L-carnitine supplementation. Pivalic moiety presence in compounds is responsible for L-carnitine reduction, as the similar effect was observed in HIV positive patients during ADV treatment, which contains the pivalic moiety as well [47]. Hypophosphatemia (1.9 mg/dl and 1.6 mg/dl) with no clinical signs and returning to normal in following 4 weeks, occurred in 2 patients receiving besiﬁvir (n = 75).

The efﬁcacy of besiﬁvir was also proven at reducing viral load in CHB patients with lamivudine-resistant virus who received LB 80380 in five ascending doses (30, 60, 90, 150, 240 mg), together with lamivudine for the ﬁrst 4 weeks, then for the 8 weeks alone, followed by 24 weeks of adefovir, showing the dose-proportional effect in decreasing HBV DNA level, safety and good toleration up to doses of 240 mg [48].

**Conclusions**

Satisfactory CHB therapy with currently available regimens is not available, neglecting to mention HBV eradication. Changing this condition is given by the wide display of new drugs presented on the horizon.
The subsequent steps of clinical investigations come up with contradictory data between in vitro and in vivo studies, which requires further examination. Presumably, a successful CHB treatment in the future will be a complex therapy including cccDNA silencing strategies, anti-viral agents, and immunomodulators.

Disclosure

Authors report no conflict of interest.

References

1. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 385:117-171.
2. Liang T, Block T, McMahon B. Present and future therapies of hepatitis B: from discovery to cure. Hepatology 2015; 62:1893-1908.
3. European Association For the Study of the Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57:167-185.
4. Xia Y, Stadler D, Ko C, et al. Analyses of HBV cccDNA Quantification and Modification. Methods Mol Biol 2017; 1540:59-72.
5. Chisari FV, Isogawa M, Wieland SF. Pathogenesis of Hepatitis B Virus Infection. Pathol Biol (Paris) 2010; 58:258-266.
6. Moucari R, Korevaar A, Lada O, et al. High rates of HBsAg seroconversion in HBeAg-positive chronic hepatitis B patients responding to interferon: a long-term follow-up study. J Hepatol 2009; 50:1084-1092.
7. Rollier CS, Reyes-Sandoval A, Cotthingam MG, et al. Viral vectors as vaccine platforms: deployment in sight. Curr Opinion Immunol 2011; 23:377-382.
8. Martin P, Dubois C, Jacquier E, et al. TG1050, an immunotherapeutic agent recognized mice previously infected with hepatitis B virus. J Hepatol 2013; 58:861-867.
9. Cai G, Karni A, Oliveira EM, et al. PD-1 ligands, negative regulators of activation of naive, memory, and recently activated human CD4+ T cells. Cell Immunol 2004; 230:89-98.
10. Sherman AC, Trehanpati N, Daucher M, et al. Augmentation of hepatitis B virus-specific cellular immunity with programmed death receptor-1/programmed death receptor-L1 blockade in hepatitis B virus and HIV/hepatitis B virus coinfected patients treated with adefovir. AIDS Res Hum Retroviruses 2013; 29:665-672.
11. Lucifora J, Xia Y, Reisinger F, et al. Specific and nonhepatotoxic destruction of nuclear hepatitis B virus cccDNA. Science 2014; 343:1221-1228.
12. Boni C, Fiscaro P, Valdatta C, et al. Characterization of Hepatitis B Virus (HBV)-Specific T-Cell, Dysfunction in Chronic HBV Infection. J Virol 2007; 81:4215-4225.
13. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006; 439:682-687.
14. Cai G, Karni A, Oliveira EM, et al. PD-1 ligands, negative regulators of activation of naive, memory, and recently activated human CD4+ T cells. Cell Immunol 2004; 230:89-98.
15. Sherman AC, Trehanpati N, Daucher M, et al. Augmentation of hepatitis B virus-specific cellular immunity with programmed death receptor-1/programmed death receptor-L1 blockade in hepatitis B virus and HIV/hepatitis B virus coinfect patients treated with adefovir. AIDS Res Hum Retroviruses 2013; 29:665-672.
16. Gibson SJ, Lindh JM, Riter TR, et al. Plasmacytid dendritic cells produce cytokines and mature in response to the TLR7 agonists, imiquimod and resiquimod. Cell Immunol 2002; 218:74-86.
17. Barbalat R, Ewald SE, Moucheess ML, et al. Nucleic acid recognition by the innate immune system. Annu Rev Immunol 2011; 29:185-214.
18. 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 Chimpanzees. Gastroenterology 2013; 144:1508-1517.e10.
19. Lipatkin U, Wolfgang G, Tumas D, et al. Safety, pharmacokinetics and pharmacodynamics of GS-9620, an oral Toll-like receptor 7 agonist. Antivir Ther 2013; 18:409-418.
20. Laity JH, Lee BM, Wright PE. Zinc finger proteins: new insights into structural and functional, Diversity. Curr Opin Struct Biol 2001; 11:39-46.
21. Zimmerman KA, Fischer KP, Joyce MA, et al. Zinc finger proteins designed to specifically target duck hepatitis B virus covalently closed circular DNA inhibit viral transcription in tissue culture. J Virol 2008; 82:8013-8021.
22. Cradick TJ, Kekk, K, Bradshaw S, et al. Zinc-finger nucleases as a novel therapeutic strategy for targeting hepatitis B virus DNAAs. Mol Ther 2010; 18:947-954.
23. Chen J, Zhang W, Lin J, et al. An Efficient Antiviral Strategy for Targeting Hepatitis B Virus Genome Using Transcription Activator-Like Effector Nucleases. Mol Ther 2014; 22:303-312.
24. Bloom K, Ely A, Mussolino C, et al. Inactivation of Hepatitis B Virus Replication in Cultured Cells and In Vivo with Engineered Transcription Activator-Like Effector Nucleases. Mol Ther 2013; 21:1889-1897.
25. Makarova KS, Haft DH, Barrangou R, et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol 2011; 9:467-477.
26. Lin SR, Yang HC, Kuo YT, et al. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates in Vivo. Mol Ther Nucleic Acids 2014; 3:e186.
27. Katen SP, Chirapu SR, Finn MG, et al. Trapping of Hepatitis B Virus capsid assembly intermediates by phenylpropanamide assembly accelerators. ACS Chem Biol 2010; 5:1125-1136.
28. King RW, Ladner SK, Miller TJ, et al. Inhibition of human hepatitis B virus replication by AT-61, a phenylpropanamide derivative, alone and in combination with (-)-β-l-2',3'-dideoxy-3'-thiacytidine. Antimicrob Agents Chemother 1998; 42:3179-3186.
29. Campagna MR, Liu F, Mao R, Mills C, Cai D, Guo F, Zhao X, Ye H, Cuconati A, Guo H, Chang J, Xu X, Block TM, Guo JT. Sulfamoylbenzamide derivatives inhibit the assembly of hepatitis B virus nucleocapsids. J Virol 2013; 87:6931-6942.
30. Stray SJ, Zlotnick A. BAY 41-4109 has multiple effects on Hepatitis B virus nucleocapsids. J Virol 2013; 87:6931-6942.
31. Ni Y, Lempp FA, Mehrle S, et al. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. Gastroenterology 2014; 146:1070-1083.
32. Blank A, Markert C, Hohmann N, et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor Myrcludex B. J Hepatol 2016; 65:483-489.
33. Volz T, Allweiss L, Ben M'Barek M. The entry inhibitor Myrcludex B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. J Hepatol 2015; 58:861-867.
34. Zizzuto M, Smedile A. Pegylated interferon therapy of chronic hepatitis D: in need of revision. Hepatology 2015; 61:1109-1111.
35. Bogomolov P, Alexandrov A, Voronkova N, et al. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: First results of a phase Ib/IIa study. J Hepatol 2016; 65: 490-498.
36. Ebert G, Poeck H, Lucifora J, et al. 5’ Triphosphorylated small interfering RNAs control replication of hepatitis B virus and induce an interferon response in human liver cells and mice. Gastroenterology 2011; 141: 696-706.e3.
37. Giladi H, Ketzinel-Gilad M, Rivkin L, et al. Small interfering RNA inhibits hepatitis B virus replication in mice. Mol Ther 2003; 8: 769-776.
38. Lanford R, Wooddell CI, Chavez D, et al. ARC-520 RNAi therapeutic reduces HBV DNA, s and e antigen in a chimpanzee with a very high viral titer. Hepatology 2013; 58: 707A.
39. Bam RA, Yant SR, Cihlar T. Tenofovir alafenamide is not a substrate for renal organic anion transporters (OATs) and does not exhibit OAT-dependent cytotoxicity. Antivir Ther 2014; 19: 687-692.
40. Murakami E, Wang T, Park Y, et al. Implications of efficient hepatic delivery by tenofovir alafenamide (GS-7340) for hepatitis B virus therapy. Antimicrob Agents Chemother 2015; 59: 3563-3569.
41. Gibson AK, Shah BM, Nambiar PH, et al. Tenofovir Alafenamide: A Review of Its Use in the Treatment of HIV-1 Infection. Ann Pharmacother 2016; 50: 942-952.
42. Agarwal K, Fung SK, Nguyen TT, et al. Twenty-eight day safety, antiviral activity, and pharmacokinetics of tenofovir alafenamide for treatment of chronic hepatitis B infection. J Hepatol 2015; 62: 533-540.
43. Yuen MF, Lee SH, Kang HM, et al. Pharmacokinetics of LB80331 and LB80317 following oral administration of LB80380, a new antiviral agent for chronic hepatitis B (CHB), in healthy adult subjects, CHB patients, and mice. Antimicrob Agents Chemother 2009; 53: 1779-1785.
44. Fung J, Lai CL, Yuen MF. LB80380: a promising new drug for the treatment of chronic hepatitis. Expert Opin Investig Drugs 2008; 17: 1581-1588.
45. Yuen MF, Kim J, Kim CR, et al. A randomized placebo-controlled, dose-finding study of oral LB80380 in HBeAg-positive patients with chronic hepatitis B. Antivir Ther 2006; 11: 977-983.
46. Lai CL, Ahn SH, Lee KS, et al. Phase IIb multicentred randomized trial of besifovir (LB80380) versus entecavir in Asian patients with chronic hepatitis B. Gut 2014; 63: 996-1004.
47. Deeks SG, Collier A, Lalezari J, et al. The safety and efficacy of adefovir dipivoxil, a novel anti-human immunodeficiency virus (HIV) therapy, in HIV-infected adults: a randomized, double-blind, placebo-controlled trial. J Infect Dis 1997; 176: 1517-1523.
48. Yuen MF, Han KH, Um SH, et al. Antiviral activity and safety of LB80380 in hepatitis B e antigen-positive chronic hepatitis B patients with lamivudine-resistant disease. Hepatology 2010; 51: 767-776.