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ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, Guang Ji, MD, PhD, Professor, Institute of Digestive Diseases, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, No. 725 South Wanping Road, Shanghai 200032, China. jiliver@vip.sina.com

AIMS AND SCOPE

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MINIREVIEWS

Viral hepatitis: Innovations and expectations

Simona Leoni, Alberto Casabianca, Benedetta Biagioni, Ilaria Serio

ORCID number: Simona Leoni 0000-0001-8825-9698; Alberto Casabianca 0000-0003-2482-9422; Benedetta Biagioni 0000-0002-5152-0626; Ilaria Serio 0000-0002-6676-4148.

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Simona Leoni, Alberto Casabianca, Benedetta Biagioni, Ilaria Serio, Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna 40138, Italy

Corresponding author: Simona Leoni, MD, PhD, Doctor, Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Via Albertoni 15, Bologna 40138, Italy. simona.leoni@aosp.bo.it

Abstract

Viral hepatitis is a significant health problem worldwide, associated with morbidity and mortality. Hepatitis B, C, D, and occasionally E viruses (HBV, HCV, HDV, and HEV) can evolve in chronic infections, whereas hepatitis A virus (HAV) frequently produces acute self-limiting hepatitis. In the last years, different studies have been performed to introduce new antiviral therapies. The most important goal in the treatment of viral hepatitis is to avoid chronic liver disease and complications. This review analyzes currently available therapies, in particular for viruses associated with chronic liver disease. The focus is especially on HBV and HCV therapies, investigating new drugs already introduced in clinical practice and clinical trials. We also describe new entry inhibitors, developed for the treatment of chronic HDV and HBV and currently available treatments for HEV. The last drugs introduced have shown important efficacy in HCV, with achievable target HCV elimination by 2030. Concurrently, renewed interest in curative HBV therapies has been registered; current nucleotide/nucleoside analogs positively impact liver-related complications, ensuring high safety and tolerability. Novel approaches to HBV cure are based on new antivirals, targeting different steps of the HBV life cycle and immune modulators. The improved knowledge of the HDV life cycle has facilitated the development of some direct-acting agents, as bulevirtide, the first drug conditionally approved in Europe for HDV associated compensated liver disease. Further studies are required to identify a new therapeutic approach in hepatitis E, especially in immunosuppressed patients.

Key Words: Viral hepatitis; Chronic liver disease; Treatments; Antiviral; Immunotherapy; Vaccination

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Core Tip: Viral hepatitis is a known worldwide health problem, with a risk of evolution in chronic liver disease. Novel therapies have shown increased efficacy in curing the hepatitis C virus (HCV), with the goal of HCV elimination by 2030. New concurrent interest in hepatitis B virus (HBV) curative therapies has been recently registered: New antivirals targeting different steps of HBV life cycle and immune modulators. In hepatitis D, the improved knowledge of the life cycle has facilitated the development of some direct-acting agents, more effective than interferon-based therapies. New studies are required to improve the treatment of hepatitis E.

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INTRODUCTION

Viral hepatitis represents a health problem that affects millions of people worldwide and is associated with high mortality. Except for hepatitis A virus (HAV), all hepatotropic viruses, including hepatitis B, C, D, and E viruses (HBV, HCV, HDV, and HEV), can produce chronic infections, whereas HAV causes acute self-limiting hepatitis that normally resolves spontaneously. In this review, we provide a brief outline of currently available therapies for major hepatotropic viruses associated with chronic liver disease. We focus especially on therapies that halt HBV progression and achieve a definitive HCV cure, investigating new drug clinical trials. We also describe bulevirtide, an entry inhibitor, developed for the treatment of chronic HDV and HBV infections and approved in the European Union for the therapy of chronic HDV infection in patients affected by liver disease, without decompensation. We describe currently available treatments for HEV with their limitations, pointing out the emergent need for novel antivirals not only safe but above all effective particularly for patients not eligible for interferon (IFN)-based regimes.

HEPATITIS B

HBV infection is the main cause of chronic liver disease in the world, affecting 257 million people globally, and is responsible for about 54% of the cases of hepatocellular carcinoma (HCC)[1].

The current goal for HBV treatment is to suppress viral replication, halt disease progression, and prevent cirrhosis development, liver failure, and HCC. The approved agents for the treatment of chronic HBV infection are not curative and can be classified into two categories: Pegylated IFN (peg-IFN) and nucleotide or nucleoside analogs (NAs)[2].

In particular, there are eight approved drugs: IFN and peg-IFN, lamivudine, adefovir, telbivudine, entecavir, tenofovir disoproxilfumarate (TDF), and tenofovir alafenamide fumarate (TAF). Besifovir dipivoxil is only approved in Korea. Their action is limited to suppress HBV replication and decelerate progression to cirrhosis and HCC.

Current and future aims of treatment

The current aim of antiviral treatment is the induction of two different responses: Virologic and biochemical[3].

Virologic response: A virologic response during NA therapy is defined as undetectable HBV-DNA. In the course of IFN treatment, a virologic response is obtained if serum HBV-DNA level falls below 2000 IU/mL, 6 mo after the start of treatment and at the end of treatment.

Biochemical response: A biochemical response is obtained when alanine aminotransferase falls into the normal range.

Functional cure: Corresponding to a durable hepatitis B surface antigen (HBsAg) loss, with or without seroconversion, is achieved rarely in the natural history of chronic hepatitis B: In only 10%-20% of Caucasian patients and less than 5% Asian patients[4]. HBsAg seroclearance correlates with a reduced risk of HCC, therefore it represents an advisable goal of treatment. The functional cure aspires to sterilize cells neither from integrated HBV-DNA nor from covalently closed circular DNA (cccDNA), a persistent form of the HBV genome in infected hepatocytes.

Complete cure: Complete cure is a functional cure with the elimination of cccDNA, which acts as a potential reservoir for reactivation. Complete HBV elimination is difficult, due to the existence of intrahepatic cccDNA and its ability to self-replenish: cccDNA serves as a template for the transcription of pre-genomic RNA, allowing the production of viral DNA and proteins even without detectable HBV-DNA or HBsAg [5,6]. A complete HBV cure consists of a full HBV eradication through the removal of all viral elements from an infected patient. To achieve this goal, combination therapy is probably required[7]. Treatments currently available, peg-IFN and NAs, have no direct effect on cccDNA that persists in the hepatocyte nucleus.

An additional obstacle to HBV elimination is the viral DNA integration into the host genome, sufficiently intact to support the translation of viral protein[8]. Integrated HBV-DNA can be a source of circulating HBsAg; definitive clearance of HBV would require the removal of hepatocytes that harbor this DNA.

The persistence of HBV in the liver of patients who have recovered from acute HBV infection can explain the risk of HBV reactivation in the course of potent immunosuppressive therapy and the potential transmission of HBV infection when these livers are transplanted into seronegative recipients[9].

An additional limitation to HBV elimination is impaired innate and adaptive immune responses in HBV patients. Chronic HBV patients fail to unleash efficient immune responses to HBV if cytotoxic T cells are hypo-responsive to HBV itself. For a complete cure, the restoration of suppressed host immunity is required[10].

New biomarkers in diagnosis and management of HBV infection

To diagnose acute and chronic HBV infections, some tests are routinely used. The identification of HBV presence and activity is essential in the diagnostic and therapeutic algorithm of infected patients. In clinical practice, there are routinely used assays to detect and measure serum levels of HbsAg, HbeAg, anti-HBs, and HBV DNA. Some of them are strong risk predictors of the development of HCC[11].

New diagnostic tools are needed to assess the efficacy of therapy and monitor with precision the response. The most interesting includes tests to measure HBV RNA and hepatitis B core-related antigen (HBcrAg). Levels of serum RNA indicate the presence of cccDNA transcriptional activity and might be used to monitor response to treatment and identify patients eligible for the safe discontinuation of NA. Also, HBcrAg might be used as a marker of cccDNA and its quantification could help to monitor response to current therapies[12]. New biochemical tools are in development to improve the diagnostic approach to virus activity and reservoir.

Available treatments: Pros and cons

Peg-IFN has lower antiviral activity but a higher rate of HBeAg and HBsAg loss than NAs, thanks to its immunomodulatory effect. It is administered as subcutaneous injections, which is a clear drawback, once weekly. It amounts to multiple adverse effects. And its use is strictly prohibited in patients with decompensated cirrhosis and autoimmune and psychiatric illnesses. Caution is mandatory in patients with compensated cirrhosis because it may precipitate hepatic decompensation. A 1-year course of pegylated IFN results in a rate of HBeAg seroconversion and HBsAg loss of 30% and 3%, respectively, in patients who are HBeAg-positive. Undetectable HBV-DNA is achieved in 25% of cases. The response is durable and rates of HBeAg and HBsAg loss increase after the end of treatment (to about 8%). The finite duration of treatment (48-52 wk) is a strong point[13].

NAs are administered orally, have irrelevant adverse effects, and can be safely administered in patients with decompensated cirrhosis or acute liver failure[14]. Their main drawback is the need for long-term treatment in the majority of the patients.

Among NAs, ETV, TDF, and TAF are normally preferred; they boast high antiviral activity and barriers to resistance. After 1 year of treatment of HBeAg positive patients, NAs allow to get HBeAg seroconversion and HBsAg loss, respectively, in 10%-21% and < 1%-3%. Rates of undetectable HBV-DNA (61%-76%) are higher than those achieved by IFNs. HBeAg negative patients treated for a year with pegylated

IFN, achieve a higher rate of HBsAg loss (4% *vs* 1%) than the same duration of ETV, TDF, or TAF, despite a lower rate of HBV-DNA clearance (63% *vs* 94%). TAF is a prodrug of TDF, a nucleotide analog that inhibits reverse transcription of the human immunodeficiency virus (HIV) and HBV[15]. It has similar antiviral activity as TDF but its superior plasma stability and more effective active metabolites, allow the use of lower doses with similar antiviral activity and less systemic exposure. It has, therefore, a smaller damaging impact on glomerular filtration rate and bone mineral density.

What's in store for the future? Antivirals

Following the mirage of a sterilizing cure able to eliminate both cccDNA and integrated HBV-DNA, new approaches are being developed for the treatment of HBV.

Here we describe new drugs for HBV, including drugs that target different steps of the HBV life cycle and immune modulators. Some of them are under phase 2 trials.

Entry inhibitors: Some novel drugs aim to block HBV entry into hepatocytes that may protect hepatocytes not yet infected or antagonize *de novo* infections. The sodium taurocholate co-transporting polypeptide (NTCP) is the entry receptor for HBV/HDV into hepatocytes. Bulevirtide (Myrcludex B) is a peptide that mimics the NTCP-binding domain of HBV, blocking HBV and HDV entry into naïve hepatocytes[16].

Bulevirtide is approved in the European Union for the treatment of chronic HDV infection in compensated liver disease.

A multicenter, open-label phase 2 clinical trial (MYR203) assessed the efficacy of myrcludex B in combination with peg-IFNα2a in HDV/HBV co-infected patients. Sixty co-infected patients were randomized to receive peg-IFN or peg-IFN plus bulevirtide, or bulevirtide for 48 wk. Among the 60 patients, 27% of them in the peg-IFN plus bulevirtide group achieved HBsAg loss at 48 wk of treatment[17].

NTCP is a transporter of conjugated bile salts from the plasma into hepatocytes. Consequently, adverse events such as elevated plasma bile acids were reported in a dose-dependent manner but without clinically relevant effects (such as cholestasis and consequent pruritus or steatorrhea)[18].

Most of these studies are focused on HBV/HDV co-infection, but we expect the result of a new trial in chronic hepatitis B mono-infection.

Capsid assembly modulators: Inhibiting nucleocapsid formation is a promising strategy for HBV therapy. Nucleocapsid contains the viral DNA needed for replication. Two core protein allosteric modulators (CpAM-capsid assembly modulators) exist: Class I CpAMs lead to the formation of misassembled capsids. Class II CpAMs accelerate capsid assembly and form morphologically normal capsids that are empty.

NVR 3–778, first-in-class CpAM, has been evaluated in different studies. In a phase 1 study of HBeAg-positive patients with chronic infection, NVR 3-778 was well tolerated[19]. The largest drop in HBV-DNA levels was achieved in the cohort treated with NVR 3-778 in combination with pegIFN.

GLS4 is an inhibitor of HBV capsid assembly that induces the formation of aberrant nucleocapsid structures. A randomized, open-label phase 1b study examined the efficacy of GLS4 (at different doses) in combination with ritonavir for treating chronic HBV infection compared with entecavir alone. GLS4 120 mg administrated for 28 d, resulted in a reduction in serum HBV-DNA and HBV pre-genomic RNA (pgRNA) levels without major side effects[20].

RO7049389 disrupts HBV nucleocapsid assembly and induces the depletion of core proteins, thereby effectively inhibiting HBV replication. A multicenter, ongoing phase 1 study is investigating the efficacy of single and multiple doses of RO7049389 in healthy volunteers and chronic HBV participants. RO7049389 administration to HBV patients at different dosages, for 28 d, achieved a drop of HBV-DNA and RNA, in both HBeAg positive and negative patients with HBV-DNA levels lower than the lower limit of quantification in 81.3% of them[21].

JNJ-6379 (JNJ-56136379) is a class II CpAM that interferes with capsid assembly and inhibits *de novo* formation of cccDNA. A phase II study is ongoing. JNJ-6379 administrated for 4 wk to treatment-naïve patients with CHB, showed potent antiviral activity: HBV-DNA and HBV-RNA decreased from baseline in all patients. On day 29, 32% of 41 patients had levels of HBV-DNA below the lower limit of quantification[22]. The most interesting drop of HBV-DNA and RNA was found in the Asian cohort treated at a dose of 75 mg/d. After discontinuation of treatment, the viral load returned to baseline levels[23]. JNJ-6379 did not interfere with HBsAg or HBeAg.

ABI-H0731 (vebicorvir) is a potent and selective class II CpAM. In the 101B study, ABI-H0731 was administrated to HBeAg-positive patients for 28 d and obtained a

dose-dependent HBV-DNA reduction (maximum decline of 4.0 $\log_{10}$ IU/mL) with a parallel HBV RNA decline. No changes in HBsAg, HBeAg, or HBV core-related antigen were reported[24].

JNJ-64530440 and QL-007**,** two assembly modulators, and ABI-H2158, a core protein binding, are further interesting objects of study.

We need to further studies to establish whether CpAMs can eliminate HBsAg, HBeAg, and/or cccDNA but the combination strategy seems to be the better one.

Post-transcriptional inhibitors: Synthetic small interfering RNAs (iRNA) or silencing RNAs, are a class of non-coding RNAs that recognize complementary viral mRNA and pgRNA, inducing mRNA degradation after transcription and preventing translation [25]. In the contest of HBV infection, small iRNAs bind HBV mRNA in hepatocytes and induce its degradation. Their target is all RNA transcripts, derived from cccDNA [26].

ARC-520 was the first iRNA therapeutic targeting HBV. A single-dose phase 2 study showed that HBsAg was reduced more in HBeAg positive patients than in HBeAg negative or treatment-experienced patients[8]. To explain these findings, it should be recalled not only that HBsAg production mostly depends on integrated HBV-DNA, which is the main source in HBeAg negative and NA-experienced patients but also that ARC-520 is not able to target mRNA produced by integrated HBV-DNA.

Two randomized multicenter studies evaluated the HBsAg decrease after multiple doses of ARC-520 compared to placebo, in NA–experienced HBeAg negative or positive patients[27]. A high dose of ARC-520 (2 mg/kg) reduced HBsAg. The absolute reduction was larger in the HBeAg positive cohort, confirming that a significant proportion of HBsAg, in HBeAg negative patients, could derive from integrated HBV sequences. Ideal iRNA should target all viral transcripts. A second-generation iRNA targeting all HBV transcripts, ARC-521, reduces HBsAg and viral load as demonstrated in a phase I trial (NCT02797522). Both ARC-520 and ARC-521 studies were stopped for the lethal toxicity of specific delivery vehicles in non-human primates. A modified iRNA, JNJ-3989 (formerly ARO-HBV), is under investigation (NCT03365947). The HBsAg decline is strong in treatment naïve and experienced patients regardless of HBeAg status due to its ability to silence mRNA from cccDNA and host integrated viral DNA. ARB-1467 was tested in a phase II study, showing a consistent decline of HBsAg in 63.6% of patients (NCT02631096). An ongoing study is evaluating the efficacy of VIR-2218 in infected volunteers[28]. Preliminary results are promising to show a decline in HBsAg, but we are waiting for official data.

Inhibitors of HBsAg release: HbsAg proteins are not only incorporated into the viral envelope but also stored in non-infectious subviral envelope particles (SVP) produced by HBV, independently of viral replication. These particles, without capsid or genome, account for an important source of HBsAg in the blood and induce immune exhaustion against HBsAg. To eliminate spherical SVP is a major goal of a functional cure. Nucleic acid polymers (NAPs), such as REP 2139, target the assembly/secretion of SVPs. An open-label, phase 2 study tested the effectiveness of REP 2139 or REP 2165 combined with TDF and peg-IFN in HBeAg-negative CHB patients. The addition of NAPs to TDF and peg-IFN, without reducing the tolerability, significantly increased rates of HBsAg loss and HBsAg seroconversion during therapy[29]. After 12 mo of treatment, HBsAg levels were very low in more than half of the patients and after a temporal interval of 1 year, virologic control persisted in about half of them.

Neutralization: Lenvervimab is a recombinant human monoclonal IgG1-type anti-S antibody that binds HBsAg, inhibiting viral penetration into hepatocytes and reducing HBsAg levels. The neutralization of virions or HBsAg is obtained by immune complexes. The HBV neutralizing activity of lenvervimab had previously been demonstrated in a chimpanzee animal model[30].

In a prospective, open-label phase I trial, a single injection or 4 weekly doses of lenvervimab were administered (doses from 80.000 to 240.000 IU) to HBeAg-positive patients, reducing levels of HBsAg to below undetectable levels for up to 1 mo[31].

Inhibitors of cccDNA: As mentioned above, viral transcription requires a template, consisting of cccDNA. Curative treatment for HBV infection would require a disablement of cccDNA and several mechanisms have been studied to eradicate or silence cccDNA expression. The possibility of using sequence-specific RNA-guided nucleases (RGNs) and proteins has been explored. Among RGNs, the best present results are linked to transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated (Cas) systems[32-34].

Nucleases cleave sequences in the HBV genome, resulting in mutated cccDNA, which is, in turn, transcribed into mutated viral proteins, not adequate to viral replication. Preclinical experiments showed that the CRISPR/Cas9 system, the most efficient method for gene inactivation, elicits inactivation of cccDNA, through mutations and deletions, but the application of this technology in a clinical setting requires further studies. For the clinical application, several challenging issues must be solved, first of all, the risk of genome instability.

Table 1 summarizes the main molecules to treat hepatitis B with the synthesis of the mechanism of action.

What's in store for the future? Immunotherapy

The progression to chronic HBV infection seems to be favored by weak immune responses and labile innate immune responses. It has been demonstrated that, in "recovered" patients, the early HBsAg clearance is fostered by activation of different cellular receptors [Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)] that allow the production of antiviral cytokines (IFN-α primarily), and, activation of natural killer (NK) cells. Activated innate immunity also boosts the adaptive immune system, leading to the maturation of B- and T-cell clones that target infected hepatocytes.

The progression to chronic HBV infection is determined by dysfunctional NK cells and plasmacytoid dendritic cells (pDCs). High levels of HBsAg or HBeAg not only block maturation of antigen-presenting cells such as DCs, causing a tolerogenic environment, but also deregulate the production of IFN and other cytokines by NK cells, NK T cells, and Kupffer cells. Prolonged exposure to viral antigens could be associated with functionally impaired immune response against the virus. Furthermore, HBV-specific cytotoxic T cell response, essential for clearance of infected hepatocytes, is inadequate with a poor cytotoxic activity and impaired cytokine production. This phenomenon has been described as "T cell immune exhaustion" and is responsible for HBV persistence. Several studies have reported overexpression of programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) in CD4+ or CD8+ T cells during chronic HBV infection.

Among compounds highly active against innate immunity, some immune stimulators (such as inarigivir) and TLR agonists (such as TLR7 and TLR8) have been studied. Among immunologic agents with activity against adaptive immunity, we must remember therapeutic vaccines (*e.g.*, GS-4774) and anti-PD-1 antibodies (*e.g.*, nivolumab) that are objects of ongoing studies.

TLR agonists: TLRs constitute the first line sensors of microorganisms and activate the production of mediators including IFNs and cytokines. TLR agonists promote up-regulation of type I IFN and cytokines, stimulating innate immunity response directed against the virus. The activation of TLR mediated pathways leads to the suppression of HBV replication. As an agonist of TLR-7 involved in IFN production, GS-9620 was tested in a human hepatocyte cell line infected with HBV; it showed suppression of HBV replication but no reduction in cccDNA levels. In woodchucks and chimpanzees, the TLR-7 agonist showed similar results. When used in humans with HBV-DNA suppression in the course of NA therapy, TLR-7 agonist (vesatolimod GS-9620) did not obtain any effect on HBsAg, HBeAg, and HBV-RNA levels despite inducing an HBV specific immune response. Another agonist for TLR-8 (Selgantolimod GS-9688) was investigated in patients with CHD and chronic suppression of the viral load, showing no significant impact on HBsAg, HBV-DNA, or RNA. Now this study is in phase II [35]. Several other TLR-7 and TLR-8 and 9 agonists are now entering clinical trials.

Retinoic acid-inducible gene-1 agonists: Type III IFNs are produced in human hepatocytes against HBV, through RIG-I. Viral RNA activates this intracytoplasmic receptor, leading to the IFN response that is essential for antiviral immunity[36]. Inarigivir is an immune modulator with an antiviral effect. It acts as a RIG-I agonist, inducing type I and type III IFN production, thus boosting immune response. In the ACHIEVE study, 80 treatment naïve non-cirrhotic patients with chronic HBV infection were enrolled. They received, through a random sampling, different doses of inarigivir or placebo for 3 mo followed by tenofovir for 3 mo. Both HBeAg-positive and negative patients achieved HBV-DNA and RNA reductions in a dose-dependent manner, and an HBsAg decline of > 0.5 $\log_{10}$ at 12 or 24 wk was found in more than 20% of patients.

Immune checkpoint inhibitors: Apoptosis is a fundamental mechanism at the basis of HBV-specific CD8 T cell depletion. Interruption of this mechanism restores CD8 T-cell responses against HBV. In the setting of chronic HBV infection, inhibitory receptors, like PD-1 and programmed death-ligand 1 (PD-L1) are overexpressed on exhausted T

Table 1 New antiviral drugs for hepatitis B

	Molecule(s)	Mechanism of action	Ref.
Entry inhibitors	Bulevirtide (Myrcludex B)	Block HBV entry into hepatocytes to protect cells not yet infected or antagonize *de novo* infection	[16, 17]
Capsid assembly modulators	NVR 3-788; GLS4; RO7049389; JNJ-6379; JNJ-0440; ABI-H0731	Inhibition of nucleocapsid formation	[19-24]
Post-transcriptional inhibitors	ARC-520; ARC-521; ARB-1467	Induction mRNA degradation after transcription and translation prevention	[27, 28]
Inhibitors of HBsAg release	REP 2165; Lenvervimab	Removal of subviral envelope particle. Neutralization activity	[29-31]
Inhibitors of cccDNA	CRISP-Cas system	Disablement of cccDNA	[32-34]

HBsAg: Hepatitis B surface antigen; cccDNA: Covalently closed circular DNA; HBV: Hepatitis B virus.

cells, limiting the effector function[37]. The block of the PD-1/PD-L1 pathway seems to enhance their function. In a few words, checkpoint inhibitors may restore T cell dysfunction.

In a recent phase I pilot study, the efficacy of the PD-1 inhibitor nivolumab was investigated. Twenty-four NA-suppressed anti-HBe positive patients received nivolumab at two different dosages, combined or not with the therapeutic vaccine GS4774. Patients that received higher doses had a noticeable decline in HBsAg levels and one of the patients receiving both nivolumab and the therapeutic vaccine GS4774 lost HBsAg[38]. Exhausted CD8+ T cells present also co-inhibitory molecules such as CTLA-4 and CD244/2B4 whose blockade, *in vitro* studies, seems to achieve restoration of immune function.

Stimulator of interferon genes agonists: The use of synthetic agonists for activation of the stimulator of interferon genes (STING) seems to stimulate the production of cytokines, especially IFNs. In mouse models, intraperitoneal infusion of a STING agonist stimulated the expression of IFN genes and mitigated HBV replication in mouse hepatocytes[39]. Such agonists may be exploited for the development of novel anti-HBV strategies.

Problem of antiviral resistance in chronic hepatitis B

Nucleoside and nucleotide analogs inhibit the viral DNA polymerase, causing an early stop of HBV replication. Drug-resistant strains of HBV have mutations in the viral polymerase gene. Resistance mutations alter the interaction and the inhibition of the drug on the viral polymerase. These mutants persist even after the end of treatment and determine cross-resistance to the next drug[40]. To prevent resistance development, guidelines recommend, as the first approach, the use of NAs with a high barrier to resistance. Sequential monotherapies with a low barrier to resistance should be avoided. In all cases of treatment failure, it is essential to precociously identify possible resistance mutations without forgetting to always check compliance to NAs[2].

What's in store for the future? Therapeutic vaccination

The therapeutic vaccine has shown encouraging results in animal models; non-similar results were demonstrated in humans for both vaccines, with adjuvant or with DNA. They are developed to stimulate the host immune response to suppress HBV replication and foster HBsAg loss. Therapeutic vaccination, alone or associated with oral antiviral therapy, is not sufficient to restore HBV immunity in chronically infected patients. Combination strategy failure was demonstrated by some studies, as the randomized study of Vandepapelière *et al*[41]: The difference between the HBe seroconversion rate obtained by the co-administration of vaccine and lamivudine (18.8%) or by lamivudine alone (16.1%) was not significant[41].

Previous vaccines only targeted HBsAg, but the new ones, under development, are against multiple HBV proteins and novel adjuvants. GS-4774 and TG-1050 are precisely the results of approaches based on multiple HBV proteins.

GS-4774 is a vaccine that uses recombinant *Saccharomyces cerevisiae* yeast to express surface, core, and X proteins. Virally suppressed patients, on treatment with oral NAs, were randomized to continue antiviral therapy alone or associated with different yeast

units of GS-4774. GS-4774 did not determine significant reductions in HBsAg levels. Five HBeAg-positive patients treated with GS-4774 showed HBeAg loss while none in the control group showed seroconversion to anti-HBe. Increased production of IFN-α, TNF-α, and IL2 by CD8+ T cells was documented[42].

TG1050 is an adenovirus-based vaccine that encodes a fusion protein composed of HBV polymerase and domains of HBcAg and HBsAg. This is a T-cell-inducing vaccine and has demonstrated antiviral effects in mice. In CHB patients treated with NAs, TG1050 is safe and effective in inducing HBV-specific cellular immune response. It induced functional IFN-γ producing cells and despite that serum HBsAg did not decrease significantly following TG1050, a robust decrease of HBcAg was shown in some patients[43].

Despite unsatisfactory results, it is now clear that a therapeutic vaccine could effectively achieve a functional cure only if associated with NAs or immunomodulatory therapies.

HEPATITIS D

HDV is a highly pathogenic virus that causes acute, fulminant, and chronic hepatitis, causing cirrhosis in more than 70% of cases. HDV has been defined as a defective virus because it needs the helper function of HBV for its transmission. The two possible scenarios are simultaneous infection of HDV and HBV (coinfection) that leads to chronic hepatitis D in < 5%, or HDV superinfection in chronic hepatitis B patients (superinfection); in this case, chronic hepatitis occurs in up to 90% of patients[44,45].

Permanent HDV-RNA suppression is associated with improved clinical outcomes. The treatment for chronic HDV infection with IFN therapy achieves a virologic response rate of about 17%-47%[46,47]. This translates into a rare curative outcome. Moreover, late viral relapses frequently occur in patients with chronic HDV hepatitis who have achieved sustained virological response (SVR). The treatment of choice in patients with HDV and HBV infection with compensated liver disease is peg-IFNa for 48 wk. The serious side effects (influenza-like illness, alopecia, leucopenia, thrombocytopenia, and emotional lability) make IFN a drug with huge limits.

NA treatment is also recommended when HBV-DNA levels are constantly less than 2.000 IU/mL and in patients with advanced liver disease[2].

In the last years, many efforts have been made to identify new therapeutic targets for HDV treatment. As mentioned above, NTCP is an important receptor for HBV to enter hepatocytes. HDV virions also attack the viral receptor NTCP before membrane fusion and release of the ribonucleoprotein into the cytoplasm. The entry of HDV into new cells can be blocked by bulevirtide (Myrcludex B) according to its action on a specific receptor NTCP. Bulevirtide is a chemically synthesized peptide composed of amino acids derived from large HBV surface proteins. Bulevirtide was recently approved in the European Union for treatment of chronic hepatitis D in HDV-RNA positive adult patients when liver disease is compensated. Bulevirtide blocks the virus entry into the cells and limits the HDV replication capacity. As mentioned above, two main studies showed that bulevirtide is effective at clearing HDV-RNA from the blood. In the first study, the MYR202 study, 55 out of 90 patients who received bulevirtide and TDF lost HDV-RNA after 6 mo, compared with 1 out of 28 patients treated with TDF alone. The combination treatment was associated with a significant normalization in alanine aminotransferase and an interesting improvement of liver function, as compared to patients who received TDF alone[48].

Similar results were seen in the second study, the MYR203, where patients were randomized to bulevirtide plus peg-INF alpha-2a, bulevirtide alone, and PEG-IFN alpha-2a alone for 48 wk[17]. At 48 wk, HDV-RNA was undetectable in 80% of patients treated with bulevertide 2 mg plus peg-INF alpha-2a while lower rates were obtained in the other two groups. Final results at week 72 confirmed the effectiveness of the association strategy, showing undetectable HDV-RNA in 53.3% of cases *vs* 0% in the IFN group[49].

A fundamental step in the virus assembly process consists of the modification (prenylation) of a specific HDV protein. This protein is the large delta antigen (HDAg). Prevention of prenylation blocks the formation of viral particles or more precisely, the assembly of mature HDV. A new class of antiviral agents, capable of inhibiting prenylation, have been developed for use in humans[50]. Lonafarnib is an oral prenylation inhibitor. A proof-of-concept phase 2A double-blinded randomized placebo-controlled study showed a decline in serum HDV-RNA levels in patients treated with lonafarnib (100 mg or 200 mg twice daily) compared with placebo. The

decline in virus levels was proportional to serum drug concentration. Two-thirds of patients treated with the higher dose had a viral rebound and ALT flare after the end of treatment[51].

Triple combination therapy with lonafarnib, ritonavir, and peg-IFN for 6 mo showed antiviral efficacy in patients with chronic HDV infection, as demonstrated in a recent study (NCT03600714; n = 26)[52]. At the end of treatment (week 24), 77% of patients achieved an important goal, the decrease of HDV RNA (> 2 log).

Recent studies are investigating the capacity of NAPs to block HBsAg release from infected hepatocytes by some non-immunostimulatory mechanism. NAPs clear circulating HBsAg by hindering the release of HBV and HDV particles. The REP-2139 is the first NAP selected for human studies. Unpublished interim results from an ongoing proof of concept trial have confirmed the ability of REP 2139 to achieve clearance of HDV-RNA in 50% of treated patients up to 12 mo after the end of treatment. The adverse events (pyrexia, chills, conjunctival hyperemia, headache, and asthenia) could represent a serious limit.

Further studies are needed to assess the efficacy and tolerability of NAPs. Bulevirtide and lonafarnib are antiviral agents in an advanced phase of investigation with a proven anti-HDV activity.

HEPATITIS C

HCV infection is one of the most important causes of chronic liver disease in the world. The primary goal of HCV therapy is the infection cure, achieving an SVR consisting of undetectable HCV-RNA. SVR also means normalization of transaminase, reduction of liver necro-inflammation and fibrosis, improvement in liver function, and reduction of HCC risk[53].

The approval of direct-acting antivirals (DAAs) in 2014 revolutionized the treatment of HCV, allowing nearly all patients to be cured. DAAs are highly effective and well-tolerated and represent the gold standard for the treatment of patients with chronic infection, in all stages of liver disease[54]. Most of the progression in HCV treatment is due to the possibility to treat many populations, once excluded for the discouraging side effects of IFN-based therapies. At present, high SVR rates are achieved in cirrhotic patients, HIV/HCV co-infected patients with chronic renal failure, or transplant patients. The efficacy is not undermined by the short duration of DAA therapy.

Thanks to the IFN-free regimens developed, the World Health Organization (WHO)'s goal for HCV infection is a 90% reduction in the incidence of new infections and reduction of HCV-related deaths by approximately 65% in 2030[55,56].

There are many available EMEA- and FDA-approved DAAs for HCV treatment, divided into three major classes based on their HCV proteins targets: Protease NS3/4A inhibitors (glecaprevir, grazoprevir, paritaprevir, simeprevir, and voxilaprevir), NS5A serine protease inhibitors (PIs) (daclatasvir, elbasvir, ledipasvir, ombitasvir, pribrentasvir, and velpatasvir), NS5B RNA-dependent RNA nucleoside polymerase (sofosbuvir), and non-nucleoside polymerase (dasabuvir) inhibitors[57]. NS3/4A PIs inhibit HCV polyprotein processing; NS5A inhibitors inhibit viral replication and assembly; NS5B polymerase inhibitors block HCV-RNA replication.

The HCV drug combinations available in Europe are sofosbuvir and velpatasvir in a single tablet administered once daily; sofosbuvir, velpatasvir, and voxilaprevir available in a combination product, glecaprevir, and pibrentasvir or grazoprevir and elbasvir available in a two-drug fixed-dose combination. The non-pan-genotypic combination of grazoprevir and elbasvir can also be used in patients infected with HCV genotype 1b. Pan-genotypic HCV drugs, in particular sofosbuvir plus velpatasvir and glecaprevir plus pibrentasvir, can be used even if the virus genotype is not available.

Contraindications to current DAAs are few. The pharmacological interactions are worthy of attention: Cytochrome P450/P-gp-inducing agents are often not switchable. The use of grazoprevir, glecaprevir, or voxilaprevir is allowed only in a contest of compensated cirrhosis (Child-Pugh B or C) and in patients without previous episodes of decompensation (EASL guidelines)[54]. Patients with decompensated cirrhosis and patients with compensated cirrhosis with a medical history characterized by previous episodes of decompensation should be treated with sofosbuvir and velpatasvir and ribavirin for 3 mo. If there are contraindications to the use of ribavirin or poor tolerance, a fixed-dose combination of sofosbuvir and velpatasvir for 24 wk without ribavirin can be used.

Two innovative and promising strategies are required and expected to obtain a global HCV elimination: A deep enhancement of screening and linkage to care for so-called "hard-to-reach" populations. The strengthening of new strategies to improve access to care for vulnerable and marginalized populations with HCV infection is becoming urgent[58]. Moreover, it has been demonstrated that a drastic decrease in HCV incidence requires proper education on injection safety, appropriate screening blood transfusions, and delivery of sterile syringes to people who inject drugs[55].

Political will and financing issues, thus, are essential to achieve elimination or a significant reduction in HCV infection prevalence and incidence.

Apart from the implementation of measures to reduce the prevalence of HCV infection, a big innovation in the HCV field is linked to a paper, published in 2020, that suggests DAA therapy as the prevention of HCV transmission. A single-arm trial evaluated short-course prophylactic therapy in patients (n = 30) receiving solid organ transplants from donors with HCV infection[59].

Prophylaxis with glecaprevir-pibrentasvir combined with the HCV entry blocker ezetimibe (from 6-12 h to 7 d after transplantation) showed prevention of chronic HCV infection in all 30 transplant recipients. This innovative study could help to reduce ethical concerns regarding the transplantation of HCV-infected organs into recipients without HCV.

In conclusion, the treatment with new oral DAA combinations is efficient and safe even in special populations and in patients with advanced liver disease or severe comorbidities. Eradication of HCV by 2030 is not a utopian goal if all countries direct efforts towards the same objective: Preventing transmission measures, improving screening programs (especially in marginalized populations without contact with healthcare services or incarcerated individuals), and enforcing educational approach among people who inject drugs who represent the most vulnerable group for HCV transmission.

Problem of DAA resistance in the treatment of hepatitis C

A large part of the responsibility for DAA failure, which involves a minority of patients (4%-5%), is the inadequate adherence to therapy. In some cases, resistance-associated variants (RAVs) directly cause relapse or viral breakthrough. RAVs affect all DAA classes but mostly occur in the NS5A region. RAVs arising after treatment are more difficult to treat; however, patients who fail to achieve SVR after first-line DAA therapy, respond to sofosbuvir/velpatasvir/voxilaprevir and glecaprevir/pibrentasvir [60].

HEPATITIS E

HEV infection has recently been known to represent a major health problem in industrialized countries, not only in developing countries. This peculiar virus, transmitted as a zoonotic infection (uncooked meat consumption) or through infected blood or blood products, is endemic in most high-income countries (mainly genotypes 3 and 4) and is an important cause of acute and chronic viral hepatitis, the latter affecting immunosuppressed patients (such as solid transplant recipients, patients with hematological malignancies receiving chemotherapy, and HIV infected patients). The incidence of acute HEV infection is about 3 million human infections *per* year worldwide. In the endemic areas, HEV is transmitted predominantly by the fecal-oral route while in the non-endemic areas, HEV is a foodborne illness. Immunocompetent individuals develop self-limiting acute icteric hepatitis. It is a mild form providing long-term protection. HEV is the leading cause of 1%-4% of acute viral hepatitis cases in the general population and 30% in pregnant women. Few patients have severe illnesses leading to fulminant hepatic failure. In immunocompromised patients, acute infections progress in over 60% of cases to chronicity and in 10% to liver cirrhosis. The presence of HEV-RNA for more than 12 wk stands for chronic HEV infection. HEV chronicity is most common among liver transplant recipients. Interestingly, chronic HEV infection has been reported to be associated with a variety of extra-hepatic manifestations, for example, neurological and renal (neuralgic amyotrophy, Guillain-Barré syndrome, and membranoproliferative and membranous glomerulonephritis) [61]. HEV can also act as a potential trigger of acute-on-chronic liver failure (ACLF) that causes a worsening of the liver function with clinical complications such as ascites and encephalopathy. Acute HEV infection is responsible for 3.2% of the cases of decompensation in patients with chronic hepatic disease, as shown in a large prospective study including 343 patients, with elevated mortality[62].

In chronic HEV infection, the reference tests for the confirmatory diagnosis are the detection of HEV-RNA in serum or stool samples by reverse transcriptase-polymerase chain reaction or loop-mediated isothermal amplification for a minimum of 3–6 mo duration[63]. Antibody tests are not useful in the diagnosis of chronic HEV.

There are no approved treatments for this infection although reduction of immunosuppression can lead to HEV clearance in one-third of patients. The study of Kamar *et al*[64] showed that reduction in immunosuppressive therapy that specifically targets T cells could promote HEV eradication in transplant recipients, in the face of a high risk of graft rejection[64]. However, dose reduction of immunosuppressive medications, particularly those targeting T lymphocytes, is the first-line therapeutic approach for solid organ transplant recipients with HEV; mycophenolate mofetil seems able to suppress viral replication.

The currently recommended drug for the treatment of severe acute and chronic hepatitis E in solid transplant recipients is off-label oral ribavirin. The EASL published guidelines (2018) recommended the use of ribavirin as a standard of care; however, the optimal ribavirin treatment regimen is not known. Ribavirin inhibits HEV-RNA replication by depleting guanosine triphosphate pools.

In a large, retrospective, multicenter case series authors showed that a 3 mo course of ribavirin is a proper strategy to obtain SVR in chronic HEV infection[65]. Ribavirin can cause anemia, in up to 50% of patients. Peg-IFN-α has been reported as a possible treatment in the setting of liver transplant recipients: Peg-IFN-alpha 2a administered to three liver transplant patients with chronic HEV infection for 2 mo, resulted in viral RNA clearance. Peg-IFN-γ with or without ribavirin has also been shown to be effective in the treatment of HEV-HIV patients and non-transplant immunosuppressed patients with hematological disorders[66]. IFN can be considered in liver transplant recipients and patients undergoing hemodialysis; IFN is contraindicated in many solid-organ transplant patients (kidney, heart, and lung) because of the risk of rejection.

Based on available data, a decrease in immunosuppression (if feasible) and ribavirin at a dose of 600-800 mg *per* day for 3 mo are recommended in patients with chronic HEV hepatitis. In the case of ACLF provoked by HEV, the efficacy of ribavirin is not clear. Ribavirin is contraindicated in pregnancy due to teratogenic potential, and can be suggested for pregnant women only in the last trimester of pregnancy.

Sofosbuvir was also reported to reduce HEV viral load without achieving SVR in seven chronic HEV-infected patients. Sofosbuvir has therapeutic activity against HEV with an additive effect to ribavirin. Sofosbuvir with ribavirin, in chronic HEV infection, promotes a reduction in HEV levels and ALT during therapy, but a rise has been documented after stopping treatment, confirming its inability to provide SVR in chronic HEV infections.

Further studies are needed to assess the efficacy and safety of Hecolin, the first vaccine produced by China, not approved yet for commercialization. This vaccine (30 mcg of purified recombinant hepatitis E antigen *per* dose) given at 0, 1, and 6 mo showed 100% efficacy in phase III clinical trial[67]. The safety and efficacy of the vaccine in patients with chronic liver disease and other populations, for example, immunosuppressed patients, are needed before recommended for its widespread use.

There is a clinical need for new therapeutic options for HEV in immunosuppressed patients. Large scale studies are required to understand the impact of some promising compounds such as 2'-C-methylguanosine, which suppresses the growth of HEV in cell cultures, zinc that could act as an adjuvant therapy in ribavirin resistant/relapsed HEV infections, and silvestrol that diminished fecal HEV-RNA in mice but has not been tested on humans[68,69].

CONCLUSION

Novel DAAs have shown an unimaginable efficacy of curing HCV to the point that the WHO defined it as an achievable target elimination of HCV by 2030. To pursue this goal, marginalized and stigmatized patients should be better engaged in the healthcare system to diagnose infection and encourage treatment adherence.

Concurrently with the approval of DAAs for HCV, starting in 2014, a renewed interest in pursuing curative therapies for HBV infection has been registered. Current NAs positively impact liver-related complications, ensuring a high level of safety and tolerability. The need for long, or even lifelong, therapy and the oncogenic role of HBV, fostered by integration into the hepatocytes genome, has increased the effort to develop definitive and curative therapy. Novel approaches to HBV cure are based on

new antivirals, targeting different steps of the HBV life cycle and immune modulators. Molecules acting on the HBV cycle have obtained encouraging results. Immune-based approaches are also emerging but must be further examined. Ideal and utopistic therapy should pursue a sterilizing aim, consisting of elimination of cccDNA or silencing its activity. A combination of traditional and new anti-HBV agents may favor HbsAg clearance and eliminate the cccDNA reservoir from the patient's liver, reducing the oncogenic effect.

About HDV, the refined knowledge of its life cycle has facilitated the study of some direct-acting agents, bulevirtide, lonafarnib, and NAPs, which are more effective than IFN-based therapies. In particular, bulevirtide is the first drug approved for the treatment of HDV in adults with compensated liver disease in Europe.

Severe side effects and limited effectiveness of current HEV therapies have also made it essential to identify new therapeutic approaches for HEV, especially in immunosuppressed individuals. Further studies are required to assess the efficacy of adjuvant zinc therapy, 2'-C-methylguanosine, sofosbuvir, or the natural compound silvestrol.

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