revisionedefinitiva

by Simona Leoni

General metrics

77,159	11,546	597	46 min 11 sec	1 hr 28 min
characters	words	sentences	reading time	speaking time

Score

87
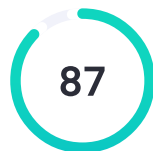
This text scores better than 87% of all texts checked by Grammarly

Writing Issues

339	✓	339
Issues left	Critical	Advanced

Unique Words

Measures vocabulary diversity by calculating the percentage of words used only once in your document

22%

unique words

Rare Words

Measures depth of vocabulary by identifying words that are not among the 5,000 most common English words.

39%

rare words

Word Length

Measures average word length

4.6

characters per word

Sentence Length

Measures average sentence length

19.3

words per sentence

revisionedefinitiva

Viral hepatitis: innovations and expectations in 2020

Viral hepatitis treatment

Simona Leoni, Alberto Casabianca, Benedetta Biagioni, Ilaria Serio

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

Author contributions: Leoni S and Serio I reviewed the literature and wrote the paper; Casabianca A and Benedetta B contributed to manuscript drafting. All authors issued final approval for the version to be submitted.

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 be evolved 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 compensated liver disease. Further studies will be 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

Leoni S, Casabianca A, Biagioni B, Serio I. Viral hepatitis: innovations and expectations in 2020. World J Gastroenterol 2021; In press

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 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 IFN-based therapies. New studies are required to improve the treatment of hepatitis E.

INTRODUCTION

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, hepatitis B, C, D, E virus (HBV, HCV, HDV, 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-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 can be classified into two categories: pegylated interferon (peg-IFN) and nucleotide or nucleoside analogs (NAs) [2].

In particular, there are 8 approved drugs: interferon and pegylated-interferon, lamivudine, adefovir, telbivudine, entecavir, tenofovir disoproxilfumarate (TDF), 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

Current antiviral treatment's aim is the induction of two different responses: virologic and biochemical [3].

Virologic response A virologic response during NAs 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, six months after the start of treatment and at the end of treatment.

Biochemical response A biochemical response is obtained when alanine aminotransferase falls into their 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 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 not a 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 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 an 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-responsiveness 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 infection 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 for 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 include tests to measure HBV RNA and hepatitis B core-related antigen (HbcrAg). Levels of serum RNA indicate the presence of cccDNA virus 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

Peginterferon 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 increases 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 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, allowing 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 in phase 2 trials.

Entry Inhibitors Bulevirtide (Myrcludex B): 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 hepatitis B and delta virus (HBV/HDV) into hepatocytes. Bulevirtide (Myrcludex B) is a peptide that mimics the NTCP-binding domain of HBV, blocks HBV and HDV entry into naive 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 weeks. Among the 60 patients, 27% of them in the peg-IFN plus bulevirtide group achieved HBsAg loss at 48 weeks off of cure [17].

NTCP is a transporter of conjugated bile salts from the plasma into hepatocytes. Consequently, although adverse events, elevated plasma bile acids were reported in a dose-dependent manner and 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 administered for 28 days, showed a reduction in serum HBV-DNA and HBV pre-genomic RNA (pgRNA) levels without major side effects [20].

RO7049389 disrupts HBV nucleocapsids 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 days, 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 weeks 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 with 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 days and obtained dose-dependent HBV-DNA reduction (maximum decline of 4.0 Log10 IU/mL) with 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 RNA (iRNA) or silencing RNA, is a class of non-coding RNA that recognize complementary viral

messenger RNA (mRNA) and pgRNA, inducing mRNA degradation after transcription and preventing translation [25]. In the contest of HBV infection, small interfering RNAs 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 in patients HBeAg positive more than HBeAg negative or treatment-experienced [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 NAs-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 NAs–experienced patients HBeAg negative or positive [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 naive and experienced patients regardless of HBeAg status due to this 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 the 12 months 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 [32,33,34].

Nucleases cleave sequences in the HBV genome, resulting in mutated cccDNA, in turn, transcribed in mutated viral proteins, not adequate to viral replication. Preclinical experiments showed that the CRISPR/Cas9 system, the most efficient method for gene inactivation, explicit 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.

In Table 1 are summarized 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, RIG-I) that allow the production of antiviral cytokines (interferon α in primis), 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 dendritic cells (DCs) causing a tolerogenic environment but also deregulating the production of IFN and other cytokines by natural killer (NK) cells, natural killer T cells, and Kupffer cells. Prolonged exposure to viral antigens could be associated with functional impairment 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 toll-like receptor 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-programmed cell death protein 1 (anti-PD-1) antibodies (e.g., nivolumab) that are objects of ongoing studies.

Toll-Like Receptor Agonists: Toll-like receptors (TLRs) constitute the first line sensors of microorganisms and activate the production of mediators: interferons and cytokines. TLR agonists promote up-regulation of type I interferon and cytokines, stimulating innate immunity response directed

against the virus. The activation of TLR mediated pathways leads to the suppression of HBV replication. 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 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 changes in 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 (RIG-I) Agonists: type III interferons are produced in human hepatocytes against HBV, through retinoic acid-inducible gene I (RIG-I). Viral RNA activates this intracytoplasmic receptor leading to the interferon response, 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 interferon 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 HBsAg decline of >0.5 Log10 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 programmed death receptor 1 (PD-1) and programmed death-ligand 1 (PD-L1) on exhausted T cells are over-expressed, 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, in two different dosages, combined or not with the therapeutic vaccine GS4774. Patients that received higher doses had a noticeable decline in Hepatitis B surface antigen 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 (STING) 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.

The problem of antiviral resistance in chronic hepatitis ìB

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 a high barrier to resistance NAs. Sequential monotherapies with a low barrier to resistance therapies should be avoided. In all cases of treatment failure, it's 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; not 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: 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].

If previous vaccines only targeted HBsAg, 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, in 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. Increase 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 serum, HBsAg did not decrease significantly following TG1050, in some patients robust decrease of HBcAg was shown [43].

Despite unsatisfactory results, it's now clear that a therapeutic vaccine could effectively, achieve a functional cure, only if associate 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 interferon 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 SVR. The treatment of choice in patients with HDV and HBV 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.

NAs 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 that 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 by the others 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 months 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 nucleic acid polymers (NAPs) to block HBsAg release from infected hepatocytes by some not 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 months 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 a sustained virological response (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 interferon-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 Interferon (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 3 major classes based on their HCV proteins targets: protease NS3/4A inhibitors (glecaprevir, grazoprevir, paritaprevir, simeprevir, voxilaprevir), NS5A serine protease inhibitors (daclatasvir, elbasvir, ledipasvir, ombitasvir, pribrentasvir, velpatasvir), NS5B RNA-dependent RNA nucleoside polymerase (sofosbuvir) and non-nucleoside polymerase (dasabuvir) inhibitors [57]. NS3/4A protease inhibitors (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 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 hours to 7 days 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), enforcing educational approach among people who inject drugs who represent the most vulnerable group for HCV transmission.

The 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
Hepatitis E virus (HEV) infection has recently been known to represent a major health problem in industrialized countries and 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, 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's a mild form

providing long-term protection. HEV is the leading cause of 1–4% of acute viral hepatitis 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 weeks 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, 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 is the detection of HEV-RNA in serum or stool samples by reverse transcriptase-polymerase chain reaction (RT-PCR) 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 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 administrated 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 the dose of 600-800 mg per day for 3 mo are recommended in patients with HEV chronic 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; 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, 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 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 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 nucleic acid polymers, 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.

REFERENCES

1 Global Burden of Disease Liver Cancer Collaboration., Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Mądry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol 2017; 3: 1683-1691 [PMID: 28983565 DOI: 10.1001/jamaoncol.2017.3055]

2 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu.; European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017; 67: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021.].]

3 Naggie S, Lok AS. New Therapeutics for Hepatitis B: The Road to Cure. Annu Rev Med 2021; 72: 93-105 [PMID: 33085923 DOI: 10.1146/annurev-med-080119-103356]

4 Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther 2010; 15: 133-143 [PMID: 20386068 DOI:

10.3851/IMP1497].]

5 Tout I, Loureiro D, Mansouri A, Soumelis V, Boyer N, Asselah T. Hepatitis B surface antigen seroclearance: Immune mechanisms, clinical impact, the importance for drug development. J Hepatol 2020; 73: 409-422 [PMID: 32333923 DOI: 10.1016/j.jhep.2020.04.013]

6 Revill PA, Chisari FV, Block JM, Dandri M, Gehring AJ, Guo H, Hu J, Kramvis A, Lampertico P, Janssen HLA, Levrero M, Li W, Liang TJ, Lim SG, Lu F, Penicaud MC, Tavis JE, Thimme R; Members of the ICE-HBV Working Groups; ICE-HBV Stakeholders Group Chairs; ICE-HBV Senior Advisors, Zoulim F. A global scientific strategy to cure hepatitis B. Lancet Gastroenterol Hepatol 2019; 4: 545-558 [PMID: 30981686 DOI: 10.1016/S2468-1253(19)30119-0.PMCID:]

7 Gao Y, Li Y, Meng Q, Zhang Z, Zhao P, Shang Q, Li Y, Su M, Li T, Liu X, Zhuang H. Serum Hepatitis B Virus DNA, RNA, and HBsAg: Which Correlated Better with Intrahepatic Covalently Closed Circular DNA before and after Nucleos(t)ide Analogue Treatment? J Clin Microbiol 2017; 55: 2972-2982 [PMID: 28747369 DOI: 10.1128/JCM.00760-17]

8 Wang X, Chi X, Wu R, Xu H, Gao X, Yu L, Liu L, Zhang M, Tan Y, Niu J, Jin Q. Serum HBV RNA correlated with intrahepatic cccDNA more strongly than other HBV markers during peg-interferon treatment. Virol J 2021; 18: 4 [PMID: 33407619 DOI: 10.1186/s12985-020-01471-2]

9 van den Berg F, Limani SW, Mnyandu N, Maepa MB, Ely A, Arbuthnot P. Advances with RNAi-Based Therapy for Hepatitis B Virus Infection. Viruses 2020; 12 [PMID: 32759756 DOI: 10.3390/v12080851].]

10 Liang TJ, Block TM, McMahon BJ, Ghany MG, Urban S, Guo JT, Locarnini S, Zoulim F, Chang KM, Lok AS. Present and future therapies of hepatitis B: From discovery to cure. Hepatology 2015; 62: 1893-1908 [PMID: 26239691 DOI: 10.1002/hep.28025]

11 Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, Ferrari C, Given BD, Hamilton J, Kanner SB, Lai CL, Lau JYN, Schluep T, Xu Z, Lanford RE, Lewis DL. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med 2017; 9 [PMID: 28954926 DOI: 10.1126/scitranslmed.aan0241]

12 Suk-Fong Lok A. Hepatitis B Treatment: What We Know Now and What Remains to Be Researched. Hepatol Commun 2019; 3: 8-19 [PMID: 30619990 DOI: 10.1002/hep4.1281]

13 Wang L, Wang K, Zou ZQ. Crosstalk between innate and adaptive immunity in hepatitis B virus infection. World J Hepatol 2015; 7: 2980-2991 [PMID: 26730277 DOI: 10.4254/wjh.v7.i30.2980]

14 Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]

15 Giersch K, Allweiss L, Volz T, Dandri M, Lütgehetmann M. Serum HBV pgRNA as a clinical marker for cccDNA activity. J Hepatol 2017; 66: 460-462 [PMID: 27826059 DOI: 10.1016/j.jhep.2016.09.028]

16 Park Y, Hong DJ, Shin S, Cho Y, Kim HS. Performance evaluation of new automated hepatitis B viral markers in the clinical laboratory: two quantitative hepatitis B surface antigen assays and an HBV core-related antigen assay. Am J Clin Pathol 2012; 137: 770-777 [PMID: 22523216 DOI: 10.1309/AJCP8QDN7NAUXJFJ]

17 Coffin CS, Zhou K, Terrault NA. New and Old Biomarkers for Diagnosis and Management of Chronic Hepatitis B Virus Infection. Gastroenterology 2019; 156: 355-368.e3 [PMID: 30472225 DOI: 10.1053/j.gastro.2018.11.037]

18 Enomoto M, Tamori A, Nishiguchi S, Kawada N. Combination therapy with a nucleos(t)ide analogue and interferon for chronic hepatitis B: simultaneous or sequential. J Gastroenterol 2013; 48: 999-1005 [PMID: 23338486 DOI: 10.1007/s00535-012-0742-5]

19 Wong GL, Tse YK, Wong VW, Yip TC, Tsoi KK, Chan HL. Long-term safety of oral nucleos(t)ide analogs for patients with chronic hepatitis B: A cohort study of 53,500 subjects. Hepatology 2015; 62: 684-693 [PMID: 25973979 DOI: 10.1002/hep.27894]

20 Ghany MG, Doo EC. Antiviral resistance and hepatitis B therapy. Hepatology 2009; 49: S174-S184 [PMID: 19399794 DOI: 10.1002/hep.22900]

21 Lee WA, He GX, Eisenberg E, Cihlar T, Swaminathan S, Mulato A, Cundy KC. Selective intracellular activation of a novel prodrug of the human immunodeficiency virus reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. Antimicrob Agents Chemother 2005; 49: 1898-1906 [PMID: 15855512 DOI: 10.1128/AAC.49.5.1898-1906.2005]

22 Agarwal K, Brunetto M, Seto WK, Lim YS, Fung S, Marcellin P, Ahn SH, Izumi N, Chuang WL, Bae H, Sharma M, Janssen HLA, Pan CQ, Çelen MK, Furusyo N, Shalimar D, Yoon KT, Trinh H, Flaherty JF, Gaggar A, Lau AH, Cathcart AL, Lin L, Bhardwaj N, Suri V, Mani Subramanian G, Gane EJ, Buti M, Chan HLY; GS-US-320-0110; GS-US-320-0108 Investigators. 96 wk treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. J Hepatol 2018; 68: 672-681 [PMID: 29756595 DOI: 10.1016/j.jhep.2017.11.039].]

23 Donkers JM, Appelman MD, van de Graaf SFJ. Mechanistic insights into the inhibition of NTCP by myrcludex B. JHEP Rep 2019; 1: 278-285 [PMID: 32039379 DOI: 10.1016/j.jhepr.2019.07.006]

24 Wedemeyer H, Schoneweis K, Bogomolov PO, Voronkova N, Chulanov V, Stepanova T, Bremer B, Allweiss L, Dandri M, Burhenne J, et al Final results of a multicenter, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of myrcludex B in with PEG-interferon Alpha 2a in patients with chronic HBV/HDV co-infection. J. Hepatol. 2019; 70: 81.

25 Bogomolov P, Alexandrov A, Voronkova N, Macievich M, Kokina K, Petrachenkova M, Lehr T, Lempp FA, Wedemeyer H, Haag M, Schwab M, Haefeli WE, Blank A, Urban S. 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 [PMID: 27132170 DOI: 10.1016/j.jhep.2016.04.016]

26 Haag M, Hofmann U, Mürdter TE, et al Quantitative bile acid profiling by liquid chromatography quadrupole time-offlight mass spectrometry: monitoring hepatitis B therapy by a novel Na-taurocholatecotransporting polypeptide inhibitor. Anal Bioanal Chem 2015; 407: 6815-25

27 Yuen MF, Gane EJ, Kim DJ, Weilert F, Yuen Chan HL, Lalezari J, Hwang SG, Nguyen T, Flores O, Hartman G, Liaw S, Lenz O, Kakuda TN, Talloen W, Schwabe C, Klumpp K, Brown N. Antiviral Activity, Safety, and Pharmacokinetics of Capsid Assembly Modulator NVR 3-778 in Patients with Chronic HBV Infection. Gastroenterology 2019; 156: 1392-1403.e7 [PMID: 30625297 DOI: 10.1053/j.gastro.2018.12.023]

28 Zhang H, Wang F, Zhu X, Chen Y, Chen H, Li X, Wu M, Li C, Liu J, Zhang Y, Ding Y, Niu J. Antiviral Activity and Pharmacokinetics of the HBV Capsid Assembly Modulator GLS4 in Patients with Chronic HBV Infection Clin Infect Dis. 2020; ciaa961. [DOI: 10.1093/cid/ciaa961. Online ahead of print.]

29 Yuen MF, Schwabe C, Tanwandee T, Jin Y, Gao L, Zhou X, Das S, Wang Y, Lemenuel-Diot A, Cosson V, et al RO7049389, a core protein allosteric

modulator, demonstrates robust decline in HBV DNA and HBV RNA in chronic HBV infected patients. Sci. HBV Cure. 2019.

30 Zoulim F, Lenz O, Vandenbossche JJ, Talloen W, Verbinnen T, Moscalu I, Streinu-Cercel A, Bourgeois S, Buti M, Crespo J, Manuel Pascasio J, Sarrazin C, Vanwolleghem T, Shukla U, Fry J, Yogaratnam JZ. JNJ-56136379, an HBV Capsid Assembly Modulator, Is Well-Tolerated and Has Antiviral Activity in a Phase 1 Study of Patients With Chronic Infection. Gastroenterology 2020; 159: 521-533.e9 [PMID: 32343960 DOI: 10.1053/j.gastro.2020.04.036]

31 Zoulim F, Yogaratnam JZ, Vandenbossche J, et al Safety, Pharmacokinetics and Antiviral Activity of Novel HBV Capsid Assembly Modulator, JNJ-56136379, in Patients with Chronic Hepatitis B The Liver Meeting; November 9-13, 2018. San Francisco, USA.

32 Yuen MF, Agarwal K, Gane EJ, et alFinal results of a phase 1B 28-day study of ABI-H0731, a novel core inhibitor in non-cirrhotic viremic subjects with chronic HBV. The Liver Meeting; November 9-13, 2018. San Francisco, USA.

33 Ma X, Lalezari J, Nguyen T, Bae H, Schiff ER, Fung S, Yuen RMF, Hassanein T, Hann HW, Elkhashab M, et al LBO-06-Interim safety and efficacy results of the ABI-H0731 phase 2a program exploring the combination of ABI-H0731 with Nuc therapy in treatment-naive and treatment-suppressed chronic hepatitis B patients. J. Hepatol. 2019; 70(Suppl. 1): e130.

34 Nayagam JS, Cargill ZC, Agarwal K. The Role of RNA Interference in Functional Cure Strategies for Chronic Hepatitis B. Curr. Hepatol. Rep. 2020; 19: 362–369.

35 Lee HW, Lee JS, Ahn SH. Hepatitis B Virus Cure: Targets and Future Therapies. Int J Mol Sci 2020; 22 [PMID: 33379331 DOI: 10.3390/ijms22010213]

36 Yuen MF, Schiefke I, Yoon JH, Ahn SH, Heo J, Kim JH, Lik Yuen Chan H, Yoon KT, Klinker H, Manns M, Petersen J, Schluep T, Hamilton J, Given BD, Ferrari C,

Lai CL, Locarnini SA, Gish RG. RNA Interference Therapy With ARC-520 Results in Prolonged Hepatitis B Surface Antigen Response in Patients With Chronic Hepatitis B Infection. Hepatology 2020; 72: 19-31 [PMID: 31654573 DOI: 10.1002/hep.31008]

37 Alexopoulou A, Vasilieva L, Karayiannis P. New Approaches to the Treatment of Chronic Hepatitis B. J Clin Med. 2020;9(10):3187. [DOI:10.3390/jcm9103187]

38 Bazinet M, Pântea V, Cebotarescu V, Cojuhari L, Jimbei P, Albrecht J, Schmid P, Le Gal F, Gordien E, Krawczyk A, Mijočević H, Karimzadeh H, Roggendorf M, Vaillant A. Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and REP 301-LTF): a non-randomised, open-label, phase 2 trial. Lancet Gastroenterol Hepatol 2017; 2: 877-889 [PMID: 28964701 DOI: 10.1016/S2468-1253(17)30288-1]

39 Bazinet M, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, Jimbei P, Iarovoi L, Smesnoi V, Musteata T, Jucov A, Dittmer U, Krawczyk A, Vaillant A. Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy. Gastroenterology 2020; 158: 2180-2194 [PMID: 32147484 DOI: 10.1053/j.gastro.2020.02.058]

40 Kim SH, Shin YW, Hong KW, Chang KH, Ryoo KH, Paik SH, Kim JM, Brotman B, Pfahler W, Prince AM. Neutralization of hepatitis B virus (HBV) by human monoclonal antibody against HBV surface antigen (HBsAg) in chimpanzees. Antiviral Res 2008; 79: 188-191 [PMID: 18479762 DOI: 10.1016/j.antiviral.2008.03.006]

41 Lee HW, Park JY, Hong T, Park MS, Ahn SH. Efficacy of Lenvervimab, a Recombinant Human Immunoglobulin, in Treatment of Chronic Hepatitis B

Virus Infection. Clin Gastroenterol Hepatol 2020; 18: 3043-3045.e1 [PMID: 31589980 DOI: 10.1016/j.cgh.2019.09.038]

42 Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. Breaking the code of DNA binding specificity of TAL-type III effectors. Science 2009; 326: 1509-1512 [PMID: 19933107 DOI: 10.1126/science.1178811]

43 Cradick TJ, Keck K, Bradshaw S, Jamieson AC, McCaffrey AP. Zinc-finger nucleases as a novel therapeutic strategy for targeting hepatitis B virus DNAs. Mol Ther 2010; 18: 947-954 [PMID: 20160705 DOI: 10.1038/mt.2010.20]

44 Liu X, Hao R, Chen S, Guo D, Chen Y. Inhibition of hepatitis B virus by the CRISPR/Cas9 system via targeting the conserved regions of the viral genome. J Gen Virol 2015; 96: 2252-2261 [PMID: 25904148 DOI: 10.1099/vir.0.000159]

45 Bengsch B, Thimme R. For whom the interferons toll - TLR7 mediated boosting of innate and adaptive immunity against chronic HBV infection. J Hepatol 2018; 68: 883-886 [PMID: 29408443 DOI: 10.1016/j.jhep.2018.01.025]

46 Menne S, Tumas DB, Liu KH, et al Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. J Hepatol. 2015; 62: 1237-1245. [DOI:10.1016/j.jhep.2014.12.026]

47 Janssen HLA, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen AH, Joshi A, Woo J, Lau AH, Gaggar A, Subramanian GM, Yoshida EM, Ahn SH, Tsai NCS, Fung S, Gane EJ. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. J Hepatol 2018; 68: 431-440 [PMID: 29104121 DOI: 10.1016/j.jhep.2017.10.027]

48 Lee HW, Lee JS, Ahn SH. Hepatitis B Virus Cure: Targets and Future Therapies. Int J Mol Sci. 2020; 22: 213. [DOI:10.3390/ijms22010213]

49 Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, Watanabe T, Iijima S, Sakurai Y, Watashi K, Tsutsumi S, Sato Y, Akita H, Wakita T, Rice CM, Harashima H, Kohara M, Tanaka Y, Takaoka A. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity 2015; 42: 123-132 [PMID: 25557055 DOI: 10.1016/j.immuni.2014.12.016]

50 Yuen MF, Chen CY, Liu CJ, Jeng RJ, Elkhashab M, Coffin C, Kim W, Greenbloom S, Ramji A, Lim YS, et al GS-12-Ascending dose cohort study of inarigivir - A novel RIG I agonist in chronic HBV patients: Final results of the ACHIEVE trial. J. Hepatol. 2019; 70: e47–e48.

51 Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, Laccabue D, Zerbini A, Cavalli A, Missale G, Bertoletti A, Ferrari C. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol 2007; 81: 4215-4225 [PMID: 17287266 DOI: 10.1128/JVI.02844-06]

52 Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, Möller I, Seiz P, Glebe D, Wang B, Yang D, Lu M, Roggendorf M. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. PLoS Pathog 2014; 10: e1003856 [PMID: 24391505 DOI: 10.1371/journal.ppat.1003856]

53 Schurich A, Khanna P, Lopes AR, Han KJ, Peppa D, Micco L, Nebbia G, Kennedy PT, Geretti AM, Dusheiko G, Maini MK. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. Hepatology 2011; 53: 1494-1503 [PMID: 21360567 DOI: 10.1002/hep.24249]

54 He J, Hao R, Liu D, Liu X, Wu S, Guo S, Wang Y, Tien P, Guo D. Inhibition of hepatitis B virus replication by activation of the cGAS-STING pathway. J Gen Virol 2016; 97: 3368-3378 [PMID: 27902332 DOI: 10.1099/jgv.0.000647]

55 Gupta N, Goyal M, Wu CH, Wu GY. The Molecular and Structural Basis of HBV-resistance to Nucleos(t)ide Analogs. J Clin Transl Hepatol 2014; 2: 202-211 [PMID: 26357626 DOI: 10.14218/JCTH.2014.00021].]

56 Vandepapelière P, Lau GK, Leroux-Roels G, Horsmans Y, Gane E, Tawandee T, Merican MI, Win KM, Trepo C, Cooksley G, Wettendorff M, Ferrari C; Therapeutic HBV Vaccine Group of Investigators. Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. Vaccine 2007; 25: 8585-8597 [PMID: 18031872 DOI: 10.1016/j.vaccine.2007.09.072]

57 Gaggar A, Coeshott C, Apelian D, Rodell T, Armstrong BR, Shen G,Subramanian GM, McHutchison JG. 2014. Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vac-cine, in healthy subjects: a randomized study. Vaccine 2014; 32: 4925–4931.

58 Zoulim F, Fournier C, Habersetzer F, Sprinzl M, Pol S, Coffin CS, Leroy V, Ma M, Wedemeyer H, Lohse AW, Thimme R, Lugardon K, Martin P, Bastien B, Sansas B, Adda N, Halluard C, Bendjama K, Brandely M, Inchauspém G. Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial. Hum Vaccin Immunother. 2020; 16: 388–399 [DOI: 10.1080/21645515.2019.1651141]

59 Farci P, Niro GA. Clinical features of hepatitis D. Semin Liver Dis 2012; 32: 228-236 [PMID: 22932971 DOI: 10.1055/s-0032-1323628]

60 Botelho-Souza LF, Vasconcelos MPA, Dos Santos AO, Salcedo JMV, Vieira DS. Hepatitis delta: virological and clinical aspects. Virol J 2017; 14: 177 [PMID: 28903779 DOI: 10.1186/s12985-017-0845-y]

61 Wranke A, Hardtke S, Heidrich B, Dalekos G, Yalçin K, Tabak F, Gürel S, Çakaloğlu Y, Akarca US, Lammert F, Häussinger D, Müller T, Wöbse M, Manns

MP, Idilman R, Cornberg M, Wedemeyer H, Yurdaydin C. Ten-year follow-up of a randomized controlled clinical trial in chronic hepatitis delta. J Viral Hepat 2020; 27: 1359-1368 [PMID: 32707605 DOI: 10.1111/jvh.13366]

62 Triantos C, Kalafateli M, Nikolopoulou V, Burroughs A. Meta-analysis: antiviral treatment for hepatitis D. Aliment Pharmacol Ther 2012; 35: 663-673 [PMID: 22273482 DOI: 10.1111/j.1365-2036.2012.04993.x]

63 European Medicines Agency. Hepcludex (bulevirtide) powder for solution for injection: EU summary of product characteristics. 2020. https://ec.europa.eu. Accessed 31 Aug 2020.

64 European Medicines Agency. Hepcludex: EU assessment report. 2020. https://www.ema.europa.eu. Accessed 31 Aug 2020.

65 Glenn JS. Prenylation of HDAg and antiviral drug development. Curr Top Microbiol Immunol 2006; 307: 133-149 [PMID: 16903224 DOI: 10.1007/3-540-29802-9_7]

66 Koh C, Canini L, Dahari H, Zhao X, Uprichard SL, Haynes-Williams V, Winters MA, Subramanya G, Cooper SL, Pinto P, Wolff EF, Bishop R, Ai Thanda Han M, Cotler SJ, Kleiner DE, Keskin O, Idilman R, Yurdaydin C, Glenn JS, Heller T. Oral prenylation inhibition with lonafarnib in chronic hepatitis D infection: a proof-of-concept randomised, double-blind, placebo-controlled phase 2A trial. Lancet Infect Dis 2015; 15: 1167-1174 [PMID: 26189433 DOI: 10.1016/S1473-3099(15)00074-2]

67 Yurdaydin C, Keskin O, Kalkan Ç, Karakaya F, Çalişkan A, Karatayli E, Karatayli S, Bozdayi AM, Koh C, Heller T, Idilman R, Glenn JS. Optimizing lonafarnib treatment for the management of chronic delta hepatitis: The LOWR HDV-1 study. Hepatology 2018; 67: 1224-1236 [PMID: 29152762 DOI: 10.1002/hep.29658]

68 Koh C, Hercun J, Rahman F, et al A phase 2 study of peginterferon lambda, lonafarnib and ritonavir for 24 wk.

69 Polaris Observatory HCV Collaborators.. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modeling study. Lancet Gastroenterol Hepatol 2017; 2: 161-176 [PMID: 28404132 DOI: 10.1016/S2468-1253(16)30181-9]

70 Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C, De Ledinghen V, Larrey D, Haour G, Bronowicki JP, Zoulim F, Asselah T, Marcellin P, Thabut D, Leroy V, Tran A, Habersetzer F, Samuel D, Guyader D, Chazouilleres O, Mathurin P, Metivier S, Alric L, Riachi G, Gournay J, Abergel A, Cales P, Ganne N, Loustaud-Ratti V, D'Alteroche L, Causse X, Geist C, Minello A, Rosa I, Gelu-Simeon M, Portal I, Raffi F, Bourliere M, Pol S; French ANRS CO22 Hepather cohort. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet 2019; 393: 1453-1464 [PMID: 30765123 DOI: 10.1016/S0140-6736(18)32111-]

71 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu.; European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2018. J Hepatol 2018; 69: 461-511 [PMID: 29650333 DOI: 10.1016/j.jhep.2018.03.026]

72 World Health Organization. Global health sector strategy on viral hepatitis 2016-2021 https://apps.who.int/iris/bitstream/handle/10665/246177/WHO-HIV-2016.06-eng.pdf;jsessionid=60A93ADD1A191FF6A0FA823314D24C43?sequence=1 (WHO, 2016).

73 Sullivan JC, De Meyer S, Bartels DJ, Dierynck I, Zhang EZ, Spanks J, Tigges AM, Ghys A, Dorrian J, Adda N, Martin EC, Beumont M, Jacobson IM, Sherman KE, Zeuzem S, Picchio G, Kieffer TL. Evolution of treatment-emergent resistant

variants in telaprevir phase 3 clinical trials. Clin Infect Dis 2013; 57: 221-229 [PMID: 23575197 DOI: 10.1093/cid/cit226]

74 Zając M, Muszalska I, Sobczak A, Dadej A, Tomczak S, Jelińska A. Hepatitis C - New drugs and treatment prospects. Eur J Med Chem 2019; 165: 225-249 [PMID: 30685524 DOI: 10.1016/j.ejmech.2019.01.025]

75 Dore GJ, Bajis S. Hepatitis C virus elimination: laying the foundation for achieving 2030 targets. Nat Rev Gastroenterol Hepatol 2021; 18: 91-92 [PMID: 33257831 DOI: 10.1038/s41575-020-00392-3]

76 Feld JJ, Cypel M, Kumar D, Dahari H, Pinto Ribeiro RV, Marks N, Kamkar N, Bahinskaya I, Onofrio FQ, Zahoor MA, Cerrochi O, Tinckam K, Kim SJ, Schiff J, Reichman TW, McDonald M, Alba C, Waddell TK, Sapisochin G, Selzner M, Keshavjee S, Janssen HLA, Hansen BE, Singer LG, Humar A. Short-course, direct-acting antivirals and ezetimibe to prevent HCV infection in recipients of organs from HCV-infected donors: a phase 3, single-centre, open-label study. Lancet Gastroenterol Hepatol 2020; 5: 649-657 [PMID: 32389183 DOI: 10.1016/S2468-1253(20)30081-9]

77 Parlati L, Pol S. Direct acting antivirals failure: cause and retreatment options. Expert Rev Gastroenterol Hepatol 2018; 12: 1245-1250 [PMID: 30791789 DOI: 10.1080/17474124.2018.1541237]

78 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu.; European Association for the Study of the Liver. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol 2018; 68: 1256-1271 [PMID: 29609832 DOI: 10.1016/j.jhep.2018.03.005]

79 Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology 2012; 55: 988-997 [PMID: 22121109 DOI: 10.1002/hep.25505]

80 Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. J Hepatol 2008; 48: 494-503 [PMID: 18192058 DOI: 10.1016/j.jhep.2007.12.008]

81 Webb GW, Dalton HR. Hepatitis E: an expanding epidemic with a range of complications. Clin Microbiol Infect 2020; 26: 828-832 [PMID: 32251845 DOI: 10.1016/j.cmi.2020.03.039]

82 Blasco-Perrin H, Madden RG, Stanley A, Crossan C, Hunter JG, Vine L, Lane K, Devooght-Johnson N, Mclaughlin C, Petrik J, Stableforth B, Hussaini H, Phillips M, Mansuy JM, Forrest E, Izopet J, Blatchford O, Scobie L, Peron JM, Dalton HR. Hepatitis E virus in patients with decompensated chronic liver disease: a prospective UK/French study. Aliment Pharmacol Ther. 2015; 42: 574–581

83 Kamar N, Rostaing L, Legrand-Abravanel F, Izopet J. How should hepatitis E virus infection be defined in organ-transplant recipients? Am J Transplant 2013; 13: 1935-1936 [PMID: 23659713 DOI: 10.1111/ajt.12253]

84 Kamar N, Abravanel F, Selves J, Garrouste C, Esposito L, Lavayssière L, Cointault O, Ribes D, Cardeau I, Nogier MB, Mansuy JM, Muscari F, Peron JM, Izopet J, Rostaing L. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. Transplantation 2010; 89: 353-360 [PMID: 20145528 DOI: 10.1097/TP.0b013e3181c4096c]

85 Kamar N, Rostaing L, Abravanel F, Garrouste C, Esposito L, Cardeau-Desangles I, Mansuy JM, Selves J, Peron JM, Otal P, Muscari F, Izopet J. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. Clin Infect Dis 2010; 50: e30-e33 [PMID: 20113176 DOI: 10.1086/650488]

86 Jagjit Singh GK, Ijaz S, Rockwood N, Farnworth SP, Devitt E, Atkins M, Tedder R, Nelson M. Chronic Hepatitis E as a cause for cryptogenic cirrhosis in HIV. J Infect 2013; 66: 103-106 [PMID: 22166370 DOI: 10.1016/j.jinf.2011.11.027]

87 Cornberg M, Pischke S, Müller T, Behrendt P, Piecha F, Benckert J, Todt D, Steinmann E, Papkalla A, von Karpowitz M, Koch A, Lohse A, Hardtke S, Manns MP, Wedemeyer H. Sofosbuvir monotherapy fails to achieve HEV RNA elimination in patients with chronic hepatitis E - The HepNet SofE pilot study. J Hepatol 2020; 73: 696-699 [PMID: 32624195 DOI: 10.1016/j.jhep.2020.05.020]

88 Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wang H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Yan Q, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010; 376: 895-902 [PMID: 20728932 DOI: 10.1016/S0140-6736(10)61030-6]

89 Nishiyama T, Kobayashi T, Jirintai S, Nagashima S, Primadharsini PP, Nishizawa T, Okamoto H. Antiviral candidates against the hepatitis E virus (HEV) and their combinations inhibit HEV growth in vitro. Antiviral Res 2019; 170: 104570

90 Horvatits T, Schübel N, Kamar N, Polywka S, Lütgehetmann M, Rutter K, zur Wiesch JS, Manns MP, Lohse A, Roque-Afonso AM, Pischke S, Behrendt P, Mallet V. SAT-208-Zinc/Ribavirin: A possible treatment option in chronically HEV genotype 3 infected patients without SVR under ribavirin monotherapy. J Hepatol 2019; 70 Suppl: e721-e722

91 Todt D, Moeller N, Praditya D, Kinast V, Friesland M, Engelmann M, Verhoye L, Sayed IM, Behrendt P, Dao Thi VL, Meuleman P, Steinmann E. The natural

compound silvestrol inhibits hepatitis E virus (HEV) replication in vitro and in vivo. Antiviral Res 2018; 157: 151-158

Footnotes

Conflict-of-interest statement: Dr. Leoni has nothing to disclose. Dr. Serio has nothing to disclose. Dr. Casabianca has nothing to disclose. Dr. Biagioni has nothing to disclose.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

Manuscript source: Invited Manuscript

Peer-review started: April 30, 2021

First decision: June 30, 2021

Article in press:

Specialty type: Gastroenterology and Hepatology

Country/Territory of origin: Italy

Peer-review report's scientific quality classification

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

P-Reviewer: Gupta MK S-Editor: Wang JL L-Editor: P-Editor: