

# A Focused Review on Recent Advances in the Diagnosis and Treatment of Viral Hepatitis

Wei Zhang<sup>a</sup>, Mahmoud Aryan<sup>b</sup>, Steve Qian<sup>c</sup>, Roniel Cabrera<sup>a</sup>, Xiuli Liu<sup>d, e</sup>

## Abstract

The global burden of viral hepatitis remains substantial despite advances in antiviral therapy and effective vaccines. There are five hepatitis viruses (hepatitis A, B, C, D, and E). Mortality related to hepatitis B virus and hepatitis C virus infections is among the top four global infectious diseases, together with human immunodeficiency virus infection, malaria, and tuberculosis. Of those deaths, approximately 47% are attributable to hepatitis B virus, 48% to hepatitis C virus and the remainder to hepatitis A virus and hepatitis E virus. Ending hepatitis epidemics as a major public health threat is feasible with the tools and approaches currently available. Effective vaccines are available for preventing viral hepatitis A, B and E infections. New oral, well-tolerated treatment regimens for chronic hepatitis C patients can achieve cure rates of over 90%. Effective treatment is also available for people with chronic hepatitis B virus infection; although for most people such treatment needs to be long-term, and recent advanced aim at a “functional cure” of hepatitis B. In this review article, we discuss the most recent advances of the diagnosis and treatment of viral hepatitis.

**Keywords:** Cirrhosis; Direct antiviral agents; Hepatitis A; Hepatitis B; Hepatic C; Hepatocellular carcinoma; Hepatitis D; Hepatitis E

## Introduction

The global burden of viral hepatitis remains substantial despite major advances in prevention and treatment in recent years. Viral hepatitis caused 1.34 million deaths in 2015, which is on a par with deaths caused by tuberculosis, malaria, and human

immunodeficiency virus (HIV) [1]. Five liver specific (hepatotropic) viruses are responsible for most cases of viral hepatitis. These are the hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV). Of these, HBV and HCV cause 96% of the mortality from viral hepatitis. The main liver complications include cirrhosis and hepatocellular carcinoma (HCC). It was reported that in 2015, an estimated 257 million people were living with chronic HBV infection, and 71 million people with chronic HCV infection [2, 3]. In 2016, the World Health Organization (WHO) committed to eliminating viral hepatitis as a public health threat by 2030 (defined as a 65% reduction in mortality and a 90% reduction in incidence compared with the 2015 baseline) [2, 3]. However, the goal is challenging due to lack of diagnosis in most patients and limited access to treatment. The epidemiology, risk for chronicity, risk for liver complications, and treatments vary considerably in the five viruses. Acute infection occurs with all five viruses, while chronic infection occurs mainly in HBV, HCV and HDV. This review provides a review of recent advances in the diagnosis and treatment of viral hepatitis focusing on rapid diagnostic modalities, new treatments developed and currently being tested in clinical trials, development of vaccines to treat chronic hepatitis B (CHB) and use of vaccine to prevent HEV in some parts of the world.

## HAV

HAV is one of the most common infectious causes of acute hepatitis worldwide. According to the WHO, there are an estimated 1.4 million cases every year [4]. Transmission occurs primarily through fecal-oral routes via contamination of food or water, or through close contact with infected persons. Historically, epidemics have occurred in developing countries in areas of poor sanitation. However, owing to the efficacy of the HAV vaccine as well as improved sanitary measures, the epidemiology of HAV infection has seen a shift in recent years [5]. Increasingly, epidemics now occur in high-risk adults, namely the homeless, illicit drug users, and men who have sex with men. Since 2016, over 31,000 cases have been documented in this population in the United States alone [6].

## Diagnosis

Definitive diagnosis of acute hepatitis A infection has clas-

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<sup>a</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Florida, Gainesville, FL, USA

<sup>b</sup>Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

<sup>c</sup>Department of Internal Medicine, University of Florida, Gainesville, FL, USA

<sup>d</sup>Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, USA

<sup>e</sup>Corresponding Author: Xiuli Liu, Department of Pathology, Immunology and Laboratory Medicine, University of Florida, PO Box 100275, 1600 SW Archer Road, Gainesville, FL 32610, USA. Email: xiuliliu@ufl.edu

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sically been made by detection of serum immunoglobulin M (IgM) anti-HAV antibodies via enzyme immunoassay (EIA). However, as EIA testing requires infrastructure and is time-consuming, there has been increased interest in point of care (POC) testing. More rapid diagnostic testing could potentially facilitate identification of susceptible individuals during outbreaks, as well as provide those in resource-poor areas a means for diagnosis. A study in Brazil showed that a commercial rapid immunochromatographic test for HAV IgM had 81% sensitivity and 100% specificity [7]. Another study on 5,438 patients in Puerto Rico demonstrated efficacy of a rapid salivary test for HAV IgG, with sensitivity comparable to human plasma analyses [8].

**Treatment**

As hepatitis A is typically self-limiting, treatment is primarily supportive. Up to 10-15% of patients will have relapsing illness up to 6 months after resolution of initial illness. However, hepatitis A does not progress to chronic liver disease and less than 1% of cases lead to acute liver failure [9]. In those who develop acute liver failure, however, approximately 30% will require transplant or die. Currently, there are no approved antiviral therapies for HAV infection, although one 2018 study has shown that sofosbuvir inhibits HAV replication *in vitro* [10].

**Vaccination and prophylaxis**

Vaccination is recommended for all children 12 months and older, travelers to endemic regions, those with chronic liver disease, men who have sex with men, and illicit drug users. In those with suspected exposure, post-exposure prophylaxis with the vaccine is recommended over the immune globulin [11]. However, for patients 41 years and older, children less than 12 months of age, and the immunocompromised, the immunoglobulin remains recommended due to increased risk for adverse effects with the vaccine, and limited data on efficacy for the vaccine [12].

**HBV**

CHB infection is a common cause of liver disease globally, with a disproportionately high burden in the African Region and the Western Pacific Region. Although vaccination has substantially reduced HBV transmission, coverage with the initial birth dose vaccination was still low at 39%. In addition, despite affordable and effective treatment, only 9% of people infected with HBV were diagnosed in 2015, and only 8% of those meeting criteria for therapy were on treatment [13, 14].

HBV is a partially double-stranded hepatotropic DNA virus that belongs to the Hepadnaviridae family. The presence of the HBV surface antigen (HBsAg) establishes the diagnosis of HBV. Chronic infection is defined by the presence of HBsAg

in the serum for over 6 months. In 2015, the global prevalence of CHB infection was 3.5% with the highest areas in the African (6.1%) and Western Pacific regions (6.2%) [15]. In most countries where HBV is endemic, perinatal transmission is the most important cause of infection. The risk of developing chronic HBV infection after perinatal exposure was 90% in newborns of HBV-infected mothers. Other routes of transmission include direct contact with infected blood, unprotected sex, use of illegal drugs, or needles and other medical/dental equipments or procedures that are contaminated or not sterile. HBV screening is recommended in high-risk groups (Table 1).

**Diagnosis**

Traditional serological tests include HBsAg, hepatitis B E antigen (HBeAg), hepatitis B core antibody (anti-HBc), and hepatitis B DNA level. The IgM subtype of anti-HBc is indicative of acute infection or reactivation, whereas the IgG subtype is indicative of chronic infection or previous infection. Quantitative HBsAg, although not commercially available in the United States, was found to predict HBsAg seroclearance after the cessation of antiviral treatment when the serum HBsAg level is < 100 to 200 IU/mL. Hepatitis B core-related antigen (HBcrAg) is a novel biomarker that has an important role in CHB. HBcrAg is a common amino acid sequence shared by hepatitis B core antigen, HBeAg, and a truncated 22 kDa precore protein (p22Cr) [16]. It was reported to correlate with the levels and transcriptional activities of intrahepatic covalently closed circular DNA (cccDNA). A complete HBV cure is currently not possible because of the presence of the cccDNA. HBcrAg has been also shown to correlate with intrahepatic viral RNA levels in Asian patients treated with nucleos(t)ide analog (NA) [16]. Hence, HBcrAg could be used as a surrogate of cccDNA in the liver. It can be useful in the evaluation of new antiviral therapies aiming at a functional cure of HBV infection either by directly or indirectly targeting the intrahepatic cccDNA pool. Another report showed that patients with persistently high on-treatment HBcrAg levels were more likely to develop HCC despite sustained viral suppression via long-term NA treatment, indicating that HBcrAg could potentially be used to predict risk of malignancy in CHB patients.

Early diagnosis remains a significant challenge of HBV infection especially in high-risk groups and in resource-limited regions. There is unmet need to scale up screening for HBV in such population. The barriers of early diagnosis include limited access to healthcare, cost, and limited laboratory resources. This is especially important in many low-resource and geographically isolated regions. POC tests (also known as rapid diagnostic tests, RDTs) are simplified tests that have the potential to overcome the barriers [17]. POCs require only small amounts of body fluids (e.g., a finger-prick blood sample), are easy to use with minimal training, take short turn-around time (TAT, 20 - 30 min) and may achieve same-day “test and treat”. A key benefit of POCs is to engage hard-to-reach communities for testing, such as using HBsAg POC tests for HBV screening in remote areas, or harm reduction programs. So far, only three POCs for detecting HBsAg have been prequalified by

**Table 1.** High-Risk Groups for Hepatitis Viruses

At-risk group	Virus
Individuals who have ever injected drugs	HAV, HBV, HCV, HEV
Individuals suffering from homelessness	HAV, HBV, HCV
Men who have sex with men	HAV, HBV, HCV
Patients with end-stage renal disease, including predialysis, hemodialysis, peritoneal dialysis and home dialysis	HBV, HCV, HEV
Sexually active individuals (more than one partner in the past 6 months)	HBV, HCV
Individuals living in close household contact with or have sexual contacts with active infected patients	HBV, HCV
Health care providers and public safety workers at risk for occupational exposure to blood or blood-contaminated body fluid	HBV, HCV
Inmates and staff of correctional facilities	HBV, HCV
All pregnant women	HBV, HEV
Exposure to undercooked meat, fish, or shellfish	HAV, HEV
Individuals born in or adopted from countries where hepatitis B is common (Asia, Africa, South America, Pacific Islands, Eastern Europe, and the Middle East)	HBV
Individual born or adopted in areas where hepatitis C is common (Central Asia, East Asia, North Africa, and West Africa)	HCV
Individuals with chronic HBV infection	HDV, HEV
Those with blood transfusion or organ donation prior to 1992	HCV
Individuals who have ever injected drugs who are HBsAg+	HDV
Men who have sex with men who are HBsAg+	HDV
Individuals who are HBsAg+ and have elevated liver function tests	HDV
Individuals who are HBsAg+ and have immigrated from areas of high HDV endemicity	HDV

HAV: hepatitis A virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus; HEV: hepatitis E virus; HBsAg: HBV surface antigen.

the WHO. Typically, POC tests have lower accuracy than traditional laboratory-based tests. However, recent studies show that the sensitivity and specificity have been excellent at over 90% [17]. Other limitations of POCs include issues regarding regulatory process, procurement and storage management for POC tests, as well as costs when implementing POCs for HBV in different settings.

## Treatment

Currently approved treatments of CHB include two formulations of interferon, standard and pegylated, and six NAs [18]. Of these, pegylated interferon alpha (Peg-IFN- $\alpha$ ), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) are recommended as first-line treatment. While Peg-IFN is rarely used, the three NAs are the preferred choice. TAF is a new oral prodrug of tenofovir (TFV), a nucleotide analog that inhibits reverse transcription of HBV DNA. TAF has greater plasma stability than TDF, which can enhance delivery of the active metabolite, tenofovir diphosphate, to hepatocytes more efficiently than TDF, thus reducing the circulatory levels of tenofovir. The two large, ongoing, randomized, double-blind, international phase III trials (GS-US-320-0110 and GS-US-320-0108) both showed that TAF had similar antiviral efficacy in suppressing HBV replication compared to TDF, did not induce virologic resistance, but

was associated with significantly less bone and renal toxicity in both HBeAg+ and HBeAg- CHB patients after 2 years of treatment [19, 20].

## Treatment guidelines

Current guidelines recommend antiviral treatment in patients with cirrhosis and detectable viremia, regardless of alanine aminotransferase (ALT) or HBV DNA levels. For non-cirrhotic patients, all guidelines recommend treatment if they are at immune active phase which indicates ongoing liver injury and has the highest risk of developing cirrhosis. However, there is slight difference between them. The European Association for the Study of the Liver (EASL) guidelines use HBV DNA cutoff of 2,000 IU/mL regardless of HBeAg status, whereas the American Association for the Study of Liver Diseases (AASLD) and the Asian Pacific Association for the Study of the Liver (APASL) guidelines suggest cutoff of 20,000 IU/mL for HBeAg-positive and 2,000 IU/mL for HBeAg-negative patients [18, 21-23]. The AASLD and APASL guidelines use ALT > 2 upper limit normal (ULN) or histologic evidence of moderate or severe necroinflammation or significant fibrosis for initiating treatment, while the EASL guidelines use ALT > ULN with histologic evidence of moderate necroinflammation and/or moderate fibrosis or liver stiffness > 9 kPa or if histology is not available, ALT > 2 ULN and HBV DNA > 20,000

**Table 2.** Guidelines for Chronic Hepatitis B Treatment

	HBV cirrhosis	HBV non-cirrhotic HBeAg+	HBV non-cirrhotic HBeAg-	Other indications to initiate treatment
EASL	Treat	HBV DNA cutoff of 2,000 IU/mL	HBV DNA cutoff of 2,000 IU/mL	ALT > 40 U/L with histologic evidence of moderate necroinflammation and/or moderate fibrosis ALT > 40 U/L with histologic evidence of moderate necroinflammation and/or liver stiffness > 9 kPa ALT > 80 U/L and HBV DNA > 20,000 IU/mL, if histology is not available.
AASLD	Treat	HBV DNA cutoff of 20,000 IU/mL	HBV DNA cutoff of 2,000 IU/mL	ALT > 70 U/L (for male) and ALT > 50 U/L (for female) or histologic evidence of moderate or severe necroinflammation or significant fibrosis for initiating treatment
AASLD	Treat	HBV DNA cutoff of 20,000 IU/mL	HBV DNA cutoff of 2,000 IU/mL	ALT > 70 U/L (for male) and ALT > 50 U/L (for female) or histologic evidence of moderate or severe necroinflammation or significant fibrosis for initiating treatment

HBV: hepatitis B virus; HBeAg: hepatitis B E antigen; ALT: alanine aminotransferase; EASL: European Association for the Study of the Liver; AASLD: American Association for the Study of Liver Diseases.

IU/mL to initiate treatment. The AASLD guidelines used the cutoff ULN for ALT at 35 U/L for male patients and 25 U/L for female patients, while the EASL and APASL use 40 U/L as a cutoff for both genders. The EASL, AASLD, and APASL guidelines for treating chronic HBV are summarized in Table 2.

*Conventional antivirals*

Antiviral therapy is effective in suppressing viral replication, reversing hepatic inflammation and fibrosis, preventing progression to cirrhosis and liver failure, and decreasing risk of HCC and liver-related mortality. However, it has limitations in that it does not eradicate cccDNA or integrated HBV DNA with low rate of HBsAg loss particularly with non-A genotypes. In addition, long-term therapy may reduce but not eliminate the risk of liver cancer. Therefore, new therapies for HBV that can achieve sustained suppression and HBsAg loss after a limited course of therapy are needed [24, 25].

There are different definitions of HBV cure. Partial cure is defined as normalization of liver enzymes and suppression of HBV DNA without loss of HBsAg. This could be achieved by current antiviral therapy, however, requires long-term therapy and does not eliminate the risk of malignancy. Complete cure is defined as loss of HBsAg with undetectable cccDNA and integrated HBV DNA. Complete cure is difficult to achieve as the HBV DNA is integrated into hepatocytes, unless patients are being treated early before integration has occurred. Functional cure is defined as loss of HBsAg with detectable but inactive cccDNA and integrated HBV DNA [26, 27]. This is considered as more reasonable and realistic goal of HBV cure. Currently, presumed pathways to achieve functional cure include therapies that can inhibit viral replication, lower viral antigen burden, and boost immune response. In the past few years, due to a better understanding of viral life cycle and viral pathogenetic mechanisms, new therapeutic viral targets have been identified that resulted in developments of several compounds.

*New therapeutic strategies*

1) RNA interference (RNAi)

RNAi is a highly specific and efficient method of post-transcriptional gene silencing [28]. The synthetic small interfering RNA (siRNA) can be used to inhibit HBV replication resulting in decreased expression of HBsAg and HBeAg (tolerogenic antigens). The main limitation of this drug is the route of delivery as it is digested rapidly in the gut and intravenous injection can cause infusion reaction. Recently subcutaneous injections that target the liver were developed. Arrowhead’s siRNA ARC520 was the first siRNA drug designed to reduce all RNA transcripts derived from covalently closed circular DNA, leading to a reduction in viral antigens and HBV DNA. In two randomized phase 2 multidose studies in HBeAg positive and HBeAg negative, NA-experienced patients with CHB infection, multiple doses of 2 mg/kg ARC520 significantly reduced HBsAg in both patient groups compared to placebo, and antigen reductions were sustained for a long period of time; however, absolute reductions were generally moderate [29]. Due to safety concern of the drug delivery system, the clinical trials of ARC520 were terminated. A modified RNAi, JNJ-3989 (formerly ARO-HBV), was developed to contain two RNAi that are both conjugated to N-acetyl galactosamine to facilitate uptake by the liver. In a recent phase 2 study of 40 HBeAg+ or HBeAg- NA-naive or NA-experienced patients, JNJ-3989 at a dose of 100 - 400 mg in combination with an NA was well tolerated. The maximum reduction in HBsAg > 1 log<sub>10</sub> IU/mL was achieved in 98% patients and 17 (43%) patients had sustained HBsAg suppression at 9 months after the last dose of JNJ-3989 [30]. Liver-targeted antisense oligonucleotides, RNA destabilizers and locked nucleic acids are alternative approaches to blocking viral protein expression. In a recent phase 2 randomized clinical trial in 66 HBeAg+ and HBeAg- NA-suppressed patients, GSK3389404 at a maximum weekly dose of 120 mg resulted in an average HBsAg reduction of 0.75

log IU/mL compared to 0.02 log IU/mL in placebo by day 85 without major side effects [31, 32].

## 2) Core protein allosteric modulators (CpAMs)

HBV core protein is key in packing pgRNA and interaction with HBsAg. CpAMs were developed to cause aberrant non-capsid polymers or assembly of empty capsids, thus decreasing the viral replication. Two classes of CpAMs have been discovered: the heteroaryldihydropyrimidines (HAPs) (type I CpAMs or CpAM-A) and the phenylpropenamides (PPAs) (type II CpAMs or CpAM-N). In a phase 1 study, a type I CpAM RO7049389 administration resulted in a robust decline in median HBV DNA (2.7 to 3.0 log<sub>10</sub> IU/mL) at 28 days. About 81% of patients attained HBV DNA levels lower than the lower limit of quantitation [33]. However, no HBsAg change was observed during 4 weeks of treatment and viral rebound was observed after stopping treatment. In another phase 1 clinical trial using a type II CpAM ABI-H0731 in combination with ETV showed that the drug was well tolerated with a decrease in HBV DNA and HBV RNA levels [34]. A recent phase 1 study using a different type II CpAM JNJ-6379 showed that all doses of JNJ-6379 were well tolerated, showed dose-dependent pharmacokinetics, and had potent antiviral activity (decreases in HBV DNA and HBV RNA) in treatment-naïve patients with chronic HBV infection [35]. A triple combination of an RNAi (JNJ-3989 200 mg every 4 weeks for three doses), a CpAM (JNJ-6379 250 mg daily for 12 weeks) and an NA (daily) in patients with CHB was well tolerated and all patients achieved robust reductions in HBsAg (> 1.0 log<sub>10</sub> IU/mL), HBV DNA, and HBV RNA regardless of HBeAg status [36].

## 3) HBV attachment/entry inhibitors

Bulevirtide (Myreludex) is a first-in-class entry inhibitor that binds to sodium taurocholate cotransporting polypeptide (NTCP) surface receptors that HBV uses to enter hepatocytes. Bulevirtide was recently approved in the European Union (EU) for the treatment of chronic HDV infection in HDV RNA positive adult patients with compensated liver disease. So far, most studies are on chronic hepatitis D infection; however, in a phase 2 randomized clinical study on HBV/HDV co-infection, the combination of Bulevirtide and Peg-IFN achieved a decline of HBsAg > 1.0 log<sub>10</sub> IU/mL or undetectable HBsAg in 40% of the patients, in addition to high rate of HDV RNA suppression. An ongoing trial in CHB mono-infection is expected to have results soon (NCT02888106) [37].

## 4) Inhibition of HBsAg release

Nucleic acid polymers (NAPs) inhibit assembly and secretion of HBV subviral particles. As subviral particles account for greater than 99.99% of HBsAg in the blood, NAPs constitute an effective means of clearing HBsAg from the serum of pa-

tients with chronic HBV infection [38]. In an open-label, phase 2 study of the safety and efficacy of the NAPs REP 2139 or REP 2165 combined with TDF and pegylated interferon alpha-2a (Peg-IFN-2a) in HBeAg- CHB patients, the addition of either NAP significantly increased rates of HBsAg loss and HBsAg seroconversion during therapy and functional cure after therapy [39].

## 5) Neutralization

Hepabig gene (lenervimab) is a recombinant hepatitis B immunoglobulin that binds to HBsAg to cause neutralization of circulating virions or surface antigens through formation of immune complexes. It can also inhibit re-entry of the virus by binding to HBsAg. In a prospective, open-label dose escalation phase I trial, single and multiple doses of lenervimab were administered in four different doses (80,000 IU, 120,000 IU, 180,000 IU or 240,000 IU) to CHB patients, and virological suppression occurred in HBeAg- CHB patients with or without NA therapy [40]. Lenervimab was well-tolerated and reduced levels of HBsAg to below undetectable levels for up to 1 month in patients with CHB infection. A double-blind, randomized, phase IIa study to evaluate its efficacy and safety in CHB patients on NAs is ongoing.

## 6) Toll-like receptor (TLR) agonists

TLRs constitute the first line of defense against invading microorganisms. The activation of TLR-mediated pathways results in suppression of HBV replication and restoration of HBV-specific adaptive immunity. In a double-blind, randomized, placebo-controlled phase II study, chronic HBV patients receiving once-weekly oral vesatolimod (GS-9620, TLR-7 agonist) induced an HBV-specific immune response, but without a significant decline in HBsAg [41]. Another phase 2 clinical study showed that selgantolimod (GS-9688, TLR-8 agonist) up to 3 mg once weekly for 24 weeks was safe and well tolerated and resulted in modest decline in HBsAg levels from baseline (5% rate of HBsAg loss, 16% of HBeAg loss) in virally suppressed CHB patients [42]. The HBsAg decline was sustained off-treatment for 24 weeks at week 48. Another phase 2 randomized double-blinded placebo-controlled study on viremic patients with CHB, weekly oral selgantolimod for 24 weeks with TAF followed by TAF for an additional 24 weeks resulted in a decline in HBsAg levels to > 0.3 log<sub>10</sub> IU/mL in the selgantolimod plus TAF group only, although no patients achieved the primary endpoint of HBsAg decline > 1 log<sub>10</sub> IU/mL [43].

## 7) Retinoic acid-inducible gene-1 (RIG-I) agonists

The RIG-I agonist SB9200 (inarigivir), a nucleotide-binding oligomerization domain-containing protein 2, is an oral HBV antiviral with both direct activity and immune-modulating activity via RIG. In the ACHIEVE study (Study Evaluating the

Safety, Pharmacokinetics, and Antiviral Efficacy of SB 9200 in Subjects Infected with Chronic HBV), 80 treatment-naïve non-cirrhotic CHB patients received ascending dose inarigivir for 12 weeks followed by a switch to 12 weeks of TDF 300 mg daily. HBV DNA and RNA reductions were achieved in both HBeAg<sup>+</sup> and HBeAg<sup>-</sup> patients in a dose-dependent manner, with more significant reduction in HBeAg<sup>-</sup> patients than HBeAg<sup>+</sup> patients, that being greater in the latter. An HBsAg reduction of > 0.5 log<sub>10</sub> at either 12 or 24 weeks was seen in 22% of patients with a mean reduction of 0.8 log<sub>10</sub> and a maximal reduction of 1.4 log<sub>10</sub>. Unlike HBV DNA, HBsAg decline was not dose-dependent. Further studies at doses of up to 400 mg daily in combination with TDF or added to NA-suppressed patients are under way [44].

### 8) Therapeutic vaccination

GS-4774 is a therapeutic vaccine engineered to restore the HBV-specific T-cell immune response to suppress HBV replication and reduce the number of cells containing HBV. In a phase 2 open-label study evaluating the safety and efficacy of GS-4774 in combination of TDF in treatment-naïve CHB patients, although it can increase production of interferon (IFN)- $\gamma$ , tumor necrosis factor (TNF), and interleukin 2 (IL-2) by CD8<sup>+</sup> T cells, it failed to reduce levels of HBsAg [45]. However, the strong immune stimulatory effect on CD8<sup>+</sup> T cells might be used in combination with other antiviral agents to boost the antiviral immune response.

TG1050 is another multi-antigenic adenovirus-based T-cell-inducing vaccine. In a phase 1 clinical trial assessing the safety and efficacy of TG1050 in virally suppressed CHB patients, single or multiple doses of TG1050 were able to induce HBV-specific cellular immune response. Overall, minor decreases of HBsAg were observed while a number of vaccinees reached unquantifiable HBcrAg by end of the study [46].

### 9) Checkpoint inhibitors

Programmed cell death protein 1 (PD-1), a checkpoint protein, is highly expressed on HBV-specific T cells and is associated with the dysfunctional T-cell responses in CHB patients. Checkpoint inhibitors direct against PD-1 and restore T-cell dysfunction [47]. Recently, a phase Ib pilot study evaluated anti-PD-1 (nivolumab) treatment with or without GS-4774 (therapeutic vaccine) in virally suppressed HBeAg<sup>-</sup> CHB patients. Patients receiving the higher dose (0.3 ng/kg) showed a significant HBsAg reduction from baseline, with three patients experiencing declines of > 0.5 log<sub>10</sub> by the end of study and one patient had loss of HBsAg [48].

Overall, advances have been made in recent years to target a functional cure of CHB. However, monotherapy will unlikely achieve the goal due to complexity of the virus. A better chance of functional cure of HBV infection may come from a combination of new drugs that act via different mechanisms. Currently, the approach is to use a combination of multiple drugs including a backbone of an NA, one or more new direct

acting antiviral drugs, and at least one immunomodulator [25]. Further studies are needed to demonstrate the safety and efficacy of these combinations. In addition, more precision lab tests (such as HBV DNA and measurement of HBcrAg) are being developed and validated to differentiate disease states and/or define stopping rules to accommodate the rapid advances in CHB treatments.

## HCV

HCV is an enveloped RNA virus from the Flaviviridae family and genus hepacivirus. Currently, there are seven recognized HCV genotypes based on structural diversity of the viral genome (30-35% nucleotide variability). This classification is further divided into 67 sub-genotypes (< 15% nucleotide variability) [49]. Some of these genotypes have different geographic distributions; however, genotypes 1 and 3 have the highest incidence worldwide [50]. A combination of polymerase chain reaction (PCR) and phylogenetic analysis is often implemented to determine the HCV genotype of each patient. Different HCV genotypes have shown variable responses to different treatment regimens; therefore, genotype analysis has routinely been used as a strategy to guide focused treatment.

### Diagnosis

Diagnostic accuracy of HCV testing has improved with the development of the third-generation HCV EIA. This test is nearly 99% sensitive and specific for determining whether a patient has ever been infected with HCV, but it does not differentiate between acute versus chronic infections [51]. Patients who have a positive HCV EIA undergo quantitative HCV RNA testing using nucleic acid amplification technology (NAAT) to diagnose active infection [52]. In instances where HCV RNA testing is unavailable, detection and quantification of HCV core antigen can be used in aiding diagnosis [53]. Furthermore, the development of POC tests that serve as anti-HCV rapid diagnostic tests have been used as effective alternatives to the traditional method HCV testing, with some tests having sensitivity as high as 99% [54]. These POC tests are implemented in areas with health care structures that cannot support the more complex EIA and NAAT testing.

### Treatment

#### *Historic treatment option*

Early/previous treatment options for HCV revolved around immunomodulatory agents including IFN and pegylated IFN therapy to induce a sustained viral response (SVR). SVR refers to achieving an undetectable HCV viral load at least 12 - 24 weeks following treatment cessation [55]. Ribavirin, a guanosine nucleoside analogue, was also introduced to be used alongside IFN therapy for HCV treatment [56]. Although these therapies provided initial treatment, they had several side ef-

**Table 3.** Class I Evidence (Beneficial, Effective, and Useful Treatment) With Data From Multiple Randomized Clinical Trials, Meta-Analysis or Equivalent

Patient population	First line treatment regimens
Genotype 1a without cirrhosis	Elbasvir/grazoprevir, glecaprevir/pibrentasvir, ledipasvir/sofosbuvir, sofosbuvir/velpatasvir
Genotype 1a + compensated cirrhosis	Elbasvir/grazoprevir, ledipasvir/sofosbuvir, sofosbuvir/velpatasvir, glecaprevir /pibrentasvir
Genotype 1b without cirrhosis	Elbasvir/grazoprevir, glecaprevir/pibrentasvir, ledipasvir/sofosbuvir, sofosbuvir/velpatasvir
Genotype 1b + compensated cirrhosis	Elbasvir/grazoprevir, ledipasvir/sofosbuvir, sofosbuvir/velpatasvir
Genotype 2 without cirrhosis	Glecaprevir/pibrentasvir, sofosbuvir/velpatasvir
Genotype 2 + compensated cirrhosis	Sofosbuvir/velpatasvir
Genotype 3 without cirrhosis	Glecaprevir/pibrentasvir, sofosbuvir/velpatasvir
Genotype 3 + compensated cirrhosis	Sofosbuvir/velpatasvir <sup>a</sup>
Genotype 4 without cirrhosis	Elbasvir/grazoprevir, glecaprevir/pibrentasvir, ledipasvir/sofosbuvir, sofosbuvir/velpatasvir
Genotype 4 + compensated cirrhosis	Sofosbuvir/velpatasvir
Genotypes 5 + 6 <sup>b</sup>	Glecaprevir/pibrentasvir

<sup>a</sup>Patients without baseline NS5A RAS Y93H for velpatasvir. <sup>b</sup>Patients both with and without cirrhosis.

fects and only maintained an SVR in 40-50% of patients [57].

#### Direct antiviral agents (DAAs)

Treatment of HCV has expanded over time with the development of medical therapy to achieve SVR. With the development of DAAs, treatment of HCV has evolved to targeting specific components that dictate HCV processing and replication [50]. Specific DAA treatment regimens that have been approved as first-line therapy for each HCV genotype are included in Table 3 [58].

##### 1) NS3/4A protease inhibitors

The HCV multifunctional protein complex known as NS3/4A is responsible for cleaving precursor proteins into the nonstructural protein products NS3, NS4A, NS4B, NS5A, and NS5B [59]. This protease complex is essential for viral replication and serves as a target for medical therapy. Boceprevir and telaprevir were developed first and are classified as first-generation inhibitors of NS3/4A protease. These medications are no longer used in clinical practice. Simeprevir, paritaprevir, grazoprevir, glecaprevir and voxilaprevir are second-generation protease inhibitors that are currently used in clinical practice [60]. These medications are often combined with other drugs with different mechanisms of action including pegylated IFN and ribavirin. Selection of these specific drug regimens is tailored to various HCV genotypes while taking into account of patient's co-morbidities [50, 61].

##### 2) NS5A inhibitors

NS5A is a nonstructural protein that is involved in the process of HCV replication. Ledipasvir, ombitasvir, daclatasvir, elbasvir, velpatasvir and pibrentasvir were developed as medications

that inhibit hyperphosphorylation of NS5A, thereby halting key steps in viral replication [62]. These drugs are also used in combination with other HCV medications and have proven to be effective towards achieving SVR in several patient populations.

##### 3) NS5B inhibitors

Nucleotide and non-nucleoside NS5B inhibitors act upon the catalytic site of action of NS5B, an RNA-dependent RNA polymerase that replicates the HCV genome [63]. Sofosbuvir was developed as a nucleotide analog that acts directly on the active side of NS5B polymerase and gets incorporated into the RNA chain to induce chain termination. Dasabuvir is a non-nucleoside inhibitor of NS5B that gets activated by a cellular kinase and targets an allosteric site to halt viral replication [63].

#### HCV screening and treatment in specific patient populations

##### 1) IV drug users

People who inject drugs (PWID) remain a high-risk group for HCV infection with a global estimate of over 50-60% of this population being HCV positive [64, 65]. The sharing of injection drug use equipment is one of the primary modes of viral transmission amongst this group. PWID are the population also most at risk for HCV infection [64]. A large proportion of people in prisons or other closed detention centers consist of PWID. The criminalization of injection drug use has made these custodial settings highly prevalent in HCV with high risk for further HCV transmission [66].

In response to the increased prevalence of HCV in PWID, the WHO has recommended yearly screening for HCV in this group [67]. Despite these recommendations, there have been lower rates of HCV testing and treatment among PWID. This has been thought to be secondary to decreased level of risk

awareness, difficulty finding access or availability of HCV testing, social stigma, as well as potential discrimination on behalf of healthcare providers [68].

## 2) HCV/HIV co-infection

There are about 37 million people infected with HIV globally with around 20-30% of them also being co-infected with HCV [69]. HCV/HIV co-infected individuals are at increased risk of liver-related mortality with these patients typically having a higher viral load and a higher chance of developing chronic infection associated with the rapid development of advanced liver disease [70, 71]. Treatment of HCV/HIV co-infection remains a challenge because of the side-effects and drug-drug interactions between HCV DAA and HIV anti-retroviral therapy. Typically, interruption of anti-retroviral therapy is not recommended prior to initiation of HCV DAA therapy [70]. Treatment of these patients oftentimes requires collaboration with an HIV or infectious disease practitioner.

## 3) HCV in chronic kidney disease (CKD)/end-stage renal disease (ESRD)

HCV has been shown to be associated with syndromes of glomerulonephritis as well as the development of CKD. Patients with CKD who develop HCV have a higher mortality rate and have a higher likelihood of developing ESRD. This is seen with a higher incidence of dialysis patients being positive for HCV. Treatment of HCV in ESRD and CKD patients is increasingly important to prevent further complications and improve long-term survivability [72-74]. Most DAAs are metabolized through the liver with only minor renal clearance and routinely do not require an adjustment in dose for CKD/ESRD patients. In this patient population, both duration as well as composition of treatment regimens vary based on HCV genotypes as well as glomerular filtration rate (GFR) with elbasvir/grazoprevir, glecaprevir/pibrentasvir, and sofosbuvir regimens being among some of the medications deemed safe and tolerable in patients with GFR < 30 (CKD4, CKD5, or hemodialysis patients) [75].

## 4) HCC risk in patients with chronic HCV following treatment

As HCV leads to hepatic fibrosis and cirrhosis, HCV infection remains a major risk factor for the development of HCC. Although DAAs have been used to induce SVR in HCV infection, patients remain at risk for HCC following treatment. It has been shown that the absolute risk of HCC development following HCV treatment can be as high as 3.5% within 12 months of completing treatment [76-78]. Additionally, certain genotypes have been shown to be associated with an increased incidence of HCC including genotypes 3 and 6. Other risk factors for increased HCC incidence in these patients include co-infection with either HIV or HBV, smoking and alcohol use, as well as diabetes and obesity [79]. Given that patients with chronic HCV

remain at increased risk of HCC, HCC surveillance remains important in these patients with advanced fibrotic or cirrhotic liver morphology even after treatment with DAAs therapy [78].

## *HCV treatment failure*

With the various HCV genotypes in addition to the underlying error prone replication pattern of HCV, treatment failure can be encountered. Clinical trials have shown that DAA treatment failure or relapse occurs in < 10% of cases. One study with 3,830 patients with either cirrhosis or advanced fibrosis showed that 139 (3.6%) of patients failed to achieve SVR following DAA therapy [80]. Viral variants with polymorphisms referred to as resistant-associated substitutions (RASs) have been shown to induce HCV treatment failure. RASs occur in a minority of patients, and sub-therapeutic DAA levels can make one more prone to developing RASs. RASs in HCV proteins NS5A and NS3 have been seen in the setting of previous exposure to NS5A and NS3 DAAs. Such drug resistance impedes one's ability to mount an SVR [81]. In instances where DAAs containing NS5A inhibitors fail, the AASLD and IDSA recommend testing for NS5A RASs [73]. Furthermore, RASs testing has also been proposed in various HCV genotypes (genotype 1a and 3) prior to initiation of DAA treatment [60, 81].

In addition to primary drug resistance due to RASs, treatment failure has also been associated with medication side-effects, drug-drug interactions, and other co-morbidities. DAAs and other antiviral treatment regimens have been shown to be associated with lactic acidosis in addition to hepatic decompensation that has led to mortality and overall treatment failure [82]. Other reported complications hindering achievement of SVR include severe pulmonary toxicity and pulmonary hypertension [83]. Patients with HIV as well as active illicit drug use, mental illness and advanced liver fibrosis are more prone to treatment failure and require more targeted and enhanced DAA treatment regimens to ensure SVR achievement [84].

## **HCV liver transplant complications**

### *Recurrence of HCV in liver allografts*

Patients with a history HCV with an active pre-transplant viremia commonly develop HCV recurrence following liver transplant. Infection of the donated allograft occurs upon reperfusion and requires histological evidence for diagnosis [85, 86]. The clinical presentation of HCV recurrence varies from chronic infection to fibrosing cholestatic hepatitis [87]. Fibrosing cholestatic hepatitis refers to a severe pattern of recurrent disease associated with extremely high levels of HCV RNA that leads to graft loss within a few weeks of onset and typically occurs in < 10% of cases [87, 88].

The course of chronic HCV is typically more aggressive in immunocompromised patients than in immunocompetent patients [89]. Almost one-third of chronically infected patients develop allograft cirrhosis within 5 - 7 years following transplant [90]. Antiviral therapy has been used to modify the



course of recurrent HCV-graft disease to achieve SVR with the underlying goal to achieve histological and necro-inflammatory allograft recovery [87, 91]. Medical treatment remains a challenge as DAA in addition to other antiviral therapies (interferon and ribavirin) has drug-drug interactions with immunosuppressant agents used in transplant patients [60]. Despite these challenges, overall graft survival among HCV-positive recipients has increased substantially since the introduction of DAA therapy [92]. In patients with histopathological evidence of established graft cirrhosis, the only treatment option is re-transplant which accounts for only 10% of all liver transplants and has overall worse outcomes than primary transplants. Although the high cost, historically poor outcomes, and limited availability of hepatic transplants have made re-transplant a contested topic, the application of DAAs during re-transplant has led to overall improved patient and graft survival rates, similar to those of non-HCV re-transplant recipients [93].

The timing of HCV treatment in association with liver transplant is an important consideration due to the variable outcomes for specific populations. Patients with chronic HCV with a MELD score < 16 typically undergo DAA therapy to achieve SVR prior to liver transplant given the mortality benefit as well as possible transplant delisting secondary to clinical improvement [94]. Those with MELD scores between 16 and 20 require individualized treatment regimens regarding when to initiate DAA given the potential to precipitate liver failure or other complications with antiviral therapy. DAA therapy in those with MELD > 20 has been shown to have optimal benefit when initiated following liver transplantation at the earliest point of clinical stability (2 - 4 weeks post-transplant) [94, 95].

#### *Use of HCV seropositive organs for transplantation*

Given the growing need for liver transplants across the globe, HCV seropositive viremic and non-viremic individuals have been used as organ donors for patients requiring liver transplant. Data have shown that HCV seronegative, non-viremic recipients showed promising outcomes and maintained SVR with DAA-based treatment regimens following transplant [92, 96, 97]. As HCV mortality continues to rise globally, DAAs have been shown to increase post-transplant survival and remain effect tools to guide liver transplant planning and treatment [98].

#### **HCV global elimination**

According to the WHO, there is a global estimate of 71 million chronic HCV cases with a growing mortality of over 400,000 deaths annually. Most of these deaths are related to the developed cirrhosis as well as HCC [3, 99]. Various HCV genotypes are more prevalent in different geographic territories with the highest prevalence of infection being spread across areas of central and eastern Asia as well as north and West Africa [2]. High risk groups include injection drug users, people with high-risk sexual practices (multiple sexual partners, men who have sex with men), healthcare workers who suffer needle-stick accidents, patients who have undergone certain

procedures such as hemodialysis in facilities with inadequate infectious control, as well as those who have had blood transfusions or organ donations prior to 1992 [69, 70].

In contrast to other infectious diseases such as tuberculosis (TB), HIV, and malaria that have seen a steady decline in prevalence and mortality over the past decade, HCV infection remains on the rise and is a significant global health problem moving forward [100]. Despite its high prevalence, it is estimated that only 20% of infected individual have been diagnosed and only 7% have received treatment worldwide [2, 3]. HCV remains the leading cause of HCC in the United States and is one of the most common causes of HCC worldwide [78].

With the development of DAAs, the WHO developed the Global Health Sector Strategy for Viral Hepatitis in order to target the spread of HCV with the goal of potentially eliminating HCV as a major global threat by 2030. Most HCV carriers remain undiagnosed until they begin to develop symptoms of cirrhosis or are found to abnormal liver function tests [101]. Endeavors towards HCV elimination have placed emphasis on wider and more prevalent testing in order to diagnose patients unaware of their HCV status. The goal of more widespread testing in high-risk populations serves to prevent further transmission, thereby leading to overall incidence reduction. The WHO has also proposed focused testing in populations with increased HCV risk and prevalence (anti-HCV antibodies in > 2-5% of the general population) as well as groups with sign and symptoms consistent with potential HCV infection. Undiagnosed patients may miss the benefits of HCV therapy: a potential 65% reduction in liver-related death [2, 3, 101]. Countries across the globe such Portugal, Egypt, Netherlands, France, and the United States have specific implemented guidelines to direct HCV testing per the demographic and geographic distribution of their high-risk populations [1, 102].

Despite expanding HCV testing globally, achieving the WHO's goal of HCV elimination by 2030 remains a challenge with several obstacles hindering global elimination. The diagnostic algorithm being a traditional two-test process provides limitations to those lost to follow-up [103]. These patients may ultimately suffer HCV-related mortality. Transitioning to a one-step method of diagnosis may provide more comprehensive treatment. In addition, several nations with high HCV prevalence lack financial, laboratory, or medical resources to comply with the WHO's targets [104]. At the individual level, certain at-risk groups do not have the financial capacity or community access to seek HCV care [105]. Furthermore, the social stigma towards HCV and lack of public awareness towards the global threat of HCV impede further upscaling of HCV diagnosis and treatment [103] (Table 4).

#### **HDV**

HDV is a single-stranded RNA virus of the genus Deltavirus. It uses the HBsAg as a viral envelope and shares the same hepatocyte receptor for viral entry [106]. Eight HDV genotypes have been identified. Two forms of infection exist including co-infection with HBV that can be self-limiting and superinfection in a patient with known HBV infection, which usually leads to chronicity in most cases. Among HBsAg+ people,

**Table 4.** Summary of the Diagnostic and Therapeutic Strategies for Hepatitis Viruses

Virus	Diagnostic testing modalities	Treatment strategies
HAV	IgM anti-HAV antibodies via EIA Rapid immunochromatographic test IgG anti-HAV rapid salivary test	Supportive care
HBV	HBsAg, HBeAg, and IgM/IgG anti-HBc antibodies via EIA Quantitative HBV DNA level via NAAT Quantitative HBsAg HBcrAg POC HBsAg	Interferon formulations Nucleos(t)ide analogs
HCV	IgG anti-HCV antibodies via EIA Quantitative HCV RNA level via NAAT POC anti-HCV	DAAT Interferon formulations
HDV	Total anti-HDV antibody Quantitative HDV RNA level via NAAT	No FDA approved formulations Bulevirtide (myrcludex): viral entry inhibitor approved in Europe
HEV	Anti-HEV IgG/IgM antibodies via EIA Quantitative HEV RNA via NAAT POC anti-HEV1 and anti-HEV3 IgM	Ribavirin ± pegylated interferon HEV vaccine in China

HAV: hepatitis A virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus; HEV: hepatitis E virus; EIA: enzyme immunoassay; IgM: immunoglobulin M; HBsAg: HBV surface antigen; HBeAg: hepatitis B E antigen; HBcrAg: Hepatitis B core-related antigen; NAAT: nucleic acid amplification technology; POC: point of care.

estimated HDV prevalence is 4.5% (95% confidence interval (CI) 3.6 - 5.7). HDV prevalence is higher in people who inject drugs and who have HCV or HIV. It causes an estimated 18% of cirrhosis and 20% of HCC associated with HBV [106].

**Diagnosis**

AASLD recommended testing HBsAg+ persons at risk for HDV, including those with HIV infection, persons who inject drugs, men who have sex with men and immigrants from areas of high HDV endemicity [107]. For patients with CHB infection with elevated liver enzymes but undetectable HBV DNA, testing HDV infection is also recommended [107]. While EASL and APASL recommend screening all patients with CHB. Patients should be tested for total anti-HDV antibody with follow-up HDV RNA testing to confirm active hepatitis D infection if total anti-HDV antibody is positive. Despite the recommendations, HDV screening is low in the United States. One Veterans Administration (VA) study in 2017 showed that only 8.5% of HBsAg+ patients ever underwent anti-HDV antibody screening [108].

**Treatments**

*Viral entry inhibitors*

There are no currently Food and Drug Administration (FDA)-approved therapies in the United States. However, in Europe, a viral entry inhibitor bulevirtide (myrcludex) was recently approved in July 2020 as the first drug for the treatment of chronic HDV infection in HDV RNA positive adult patients with compensated liver disease [109]. The approved dose is 2 mg self-ad-

ministered as a once-daily injection with or without NAs such as TDF. Bulevirtide is an entry inhibitor that binds to NTCP surface receptors that are shared by HBV and HCV. Two randomized controlled studies using bulevirtide in combination with either Peg-IFN or TDF. In the phase II MYR202 (NCT03546621) trial in patients with chronic hepatitis D with liver cirrhosis, or who failed previous IFN therapy, or for whom such therapy was contraindicated (including history of IFN intolerance), treatment with bulevirtide 2 mg plus tenofovir was associated with a significantly increased proportion of patients achieving undetectable HDV RNA or decrease by  $\geq 2 \log_{10}$  from baseline to week 24 than tenofovir alone (53.6% vs. 3.6% of patients, respectively) [110]. However, HDV RNA relapse was reported in 60% of HDV RNA responders in the bulevirtide 2 mg plus tenofovir arm at 12 weeks’ treatment-free follow-up, indicating a long-term use of bulevirtide is needed for sustained suppression of hepatitis D. In the phase II MYR 203 (NCT02888106) study, serum HDV RNA was undetectable in more bulevirtide 2 mg plus Peg-IFN- $\alpha$ -2a recipients than bulevirtide or Peg-IFN- $\alpha$ -2a recipients (80% vs. 13.3% vs. 13.3% of patients, respectively) at 48 weeks and the effects maintained for up to 72 weeks (24 weeks’ treatment-free follow-up). Interestingly, HBsAg levels declined (by  $> 1 \log$ ) or were undetectable in 46.7% of bulevirtide 2 mg plus Peg-IFN- $\alpha$ -2a recipients. No changes were seen in monotherapy recipients [37].

*IFN and antiviral agents*

The Hep-Net/International Delta Hepatitis Intervention Trial (HIDIT-1) studied combination of Peg-IFN and adefovir in chronic hepatitis D patients. Peg-IFN with or without adefovir groups achieved a 2.5 log decline in median HDV RNA at 48 weeks of treatment, while no changes was noted in adefovir

group [111]. In a follow-up HIDIT-2 study when adefovir was replaced by tenofovir and the treatment was extended to 96 weeks, no difference was noted between Peg-IFN with tenofovir group or Peg-IFN alone group [112]. This indicated that the combination of NAs with IFN does not seem to provide additional benefit in chronic HDV infection.

#### *The prenylation inhibitor*

The prenylation inhibitor, lonafarnib (LNF) can prevent proper interaction of large HDAg (L-HDAg) with HBsAg in HDV. In the four series of LOWR HDV (LONafarnib With and without Ritonavir in HDV) studies, combinations of low dose LNF (oral 25 or 50 mg twice daily) with low dose ritonavir (oral 100 mg twice daily) with Peg-IFN- $\alpha$ -2a resulted in 88.9% (8/9) decline of HDV RNA to undetectable or a greater than or equal to 2  $\log_{10}$  IU/L decline in serum HDV RNA by week 24 [113]. A phase 3 study for HDV (Delta Liver Improvement and Virologic Response, D-LIVR) (NCT03719313), studying LNF with RTV with or without Peg-IFN- $\alpha$ -2a in 400 subjects is ongoing.

#### *IFN lambda-1a*

Peg-IFN-lambda-1a is a type-III IFN that has demonstrated antiviral activity against HBV and HCV, but also showed antiviral activity against HDV in a recent clinical study. In this study, Peg-IFN lambda monotherapy (120 or 180 mg weekly) for 48 weeks showed that both doses have antiviral activity against HDV. When giving high dose, 64% patients achieved either a 2  $\log_{10}$  decline in HDV RNA or undetectable at the end of therapy, which was sustained in seven out of 14 (50%) subjects 24 weeks after therapy [114]. Currently, an open-label clinical trial exploring lambda IFN in combination with LNF and ritonavir (RTV) for 24 weeks is ongoing (NCT03600714).

#### *Nucleic acid polymers (NAPs)*

NAPs are another type of therapy that is being studied. NAPs are phosphorothioated oligonucleotides that can suppress HBV replication. It is presumed that they can also inhibit the HDV replication cycle. In a phase 2, proof-of-concept study (NCT02233075) studying patients with compensated hepatitis D, the NAP REP 2139-Ca was given as an intravenous infusion once weekly, with add-on Peg-IFN starting at week 15 for another 15 weeks. REP 2139-Ca demonstrated antiviral effects against both HBV and HDV. About 75% (9/ 12) subjects became HDV RNA negative in serum with a mean HDV RNA decline of 5.34  $\log_{10}$  IU/L [115]. A follow-up study (NCT02876419) exploring the durability of these responses through 3 years of follow-up is currently ongoing.

## **HEV**

HEV is a single-stranded, non-enveloped, RNA icosahedral

virus. There are eight genotypes currently identified, with the HEV1, HEV2, HEV3, and HEV4 genotypes being the most frequently seen in humans. HEV1 and HEV2 contaminate water supplies and are associated with severe hepatitis in pregnant women and children in developing countries [116]. HEV3 and HEV4 are zoonotic, but infection in humans can occur with fecal contamination of water or undercooked meat. There are estimated 20 million HEV1 and HEV2 infections annually, with 3.3 million symptomatic cases, leading to over 60,000 deaths [116].

Diagnosis of HEV is usually carried out with direct testing via PCR of HEV RNA or with indirect testing via detection of anti-HEV IgM or IgG through EIA. Increasingly, POC testing is showing promise as tool for early detection. In 2016, an Italian study found the sensitivity of two rapid tests for anti-HEV1 and anti-HEV3 IgM to be 96% and 93%, and the specificity for both to be 100% [117]. A 2017 study tested a saliva-based EIA against commercial plasma and serum-based kits and found similar sensitivity and specificities [118].

While the majority of HEV infection cases are asymptomatic, 5-30% progress to acute hepatitis [119]. Particular attention should be paid to affected pregnant women and the immunocompromised, as they are at higher risk for developing complications including fulminant and chronic hepatitis.

## **Manifestations and treatment in pregnant women**

HEV1 and HEV2 are well-documented causes of fulminant hepatitis in pregnant women, particularly those in the third trimester. The HEV1 genotype poses the highest risk, with a systematic review showing median maternal fetal, and neonatal case-fatality rates of 26%, 33%, and 8%, respectively [120]. One proposed mechanism for the higher virulence of HEV1 in pregnant women may be attributed to HEV1's greater affinity towards stromal cells, the decidua basalis, and the fetal placenta. An *ex vivo* model in 2018 demonstrated that proliferation of HEV1 along the maternal-fetal barrier led to release of pro-inflammatory cytokines, cellular death, and necrosis [121]. Hormonal changes during pregnancy may also play a role, as pregnant women who developed fulminant hepatitis had higher serum levels of estrogen, progesterone, and beta-human chorionic gonadotropin ( $\beta$ -hCG) [122].

Currently, the standard of care for acute HEV is ribavirin with or without Peg-IFN, although there are no clear guidelines on the dosage and duration of treatment. As both are contraindicated in pregnancy due to teratogenicity, focus should be on prevention. In those who are appropriate candidates, liver transplant should be considered early in the course of disease [123].

## **Treatment in the immunocompromised hepatitis E patients**

In the immunocompromised, acute HEV infection may lead to chronic hepatitis and cirrhosis. Confirmation of infection usually is performed via direct testing via PCR of HEV RNA, as IgM and IgG may both be negative in the setting of immu-

nosuppression [124].

In solid organ-transplant recipients, the first step in treatment is a dose reduction in immunosuppressive medications, with studies showing a nearly 33% rate of complete remission by this method alone [125]. In other patients or in those without adequate response to a reduction in immunosuppression, ribavirin has been shown to be effective. In one study, 78% of patients achieved sustained virologic clearance after a median of 3 months of treatment at 600 mg/day [126].

In patients with ribavirin-resistant infections, pegylated IFN-alpha has also been shown in a few small studies to be efficacious, although its contraindication in non-liver transplant patients due to risk of organ rejection, as well as the limited amount of data on its efficacy, limits its use [127, 128]. Several studies have demonstrated that while sofosbuvir has antiviral activity against HEV, treatment does not lead to sustained virologic response [129-131]. Other agents including 2'-C-methylguanosine, silvestrol, and compounds GPC-N114 and NITD00840 have shown promise in *in vitro* studies [131, 132]. However, further investigation and clinical trials are required before they can be considered for use in humans.

### HEV vaccine

In 2011, a recombinant hepatitis E vaccine (HEV 239, Hecolin) was approved for use in those aged 16 - 65 years in China after a phase 3 trial involving over 110,000 participants and showed over 99% efficacy against the HEV1 and HEV4 genotypes with few adverse events [133]. While the vaccine has not yet gained approval in other countries, in 2020, the Norwegian Institute of Public Health completed recruitment for a phase IV study investigating the efficacy of the vaccine in pregnant women in Bangladesh (NCT02759991) [134]. A recent clinical trial in China also demonstrated the safety of the vaccine in those over 65 years old [135]. A National Institute of Allergy and Infectious Diseases (NIAID)-sponsored phase I study in the United States is ongoing (NCT03827395).

### HGV

Hepatitis G virus (HGV) is a newly discovered and uncommonly reported virus. HGV has a dsRNA structure and is a member of the Flaviviridae family, with an overall design and epidemiological spread similar to that of HCV [136]. The clinical significance of this virus in relationship to hepatic disease and other manifestations remains unclear. There have been no reported definitive downstream impacts of this virus to date [137]; however, case reports and studies have proposed potential associations of this virus with clinic symptoms or other infections. Bhattarai et al reported that HGV may impact or even interfere with HIV replication mechanisms [138]. Co-infection of HGV with HBV, HCV, and HDV has been more frequently detected than mono-infection alone. Additionally, despite the majority of HGV-positive cases being clinically asymptomatic, a minority of HGV-positive patients can present with hepatic disease [136]. HGV has also been reported in cerebrospinal fluid (CSF) analy-

sis in the setting of HIV infection [139]. Although our knowledge on this virus remains limited, there are concerns that HGV may alter clinical course in various patient populations. Furthermore, there is concern that medical systems are not appropriately screening for this virus in populations at risk. Wang et al demonstrated through meta-analysis that pooled prevalence of HGV in donor blood is between 3% and 4% in China [140]. This percentage is higher than any other hepatitis virus, and certain groups have proposed increased screening for this virus. Clinical significance of HGV remains unclear and further research is needed to better understand the impact of this virus both as a single agent as well as a co-infection with other viruses.

### Conclusion

Viral hepatitis epidemics and pandemics take a heavy toll on lives, communities and health systems. Viral hepatitis is also a growing cause of mortality among people living with HIV. The five hepatitis viruses (A, B, C, D and E) are very different, with different modes of transmission, affecting different populations and resulting in different health outcomes. An effective response requires a range of common actions for prevention, diagnosis, treatment, surveillance, and screening, while at the same time delivering tailored interventions for each of the viruses. Acute hepatitis A infection requires prompt diagnosis and treatment is often supportive. Acute hepatitis B and C usually is undiagnosed until it becomes chronic. Acute hepatitis E is more unique in pregnant patients and can cause significant mortality in this population. Worldwide, most of hepatitis-associated morbidity (96%) and mortality (91%) is caused by HBV and HCV infections, because these two viruses cause chronic, life-long infections, resulting in irreversible liver damage that leads to cirrhosis and in some cases, HCC. Hepatitis C treatment is the biggest advancement of viral hepatitis in the past decades, although minor issue is still present. In contrast, despite the recent advances in understanding HBV, significant obstacles exist due to its integration to the host DNA. The first approved medication in EU for hepatitis D is promising. In the next decade, scientific advances in HBV virology and immunology are essential to achieve functional cure for patients with CHB. Overall, for the hepatitis elimination targets to be reached by 2030, substantially more resources and additional innovations are needed to make these interventions widely available. Region-specific strategies are needed that reflect regional epidemiology and disease burden and are supported by a national elimination plan and provision of sufficient resources for implementation.

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None of the authors have relevant conflict of interest or competing interest to declare related to this review.

## Author Contributions

All authors were involved in idea conception, in-depth literature review, manuscript writing, approval of the final manuscript, and take full responsibility for the manuscript.

## Data Availability

All authors agree to allow this data to be open to the public of accessing.

## Abbreviations

AASLD: American Association for the Study of Liver Diseases; ALT: alanine aminotransferase; Anti-HBc: hepatitis B core antibody; APASL: Asian Pacific Association for the Study of the Liver; cccDNA: covalently closed circular DNA; CHB: chronic hepatitis B; CKD: chronic kidney disease; CpAM: core protein allosteric modulators; CSF: cerebrospinal fluid; D-LIVR: Delta Liver Improvement and Virologic Response; DAA: direct anti-viral agents; EASL: European Association for the Study of the Liver; EIA: enzyme immunoassay; ESRD: end-stage renal disease; ETV: entecavir; EU: European Union; GFR: glomerular filtration rate; HAPs: Heteroaryldihydropyrimidines; HAV: hepatitis A virus; HBcrAg: hepatitis B core-related antigen; HBeAg: hepatitis B E antigen; HBsAg: HBV surface antigen; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HDV: hepatitis D virus; HEV: hepatitis E virus; HIDIT-1: Hep-Net/International Delta Hepatitis Intervention Trial; HIV: human immunodeficiency virus; IgM: immunoglobulin M; IL2: interleukin 2; L-HDag: large HDag; LNF: lonafarnib; LOWR HDV: LOnafarnib With and without Ritonavir in HDV; NA: nucleos(t)ide analog; NAAT: nucleic acid amplification technology; NAs: nucleic acid polymers; NAs: nucleic acid polymers; NIAID: National Institute of Allergy and Infectious Diseases; NTCP: sodium taurocholate cotransporting polypeptide; p22Cr: 22 kDa precore protein; PCR: polymerase chain reaction; PD-1: programmed cell death protein 1; Peg-IFN-2a: pegylated interferon alpha-2a; Peg-IFN-a: pegylated interferon alpha; POC: point of care; PPAs: phenylpropanamides; PWID: people who inject drugs; RASs: resistant-associated substitutions; RDTs: rapid diagnostic tests; RIG-I: retinoic acid-inducible gene-1; RNAi: RNA interference; RTV: ritonavir; siRNA: small interfering RNA; SVR: sustained viral response; TAF: tenofovir alafenamide; TAT: turn-around time; TB: tuberculosis; TDF:

tenofovir disoproxil fumarate; TFV: tenofovir; TLRs: Toll-like receptors; TNF: tumor necrosis factor; ULN: upper limit normal; VA: Veterans Administration; WHO: World Health Organization

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