Hepatitis Delta: Current Knowledge and Future Directions

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Abstract: Hepatitis delta virus (HDV) infection is caused by a unique circular RNA virus that relies on both the hepatitis B virus (HBV) antigen and human host polymerases for its transmission and replication. HDV infection can be acquired simultaneously with HBV as a coinfection or as a superinfection in patients already chronically infected with HBV. Chronic HDV is the most severe and progressive form of viral hepatitis–induced liver disease, accounting for significant morbidity and mortality worldwide. Despite the severity of disease and poor clinical outcomes, there are few therapeutic options for the treatment of HDV infection. This article discusses the epidemiology of HDV globally and in the United States, the diagnosis and clinical course of HDV infection, and the current and future therapeutic options for the management of HDV infection.

Keywords
Hepatitis delta virus, hepatitis delta antigen, hepatitis B virus, bulevirtide, lonafarnib, epidemiology

Almost 50 years after its discovery and despite tremendous progress in the management of chronic liver disease owing to hepatitis B virus (HBV) and hepatitis C virus advances, hepatitis delta virus (HDV) infection continues to affect 48 to 72 million people globally, remaining a significant burden of liver disease worldwide.[1,2] HDV infection is caused by a defective satellite virus that requires hepatitis B surface antigen (HBsAg) from HBV to infect humans. Eight distinct genotypes of HDV exist, with genotype 1 being the most ubiquitous worldwide.[2] Transmission occurs through parenteral exposure from infected individuals, and can occur as either a coinfection when acquired simultaneously with HBV or as a superinfection in chronic carriers of HBV.[3] Infection with hepatitis delta leads to the most severe form of viral hepatitis–induced liver disease, with the most rapid progression to cirrhosis and hepatocellular carcinoma.[4-6]

Despite the severity of clinical outcomes in HDV infection, there remains a paucity of data on its epidemiology as well as limited therapeutic options. For the first time in almost 3 decades since the discovery of interferons (IFNs), new therapies specifically for HDV are becoming commercially available to clinicians and their patients. This article aims to provide an overview of current global and US epidemiology of HDV

Table 1. Country-Specific Seroprevalence of HDV Among HBsAg-Positive Carriers

Country	Study Design	Number of HBsAg-Positive Participants Screened	Seroprevalence of Anti-HDV Antibodies in HBsAg-Positive Cohort	Seroprevalence of Active HDV Infection in HBsAg-Positive Cohort[a]
Africa				
Central African Republic[90]	Prospective	181	10.00%	–
Egypt[91]	Prospective	763	3.50%	36.40%
Ethiopia[92]	Prospective	1267	1.50%	0.90%
Libya[93]	Cross-sectional	162	2.50%	–
Malawi[94]	Systematic review	133	1.50%	0%
Nigeria[95]	Cross-sectional	188	4.90%	9.00%
Somalia[96]	Cross-sectional	52	50%	–
Tanzania[97]	Cross-sectional	118	0.60%	–
Tunisia[98]	Retrospective	1615	2.00%	69.70%
Americas				
Brazil[99]	Cross-sectional	787	13.50%	–
Colombia[100]	Cross-sectional	173	5.20%	–
United States Patel et al[28] Safaie et al[26]	 Cross-sectional Retrospective	 113 121	 42.00% 3.30%	 – –
Asia				
Afghanistan[101]	Cross-sectional	234	2.20%	–
China[102]	Retrospective	3065	1.37%	–
Georgia[103]	Cross-sectional	188	–	2.30%
India[104]	Retrospective	120	0.83%	100%
Iran[105]	Systematic review	5700	6.61%	–
Israel[106]	Retrospective	8969	6.50%	23.00%
Japan[107]	Prospective	199	21.10%	–
Pakistan[108]	Retrospective	96	88.81%	30.00%
Taiwan[109]	Prospective	2562	14.50%	–
Turkey[110]	Retrospective	282	45.50%	56.90%
Vietnam[111]	Prospective	266	–	15.40%
Europe				
Germany[112]	Review	–	0%-7.40%	64.50%
Greece[4]	Prospective	4673	4.20%	–
Italy[113]	Prospective	786	10.00%	77.80%
Slovenia[114]	Retrospective	1305	0.23%	66.66%
Switzerland Vieira Barbosa et al[115] Genné, Rossi[116]	 Retrospective Cross-sectional	 648 1699	 7.10% 5.90%	 70.00% –
United Kingdom[20]	Retrospective	962	8.50%	–
Oceania				
Australia[60]	Retrospective	4407	4.10%	–

HBsAg, hepatitis B surface antigen; HDV, hepatitis delta virus.
[a]Based on the subset of HBsAg-positive individuals tested for HDV RNA after confirmation of presence of anti-HDV antibodies.

infection, its clinical course, and future horizons in disease management and treatment.

Epidemiology of Hepatitis Delta

Accurate global and country-specific prevalence of hepatitis delta remains elusive. Table 1 summarizes a selection of published country-specific estimates. Recent meta-analyses estimate that roughly 1% of the global general population and from 5% to 15% of HBV carriers have antibodies to HDV (anti-HDV).[1,2] HDV is not distributed uniformly across the globe. Historically, HDV has been thought to be endemic mainly to Central Africa, the Amazon River Basin in South America, Eastern and Mediterranean Europe, the Middle East, and certain parts of Asia. Today, countries in Africa and Asia, such as Mongolia and Niger, carry the largest global burden of HDV infection.[1,2,7] Universal HBV vaccination efforts initially led to a marked decline in HDV seroprevalence in younger HBsAg carriers from developed countries.[2,8,9] However, increased migration flows in the past decades have led to an increase in the spread of HDV infection, even in historically nonendemic regions of the world.[9,10] In many countries, the main reservoirs for HDV are the local aging population with advanced liver disease and young immigrants from endemic countries with active chronic HDV infection.[9] Immigration has also contributed to changing patterns in HDV genotype distribution worldwide. Although genotype 1 remains the most common, genotypes 5 to 7, which were once confined to African countries, have also been reported in certain regions of Europe.[2] HDV genotypes 2 and 4 continue to be found primarily in Russia and East Asian countries.[11-13] Genotype 3, which leads to a more aggressive form of HDV with higher likelihood of fulminant hepatitis, is mainly found in South America.[14-16]

Risk factors for HDV infection include injection drug use, particularly in regions with low endemicity; history of HIV or hepatitis C virus infection; and exposure to blood or other bodily fluids of infected persons.[1,2,17,18] For medical and dental professionals, exposure to the blood, saliva, and nasopharyngeal secretions of an infected patient with HDV is a notable source of infection.[19] In highly endemic regions as well as in the aging population, intrafamilial transmission and iatrogenic spread (eg, reusing needles) have also been shown to be routes of transmission.[1,2,20,21] Given that HDV infection is typically associated with low HBV viremia (and low viremia is unlikely to lead to HBV transmission and therefore no HDV transmission), HDV is not commonly transmitted vertically from mother to offspring.[22,23] Sexual transmission of HDV is also considered to be infrequent, although not as rare as vertical transmission.[24]

Hepatitis Delta in the United States

Although HDV infection is considered relatively uncommon in the United States, surveillance data remain limited. Currently, the American Association for the Study of Liver Diseases (AASLD) guidelines recommend screening for high-risk HBsAg carriers rather than all patients with HBV infection,[25] and HDV screening is rarely conducted even for persons who fall within the guidelines.[26,27] Surveillance data are further limited by HDV infection not being a nationally reportable condition like other viral hepatitides and by clinicians in the United States having varied availability of RNA assays to quantify viral load.[26,27]

A study examining data from a representative sample of the American noninstitutionalized population in the National Health and Nutrition Examination Survey estimated that 42% of adult HBsAg carriers in the United States are living with past or ongoing HDV infection.[28] This estimate is not only significantly higher than previous reports using National Health and Nutrition Examination Study data[29] but also higher than studies in a variety of populations, including individuals who use injection drugs as well as health care–based and nationwide veteran cohorts. Most studies estimate the seroprevalence of hepatitis delta to be between 0.02% and 11% among HBsAg-positive Americans.[27,29-31] Aside from differing study populations, disparities in approximating the prevalence of HDV infection may also be attributed to the use of the DiaSorin anti-HDV enzyme-linked immunosorbent assay, which has a lower specificity than the quantitative microarray antibody capture assay.[32]

In the United States, HDV is predominantly found in high-risk groups such as persons who inject drugs or who immigrated from an HDV-endemic country.[18,31,33-35] Given inconsistent testing for HDV as well as emerging therapies for its treatment, more accurate surveillance data are needed worldwide and in the United States to implement proper policies for prevention, diagnosis, and management of hepatitis delta.

Basic Virology

HDV is the smallest animal virus, measuring 36 nm in diameter and possessing a single-stranded, circular RNA genome that structurally resembles that of plant viroids.[36,37] The viral genome forms a rod-like conformation owing to a high degree of self-complementarity,[38] and only encodes 1 protein, a hepatitis D antigen (HDAg) composed of 2 isoforms, a small 24 kilodalton (kDa) HDAg (S-HDAg) and a large 27 kDa HDAg (L-HDAg).[38,39] Each isoform is coexpressed in an infected individual and has its own specific functions. Genetic studies of the hepatitis delta viral genome have revealed

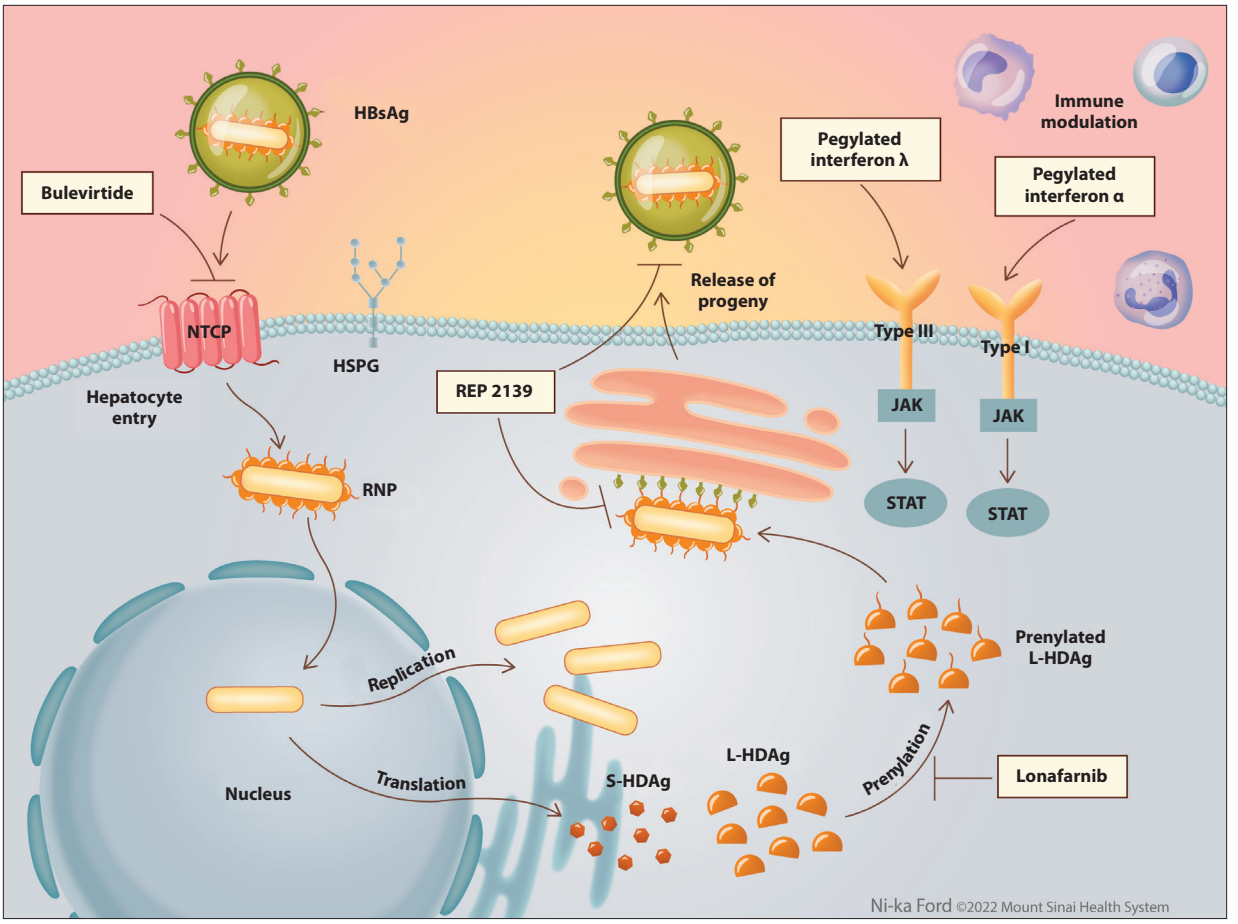

Figure. Hepatitis delta virus life cycle and therapeutic targets.

HBsAg, hepatitis B surface antigen; HSPG, heparan sulfate proteoglycans; JAK, Janus kinase; L-HDAg, large hepatitis D antigen; NTCP, sodium taurocholate cotransporting polypeptide; RNP, ribonucleoprotein; S-HDAg, small hepatitis D antigen; STAT, signal transducer and activator of transcription.

significant sequence heterogeneity among different HDV isolates, leading to a broad classification into 8 distinct HDV genotypes.[40]

The outer HBsAg glycoprotein of the HDV viral particle facilitates entry and exit into the host hepatocyte (Figure). The HDV entry into a new hepatocyte is facilitated by the binding preferences of HBsAg. First, a HBsAg loop attaches in a low-affinity manner to heparan sulfate proteoglycans on the hepatocyte surface. Then, the HBsAg pre-S1 domain binds to a hepatocyte bile acid transporter, sodium taurocholate cotransporting polypeptide (NTCP), in a high-affinity fashion, allowing HDV to enter the cell.[41]

Replication begins when the HDV RNA is translocated from the hepatocyte nucleus into the nucleolus, where human DNA polymerase II (Pol II), and likely DNA polymerase I, transcribes a long antigenomic strand of RNA.[42] A ribozyme cleaves the strand into a monomer, allowing for annealment into a circular form.[43,44] The genomic replication process is completed after the antigenomic HBV RNA returns to the nucleus, where it is then transcribed back into genomic HBV RNA.[45]

Subsequently, the HDAg proteins are encoded. First, Pol II transcribes a messenger RNA strand from genomic HDV RNA that encodes for the 195 amino acid (AA) S-HDAg protein.[42] S-HDAg has been shown to have a positive feedback mechanism and promote more viral replication via recruitment of Pol II.[46,47] Creation of L-HDAg, however, involves a more complex alteration of the replication process. The human adenosine deaminase acting on RNA 1 alters the UAG stop codon on the antigenic HDV RNA, changing the adenosine to inosine.[48,49] When the antigenomic HDV RNA is transcribed back into the genomic HDV RNA, the inosine is read as a guanine and translation is no longer halted at that site. The messenger RNA from this altered genomic strand

is then translated into a longer, 214 AA L-HDAg protein. Within the additional 19 AA at the C-terminal of L-HDAg resides a posttranscription modification signal. A hepatocyte farnesyltransferase recognizes this signal, adding a farnesyl lipid group to the L-HDAg protein in a process called farnesylation.[50] This lipid group is essential for viral assembly, helping the HDV riboprotein complex associate with the HBsAg proteins that will make up the viral envelope necessary for export out of the hepatocyte.[51,52]

HDV's use of host cell machinery for viral replication and assembly as well as the lipoprotein envelope of HBsAg for viral packaging and translocation into host cells results in a paucity of specific enzymatic functions for therapeutic target.[53] Despite elucidation of the major steps of the viral life cycle, there are still many unknown facets, and future investigations on viral life cycle will help guide pharmacotherapeutic development.

Diagnosis of Hepatitis Delta

Prior to making the diagnosis of HDV infection, confirmation of the presence of HBV infection by serologic markers (ie, HBsAg) is needed. Suppression of HBV replication may be present during active HDV infection, so low levels of HBV DNA should not preclude testing for HDV.[22] Recommendations for whom to screen for HDV varies by the governing body. For instance, both the European Association for the Study of the Liver and the Asian Pacific Association for the Study of the Liver recommend screening for HDV in all persons with acute or chronic HBV infection.[54,55] However, the AASLD recommends screening only at-risk HBsAg carriers, such as individuals who use injection drugs or are HIV-positive; people from HDV-endemic regions of the world; and HBsAg carriers with low HBV DNA levels but persistently elevated alanine aminotransferase (ALT) levels regardless of whether they are on antinucleoside therapy.[22,25] Despite these recommendations, screening for HDV is often not performed,[4,27,31] highlighting the potential benefit of implementing automated or reflex testing.[4,56]

Most diagnostic testing for hepatitis delta captures chronic rather than acute HDV infections. There are 2 main blood tests used in clinical practice to screen for HDV infection: anti-HDV antibodies and HDV RNA. Hepatitis delta is initially identified through detection of total antibodies to HDV, with a positive test not distinguishing between a past or current infection. To confirm active infection and a clinical diagnosis, molecular hybridization or reverse transcription polymerase chain reaction can be used to detect the presence of HDV RNA qualitatively or quantitatively.[17,56] Although time-consuming and not routinely used in the clinical setting, liver biopsy with immunohistochemical staining for HDAg is a suitable alternative to HDV RNA detection.[17,57] However, confirmatory testing remains a challenge in many regions of the world. Qualitative assessments of HDV RNA, although not as informative of disease activity, may be more readily available than quantitative ones. Quantitative HDV RNA tests are becoming more available across research and clinical settings.

After confirmatory testing of active, often chronic, HDV infection, differentiating between coinfection and superinfection is helpful for disease prognosis and management. Coinfection is diagnosed by simultaneous presence of serologic markers for both acute HBV and HDV infection. Depending on the timing of testing, immunoglobulin M antibody to hepatitis B core antigen (anti-HBc IgM) is usually the first to appear in serum followed by anti-HDV IgM 2 weeks after symptom onset.[57] Establishing a diagnosis of HDV superinfection requires a positive test for anti-HDV IgM with presence of HDV RNA in addition to a negative test for anti-HBc IgM in HBsAg carriers. In addition, upon the development of a new positive HDV antibody test, superinfection can be suspected in those with known chronic HBV with previously negative HDV testing.

Clinical Course of Hepatitis Delta

As discussed previously, HDV infection can occur as either a coinfection or a superinfection with HBV. The initial clinical course for acute HDV is indistinguishable from that of other viral hepatitides. Symptoms typically begin 3 to 7 weeks following initial infection, and can be nonspecific, including fatigue, anorexia, nausea, and abdominal pain.[17] In addition, patients can be completely asymptomatic, even during acute infection. Coinfection resolves in 80% of cases.[1] On the other hand, the vast majority of superinfections progress to chronic HDV infection.[1]

The chronic form of HDV infection is associated with worse clinical outcomes than HBV monoinfection, often rapidly progressing to more severe liver disease. HDV infection also increases the risk of developing hepatocellular carcinoma and cirrhosis, which is already elevated from HBV infection.[1,4,6,58,59] The mechanism by which this occurs remains unclear. Studies have also shown that HDV infection increases risk of undergoing liver transplant.[18,60] In patients who progress to requiring salvage therapy, contemporary posttransplant outcomes are similar to those in HBV-monoinfected patients.[61] Variations in the severity of HDV infection may also be influenced by viral genotype. As mentioned previously, HDV genotype 3, predominant in the Amazon River Basin in Brazil, has a high chance of progression to

fulminant hepatitis,[14,62] whereas genotypes 2 and 5 may present with overall more favorable disease outcomes than genotype 1.[63]

Current Available Treatments

Although antiviral polymerase treatments (ie, nucleoside analogues) are being used for treating HBV, they do not have specific efficacy for HDV. There is therefore no indication for isolated use of nucleoside analogues for the treatment of HDV, although control of active HBV infection and reduction of HBsAg levels would minimize the ability of HDV to complete its viral replication cycle. Outside the typical use of nucleoside analogues for the treatment of HBV, the AASLD recommends nucleoside analogue therapy for patients with HDV and low or suppressed HBV replication if they also have cirrhosis.[25] The European Association for the Study of the Liver recommends considering nucleoside analogue treatment in patients with HDV if they have HBV DNA levels greater than 2000 IU/mL.[55]

To date, the only strategy that has led to a viral response is an immune-mediated approach targeting IFN α. As a result, current society guidelines, including those by the AASLD, recommend the use of pegylated IFN (PEG-IFN) α for the treatment of chronic HDV infection, despite the drug not having US Food and Drug Administration (FDA) approval for this use.[25] Furthermore, although it is the only treatment option currently available in the United States, use of PEG-IFN α outside a clinical trial is limited by its significant safety profile as well as somewhat limited efficacy. PEG-IFN α for the treatment of HDV has been studied for treatment durations and follow-up periods ranging from 1 to 5 years as well as in combination with nucleoside/nucleotide analogues and on its own. HDV virologic response ranged from 23% to 48%, with ALT response occurring in up to 71% of study participants.[64]

Future Horizons

Exciting developments have been made over the past decade for the treatment of HDV. Current therapeutic approaches address distinct steps in the viral life cycle of HDV. As discussed, the pre-S1 domain of HBsAg binds to the bile acid transporter NTCP and allows for the entry of HDV into the host hepatocyte. Bulevirtide (BLV; Hepcludex, Gilead), formerly known as Myrcludex B, is a synthetic lipopeptide version of the pre-S1 domain, and competes with the HBsAg particle for binding to NTCP, thereby blocking viral entry.[65] Another key piece of the HDV life cycle is the farnesylation of L-HDAg, which facilitates the association of the HDV riboprotein complex with HBsAg to form the complete viral particle. Lonafarnib (LNF; Zokinvy, Eiger) is a farnesyltransferase inhibitor first developed for potential oncologic use and has since been investigated in other farnesylation-dependent pathologies such as HDV and progeria, for which it is now approved for use by the FDA.[66] Nucleic acid polymers are antiviral agents that block the release of HBsAg and thus interfere with viral export. Finally, PEG-IFN λ, an IFN with fewer side effects than PEG-IFN α owing to its more specific binding to an IFN receptor highly expressed on hepatocytes, is under investigation for the treatment of HDV. In addition, the FDA has approved an important surrogate treatment endpoint that consists of a greater than or equal to 2 $\log_{10}$ decline in HDV viral load and ALT normalization on treatment.[67] The following sections provide a brief summary of data surrounding the use of these agents, with additional information in Table 2.

Bulevirtide

BLV has been under investigation for the treatment of HDV over the past decade. In 2011, the initial trial (EudraCT2010-022776-31) began to evaluate the safety, tolerability, and pharmacokinetics of BLV (n=46; phase 1), with both subcutaneous (SC) and intravenous (IV) routes of drug administration resulting in no serious side effects.[68] Subsequently, the MYR-201 study (n=24; phase 1b/2a; NCT02881008) compared tolerability as well as efficacy of patients taking BLV 2 mg SC daily, PEG-IFN α, or a combination of the two.[69] The combination regimen had the most impressive decline in HDV RNA, with the majority of patients achieving HDV RNA negativity after 24 weeks of treatment.

In the next study in the series, MYR-202 (n=120; phase 2b; NCT03546621), patients were pretreated with oral tenofovir disoproxil fumarate (TDF) 245 mg daily for 12 weeks and then started on a combination regimen of TDF with varying doses of BLV for 24 weeks.[70] Patients receiving BLV experienced a decline in ALT levels and liver stiffness, and the 10 mg regimen resulted in the largest decline in HDV RNA. However, after 12 weeks posttreatment and return to TDF monotherapy, the majority of BLV patients who had initially responded to treatment had a relapse in HDV RNA levels.

MYR-203 (n=60; phase 2; NCT02888106), similarly to MYR-201, compared different combinations of PEG-IFN α with BLV as well as BLV alone and PEG-IFN α alone for 48 weeks.[71] The patients were followed for an additional 24 weeks posttreatment to assess viral relapse. Consistent with the findings in MYR-201, the combination of BLV and PEG-IFN α proved synergistic and efficacious, with the majority of side effects related to PEG-IFN α. The higher-dose BLV combination regimen,

Table 2. Treatments for HDV Under Development

Study (Phase) and Number Enrolled	Therapeutic Regimen(s)	Viral Response	Biochemical or Physiologic Response	Adverse Effects
MYR-201 (1b/2a)[69] n=24	(A) BLV 2 mg SC QD for 24 weeks (B) BLV 2 mg SC QD + PEG-IFN α 180 μg SC weekly for 24 weeks (C) PEG-IFN α 180 μg SC weekly for 24 weeks	HDV RNA undetectable at 24 weeks: – 29% of group A – 71% of group B – 86% of group C	ALT normalization at 24 weeks: – 75% of group A – 14% of group B – 13% of group C	Thrombocytopenia, lymphopenia, eosinophilia, neutropenia, increase in conjugated bilirubin
MYR-202 (2b)[70] n=120	All groups: pretreatment with TDF 245 mg PO QD for 12 weeks (A) TDF continued + BLV 2 mg SC QD for 24 weeks (B) TDF continued + BLV 5 mg SC QD for 24 weeks (C) TDF continued + BLV 10 mg SC QD for 24 weeks (D) TDF continued for 24 weeks	HDV RNA reduction by 2 $\log_{10}$ or negativity at 24 weeks: – 46% of group A – 47% of group B – 77% of group C – 3% of group D Relapse at 12 weeks posttreatment: – 60% of group A responders – 80% of group B responders – 83% of group C responders	ALT normalization at 14 weeks: – 43% of group A – 50% of group B – 40% of group C – 7% of group D Mean liver stiffness at 24 weeks declined significantly in all BLV groups	Bilirubin elevations, posttreatment hepatitis exacerbations that self-resolved
MYR-203 (2)[71] n=60	(A) BLV 2 mg SC QD for 48 weeks (B) BLV 2 mg SC QD + 180 μg PEG-IFN α SC weekly for 48 weeks (C) BLV 5 mg SC QD + 180 μg PEG-IFN α SC weekly for 48 weeks (D) 180 μg PEG-IFN α SC weekly for 48 weeks	Median HDV RNA $\log_{10}$ reduction at 48 weeks: – 1.30 in group A – 4.81 in group B – 5.59 in group C – 2.84 in group D Greatest persistent reduction of HDV RNA from baseline to 24 weeks posttreatment: – 4.04 in group B	ALT normalization at 48 weeks: – 27% of group A – 27% of group B – 47% of group C – 67% of group D ALT normalization at 24 weeks posttreatment: – 47% of group B Decline in all other groups	Bilirubin elevations
MYR-204 (2b)[72] n=175	(A) BLV 2 mg SC QD + 180 μg PEG-IFN α weekly for 48 weeks, followed by another 48 weeks of only BLV 2 mg SC QD (B) BLV 10 mg SC QD + 180 μg PEG-IFN α weekly for 48 weeks, followed by another 48 weeks of only BLV 10 mg SC QD (C) BLV 10 mg SC QD for 96 weeks (D) PEG-IFN α 180 μg weekly for 48 weeks	HDV RNA undetectable or 100-fold drop: – 88% in group A – 92% in group B – 72% in group C – 38% in group D Mean HDV RNA $\log_{10}$ reduction at 24 weeks: – 3.78 in group A – 4.11 in group B – 2.68 in group C – 2.01 in group D	ALT normalization at 24 weeks: – 30% of group A – 24% of group B – 50% of group C – 13% of group D	Mainly owing to PEG-IFN α
MYR-301 (3)[73] n=150	(A) BLV 2 mg SC QD for 144 weeks (B) BLV 10 mg SC QD for 144 weeks (C) 48 weeks of observation, followed by BLV 10 mg SC QD	HDV RNA 100-fold reduction at 24 weeks: – 55.1% in group A – 68% in group B – 4% in group C	ALT normalization: – 53.1% of group A – 38% of group B – 5.9% of group C	Study in progress; not reported yet

(Table continues on following page)

Table 2. *(Continued)* Treatments for HDV Under Development

Study (Phase) and Number Enrolled	Therapeutic Regimen(s)	Viral Response	Biochemical or Physiologic Response	Adverse Effects
LOWR-1 (2)[76] n=20	(A) LNF 200 mg BID for 12 weeks (B) LNF 300 mg BID for 12 weeks (C) LNF 100 mg TID for 5 weeks (D) LNF 100 mg BID + PEG-IFN α 180 μg weekly for 8 weeks (E) LNF 100 mg BID + RTV 100 mg QD for 8 weeks	Groups D and E exhibited the greatest drops in HDV RNA after 8 weeks of therapy (>2 $\log_{10}$) HDV RNA levels returned to pretreatment levels by 12 weeks posttreatment for majority of responders	ALT normalization occurred in groups D and E	High doses of LNF in monotherapy poorly tolerated Anorexia, nausea, diarrhea, weight loss
LOWR-2 (2)[77] n=55	10 regimens, including 5 high-dose regimens of LNF + RTV, 3 low-dose PO regimens of LNF + RTV, and 2 low-dose combination regimens of LNF + RTV + PEG-IFN α	Combination therapy with PEG-IFN α showed most robust HDV RNA reduction	ALT normalization at 24 weeks in most patients on low-dose combination therapy	Anorexia, nausea, diarrhea, weight loss, thrombocytosis, anemia
LOWR HDV-3 (2)[78] n=21	(A) LNF 100 mg BID for 28 days (B) LNF 200 mg BID for 28 days	HDV RNA reduction ($\log_{10}$) at 28 days: – 0.73 in group A – 1.54 in group B – 0.13 in placebo group At 4 weeks posttreatment, HDV RNA levels returned to baseline in groups A and B	No significant changes in ALT	All regimens safe and tolerable
LOWR HDV-4 (2)[79] n=15	Dose-escalation from LNF 50 mg BID + RTV to LNF 100 mg BID + RTV over 24 weeks	HDV RNA reduction (>2 $\log_{10}$) at 24 weeks: 27% (4/15) HDV RNA reduction (>2 $\log_{10}$) at 48-week follow-up: 20% (3/15)	ALT normalization at 24 weeks: 53% ALT normalization at 48-week follow-up: 0%	>50% of participants required dose reduction owing to GI side effects 13% discontinued
REP 301 (2)[84] n=12	3 stages: (1) REP 2139 500 mg IV weekly for 15 weeks (2) Combination therapy: REP 2139 250 mg IV + PEG-IFN α 180 μg SC weekly for 15 weeks (3) Monotherapy with PEG-IFN α 180 μg weekly for 33 weeks	HDV RNA undetectable by end of treatment: 75% (9/12) HDV RNA remained undetectable at 1-year follow-up: 78% (7/9)	ALT normalization at 1-year follow-up: – 75% (9/12)	Anemia, neutropenia, thrombocytopenia, ALT/AST elevations, bilirubin elevations
LIMT (2)[87] n=33	(A) PEG-IFN λ 180 μg SC weekly for 48 weeks (B) PEG-IFN λ 120 μg SC weekly for 48 weeks	HDV RNA reduction (>2 $\log_{10}$) at 48 weeks: – 50% of group A – 21% of group B HDV RNA reduction (>2 $\log_{10}$) at 24 weeks posttreatment: – 36% of group A – 10% of group B	ALT normalization at 48 weeks: – 14% of group A – 11% of group B ALT normalization at 24 weeks posttreatment: – 36% of group A – 26% of group B	Dizziness, headache, ALT/AST elevations, bilirubin elevations, flu-like symptoms

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BID, twice daily; BLV, bulevirtide; GI, gastrointestinal; HDV, hepatitis delta virus; IV, intravenous; LNF, lonafarnib; PEG-IFN, pegylated interferon; PO, orally; QD, once daily; RTV, ritonavir; SC, subcutaneous; TDF, tenofovir disoproxil fumarate; TID, 3 times daily.

containing 5 mg of BLV, showed the greatest decrease in HDV RNA immediately posttreatment. Furthermore, the lower-dose BLV combination regimen, containing 2 mg of BLV, resulted in the greatest persistent reduction of HDV RNA from baseline to 24 weeks posttreatment cessation.

A follow-up study, MYR-204 (n=175; phase 2b; NCT03852433), has been in progress since 2019 to evaluate higher doses and longer courses of the BLV and PEG-IFN α combination.[72] Although the high-dose combination regimen, containing 10 mg of BLV, again demonstrated the greatest decline in HDV RNA at 24 weeks, the BLV 10 mg monotherapy group resulted in the most patients achieving normalization of their ALT levels in conjunction with a significant serologic response (≥2 $\log_{10}$ decrease from baseline or undetectable HDV RNA).

Finally, a phase 3 trial (MYR-301; n=150; NCT03852719) was initiated in 2019 to assess the combined response measure used in MYR-204, namely to determine if patients demonstrated both a decrease in HDV RNA as well as normalization of ALT levels.[73] The experimental groups will receive 2 different doses of BLV for 144 weeks and be compared with a delayed treatment group that receives BLV 10 mg SC daily after an observational 48-week period. This study also aims to measure liver stiffness using elastography every 48 weeks for 240 weeks to better characterize the overall impact of BLV on the liver. Preliminary data at 24 weeks of treatment showed significant decreases in HDV RNA in both experimental arms. The virologic response was slightly greater in the higher-dose regimen, but the 2 mg group demonstrated a faster and more sustained reduction in ALT levels. By 24 weeks, 53.1% of the 2 mg group had normal ALT levels compared with 38% of the 10 mg group and 5.9% of the control group. Further data evaluating sustained virologic and biochemical response, as well as changes in markers of liver disease such as liver stiffness or evidence of portal hypertension, will become available in the near future.

With promising early data from these investigations, BLV has already been conditionally approved as Hepcludex at a dose of 2 mg SC per day for the treatment of chronic HDV in adult patients with compensated liver disease and positive HDV viremia by the European Medicines Agency, and is being evaluated by the FDA for approval in the United States.[67] Preliminary data from its use in Europe appear to confirm the previously stated study findings. When evaluating 133 patients receiving either BLV monotherapy or BLV with weekly PEG-IFN α, the combination group saw a higher proportion of greater than 2 $\log_{10}$ decline of HDV RNA (94% in combination vs 68% in monotherapy) after 48 weeks of treatment.[74] However, ALT normalization was higher in the monotherapy group (49% in monotherapy vs 36% in combination). Smaller studies of monotherapy in Italy, Austria, and Germany demonstrated similar findings. Longer-term follow-up data will help determine the best treatment regimen as well as which outcomes are ultimately most beneficial to patients.

Lonafarnib

As discussed previously, LNF is a farnesyl transferase inhibitor initially developed for potential oncologic applications. Its use for HDV was first trialed in a proof-of-concept study (NCT01495585) that began enrollment in 2012 and demonstrated an inverse correlation between LNF serum concentrations and HDV RNA serum levels.[75]

Successive clinical trials have identified the dose, duration, and supplemental medications to maximize efficacy and minimize adverse effects of LNF treatment, which are predominantly gastrointestinal (GI) (anorexia, nausea, diarrhea, and weight loss). The LOWR-1 trial (n=20; phase 2; NCT02430181) compared a variety of combinations of LNF dosing with or without ritonavir (RTV) or PEG-IFN α.[76] Although higher doses of LNF led to a more rapid reduction in HDV RNA, the higher doses were more poorly tolerated. Combining a low-dose LNF (100 mg twice daily [BID]) with RTV or PEG-IFN α allowed for a similar reduction in HDV RNA while minimizing adverse effects. However, this combination regimen proved ineffective in the long term, as the majority of patients' HDV RNA levels returned to pretreatment levels by 24 weeks posttreatment.

The LOWR-2 study (n=55; phase 2; NCT02430194) further evaluated 10 combination regimens of LNF, RTV, and PEG-IFN α and found significant GI side effects with higher doses of LNF, as well as thrombocytosis and anemia. The study also determined that an all-oral regimen of lower-dose LNF and RTV was much better tolerated and effective at reducing HDV RNA, but that addition of PEG-IFN α was important for significantly reducing viral load.[77] The LOWR HDV-3 study (n=21; phase 2) removed PEG-IFN α from the regimen and assessed the side effect profile of varying doses of once-daily LNF with RTV 100 mg and found all regimens to be safe and tolerable.[78] The LOWR HDV-4 study (n=15; phase 2; NCT01495585) had similar goals, but instead examined an escalating dose regimen of LNF.[79] One-half of the study participants required a dose reduction owing to GI side effects, and only one-third of patients were able to reach the goal of receiving LNF 100 mg and RTV 100 mg BID.

Although these studies were predominantly designed to identify tolerable dosing regimens, many also evaluated

HDV viral load response to treatment with the common endpoint of $\log_{10}$ decrease in viral load by 2 or more. Some treatment courses met this goal, but many that monitored patients posttreatment, such as those used in LOWR-1 and LOWR-3, found evidence of viral relapse. There are now multiple phase 3 trials initiated to further assess the efficacy of LNF and monitor for a sustained response. The sixth iteration of the LOWR series (the fifth was withdrawn owing to challenges in setting up the study) began recruiting in 2021 (goal n=30; NCT05229991).[80,81] A once-daily dose of LNF 50 mg with RTV 200 mg will be given for 48 weeks, with a 24-week follow-up period. The 2 primary outcome measures will be change in HDV viral load from baseline to the end of treatment and at the end of the follow-up period. D-LIVR, which has a separate matrix design and is partially double-blind and larger (goal n=400; NCT03719313), is in progress to evaluate the efficacy of LNF 50 mg BID and RTV 100 mg BID with and without PEG-IFN α.[82] Flexibility in dosage and formulation of LNF will ultimately ameliorate adherence and tolerance in patients.

Nucleic Acid Polymers

Nucleic acid polymers have also been evaluated for the treatment of HDV. REP 2139 is a nucleic acid polymer shown to clear HBsAg by blocking its release. It was evaluated in a phase 2 study in which 12 HDV patients were treated with 15 weeks of REP 2129 as monotherapy, followed by add-on PEG-IFN α for 15 weeks and then PEG-IFN α monotherapy for another 33 weeks.[83] The study showed impressive HDV suppression rates of more than 80% during treatment. Furthermore, viral response was maintained in more than 50% of patients after 3.5 years of follow-up. However, further larger studies are needed to validate these data.[84]

Pegylated Interferon Lambda

PEG-IFN λ is a novel type 3 IFN that binds to a unique receptor highly expressed on hepatocytes but much less so on extrahepatic cells such as hematopoietic and central nervous system cells. Its potential therapeutic use has been explored, first in HBV and hepatitis C virus. PEG-IFN λ has been previously shown to have a more tolerable and safe side effect profile than PEG-IFN α.[85,86] Its use for HDV was first investigated in 2016 in the LIMT study (n=33; phase 2; NCT02765802).[87] LIMT randomized HDV patients to 2 doses of PEG-IFN λ for 48 weeks; all patients received tenofovir or entecavir as well. At the end of treatment, 50% of patients in the high-dose (180 μg; n=14) and 21% of patients in the low-dose (120 μg; n=19) groups achieved a greater than 2 $\log_{10}$ decline in HDV RNA. At 24 weeks posttreatment, 36% of patients in the high-dose group had a sustained response, compared with 10% of patients in the low-dose group. Within the high-dose group, 4 out of 5 patients with a sustained response also had ALT normalization at 72 weeks. The medication was generally better tolerated than PEG-IFN α; however, hyperbilirubinemia and jaundice were noted predominantly among Pakistani patients, which will likely require further study.

The phase 3 study LIMT-2 (goal n=150; NCT05070364) will begin in 2022.[88] This study will compare 48 weeks of treatment with the higher dose (180 μg weekly), followed by an additional 24 weeks of no treatment, and plans to provide the treatment regimen to the control group after 12 weeks of monitoring. All patients will receive additional treatment for HBV throughout the study period.

Combination Therapy

Given the unique mechanisms of each of the new therapeutics being evaluated, researchers are investigating potential synergistic effects on viral response. The first of these trials, LIFT HDV (n=26; phase 2a; NCT03600714), began in 2018 and mirrors the earlier LOWR trials and the ongoing D-LIVR trial discussed previously.[89] This study combined weekly PEG-IFN λ 180 μg with LNF 50 mg BID and RTV 100 mg BID for 24 weeks of treatment. Initial end-of-treatment data showed 25 of 26 patients (96%) achieving a greater than 2 $\log_{10}$ decline of HDV RNA, with mostly mild to moderate adverse effects. These promising results highlight the need for future investigations to determine the optimal regimens for HDV. Combining medications with distinct therapeutic mechanisms may better aid in achieving the ultimate goal of finite treatment duration and superior antiviral efficacy.

Conclusion

Although HDV is the most severe form of viral hepatitis, accurate data regarding its epidemiology and disease course are still somewhat limited as a result of suboptimal HDV screening practices. However, a deepened understanding of the viral life cycle of hepatitis delta has facilitated the development of promising therapeutics that may lead to effective and tolerable long-term treatments for patients living with HDV. With the advent of these new therapies, there is renewed hope for combating this devastating chronic liver disease that affects millions of people worldwide. Future research in the field should focus on improving the epidemiologic understanding of HDV in order to better identify patients at highest risk for disease and severe outcomes, as well as determine the most effective, tolerable combination treatment to help those patients.

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Dr Kushner has served on advisory boards for Gilead Sciences and AbbVie. The other authors have no relevant conflicts of interest to disclose.

References

1. Miao Z, Zhang S, Ou X, et al. Estimating the global prevalence, disease progression, and clinical outcome of hepatitis delta virus infection. *J Infect Dis*. 2020;221(10):1677-1687.
2. Chen HY, Shen DT, Ji DZ, et al. Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. *Gut*. 2019;68(3):512-521.
3. World Health Organization. Hepatitis D. https://www.who.int/news-room/fact-sheets/detail/hepatitis-d. Updated June 24, 2022.
4. Manesis EK, Vourli G, Dalekos G, et al. Prevalence and clinical course of hepatitis delta infection in Greece: a 13-year prospective study. *J Hepatol*. 2013;59(5):949-956.
5. Jang TY, Wei YJ, Liu TW, et al. Role of hepatitis D virus infection in development of hepatocellular carcinoma among chronic hepatitis B patients treated with nucleotide/nucleoside analogues. *Sci Rep*. 2021;11(1):8184.
6. Alfaiate D, Clément S, Gomes D, Goossens N, Negro F. Chronic hepatitis D and hepatocellular carcinoma: a systematic review and meta-analysis of observational studies. *J Hepatol*. 2020;73(3):533-539.
7. Da BL, Heller T, Koh C. Hepatitis D infection: from initial discovery to current investigational therapies. *Gastroenterol Rep (Oxf)*. 2019;7(4):231-245.
8. Wedemeyer H, Manns MP. Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead. *Nat Rev Gastroenterol Hepatol*. 2010;7(1):31-40.
9. Ciancio A, Rizzetto M. Chronic hepatitis D at a standstill: where do we go from here? *Nat Rev Gastroenterol Hepatol*. 2014;11(1):68-71.
10. Sagnelli C, Sagnelli E, Russo A, Pisaturo M, Occhiello L, Coppola N. HBV/HDV co-infection: epidemiological and clinical changes, recent knowledge and future challenges. *Life (Basel)*. 2021;11(2):169.
11. Joseph K, Shabangu CS, Jang T-Y, et al. The prevalence and serological association of hepatitis D virus genotypes in Taiwan. *Pathogens*. 2021;10(10):1227.
12. Ma SP, Sakugawa H, Makino Y, Tadano M, Kinjo F, Saito A. The complete genomic sequence of hepatitis delta virus genotype IIb prevalent in Okinawa, Japan. *J Gen Virol*. 2003;84(pt 2):461-464.
13. Ivaniushina V, Radjef N, Alexeeva M, et al. Hepatitis delta virus genotypes I and II cocirculate in an endemic area of Yakutia, Russia. *J Gen Virol*. 2001;82 (pt 11):2709-2718.
14. Casey JL, Brown TL, Colan EJ, Wignall FS, Gerin JL. A genotype of hepatitis D virus that occurs in northern South America. *Proc Natl Acad Sci USA*. 1993;90(19):9016-9020.
15. Casey JL. Hepatitis delta virus. Genetics and pathogenesis. *Clin Lab Med*. 1996;16(2):451-464.
16. Nakano T, Shapiro CN, Hadler SC, et al. Characterization of hepatitis D virus genotype III among Yucpa Indians in Venezuela. *J Gen Virol*. 2001;82(pt 9): 2183-2189.
17. Pascarella S, Negro F. Hepatitis D virus: an update. *Liver Int*. 2011;31(1):7-21.
18. Wasuwanich P, Striley CW, Kamili S, Teshale EH, Seaberg EC, Karnsakul W. Hepatitis D-associated hospitalizations in the United States: 2010-2018. *J Viral Hepat*. 2022;29(3):218-226.
19. Dahiya P, Kamal R, Sharma V, Kaur S. "Hepatitis" - prevention and management in dental practice. *J Educ Health Promot*. 2015;4:33.
20. Cross TJS, Rizzi P, Horner M, et al. The increasing prevalence of hepatitis delta virus (HDV) infection in South London. *J Med Virol*. 2008;80(2):277-282.
21. Niro GA, Casey JL, Gravinese E, et al. Intrafamilial transmission of hepatitis delta virus: molecular evidence. *J Hepatol*. 1999;30(4):564-569.
22. Krogsgaard K, Kryger P, Aldershvile J, Andersson P, Sørensen TIA, Nielsen JO. δ-infection and suppression of hepatitis B virus replication in chronic HBsAg carriers. *Hepatology*. 1987;7(1):42-45.
23. Sellier PO, Maylin S, Brichler S, et al. Hepatitis B virus-hepatitis D virus mother-to-child co-transmission: a retrospective study in a developed country. *Liver Int*. 2018;38(4):611-618.
24. Weisfuse IB, Hadler SC, Fields HA, et al. Delta hepatitis in homosexual men in the United States. *Hepatology*. 1989;9(6):872-874.
25. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*. 2018;67(4):1560-1599.
26. Safaie P, Razeghi S, Rouster SD, Privitera I, Sherman KE. Hepatitis D diagnostics: utilization and testing in the United States. *Virus Res*. 2018;250:114-117.
27. Kushner T, Serper M, Kaplan DE. Delta hepatitis within the Veterans Affairs medical system in the United States: prevalence, risk factors, and outcomes. *J Hepatol*. 2015;63(3):586-592.
28. Patel EU, Thio CL, Boon D, Thomas DL, Tobian AAR. Prevalence of hepatitis B and hepatitis D virus infections in the United States, 2011-2016. *Clin Infect Dis*. 2019;69(4):709-712.
29. Njei B, Do A, Lim JK. Prevalence of hepatitis delta infection in the United States: National Health and Nutrition Examination Survey, 1999-2012. *Hepatology*. 2016;64(2):681-682.
30. Gish RG, Yi DH, Kane S, et al. Coinfection with hepatitis B and D: epidemiology, prevalence and disease in patients in Northern California. *J Gastroenterol Hepatol*. 2013;28(9):1521-1525.
31. Kucirka LM, Farzadegan H, Feld JJ, et al. Prevalence, correlates, and viral dynamics of hepatitis delta among injection drug users. *J Infect Dis*. 2010;202(6):845-852.
32. Chen X, Oidovsambuu O, Liu P, et al. A novel quantitative microarray antibody capture assay identifies an extremely high hepatitis delta virus prevalence among hepatitis B virus-infected Mongolians. *Hepatology*. 2017;66(6):1739-1749.
33. Da BL, Rahman F, Lai WC, Kleiner DE, Heller T, Koh C. Risk factors for delta hepatitis in a North American cohort: who should be screened? *Am J Gastroenterol*. 2021;116(1):206-209.
34. Rosenblum L, Darrow W, Witte J, et al. Sexual practices in the transmission of hepatitis B virus and prevalence of hepatitis delta virus infection in female prostitutes in the United States. *JAMA*. 1992;267(18):2477-2481.
35. Lettau LA, McCarthy JG, Smith MH, et al. Outbreak of severe hepatitis due to delta and hepatitis B viruses in parenteral drug abusers and their contacts. *N Engl J Med*. 1987;317(20):1256-1262.
36. Kos A, Dijkema R, Arnberg AC, van der Meide PH, Schellekens H. The hepatitis delta (delta) virus possesses a circular RNA. *Nature*. 1986;323(6088):558-560.
37. Makino S, Chang M-F, Shieh C-K, et al. Molecular cloning and sequencing of a human hepatitis delta (delta) virus RNA. *Nature*. 1987;329(6137):343-346.
38. Abbas Z, Afzal R. Life cycle and pathogenesis of hepatitis D virus: a review. *World J Hepatol*. 2013;5(12):666-675.
39. Alves C, Freitas N, Cunha C. Characterization of the nuclear localization signal of the hepatitis delta virus antigen. *Virology*. 2008;370(1):12-21.
40. Le Gal F, Gault E, Ripault M-P, et al. Eighth major clade for hepatitis delta virus. *Emerg Infect Dis*. 2006;12(9):1447-1450.
41. Yan H, Zhong G, Xu G, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *eLife*. 2012;1:e00049.
42. Chang J, Nie X, Chang HE, Han Z, Taylor J. Transcription of hepatitis delta virus RNA by RNA polymerase II. *J Virol*. 2008;82(3):1118-1127.
43. Macnaughton TB, Wang YJ, Lai MM. Replication of hepatitis delta virus RNA: effect of mutations of the autocatalytic cleavage sites. *J Virol*. 1993;67(4):2228-2234.
44. Reid CE, Lazinski DW. A host-specific function is required for ligation of a wide variety of ribozyme-processed RNAs. *Proc Natl Acad Sci USA*. 2000;97(1):424-429.
45. Huang WH, Chen YS, Chen PJ. Nucleolar targeting of hepatitis delta antigen abolishes its ability to initiate viral antigenomic RNA replication. *J Virol*. 2008;82(2):692-699.
46. Chao M, Hsieh SY, Taylor J. Role of two forms of hepatitis delta virus antigen: evidence for a mechanism of self-limiting genome replication. *J Virol*. 1990;64(10):5066-5069.
47. Yamaguchi Y, Filipovska J, Yano K, et al. Stimulation of RNA polymerase II elongation by hepatitis delta antigen. *Science*. 2001;293(5527):124-127.
48. Luo GX, Chao M, Hsieh SY, Sureau C, Nishikura K, Taylor J. A specific base transition occurs on replicating hepatitis delta virus RNA. *J Virol*.

1990;64(3):1021-1027.
49. Polson AG, Bass BL, Casey JL. RNA editing of hepatitis delta virus antigenome by dsRNA-adenosine deaminase. *Nature*. 1996;380(6573):454-456.
50. Glenn JS, Watson JA, Havel CM, White JM. Identification of a prenylation site in delta virus large antigen. *Science*. 1992;256(5061):1331-1333.
51. Chang FL, Chen PJ, Tu SJ, Wang CJ, Chen DS. The large form of hepatitis delta antigen is crucial for assembly of hepatitis delta virus. *Proc Natl Acad Sci USA*. 1991;88(19):8490-8494.
52. Lee CZ, Chen PJ, Chen DS. Large hepatitis delta antigen in packaging and replication inhibition: role of the carboxyl-terminal 19 amino acids and amino-terminal sequences. *J Virol*. 1995;69(9):5332-5336.
53. Rizzetto M, Alavian SM. Hepatitis delta: the rediscovery. *Clin Liver Dis*. 2013;17(3):475-487.
54. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int*. 2016;10(1):1-98.
55. 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(2):370-398.
56. Dunn R, Wetten A, McPherson S, Donnelly MC. Viral hepatitis in 2021: the challenges remaining and how we should tackle them. *World J Gastroenterol*. 2022;28(1):76-95.
57. Niro GA, Ferro A, Cicerchia F, Brascugli I, Durazzo M. Hepatitis delta virus: from infection to new therapeutic strategies. *World J Gastroenterol*. 2021;27(24):3530-3542.
58. Stockdale AJ, Kreuels B, Henrion MYR, et al. The global prevalence of hepatitis D virus infection: systematic review and meta-analysis. *J Hepatol*. 2020;73(3):523-532.
59. Ji J, Sundquist K, Sundquist J. A population-based study of hepatitis D virus as potential risk factor for hepatocellular carcinoma. *J Natl Cancer Inst*. 2012;104(10):790-792.
60. Coghill S, McNamara J, Woods M, Hajkowicz K. Epidemiology and clinical outcomes of hepatitis delta (D) virus infection in Queensland, Australia. *Int J Infect Dis*. 2018;74:123-127.
61. Kushner T, Da BL, Chan A, Dieterich D, Sigel K, Saberi B. Liver transplantation for hepatitis D virus in the United States: a UNOS study on outcomes in the MELD era. *Transplant Direct*. 2021;8(1):e1253.
62. Alvarado-Mora MV, Romano CM, Gomes-Gouvêa MS, Gutierrez MF, Carrilho FJ, Pinho JRR. Dynamics of hepatitis D (delta) virus genotype 3 in the Amazon region of South America. *Infect Genet Evol*. 2011;11(6):1462-1468.
63. Spaan M, Carey I, Bruce M, et al. Hepatitis delta genotype 5 is associated with favourable disease outcome and better response to treatment compared to genotype 1. *J Hepatol*. 2020;72(6):1097-1104.
64. Koh C, Heller T, Glenn JS. Pathogenesis of and new therapies for hepatitis D. *Gastroenterology*. 2019;156(2):461-476.e1.
65. Urban S, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. *Gastroenterology*. 2014;147(1):48-64.
66. Dhillon S. Lonafarnib: first approval. *Drugs*. 2021;81(2):283-289.
67. US Food and Drug Administration. Chronic hepatitis D virus infection: developing drugs for treatment guidance for industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chronic-hepatitis-d-virus-infection-developing-drugs-treatment-guidance-industry. Published November 2019.
68. Blank A, Markert C, Hohmann N, et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. *J Hepatol*. 2016;65(3):483-489.
69. Bogomolov P, Alexandrov A, Voronkova N, et al. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: first results of a phase Ib/IIa study. *J Hepatol*. 2016;65(3):490-498.
70. Wedemeyer H, Bogomolov P, Blank A, et al. Final results of a multicenter, open-label phase 2b clinical trial to assess safety and efficacy of MyrcludexB in combination with tenofovir in patients with HBV/HDV coinfection. *J Hepatol*. 2018;68(suppl 1):S3.
71. Wedemeyer H, Schoneweis K, Bogomolov P, et al. GS-13 Final results of a multicenter, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of Myrcludex B in cwith PEG-interferon alpha 2a in patients with chronic HBV/HDV co-infection. *J Hepatol*. 2019;70(1 suppl):E81.
72. Asselah T, Stefan Arama S, Bogomolov P, et al. Safety and efficacy of bulevirtide monotherapy and in combination with peginterferon alfa-2a in patients with chronic hepatitis delta: 24 weeks interim data of MYR204 phase 2b study. Presented at the Digital International Liver Congress; June 23-26, 2021. https://www.natap.org/2021/EASL/EASL_54.htm.
73. Wedemeyer H, Aleman S, Andreona P, et al. Bulevirtide monotherapy at low and high doses in patients with chronic hepatitis delta: 24-week interim data of the phase 3 MYR301 study. Presented at the Digital International Liver Congress; June 23-26, 2021. https://www.natap.org/2021/EASL/EASL_14.htm.
74. Lampertico P, Roulot D, Wedemeyer H. Bulevirtide with or without pegIFNα for patients with compensated chronic hepatitis delta: from clinical trials to real-world studies [published online June 22, 2022]. *J Hepatol*. doi:10.1016/j.jhep.2022.06.010.
75. Koh C, Canini L, Dahari H, et al. 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(10):1167-1174.
76. Yurdaydin C, Keskin O, Kalkan Ç, et al. Optimizing lonafarnib treatment for the management of chronic delta hepatitis: the LOWR HDV-1 study. *Hepatology*. 2018;67(4):1224-1236.
77. Yurdaydin C, Keskin O, Yurdcu E, et al. A phase 2 dose-finding study of lonafarnib and ritonavir with or without interferon alpha for chronic delta hepatitis. *Hepatology*. 2022;75(6):1551-1565.
78. Koh C, Surana P, Han T, et al. A phase 2 study exploring once daily dosing of ritonavir boosted lonafarnib for the treatment of chronic delta hepatitis —end of study results from the LOWR HDV-3 study. *J Hepatol*. 2017;66 (1 suppl):S101-S102.
79. Wedemeyer H, Port K, Deterding K, et al. A phase 2 dose-escalation study of lonafarnib plus ritonavir in patients with chronic hepatitis D: final results from the lonafarnib with ritonavir in HDV-4 (LOWR HDV-4) study. *J Hepatol*. 2017;66 (1 suppl):S24.
80. ClinicalTrials.gov. A study of lonafarnib with or without ritonavir in patients with HDV (LOWR-5). https://clinicaltrials.gov/ct2/show/NCT02968641. Identifier: NCT02968641. Updated February 16, 2021.
81. ClinicalTrials.gov. Once daily dosing of lonafarnib co-administered with ritonavir for treatment of chronic hepatitis D virus infection (LOWR6). https://clinicaltrials.gov/ct2/show/NCT05229991?cond=Hepatitis%2C+Delta&draw=5. Identifier: NCT05229991. Updated February 8, 2022.
82. ClinicalTrials.gov. Study of the efficacy and safety of lonafarnib/ritonavir with and without pegylated interferon -alfa-2a (D-LIVR). https://clinicaltrials.gov/ct2/show/NCT03719313?cond=Hepatitis%2C+Delta&draw=5. Identifier: NCT03719313. Updated October 1, 2021.
83. Bazinet M, Anderson M, Pantea V, et al. Analysis of HBsAg levels, HBsAg isoforms, HBsAg immune complexes, HBV pregenomic RNA and HBcrAg dynamics during and after NAP-based combination therapy in the REP 301-LTF and REP 401 studies. *J Hepatol*. 2020;73(suppl):S142.
84. Bazinet M, Pântea V, Cebotarescu V, et al. 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(12):877-889.
85. Chan HLY, Ahn SH, Chang TT, et al; LIRA-B Study Team. Peginterferon lambda for the treatment of HBeAg-positive chronic hepatitis B: a randomized phase 2b study (LIRA-B). *J Hepatol*. 2016;64(5):1011-1019.
86. Foster GR, Chayama K, Chuang W-L, et al. A randomized, controlled study of peginterferon lambda-1a/ribavirin ± daclatasvir for hepatitis C virus genotype 2 or 3. *Springerplus*. 2016;5(1):1365.
87. Etzion O, Sadiq Hamid S, Lurie Y, et al. PS-052 - End of study results from LIMT HDV study: 36% durable virologic response at 24 weeks post-treatment with pegylated interferon lambda monotherapy in patients with chronic hepatitis delta virus infection. *J Hepatol*. 2019;70(1 suppl):E32.
88. ClinicalTrials.gov. Phase 3 study to evaluate the efficacy and safety of peginterferon lambda for 48 weeks in patients with chronic HDV (LIMT-2). https://clinicaltrials.gov/ct2/show/NCT05070364. Identifier: NCT05070364. Updated July 14, 2022.
89. Koh C, Hercun J, Rahman F, et al. A phase 2 study of peginterferon lambda, lonafarnib and ritonavir for 24 weeks: end-of-treatment results from the LIFT HDV study. *J Hepatol*. 2020;73(suppl):S130.
90. Komas NP, Ghosh S, Abdou-Chekaraou M, et al. Hepatitis B and hepatitis D virus infections in the Central African Republic, twenty-five years after a fulminant hepatitis outbreak, indicate continuing spread in asymptomatic young adults. *PLoS Negl Trop Dis*. 2018;12(4):e0006377.
91. Elzefzafy W, Soliman R, Saleh L, et al. Seroprevalence and epidemiological characteristics of HDV infection among HBV patients in the Nile Delta, Egypt. *J Viral Hepat*. 2022;29(1):87-90.
92. Aberra H, Gordien E, Desalegn H, et al. Hepatitis delta virus infection in a large cohort of chronic hepatitis B patients in Ethiopia. *Liver Int*. 2018;38(6):1000-1009.

93. Elzouki A-N, Bashir SM, Elahmer O, Elzouki I, Alkhattali F. Prevalence and risk factors of hepatitis D virus infection in patients with chronic hepatitis B infection attending the three main tertiary hospitals in Libya. *Arab J Gastroenterol*. 2017;18(4):216-219.
94. Stockdale AJ, Mitambo C, Everett D, Geretti AM, Gordon MA. Epidemiology of hepatitis B, C and D in Malawi: systematic review. *BMC Infect Dis*. 2018;18(1):516.
95. Opaleye OO, Japhet OM, Adewumi OM, et al. Molecular epidemiology of hepatitis D virus circulating in Southwestern Nigeria. *Virol J*. 2016;13(1):61.
96. Aceti A, Mohamed OM, Paparo BS, et al. High prevalence of anti-hepatitis delta virus antibody in chronic liver disease in Somalia. *Trans R Soc Trop Med Hyg*. 1991;85(4):541-542.
97. Froeschl G, Hoelscher M, Maganga LH, et al. Hepatitis B, C and D virus prevalence in children and adults in Mbeya Region, Tanzania: results from a cohort study 2002 - 2009. *Pan Afr Med J*. 2021;39:174.
98. Yacoubi L, Brichler S, Mansour W, et al. Molecular epidemiology of hepatitis B and delta virus strains that spread in the Mediterranean North East Coast of Tunisia. *J Clin Virol*. 2015;72:126-132.
99. Braga WSM, Castilho MdaC, Borges FG, et al. Hepatitis D virus infection in the Western Brazilian Amazon - far from a vanishing disease. *Rev Soc Bras Med Trop*. 2012;45(6):691-695.
100. Alvarado-Mora MV, Fernandez MF, Gomes-Gouvêa MS, de Azevedo Neto RS, Carrilho FJ, Pinho JR. Hepatitis B (HBV), hepatitis C (HCV) and hepatitis delta (HDV) viruses in the Colombian population—how is the epidemiological situation? *PLoS One*. 2011;6(4):e18888.
101. Husseini AA, Islam Saeed KM, Yurdcu E, Bozdayı AM. Molecular epidemiology of hepatitis B virus, hepatitis C virus, and hepatitis D virus in general population of Afghanistan. *Turk J Gastroenterol*. 2020;31(9):658-666.
102. Roggenbach I, Chi X, Lempp FA, et al. HDV seroprevalence in HBsAg-positive patients in China occurs in hotspots and is not associated with HCV mono-infection. *Viruses*. 2021;13(9):1799.
103. Kasradze A, Shadaker S, Kuchuloria T, et al. The burden and epidemiology of hepatitis B and hepatitis D in Georgia: findings from the national seroprevalence survey. *Public Health*. 2020;185:341-347.
104. Ramachandran K, Agarwal R, Sharma MK, Bhatia V, Gupta E. Prevalence of hepatitis delta virus infection among hepatitis B virus-infected and exposed patients. *J Glob Infect Dis*. 2020;12(4):197-201.
105. Amini N, Alavian SM, Kabir A, Saiedi Hosseini SY, Aalaei Andabili SH. Clinical features and seroepidemiology of anti-HDV antibody in patients with chronic hepatitis B virus infection in Iran: a meta-analysis. *Hepat Mon*. 2011;11(12):960-967.
106. Shirazi R, Ram D, Rakovsky A, et al. Characterization of hepatitis B and delta coinfection in Israel. *BMC Infect Dis*. 2018;18(1):97.
107. Nakasone H, Sakugawa H, Shokita H, et al. Prevalence and clinical features of hepatitis delta virus infection in the Miyako Islands, Okinawa, Japan. *J Gastroenterol*. 1998;33(6):850-854.
108. Zaidi G, Idrees M, Malik FA, et al. Prevalence of hepatitis delta virus infection among hepatitis B virus surface antigen positive patients circulating in the largest province of Pakistan. *Virol J*. 2010;7(1):283.
109. Lin H-H, Lee SS-J, Yu M-L, et al. Changing hepatitis D virus epidemiology in a hepatitis B virus endemic area with a national vaccination program. *Hepatology*. 2015;61(6):1870-1879.
110. Bahcecioglu IH, Aygun C, Gozel N, Poyrazoglu OK, Bulut Y, Yalniz M. Prevalence of hepatitis delta virus (HDV) infection in chronic hepatitis B patients in eastern Turkey: still a serious problem to consider. *J Viral Hepat*. 2011;18(7):518-524.
111. Sy BT, Ratsch BA, Toan NL, et al. High prevalence and significance of hepatitis D virus infection among treatment-naïve HBsAg-positive patients in Northern Vietnam. *PLoS One*. 2013;8(10):e78094.
112. Sperle I, Steffen G, Leendertz SA, et al. Prevalence of hepatitis B, C, and D in Germany: results from a scoping review. *Front Public Health*. 2020;8:424.
113. Stroffolini T, Ciancio A, Furlan C, et al. Migratory flow and hepatitis delta infection in Italy: a new challenge at the beginning of the third millennium. *J Viral Hepat*. 2020;27(9):941-947.
114. Jelen MM, Hošnjak L, Štunf Š, et al. Hepatitis D virus infection in Slovenian patients with chronic hepatitis B virus infection: a national prevalence study and literature review. *Acta Dermatovenerol Alp Panonica Adriat*. 2016;25(3):49-53.
115. Vieira Barbosa J, Sahli R, Aubert V, Chaouch A, Moradpour D, Fraga M. Demographics and outcomes of hepatitis B and D: a 10-year retrospective analysis in a Swiss tertiary referral center. *PLoS One*. 2021;16(4):e0250347.
116. Genné D, Rossi I. Hepatitis delta in Switzerland: a silent epidemic. *Swiss Med Wkly*. 2011;141:w13176.