Hepatitis D infection: from initial discovery to current investigational therapies

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Abstract

Hepatitis D is the most severe form of viral hepatitis associated with a more rapid progression to cirrhosis and an increased risk of hepatocellular carcinoma and mortality compared with hepatitis B mono-infection. Although once thought of as a disappearing disease, hepatitis D is now becoming recognized as a serious worldwide issue due to improvement in diagnostic testing and immigration from endemic countries. Despite these concerns, there is currently only one accepted medical therapy (pegylated-interferon-α) for the treatment of hepatitis D with less than desirable efficacy and significant side effects. Due to these reasons, many patients never undergo treatment. However, increasing knowledge about the virus and its life cycle has led to the clinical development of multiple promising new therapies that hope to alter the natural history of this disease and improve patient outcome. In this article, we will review the literature from discovery to the current investigational therapies.

Key words: Hepatitis D; hepatitis B; epidemiology; natural history; outcome; treatment; novel therapies

Introduction

Hepatitis D is a rare form of viral hepatitis that was first described in 1977 by Rizzetto et al. through immunofluorescence detection of the delta antigen and anti-delta antibody in the serum and liver tissues of hepatitis B surface antigen (HBsAg) carriers [1]. While it has been estimated to affect 15–20 million people worldwide, more recent data suggest that the global disease burden may be closer to 62–72 million [2]. Hepatitis D virus (HDV) is an incomplete RNA virus that requires the assistance of the hepatitis B virus (HBV), specifically the HBsAg, to be infectious in humans [3]. Once chronicity is established, HDV has been described as the most severe form of viral hepatitis, with progression to cirrhosis in 10%–15% of patients within 2 years and in 70%–80% of patients within 5–10 years [4, 5]. Despite this, a lack of adequate treatment options currently exists for HDV. Current international guidelines suggest the use of pegylated-interferon-α (peg-INF-α), although sustained virological response (SVR) rates with this treatment are reported to be only 20%–30% [6–8]. In addition, even after SVR is achieved, late relapse remains an issue [9]. Recent advances in our understanding of the HDV viral cycle have led to the development of several promising investigative therapies that are under investigation.

In this piece, we review the virology, epidemiology, diagnosis, clinical presentation, natural history, outcomes and the available and investigative treatments pertaining to this disease.
Viral structure and life cycle

HDV is a small, spherical RNA virus measuring \(~36\) nm in diameter with an inner nucleocapsid that consists of a short \((\sim 1.7\)-kb\) single-stranded, circular RNA and approximately \(200\) molecules of hepatitis D antigen (HDAg) \([10, 11]\). This viral genome is the smallest among known mammalian viruses and shares structural similarity to viroid RNAs \([12–14]\). It encodes for a single protein, the HDAg, which exists in two forms: the small HDAg (S-HDAg) and large HDAg (L-HDAg). Structurally, the two forms are identical except that the L-HDAg has an additional \(19\) amino acid sequence at the C-terminus \([15]\). The outer coat of the HDV virion consists of components taken from HBV, thus mandating a co-infective process with HBV, which includes the small, medium and large HBsAg \([3]\). The isoforms of HBsAg are embedded in a lipid envelope surrounding the HDV genome and HDV antigen isoforms. The large HBsAg then undergoes a process called myristoylation at the N-terminus to prepare for cell entry \([16–18]\). The life cycle of HDV is depicted in Figure 1. First, the HDV virion binds to the human hepatocyte through an interaction between the myristoylated N-terminus of the pre-S1 domain of the large HBsAg and the host receptor, which has been identified as the multiple transmembrane receptor sodium taurocholate cotransporting polypeptide (NTCP) located on the basolateral membrane of hepatocytes \([19, 20]\). After entry, the HDV genome is translocated to the nucleus via the HDAg \([21]\). Upon arriving in the nucleus, HDV commandeers host RNA polymerase II, a DNA-directed RNA polymerase, for transcription of HDV RNA \([22]\). Replication of the HDV occurs through a rolling-circle mechanism \([12, 23]\). The initial step is synthesis of multimeric linear transcripts from the circular genomic template. Afterwards, these multimeric linear transcripts are cleaved into monomers by autocatalytic self-cleaving sequences called ribozymes. Subsequently, monomer RNAs are ligated by the ribozyme into an antigenomic, monomeric, circular RNA that serves as a template for another round of rolling-circle replication. The finished product is a circular genomic HDV RNA \([13]\).

![Figure 1. Hepatitis D virus viral life cycle and sites of drug target.](image)

Figure 1. Hepatitis D virus life cycle and sites of drug target. 1. Hepatitis D virus (HDV) virion attaches to the hepatocyte via interaction between hepatitis B surface antigen proteins and the sodium taurocholate cotransporting polypeptide (NTCP), a multiple transmembrane transporter. 2. HDV ribonucleoprotein (RNP) is translocated to nucleus mediated by the hepatitis D antigen (HDAg). 3. HDV genome replication occurs via a ‘rolling-circle’ mechanism. 4. HDV antigenome is translated in the endoplasmic reticulum (ER) into small HDAg (S-HDAg) and large HDAg (L-HDAg). 5. L-HDAg undergoes prenylation prior to assembly. 6. S-HDAg is transported back to the nucleus where it supports HDV replication. 7. New HDAg molecules are associated with new transcripts of genomic RNA to form new RNPs that are exported to the cytoplasm. 8. New HDV RNP associates with hepatitis B virus (HBV) envelope proteins and assembled into HDV virions. 9. Completed HDV virions are released from the hepatocyte via the trans-Golgi network.
Three different RNAs are generated during the replication process: the HDV genome, the antigenome that is its exact complement and a third antigenome that contains the open reading frame (ORF) that codes for the HDAg [22]. During replication, the HDV genome and antigenome collapses into a characteristic unbranched rod-like structure [24]. Both forms of HDAg are translated in the endoplasmic reticulum from the ORF located on the antigenomic HDV RNA strand [25]. The S-HDAg plays a significant role in replication, since it is required for RNA synthesis, while the L-HDAg inhibits RNA synthesis and is required for packaging. Cellular adenosine deaminase acting on RNA (ADAR) editing the amber codon on the viral antigenome RNA enables the HDV to switch from replication to packaging [24].

The HDV RNA and HDAg proteins interact to form a ribonucleoprotein (RNP) particle at a molar ratio of 200 [12, 26]. The modification of post-translation HDAg proteins is essential for this process but is also crucial for HDV replication and HDV-virion assembly [27, 28]. One of these post-translational modification processes is called farnesylation, which is crucial for HDV-virion assembly and serves as a target for drug action. At the C-terminal of the L-HDAg is a 19-amino acid polypeptide that includes the C-terminal CXXC-box motif (where C = cysteine and X = any amino acid), which is the substrate for prenyltransferases to add a prenyl lipid group [15, 29]. Farnesyl is one of the prenyl groups that can be added via the enzyme farnesyltransferase in a process specifically called farnesylation. Farnesylation anchors the RNP to the endoplasmic reticulum membrane where HBV envelope proteins are made and then makes the RNP more lipophilic and more amenable to interactions with HBsAg.

Finally, once the RNP interacts with the envelop protein of HBV and the HDV is assembled, the HDV virion is now ready for release. The HDV virion is released via the trans-Golgi network, where it can go on to infect other hepatocytes. However, the exact mechanism of HDV-virion release remains unknown [15].

**Viral genotypes**

To date, eight distinct HDV genotypes have been recognized, with two to four subtypes per genotype characterized by >90% similarity over the entire genome sequence [2, 34, 35]. The geographical distributions of the HDV genotypes have changed over time, most probably due to human migration patterns. Currently, genotype 1 is the most prevalent worldwide and the predominant genotype in Europe and North America [36]. Genotype 2 was previously confined to Asia but has now emerged in Middle Eastern countries including Iran [37] and Egypt [38]. Genotype 3 is mainly found in the Amazon Basin in South America and is the most different of all the genotypes exhibiting ~40% divergence at the nucleic acid level [39]. Genotype 4 is predominately found in Taiwan, China and Japan [35]. Meanwhile, genotypes 5, 6, 7 and 8 have traditionally been found only in Africa, but recent reports describe the migration of genotypes 5, 6 and 7 to various parts of Europe [40–42]. Notably, central Africa is thought to be the main site of HDV diversification, with the presence of genotypes 1, 5, 6, 7 and 8 [35].

It is well known that specific HDV genotypes influence clinical outcomes. HDV genotype 3 appears to be the most pathogenic of all the HDV genotypes [43, 44]. HDV genotype 1 patients have lower rates of remission, more aggressive disease and worse outcomes than HDV genotype 2 patients [45, 46]. For example, in a study from Taiwan, Su et al. reported significantly lower rates of remission in HDV genotype 1 compared to HDV genotype 2 (15.2% vs 40.2%, P = 0.007) and more adverse outcomes (cirrhosis, hepatocellular carcinoma [HCC] or mortality) (52.2% vs 25.0%, P = 0.005) [45]. This is likely due to HDV genotype 1 being a more efficient genotype in terms of virion assembly and RNA editing than genotype 2, resulting in the secretion of more viral particles [31, 32].

**Epidemiology and risk factors**

The seroprevalence of HDV among HBsAg-positive carriers shows substantial variations worldwide. These are depicted in Table 1. Interestingly, more recent data have shown that 8% of the general Mongolian population is estimated to be positive for HDV [2]. Notably, in the USA, the prevalence of HDV among HBV carriers has been reported to range from 2% to 50%, depending on the patient population [63–66]. A large study of the US Veteran’s Affairs medical system recently reported an HDV seroprevalence of 3.4% among patients with chronic HBV who are tested for HDV [78]. A study using the National Health and Nutrition Examination Survey reported a HDV seroprevalence of 42% among HBV carriers [65]. Finally, the highest estimation of HDV seroprevalence came from a study of HBV-positive

| Endemic country | Seroprevalence | Non-endemic country | Seroprevalence |
|-----------------|---------------|---------------------|---------------|
| Brazil          | 41.9% [47]    | Australia           | 4.1%–4.8% [48, 49] |
| China           | 3.5%–13.1% [50–52] | England          | 2.6%–8.5% [53, 54] |
| Egypt           | 9.9% [38]     | Greece              | 4.2% [55]     |
| Germany         | 8%–10.9% [56, 57] | Japan             | 6% [58]      |
| India           | 10.6%–37.5% [59, 60] | Jordan           | 2% [61]      |
| Iran            | 17%–48% [37, 62] | United States      | 2%–50% [63–66]|
intravenous drug users (IVDU), which showed that the sero-
prevalence of HDV increased from 29% in 1988–1989 to 50% in
2005–2006 [66]. However, a general lack of HDV RNA validation
in these studies prevents estimation of true HDV prevalence.

Despite the various HDV epidemiological reports, there con-
tinues to be insufficient data on the true global prevalence of
HDV [2]. This is attributable to several factors. First, reported
rates are likely to be underestimations, stemming from incom-
plete population testing because of a lack of clinician awareness
resulting in a lack of diagnosis along with a lack of availability
of HDV clinical tests. In a nationwide study in the USA of 25,603
HBV patients, only 8.5% of patients were ever tested for HDV
[78]. Manesis et al. reported that only a third of Greek patients
with chronic hepatitis B (CHB) were tested for HDV [55]. El
Bouzidi et al. similarly reported that only 40% of British chronic
HBV patients were tested for anti-HDV in patients with positive
HBsAg [40]. Special focus also needs to be made for HDV testing
among patients with hepatitis C virus (HCV) and human immu-
nodeficiency virus (HIV) due to a high rate of co-infection be-
cause of shared risk factors such as transfusions, tattooing, IVDU
and high-risk sexual practices [79]. For example, reported co-infection rates with HCV have ranged from 26%–73% [42, 48,
53, 68] and HIV of 12.7% [42].

Second, studies from the same country have reported dis-
crepant numbers that may be due to significant geographical
variations even within the same country. For example, a meta-
analysis from Turkey described that the HDV seroprevalence of
west Turkey was 4.8%, while the seroprevalence of HDV in
southeast Turkey was 46.3% [80]. In addition, most of the epi-
demiologic studies on the seroprevalence of HDV among
chronic HBV patients did not require HDV polymerase chain
reaction (PCR) confirmation as an inclusion factor, further pre-
venting the estimation of the true prevalence of HDV. Finally,
the timing of the publication is also important because the se-
roprevalence of HDV declined drastically after the late 1980s
due to the implementation of HBV vaccine programs around the
world [81–83].

Despite an earlier notion that HDV was a disappearing dis-
ease in many parts of the world, this has proven to be no longer the
case. In fact, more recent data have shown that the preva-
ience of HDV has remained stable or has increased in many
endemic and non-endemic countries due to an increase in asso-
ciated risk factors such as immigration, IVDU, men-who-have-
sex-with men (MSM) and intra-familial spread [41, 67, 71, 79, 84,
85]. For example, a collection of studies from Italy reported a de-
cline in the prevalence of HDV among HBsAg-positive patients
from 23% in 1987 to 14% in 1992 to 8.3% in 1997, which was
attributed to HBV-vaccination programs [67, 86, 87]. However,
more recent data have suggested that the prevalence of HDV in
Italy has either remained stable at 8.1% [85] or has rebounded to
11.3% [84]. Lin et al. showed that the prevalence of HDV in-
creased from 38.5% to 89.8% among HIV-infected IVDU patients
with HBV from 2001 through 2012 [71]. Furthermore, outbreaks
of HDV have been especially rampant among areas of with low
social-economic levels [79, 80]. Due to immigration from en-
demic countries, multiple studies have reported an increase in
the prevalence of HDV in countries in which HDV was previ-
ously uncommon [48, 49, 53–55]. In a study by Coghill et al. from
Australia, those born in Africa were shown to have a higher risk
ratio (RR) for HDV infection (RR, 1.55; 95% confidence interval
[95% CI], 1.14–2.09) [48]. Cross et al. reported in a study from
England that over half of their HDV patients were from regions of
the world where HDV is endemic including Southern or
Eastern Europe (28.1%), Africa (26.8%) and the Middle East (7.3%)
[53]. Similarly, Manesis et al. reported that immigrants repre-
sented over half of the HDV burden in Greece [55].

Due to these reasons, HDV is now becoming a serious issue
worldwide. A recently published systematic review and meta-
analysis reported a worldwide HDV seroprevalence of 14.6% am-
ong HBV-positive patients compared with prior estimates of
approximately 5%, with a substantially higher seroprevalence
in the IVDU and high-risk sexual-behavior populations [2, 88].
These findings stress the importance of HDV awareness regard-
less of country of origin. Indeed, HDV needs to be especially
considered in high-risk populations such as among patients
who are immigrants from endemic countries, IVDU, MSM and
with positive family members.

Diagnostics

This section will focus on the different diagnostic modalities
tests that are available (Table 2). When HDV was first dis-
covered, the diagnosis of HDV relied on immunohistochemical
staining of HDAg in liver tissue that could only be obtained
through liver biopsy [1]. Testing for serum immunoglobulin M
(immunoglobulin G (IgG) anti-HDV quickly became avail-
able, which, in addition to liver chemistries and the patient’s
clinical picture, enabled classification of patients into the two
stages of HDV infection: acute and chronic HDV [89]. The use
of serum IgM/IgG anti-HDV for this purpose was not perfect, how-
ever, as anti-HDV IgM is not 100% specific to chronic HDV and
can be found in acute HDV. Likewise, anti-HDV IgM is not spe-
cific to acute HDV and can frequently be found in chronic HDV
[61, 90, 91].

For more than a decade after the discovery of HDV, testing
for the presence of HDV RNA remained sub-optimal, with tech-
niques such as the slot-blot hybridization technique [82]. In
the early 1990s, serum HDV RNA PCR techniques were developed
that enabled efficient and accurate testing of HDV in HBsAg-
positive patients [92–94]. However, these early assays only en-
abled qualitative testing of HDV and were not able to quantify
the virus in the serum. The emergence of quantification PCR
assays in the early 2000s finally enabled measurement of HDV
RNA viremia in the serum [95, 96]. Despite these advances, there
continued to be a high degree of variation in the sensitivity and
specificity of the HDV assays due to a lack of standardization.
Recent measures by the World Health Organization (WHO)
resulting in the first international external quality control for
HDV quantification have led to substantial improvements in
this area but significant assay heterogeneity of the available
assays remains [97, 98].

HDV should be highly suspected in HBV patients with persis-
tently elevated liver chemistries despite suppressed HBV viral
loads with or without nucleos(t)ide analog (NA) therapy without
a history of significant alcohol intake or metabolic syndrome.
This is due to direct suppression of HBV replication by HDV it-
self [48]. Not surprisingly, HBV viral loads do not appear to have
any correlation with serum alanine aminotransferase (ALT) lev-
els in chronic HDV [99]. However, this scenario most often
applies to hepatitis B envelope antigen (HBeAg)-negative and
patients who are HBeAg-positive may still have a high HBV viral
load despite the presence of HDV. The American Association for
the Study of Liver Diseases (AASLD) currently recommends that
HBsAg-positive patients with low or undetectable HBV DNA but
high ALT levels be considered for HDV testing while the
European Association for the Study of the Liver (EASL) and
Asian Pacific Association for the Study of the Liver do not offer
specific details regarding which patients with HBV should be
tested [6–8]. The recommended screening test for HDV is serum anti-HDV IgG followed by HDV RNA PCR if positive. Screening is also recommended in HIV-positive patients, IVDU patients, MSM, those at risk for sexually transmitted diseases and immigrants from endemic areas [6]. Nonetheless, when present, HDV is usually found in the setting of acute hepatitis or chronic liver disease and rarely in the setting of an asymptomatic HBV carrier [61].

**Acute hepatitis D**

Acute HDV occurs after an incubation period of 3–7 weeks and may present with non-specific flu-like symptoms with high levels of ALT and aspartate aminotransferase (AST) followed possibly by jaundice [100]. The presentation of acute HDV can mirror that of acute HBV infection, making it important to test for both processes in a patient with acute hepatitis. Acute HDV can either represent co-infection of HBV and HDV or super-infection of HDV. Co-infection usually leads to an acute self-limited hepatitis that cannot be distinguished clinically from acute HBV. Co-infection induces a cellular immune response followed by down-regulation of HBV replication and elimination of infected hepatocytes by cytotoxic cells [101]. Meanwhile, super-infection presents as an acute exacerbation of pre-existing chronic HBV or elevations in liver chemistries in an asymptomatic HBsAg carrier [100]. Acute co-infection of HBV and HDV rarely progresses to a chronic HDV infection, although acute HDV due to super-infection of HDV in a patient with HBV progresses to chronic HDV 80%–100% of the time [82, 101].

Clinically, patients may appear the same as in co-infection with elevated ALT levels, but the acute phase of HDV super-infection is characterized by more active HDV replication and suppression of HBV [101]. In addition to HBV viral loads being markedly suppressed, anti-hepatitis B core (Hbc) IgM are usually negative or with low titer [46, 100]. Although anti-HDV IgM levels may be a useful indicator of an acute HDV infection, it has only been described to be positive in 41% of acute cases and can also be found during flares of chronic HDV infection [61, 102]. Anti-HDV IgG may develop late in acute HDV and may persist for a lifetime even after the patient clears the virus [61, 101, 102].

Acute HDV can vary in severity from mild to fulminant hepatitis. Fulminant hepatic failure has been reported to occur in 1% of HBV/HDV co-infected patients and in 5% of HDV super-infected patients [103]. Genotype 1 HDV appears to be a risk factor for fulminant hepatic failure in acute HDV due a higher efficiency of editing, assembly and replication compared with genotype 2 [31, 32, 45, 104]. HDV may cause hepatic failure through direct cytotoxicity caused by the S-HDAg or inducing an exaggerated immune response leading to the destruction of hepatocytes by cytotoxic T cells [101, 105, 106].

**Chronic hepatitis D**

Chronic HDV is defined as the presence of HDV infection for greater than 6 months. Clinically, patients may be asymptomatic or have non-specific symptoms of fatigue, malaise and anorexia [100]. Three distinct chronic HBV/HDV patterns have been described. Most patients will have predominant HDV replication with suppressed HBV replication and are HBsAg-negative. This is because HDV tend to suppress HBV transcription as well as HBV virion release, which is hypothesized to be mediated by HDAg [107]. A similar phenomenon is seen in those with HCV/HDV co-infection as well in which HDV suppresses HCV replication [56]. In many patients with predominant HDV replication, HBV viral loads may be even undetectable, depending on the sensitivity of the assay. In a cohort of 126 chronic HDV patients, HBV viral loads could not be detected in 67% of patients [91]. Some HDV patients, however, can have similar viral loads of both viruses and rarely can patients have predominant HBV replication [108]. These patients are often HBsAg-positive. HBsAg has been reported to be positive in 9.0%–22.8% of HDV cases [44, 45, 90, 109]. Still, HBV viral loads are not suppressed as much as in the acute hepatitis stage and may reactivate in certain patients [46].

**Chronic hepatitis D outcomes**

It is well described that patients who are chronically infected with HBV/HDV have significantly worse liver disease compared to those with chronic HBV mono-infection [46, 101]. Table 3 illustrates the increased risks associated with chronic HDV compared with HBV mono-infection. Patients co-infected with chronic HBV/HDV have an accelerated progression to cirrhosis...
Although initial studies did not show an association between HDV and mortality [61], more recent studies have described an increased mortality risk in chronic HDV [42, 78, 90, 111, 112]. Fattovich et al. demonstrated, in cohort of 2000 Western European HBV-positive patients, that anti-HDV-positive patients were at a 2-fold increased risk of mortality relative to anti-HDV negative HBV patients [111]. This effect was also seen in a large cohort of HBV/HIV co-infected patients followed for a median of 8.7 years. In this study, Beguelin et al. reported a ~2-fold increased risk of mortality due to HDV [42]. The primary cause of death in HDV is most often liver failure [90]. This finding was supported by Niro et al., who found that over half of chronic HDV patients who become cirrhotic will advance to liver failure [91].

Predictors of worse outcomes in chronic HDV have generally varied between studies but have included cirrhosis at presentation [82, 91], age [44, 45, 82], male sex [91], HDV genotype 1 infection [45], country of origin [44] and positivity for serum anti-HDV IgM [116]. Multiple studies have confirmed that the HBV viral load has no impact on meaningful outcomes, although this is possibly due to the suppressive effect of HDV on HBV replication, which prevents any correlation from being obvious [45, 53, 108]. Although HBsAg-positive HDV patients typically have higher biochemical disease activity, HBsAg levels and HBV viral loads compared to HBeAg-negative HDV, long-term clinical outcomes do not differ [109]. This contrasts with studies in HBV mono-infection that have demonstrated significant associations between HBV viral loads with clinical outcomes such as histology, subsequent risk of cirrhosis and HCC [117, 118].

Meanwhile, anti-HDV IgM and HDV RNA levels have been shown to have disease activity and prognostic implications. Anti-HDV IgM levels correlate with higher biochemical activity indicators such as ALT levels as well as total histological activity index [90, 116]. In a study of 78 patients from the Hep-Net-International-Delta-Hepatitis-Intervention Trial-2 (HIDIT-2) study with long-term follow-up, only 1 out of 11 (9%) anti-HDV IgM-positive patients developed clinical decompensation compared with 26 out of 67 (39%) anti-HDV IgM-positive patients [116]. Similarly, high HDV RNA levels also correlate with disease activity [96] and are associated with an increase in the risk of HCC as well as progression to cirrhosis in non-cirrhotic patients [98]. In genotype 3, HDV RNA levels have been shown to positively correlate with neocellular inflammatory activity and fibrosis stage and negatively correlate with platelet counts. Patients with an HDV viral load of $>$2log_{10} had a significantly increased risk of advanced fibrosis (odds ratio of 6.47; 95% CI, 1.79–23.37; $P = 0.004$) [108].

### Therapies for HDV

Although there is currently no US Food and Drug Administration-approved therapy for HDV infection, IFN-α and peg-IFN-α can be used in chronic HDV, although both induce low rates (~20%–30%) of SVR, defined as an undetectable HDV RNA viral load at 6 months post cessation of treatment [36, 119, 120]. The low rates of response may be due to the interference of HDV with IFN-α intracellular signaling [121]. Notably, the interpretation of the SVR results of older therapeutic studies in HDV, especially prior to the standardization of the HDV assays by the WHO, should be made with caution, as there are wide variations between the different assays to detect and quantify HDV RNA [36, 97]. Additionally, although SVR has been used as the primary outcome in various clinical trials [122, 123], there remains uncertainty as to whether this outcome is adequate in HDV due to reported rates of late post-treatment relapse [9].

A major limitation of IFN-α-based therapies is the known side effects that accompany this therapy, which include: flu-like symptoms, myalgias, arthralgias, exacerbation of psychiatric illness, hematologic toxicity and elevations in transaminases [124]. Furthermore, the efficacy of IFN-α-based therapy is decreased in cirrhosis and is not recommended for use in patients with advanced cirrhosis (Childs B and C) due to fear of decompensation [36]. Because of these limitations, many patients with HDV never undergo treatment [48, 90]. In a study from Italy, only 30% of patients received interferon therapy with a mean follow-up of 233 months [90].

Nonetheless, IFN-α-based therapy has been shown to favorably affect the natural history of chronic HDV with substantial benefits that may become evident even years after treatment.

| Clinical outcome | Approximate relative risk increase* |
|------------------|-----------------------------------|
| Cirrhosis [58, 90, 110] | 2- to 3-fold |
| Hepatocellular carcinoma | 3- to 6-fold |
| Liver transplantation [48] | 2-fold |
| Hepatic decompensation [111] | 2-fold |
| Mortality [42, 78, 90, 111] | 2-fold |

*Compared with hepatitis B mono-infection.
cessation [125]. These benefits include improvement of liver histology (specifically activity grade and fibrosis), clearance of HDV, decreased risk of liver decompensation or need for liver transplantation and survival [125–127]. Prospective data from the Hep-Net Greece Cohort Study showed that interferon-based treatment significantly decreased disease progression in HDV-positive patients (hazard ratio [HR] = 0.14; 95% CI, 0.02–0.86; P = 0.033) [55]. This finding has been collaborated by several other studies that have shown that a lack of interferon therapy was an independent predictor of worse outcome [91]. Thus, the AASLD recommends peg-IFN-α for 12 months for those with elevated HDV RNA levels and ALT elevation [6], while EASL recommends peg-IFN-α for at least 48 weeks for those with compensated liver disease [7].

**Interferon-α monotherapy**

In chronic HDV infection, two types of IFN-α therapy have been explored: IFN-α2a and IFN-α2b. IFN-α is usually given as a subcutaneous injection three times a week. SVR rates with IFN-α are ~17% [128]. In a landmark early study that randomized 42 chronic HDV patients to either IFN-α 9 million units or 3 million units three times a week or no treatment for 48 weeks, 7/14 (50%) of the patients receiving the higher dose of IFN-α had normalization of alanine aminotransferase and clearance of HDV RNA. Treatment with the higher dose was associated with histologic improvement [129]. However, the high rate of HDV RNA clearance must be interpreted with caution in this study due to the low sensitivities of the assays used at the time as well as the size of the trial.

To improve compliance, a pegylated group was added to IFN-α to form peg-IFN-α that allowed once-a-week subcutaneous dosing [128]. Like IFN-α, there are two types of peg-IFN-α that have been investigated in trials: peg-IFN-α2a and peg-IFN-α2b, which appear to have similar efficacy. The recommended dose and duration of peg-IFN-α are 180 μg/week for 48 weeks for chronic HDV [130]. Clinical trials investigating the efficacy of peg-IFN-α are shown in Table 4. Recent studies have reported SVR rates of approximately 20%–30% after 1 year of therapy with peg-IFN-α [122]. However, as previously mentioned, relapse is common, even among patients who achieve SVR. In a study evaluating the long-term follow-up of the HIDIT-1 trial, 9 of the 16 patients who achieved SVR tested positive for HDV RNA at least once during post-SVR follow-up [9].

The optimal treatment duration of interferon therapy has continued to be undefined and treatment beyond 1 year is controversial. Although there have been reports of patients who have cleared chronic HDV infection after long-term IFN-α therapy of up to 12 years [126, 134, 135] and a study by Karaca et al., who reported that more than half of their patients who received peg-IFN-α for 2 years achieved SVR [136], further studies have shown a lack of substantial improvement in SVR rates or rates of post-treatment relapse [135, 137, 138]. In contrast, a recent retrospective study reported that maintained virological response rates, defined as remaining HDV RNA-negative 2 years after treatment discontinuation, increased with treatment duration and reached 50% at 5 years of treatment [127].

**Interferon combination therapy**

Due to the limited efficacy of interferon monotherapy in chronic HDV, several studies have evaluated the utility of combining interferon-based therapy with antiviral medications. Medications that have been studied include: ribavirin [131, 139], lamivudine [140, 141], famciclovir [142], tenofovir [138] and adefovir [122]. Overall, these studies have not been demonstrated to provide any added benefit such as improvement in virological response rates. The AASLD recommends that, since NAs have no efficacy against HDV, they should not be used in patients with suppressed or low HBV replication except in those with cirrhosis [6]. Meanwhile, EASL recommends that NA therapy should be considered in HBV/HDV co-infected patients with evidence of ongoing HBV DNA replication (>2000 IU/mL) and can be considered in those with advanced liver disease [7].

**Monitoring for response during and after interferon therapy**

During IFN-α or peg-IFN-α therapy, patients should be monitored monthly with complete blood counts and liver chemistries. In addition, patients should be tested for HBsAg and their HDV RNA and HBV DNA should be quantified at baseline and at 3-month intervals during treatment [143, 144]. After initiation of peg-IFN-α therapy, HDV RNA begins to decrease at around 1 week [145]. A negative or greater than 2log10 IU/mL decline in HDV RNA at 6 months of interferon therapy has been described to be predictive of SVR [132, 146]. Early viral kinetics may also predict the likelihood of response to interferon-α-based therapies. Mathematical modeling of HDV viral kinetic responses to peg-IFN-α therapy has demonstrated that HDV declines in a bi-phasic manner and the presence of a flat second-phase response is associated with non-response [145]. Additionally, patients with HDV replication greater than 1log10 higher than HBV replication may have a delayed HDV RNA response to peg-IFN-α therapy [147]. Finally, HBsAg titers are often correlated with HDV RNA levels and its decline in the serum may be an important early biomarker of HDV response. A HbsAg of less than 1000 IU/mL at 6 months is predictive of HDV loss [135, 148].

**Investigational therapies**

Due to the current limitations of interferon-α-based therapies, there has been substantial recent interest in novel therapies for this devastating disease. Current investigational therapeutic pipelines can be separated into the following classes: (i) interferon-lambda (IFN-λ), (ii) prenylation inhibitors

| Table 4. Clinical trials on the use of pegylated-interferon-α |
|---------------------------------------------------------------|
| **Publication** | **Publication year** | **Dose and delivery** | **Study arms and duration** | **Number of patients** | **VR** | **SVR** |
| Erhardt et al. [123] | 2006 | 1.5 mcg/kg SC/wk | peg-IFN-α for 48 weeks | 12 | NR | 17% |
| Niro et al. [131] | 2006 | 1.5 mcg/kg SC/wk | peg-IFN-α for 72 weeks ± ribavirin for 48 weeks | 38 | 13% | 21% |
| Castelnau et al. [132] | 2006 | 1.5 mcg/kg SC/wk | peg-IFN-α for 48 weeks | 14 | 57% | 43% |
| Wedemeyer et al. [122] | 2011 | 180 mcg SC/wk | peg-IFN-α ± adeovir vs placebo for 48 weeks | 90 | 23% | 28% |
| Gheorghe et al. [133] | 2011 | 1.5 mcg/kg SC/wk | peg-IFN-α for 52 weeks | 49 | 33% | 25% |

Peg-IFN-α, pegylated-interferon-α; VR, virological response; SVR, sustained virological response; NR, not reported.
Of note, clinical trials with the new investigational horizon for HDV but have yet to undergo clinical testing that target post-transcriptional gene silencing are also on the pending, although another clinical trial called the LIFT (line in HDV RNA [149]. End-of-trial data from that study are pending but patients experienced the highest evidence that another therapy, other than interferon, had efficacy in chronic HDV. However, gastrointestinal side effects such as diarrhea, nausea, anorexia, dyspepsia and weight loss were common, especially among patients in the higher-dose group, and the HDV RNA suppression was not sustained after discontinuation of therapy.

Subsequently, this trial was followed by four dose finding/optimization clinical trials termed LOWR-HDV (Lonafarnib With Ritonavir in HDV) 1, 2, 3 and 4 [151–154]. Since LNF is extensively metabolized by CYP3A4, these clinical trials included the addition of RTV—a CYP3A4 inhibitor that has been extensively used in HIV [171]. By utilizing RTV, LNF levels could be boosted in the blood while the gastrointestinal tract was exposed to lower doses of LNF. This allowed lower doses of lonafarnib, resulting in better tolerability while simultaneously increasing post-absorption LNF levels and antiviral efficacy [172].

LOWR-1 [NCT02430181] was a single-center, phase 2 pilot study performed in Ankara, Turkey that investigated varying doses of LNF Ë plus RTV Ë peg-IFN-Î± for 5–12 weeks in five groups of patients (three patients per group) [151]. The most impressive antiviral and biochemical responses were achieved in the LNF 100 mg twice daily (BID) plus RTV 100 mg daily (HDV decline 3.2log10 IU/L and LNF 100 mg BID Ë peg-IFN-Î± groups (HDV decline 3.0log10 IU/L) after 8 weeks of therapy. The rate of decline was more rapid compared with prior studies with peg-IFN-Î± alone [122]. Although no patients cleared the virus during treatment, two patients in the LNF monotherapy group achieved an undetectable HDV viral load post treatment after a transient ALT flare [151].

LOWR-2 [NCT02430194] was a phase 2, dose-ranging study that attempted to optimize dosing by evaluating 58 patients treated with 10 separate treatment arms of LNF (25 mg, 50 mg, 100 mg BID) Ë plus RTV 100 mg BID Ë peg-IFN-Î± for 12–24 weeks [173]. This study showed impressive synergistic activity of lower-dose LNF/RTV with peg-IFN-Î± resulting in an HDV decline of 5.57log10 IU/L at 24 weeks. Three of five patients became HDV RNA-negative and ALT-normalized, and the regimen was well tolerated [152]. Interestingly, from the LOWR-1 and LOWR-2 studies, 5 of 27 (18.5%) patients treated with LNF who had a detectable HDV RNA at the end of treatment subsequently experienced a post-treatment ALT flare followed by rapid clearance of HDV, suggesting immune restoration [174].

LOWR-3 [NCT02511431] is a phase 2, double-blind, placebo-controlled study that evaluated once-daily dosing in 21 patients for 12–24 weeks [153]. Patients were randomized to doses of LNF at 50, 75 or 100 mg plus RTV 100 mg once a day. Final data are pending but patients experienced the highest HDV decline with LNF 50 mg (1.93log10 IU/L) compared with LNF 75 mg (1.5log10 IU/L) and LNF 100 mg (0.29log10 IU/L) at 24 weeks.
Table 5. Clinical trials on investigative treatments for hepatitis D

| Therapeutic agents | Publication | Trial phase | Dose and delivery | Study arms and duration | Number of patients | HDV change (log10 IU/L) | HDV negative |
|--------------------|-------------|-------------|-------------------|-------------------------|--------------------|------------------------|---------------|
| Pegylated-interferon-α† | Hamid et al. [149] | 2 | peg-IFN-α 120/180 mcg SC/wk | peg-IFN-α for 48 weeks | 33 | NR | 3 of 11 |
| Lonafarnib | Koh et al. [150] | 2 | LNF 100/200 mg PO/BID | LNF for 4 weeks vs placebo | 14 | 100 mg (-0.73) 200 mg (-1.54) | NR |
| Lonafarnib ritonavir (LOWR-1)† | Yurdaydin et al. [151] | 2 | LNF 100/200/300 mg PO/BIDRTV 100 mg PO/BID | LNF + RTV ± peg-IFN-α for 5-12 weeks | 15 | LNF 100 mg BID + RTV (–3.2) LNF 100 mg BID + peg-IFN-α (–3.0) | LNF monotherapy (2 of 6)* |
| Lonafarnib ritonavir pegylated-interferon-α (LOWR-2)† | Yurdaydin et al. [152] | 2 | LNF 25/50/75/100 mg PO/BIDRTV 100 mg PO/BIDpeg-IFN-α 180 mcg SC/wk | LNF + RTV ± peg-IFN-α for 12-24 weeks | 58 | LNF 25 mg BID + RTV + peg-IFN-α (–5.57) | LNF 25 mg BID + RTV + peg-IFN-α (3 of 5) |
| Lonafarnib ritonavir (LOWR-3)† | Koh et al. [153] | 2 | LNF 50/75/100 mg PO/dailyRTV 100 mg PO/daily | LNF + RTV for 12-24 weeks | 21 | LNF 50 mg (–1.93) LNF 75 mg (–1.3) LNF 100 mg (–0.29) | NR |
| Lonafarnib ritonavir (LOWR-4)† | Wedemeyer et al. [154] | 2 | LNF 50/75/100 mg PO/BID | LNF + RTV for 24 weeks | 15 | LNF 50 mg (–1.93) LNF 75 mg (–1.3) LNF 100 mg (–0.29) | NR |
| Myrcludex B | Bogomolov et al. [155] | 1b/2a | MB 2 mg SC/daypeg-IFN-α 180 mcg SC/wk | peg-IFN-α for 48 weeks or Myrcludex B ± peg-IFN-α for 24 weeks followed by peg-IFN-α for 24-48 weeks | 24 | Myrcludex B (–1.67) Myrcludex B + peg-IFN-α (–2.6) | Myrcludex B (2 of 8) Myrcludex B + peg-IFN-α (5 of 7) |
| Myrcludex B | Wedemeyer et al. [156] | 2b | MB 2/5/10 mg SC/dayTDF 245 mg PO/day | TDF ± Myrcludex B | 120 | Myrcludex B 2 mg (–1.7) Myrcludex B 5 mg (–1.6) Myrcludex B 10 mg (–2.7) | NR |
| Nucleic acid polymer (REP2139) | Bazinet et al. [157] | 2 | REP 500/250 mg IV/weekpeg-IFN-α 180 mcg SC/wk | REP 2139 for 15 weeks followed by peg-IFN-α + REP2139 for 15 weeks followed by peg-IFN-α for 33 weeks | 12 | | 9 of 12 |

*Post-treatment result.
†Interim results; peg-IFN-α, pegylated-interferon-α; HDV, hepatitis D virus; LNF, lonafarnib; TDF, tenofovir disoproxil fumarate; NR, not reported; RTV, ritonavir; LOWR, Lonafarnib With and without Ritonavir; MB, Myrcludex B; pegylated-interferon-α, peg-IFN-α.
One patient became HDV RNA-negative during the study and 6/21 (28.6%) patients achieved a HDV RNA below 250IU/mL [153].

Finally, LOWR-4 [NCT02527707] is a phase 2, open-label, dose-titration study that treated 15 chronic HDV patients for 24 weeks [154]. Patients were started on LNF 50 mg BID and RTV 100 mg BID initially. LNF was then increased to a maximum of 100 mg BID per protocol as tolerated. Interim results have been reported describing that dose escalation was feasible and patients experienced a mean HDV decline of $1.87\log_{10}$IU/L at Week 8 [154].

In summary, the regimen that appears to have the highest efficacy in these LOWR-HDV trials is that of low-dose LNF 25 or 50 mg twice a day and ritonavir 100 mg twice a day with peg-IFN-$\alpha$ from LOWR-2. Three of five patients were HDV RNA-negative at Week 24—a response that persisted to Week 48 in the two patients who stopped treatment at Week 24 [175]. Although these results are promising and LNF has been well tolerated in the recent LOWR trials, long-term follow-up regarding therapy efficacy and safety are needed. It is encouraging to note that there has not been any evidence of viral resistance in the LNF studies thus far [151–154]. As mentioned, the LIFT trial [NCT03600714] evaluating the combination of peg-IFN-$\alpha$, LNF and RTV is ongoing. A phase 3 trial called the D-LIVR (Delta Liver Improvement and Virologic Response) study [NCT03719313] studying the efficacy and safety of LNF+RTF $\pm$ peg-IFN-$\alpha$ will begin recruiting soon.

Entry inhibitor (Myrcludex B)

Myrcludex B is a myristoylated peptide of 47-amino acids derived from the preS1-domain of the L-HBsAg protein and first-in-class HBV/HDV entry inhibitor that works by inactivating the host receptor, NTCP [20]. This receptor is crucial for viral entry and inactivation leads to a decrease in the number of infected cells (Figure 1). Entry inhibitors as a class may also have a role in eliminating intrahepatic HBV/HDV genome [176]. Initial mice studies of Myrcludex B showed that Myrcludex B significantly inhibited HDV-infection establishment. However, continuous drug administration was necessary to prevent HDV spread [177].

In a phase 1b/2a, randomized, three-arm, parallel, open-label, proof-of-concept study [NCT02637999] evaluating 24 patients with chronic HDV randomized (1:1:1) to Myrcludex B, peg-IFN-$\alpha$ or combination, Myrcludex B for 24 weeks showed efficacy as both a monotherapy as well as exhibiting a synergistic effect with peg-IFN-$\alpha$. Myrcludex B was administered as a 2-mg subcutaneous injection once daily. Although the primary endpoint—a change in HBsAg levels—was not met, both Myrcludex B monotherapy and combination therapy with peg-IFN-$\alpha$ led to significant declines in HDV RNA and ALT levels. At 24 weeks, mean HDV declines were as follows: $1.67\log_{10}$IU/L (Myrcludex B), $2.60\log_{10}$IU/L (Myrcludex B + peg-IFN-$\alpha$) and $2.20\log_{10}$IU/L (peg-IFN-$\alpha$). Two out of the eight patients in both monotherapy groups became HDV RNA-negative, while combination therapy led to five of the seven patients becoming HDV RNA-negative [155]. Myrcludex was generally well tolerated.

This study was followed by a phase 2b, multicenter, open-label study [NCT03546621] conducted in 120 patients with chronic HDV who were randomized to tenofovir $\pm$ Myrcludex B (2, 5, 10 mg) for 24 weeks. At 24 weeks, HDV declines were the most impressive at 5 and 10 mg: $1.60\log_{10}$IU/L (Myrcludex B 5 mg) and $2.70\log_{10}$IU/L (Myrcludex B 10 mg) [156, 178]. Efficacy was dose-dependent, with the best results seen at the 10-mg dose, with 76.6% of the patients in that arm achieving the primary endpoint (a decrease in HDV RNA by $2\log_{10}$ or HDV RNA negativity). However, relapse occurred in most responders (60%–83%, depending on group) after cessation of therapy [156]. A phase 2, randomized, open-label study [NCT02637999] evaluating Myrcludex B $\pm$ peg-IFN-$\alpha$ for 24 weeks followed by peg-IFN-$\alpha$ for 48 weeks vs peg-IFN-$\alpha$ for 48 weeks has been completed, with results pending.

Although these results are promising, the appearance of antibodies to Myrcludex B in some patients has been described and is likely to be concerning. Although no correlation between the appearance of antibodies to safety or efficacy has been seen, attention to this finding has to be made in future studies [155]. In addition, HDV has been shown in ‘in vitro’ and ‘in vivo’ studies to propagate during liver regeneration through clonal cell division despite the presence of Myrcludex B [179]. Furthermore, Blank et al. reported that Myrcludex B significantly increases total plasma bile-acid exposure by 19.2-fold and conjugated bile acids by 124-fold in healthy human volunteers because NTCP is also a physiological bile-acid transporter [180]. Although the patients in that study remained asymptomatic, the long-term effects of elevated bile acids in the setting are unknown [180].

Nucleic acid-based polymer (REP2139)

NAPs are phosphorothioated oligonucleotides with broad-spectrum antiviral activity against many different types of viral infections including HCV and HIV [181–184]. NAPs continue to be investigated in HBV monotherapy due to a potent effect on HBsAg secretion [185, 186]. The leading drug in this class of HDV medication is REP2139 (Replicor®), which is administered as a once-a-week intravenous infusion. In HDV, the exact mechanisms of antiviral effects of NAPs have yet to be fully elucidated, but they appear to inhibit the secretion of HBsAg envelope proteins as well as being active at viral entry (Figure 1) [185, 187]. The antiviral effects of NAPs appear to be dependent on the size and amphipathicity of the polymer. The inhibition of viral entry of NAPs appears to be at least partly due to the NAPs binding to HDV particles and preventing attachment of the virus to the cell-surface glycosaminoglycans [181].

The initial proof-of-concept study, REP301 [NCT02233075], was an open-label, non-randomized study evaluating 12 patients with chronic HDV [157]. Patients received 500 mg intravenous REP2139 weekly for 15 weeks followed by combination therapy of 250 mg intravenous REP2139 and peg-IFN-$\alpha$ for 15 weeks and then peg-IFN-$\alpha$ monotherapy for 33 weeks. REP2139 demonstrated potent efficacy on HBV and HDV. There was a mean $3.50\log_{10}$IU/L decline in HBsAg from baseline and 5 of 12 patients became HBsAg-negative, which was maintained to 1 year post treatment. Nine of 12 patients became HDV RNA-negative by the time of treatment, with 7 of 9 patients remaining HDV RNA-negative at 1.5 years of post-therapy follow-up. Mean HDV RNA decline was $5.34\log_{10}$IU/L [157, 188]. Durability of the response is being evaluated in a long-term follow-up study [NCT02876419].

REP2139 appears to be the only one of the new investigative drugs that results in a fast reduction in HBsAg [143]. Although REP2139 was well tolerated, substantial ALT flares did occur in some patients, which may limit its use in cirrhosis [187]. Other concerns with NAPs include: side effects such as hair loss, dysphagia and dysgeusia that were seen during a prior HBV trial, which was attributed to heavy-metal exposure at the trial site, as well as the possibility of late relapses, which were seen during a prior HBV trial [157, 185].
Conclusion

HDV is a rapidly progressive viral hepatitis that increases the risk of cirrhosis, HCC, hepatic decompensation, liver transplantation and mortality compared with HBV mono-infection. Due to immigration and improvements in diagnosis through the development and standardization of diagnostic tests, HDV is now becoming increasingly recognized as a problem worldwide. However, there continues to be a lack of efficacious treatment options. Interferon therapy with peg-IFN-α remains the only regularly used therapy outside of clinical trials. However, recent improvements in our understanding of the virus have led to the investigation of several new and promising therapies—some of which may eventually be approved for use in HDV.

Authors' contributions

B.D. and C.K. conceived of and designed the manuscript. B.D. and C.K. interpreted the available studies on the subject. B.D., T.H. and C.K. drafted and revised the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest

None declared.

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