Current and Future Management of Chronic Hepatitis D

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Abstract: Hepatitis D virus (HDV) is a defective RNA virus that requires the hepatitis B surface antigen (HBsAg) of the hepatitis B virus (HBV) for its assembly, release, and transmission. HDV is highly pathogenic, causing the least common, but most severe, form of chronic viral hepatitis at all ages. Although significant advances have been made in the treatment of chronic viral hepatitis, targeting HDV remains a major challenge because of the unconventional nature of this virus and the severity of its disease. The virus contains a ribonucleoprotein complex formed by the RNA genome with a single structural protein, delta antigen (HDAg), which exists in 2 forms (small and large HDAg) and is coated by HBsAg. Farnesylation of the large HDAg is essential for anchoring the ribonucleoprotein to HBsAg for the assembly of virion particles. HDV enters into hepatocytes by using the HBV receptor, the sodium taurocholate cotransporting polypeptide (NTCP). Unlike other RNA viruses, HDV does not encode its own polymerase but exploits the host RNA polymerase II for replication. Thus, in contrast to HBV and hepatitis C virus, which possess virus-specific enzymes that can be targeted by specific inhibitors, the lack of a virus-specific polymerase makes HDV a particularly challenging therapeutic target. Treatment of hepatitis D remains unsatisfactory, and interferon-α has been the only approved drug over the past 30 years. This article examines the unconventional nature of HDV, the current management of chronic hepatitis D, and how new insights from the HDV life cycle have led to the development of 3 novel classes of drugs (NTCP receptor inhibitors, farnesyltransferase inhibitors, and nucleic acid polymers) that are currently under clinical evaluation.

Hepatitis D virus (HDV) was discovered more than 40 years ago by Rizzetto and colleagues in Italy. Initially described as a new antigen-antibody system (delta/antidelta) in chronic hepatitis B surface antigen (HBsAg) carriers, subsequent transmission studies in chimpanzees conducted in the early 1980s at the National Institutes of Health demonstrated that the delta antigen (HDAg) was the internal component of a new transmissible
pathogen, the delta agent. Epidemiologic research in the 1980s showed that the delta agent was found worldwide and was a major cause of severe acute and chronic hepatitis. Because of its medical importance and unique virologic features, the delta agent was recognized in 1983 as a distinct hepatitis virus and designated HDV, and the disease it causes was designated hepatitis D. This article reviews the unconventional nature of HDV, how the dramatic change in the epidemiology of this virus has modified the clinical scenario of hepatitis D in Western countries, the current treatment challenges posed by this pathogen, and how new insights from the HDV life cycle are paving the way for the development of novel strategies for the treatment of chronic hepatitis D.

The Virus

HDV is a defective RNA virus that requires the HBsAg of the hepatitis B virus (HBV) for virion assembly, release, and transmission. The virus is a 36-nm particle. It contains in its interior a ribonucleoprotein (RNP) complex, approximately 20 nm in diameter, consisting of an RNA genome complexed with a structural protein, HDAg, surrounded by the envelope glycoprotein, HBsAg, which is the only helper function provided by HBV. In infected cells, the formation of the RNP is independent of HBV, but the RNP without the HBV envelope protein cannot egress the cell and infect other hepatocytes. Thus, HDV is a satellite virus of HBV and can only infect individuals who simultaneously acquire HBV (coinfection) or superinfect an HBsAg carrier (superinfection). Persons who have antibody to HBsAg (anti-HBs), who are immune to HBV infection, are not susceptible to HDV.

Cloning and sequencing of the HDV genome in 1986 confirmed the unique features of this virus, which has been classified as the only member of a separate genus, Delta virus. HDV is the only animal virus to possess a single-stranded circular RNA genome of negative polarity, of approximately 1700 nucleotides, which is the smallest genome in animal virology. Besides genomic RNA, in infected cells, there are 2 additional HDV-specific RNAs: the antigenomic RNA, which is the exact complementary copy of the genomic RNA but is less abundant, and the messenger RNA (mRNA), which is generated from the genomic RNA. The antigenomic RNA, which is not assembled into virions, contains the open reading frame that encodes the single structural protein of HDV, the HDAg. There are 2 forms of HDAg: the small HDAg (S-HDAg) of 195 amino acids, and the large HDAg (L-HDAg) of 214 amino acids, which contains 19 additional amino acids at the C-terminus. The L-HDAg is transcribed as a result of postranscriptional RNA editing of the antigenomic RNA strand by a host cellular enzyme, adenosine deaminase acting on RNA1 (ADAR1), which modifies the S-HDAg amber termination codon, allowing for the transcription of a longer mRNA. The L-HDAg undergoes farnesylation, which occurs at a C-terminal cysteine-containing signal domain (CXXQ) that serves as the substrate for the enzyme prenyltransferase, which catalyzes the addition of prenyl lipids to render the molecules more lipophilic and facilitate their association with membranes. This process, which is unique to the L-HDAg, is essential for anchoring the HDV RNP to the HBsAg during the assembly of HDV.

The replication of HDV occurs in the nucleus of hepatocytes, as the liver is the only organ in which HDV replicates. The mechanism of HDV RNA replication is one of the most intriguing aspects of the biology of this virus. It has been proposed that the RNA genome replicates via a double rolling circle mechanism similar to that proposed for viroids. Unlike other RNA viruses, HDV does not encode its own polymerase but exploits the host RNA polymerase II for replication. The only enzymatic activity of HDV is mediated by RNA elements termed ribozymes (RNA enzymes) that cleave the circular genomic and antigenomic RNAs, producing linear molecules.

The replication of HDV is completely autonomous from that of HBV, but the assembly, release, and propagation of infectious virions are critically dependent on HBsAg, which encapsidates the HDV RNP. Remarkably, even when HBV replication is undetectable, abundant HBsAg production may still occur, allowing for the formation of HDV virions. There are 3 forms of HBsAg envelope proteins: small (S-HBsAg), medium, and large (L-HBsAg). The production of HBsAg in HBV-infected cells far exceeds the need for HBV virion assembly, with a very high release of HBsAg empty subviral particles into serum (10^{12}-10^{13} per mL vs only 10^{8}-10^{9} HBV virions).

Being encapsidated by HBsAg, HDV enters into hepatocytes using the same receptors as those used by HBV. These include an initial low-affinity docking to heparan-sulfate proteoglycans, through the antigenic loop of the S-HBsAg, followed by high-affinity binding to the recently identified specific receptor, the sodium taurocholate cotransporting polypeptide (NTCP). Via the pre-S1 (2-48 amino acid sequence) of the L-HBsAg. Insights into the molecular biology of HDV have led to the development of novel antiviral agents capable of interfering with the life cycle of this virus.

Course of Hepatitis D

The clinical outcome of acute hepatitis D differs according to the type of infection. Whereas HBV/HDV coinfection evolves to chronicity in only 2% of cases, HDV...
superinfection results in chronic infection in at least 90% of cases. Since the earliest studies, HDV has turned out to be a highly pathogenic virus, causing the least common, but most severe, form of chronic viral hepatitis at all ages. Cirrhosis develops in approximately 70% to 80% of cases within 10 years from the onset of acute hepatitis. However, in Greece, the disease was reported to be associated with minimal liver damage and a favorable clinical course.

The lack of large prospective studies on the natural history of this disease has made it difficult to define the rate of long-term sequelae of chronic hepatitis D once cirrhosis is developed—that is, liver decompensation and hepatocellular carcinoma (HCC). Thus, most of the data have been inferred from retrospective studies, which have provided a general picture of the natural history of chronic hepatitis D. Once established, cirrhosis may be a stable disease for another decade, although later in the course of disease, a high proportion of patients die of liver decompensation or HCC unless they undergo liver transplantation. The estimated annual incidence of liver decompensation in HDV cirrhosis ranges from 2.6% to 3.6% and from 2.6% to 2.8% for HCC.

Over the past 2 decades, there has been a significant decline in the incidence of HDV infection in developed countries, especially in Southern Europe, because of universal HBV vaccination and improved socioeconomic conditions. This dramatic change in the epidemiology of HDV has resulted in a significant reduction of new cases of hepatitis D in Europe, with a preponderance of subjects who have limited effects on the expression of HBsAg, which is abundantly expressed in chronic HBsAg carriers. Therefore, HBsAg represents an ideal molecular partner for the sustained production of infectious HDV particles.

The goals of antiviral treatment in chronic hepatitis D are to eradicate HDV and HBV and to prevent the long-term sequelae of chronic hepatitis D—cirrhosis, liver decompensation, and HCC, which lead to liver-related death or the need for liver transplantation. However, these goals are not commonly achieved, and treatment of chronic hepatitis D remains unsatisfactory.

**Current Management**

**Interferon-α**

Thirty years have elapsed since interferon-α (IFN-α) was first used to treat chronic hepatitis D. Despite this long period of time and the progress made in the therapy of chronic hepatitis B and C, IFN-α still remains the only drug currently used for the treatment of HDV infection. Initial research using standard IFN-α provided evidence that the efficacy of this drug was related to the dose and duration of therapy, although a 1-year course of high-dose standard IFN-α induced only a 10% to 20% rate of sustained HDV clearance and a 10% rate of HBsAg clearance. Strategies to increase the efficacy of standard IFN-α, such as longer duration of treatment or even continuous therapy for up to 12 years, were explored, but most patients still failed to clear HDV, and the rate of relapse remained high.

Following the superior results obtained with pegylated IFN-α in chronic hepatitis B and C, its efficacy was also evaluated in chronic hepatitis D. These studies demonstrated that pegylated IFN-α, given for 1 year, was associated with a response rate that was better than that of standard IFN-α, although it rarely exceeded 25%. The response to standard IFN-α was better in patients with chronic hepatitis D than in those with cirrhosis, whereas a similar response rate was reported using pegylated IFN-α in patients with advanced fibrosis (stage ≥4 according to the Ishak score or imaging indicative of cirrhosis) vs nonadvanced liver disease in one study. The efficacy of long-term, high-dose, pegylated IFN-α was recently examined in 12 patients; clearance of HDV RNA, followed by HBsAg clearance, was achieved in only 25% of the patients.

**Combination Therapy With Standard or Pegylated Interferon-α**

The efficacy of standard IFN-α in combination with ribavirin or lamivudine was not significantly higher than that of IFN-α monotherapy in chronic hepatitis D. Similar results were obtained when pegylated IFN-α...
was used in combination with ribavirin or adefovir. In the largest randomized trial, HIDIT (Hep-Net International Delta Hepatitis Intervention Trial-1), pegylated IFN-α either alone or in combination with adefovir was compared to adefovir monotherapy. Clearance of HDV RNA was observed in 28% of the patients in the 2 pegylated IFN-α treatment arms, but in none of those who received adefovir monotherapy, 6 months after completion of treatment.

Long-Term Effects of Standard and Pegylated Interferon-α on the Natural History of Chronic Hepatitis D

Studies on the long-term effects of IFN-α treatment on the natural history of hepatitis D are limited. A prospective study of 36 patients followed for up to 20 years after 1 year of treatment showed a significant improvement in the long-term clinical outcome and survival of patients who received high doses of standard IFN-α. Reversion of advanced hepatic fibrosis occurred in some patients with an initial diagnosis of active cirrhosis. New data were obtained from the HIDIT trial using pegylated IFN-α, in which 75% of the patients were prospectively followed over a median period of 4.5 years after completion of therapy. This long-term study documented a late relapse in HDV RNA in more than half of the patients (58%) who were negative for HDV RNA 6 months after therapy. Sequencing analysis showed that the reappearance of viremia was due to reactivation of the original viral strain, raising questions as to whether HDV can be definitively cleared in patients who remain HBsAg-positive as well as concerns on the reliability and appropriateness of the use of sustained virologic response as a surrogate marker of treatment efficacy in chronic hepatitis D. However, this long-term study also documented that late and transient relapses were not associated with clinical complications during the observation period, indicating that a prolonged virologic response to pegylated IFN-α, even if not sustained, can be clinically relevant in chronic hepatitis D. Further studies are necessary to better characterize these late relapses to establish whether they are all transient, their frequency and magnitude, and their long-term impact on the natural history of chronic hepatitis D.

Some patients may become HDV RNA–negative after therapy with both standard and pegylated IFN-α. The loss of HDV RNA at the end of therapy as well as during follow-up has been associated with a favorable outcome and fewer liver-related complications. Prolonged loss of HDV RNA was associated with a favorable outcome even in the absence of HBsAg clearance. In a recent retrospective study, Yurdaydin and colleagues assessed the effects of treatment duration in patients who received more than 1 course of standard or pegylated IFN-α. The researchers found that the virologic response to IFN-α increases with treatment duration and favorably affects the natural course of chronic hepatitis D. HBsAg clearance occurred in a significantly higher proportion of patients with prolonged HDV RNA–negative response.

Monitoring Antiviral Therapy

Patients treated with standard or pegylated IFN-α should be monitored monthly with measurement of complete blood counts and serum alanine aminotransferase (ALT) levels. Serum HDV RNA and HBV DNA should be quantified at baseline and at 3-month intervals during treatment, and then every 6 months during follow-up after the completion of therapy. Quantification of serum HBsAg levels provides an additional tool for monitoring antiviral therapy. The side effects, which are typical of IFN-α treatment and are particularly common with high doses and a prolonged course of therapy, include flu-like symptoms such as fatigue and weight loss. Reasons for dose modification or cessation of therapy most often include thrombocytopenia, neutropenia, anemia, and psychiatric complications.

Predictors of Response to Interferon-α

Baseline biochemical and virologic parameters are usually not predictive of a sustained virologic response. Patients without cirrhosis respond better to IFN-α, highlighting the importance of early diagnosis and treatment in chronic hepatitis D. A negative HDV RNA result by polymerase chain reaction within 6 months of therapy is a strong predictor of sustained virologic response. Clearance of HBsAg would be desirable, raising the question of whether patients with a decline in HBsAg and loss of HDV RNA may be candidates to prolong treatment to force the elimination of the remaining HBsAg.

Current Recommendations for Treatment With Interferon-α

Only patients with compensated HDV-associated liver disease should be considered for treatment with IFN-α, whereas liver transplantation is the only therapeutic
choice for patients with advanced or decompensated liver disease. Pegylated IFN-α is the first-choice treatment for chronic hepatitis D, and a 1-year course should be offered to all IFN-α-naive patients, as well as to previous nonresponders to standard IFN-α. Monitoring serum HDV RNA and HBsAg offers clinicians the possibility to assess an early response as well as to identify patients with a delayed virologic response that might benefit from a prolonged course of therapy.

**Future Management**

New therapies are needed for the treatment of chronic hepatitis D because even with the use of pegylated IFN-α, either alone or in combination with nucleos(t)ide analogues, the overall rate of virologic response remains low, and most patients relapse after discontinuation of therapy. Long-term follow-up demonstrated that relapse is frequent even in responders unless HBsAg is cleared. Novel therapeutic strategies are, therefore, needed both to improve the efficacy of therapy and to extend treatment to patients with advanced disease for whom IFN-α is contraindicated. At present, 3 new classes of drugs that interfere with the life cycle of HDV (Figure) are under clinical evaluation. These include inhibitors of the NTCP receptor, farnesyltransferase inhibitors (FTIs), and nucleic acid polymers (NAPs).

**Myrcludex B: A Hepatitis D Virus Entry Inhibitor**

Viral entry into hepatocytes is the first step in the life cycle of HBV and HDV, and NTCP, a key bile-acid transporter in the liver, has been identified as the receptor for HBV and HDV entry. Myrcludex B (Hepatera/MYR GmbH), a myristoylated lipopeptide of 47 amino acids corresponding to the pre-S1 N-terminal segment of the L-HBsAg, inhibits HBV and HDV entry through competitive inhibition of receptor binding. This drug was reported to interfere with the formation of intrahepatic HBV covalently closed circular DNA (cccDNA) and with intrahepatic viral spreading in a humanized mouse model. Using NTCP knockout mice, Slijepcevic and colleagues confirmed the liver specificity of this agent for NTCP.

**Clinical Trials** The first study to evaluate the safety of Myrcludex B in humans was performed by Blank and colleagues in 36 healthy volunteers who received the drug either intravenously or subcutaneously at escalating doses up to 20 mg/day. Administration of the drug was associated with an increase in conjugated bile acids in humans, raising safety concerns on the consequences of its prolonged use. The concentration of Myrcludex B required for blocking HBV/HDV entry through the NTCP receptor is approximately 100 times lower than the inhibitory dose for bile-acid transport, which indicates that viral blockade can be achieved without saturating the transporter function for bile acids. The drug was well tolerated, with only 2 serious events reported, which included an increase in lipase and amylase. This study was preliminary to testing Myrcludex B in patients with hepatitis B or D.

The safety and efficacy of Myrcludex B in chronic hepatitis D was first assessed in a pilot study performed in Russia, in which a total of 24 patients were randomized into 3 groups: 8 patients sequentially received 2 mg of Myrcludex B daily for 24 weeks followed by pegylated IFN-α 2a for 48 weeks; 8 patients received Myrcludex B combined with pegylated IFN-α 2a for 24 weeks, and then pegylated IFN-α 2a monotherapy for 24 additional weeks; and 8 patients received pegylated IFN-α 2a alone for 48 weeks. The primary endpoint was HBsAg response, defined as a decline of HBsAg in serum of at least 0.5 log IU/mL. All patients were hepatitis B e antigen (HBeAg)-negative, 3 had cirrhosis, and 9 had previously been treated with pegylated IFN-α 2a. At baseline, mean serum HDV RNA was approximately 10⁴ copies/mL and mean HBsAg was approximately 10⁴ IU/mL. An interim analysis at week 24 in patients receiving only Myrcludex B showed ALT normalization in 6 patients and HDV RNA decrease greater than 1 log in 4 patients (mean change from baseline, 10^{1.67}), with clearance in 2; however, the levels of HBsAg were unchanged in all patients. After cessation of therapy, HDV RNA reappeared in all patients. In patients treated with Myrcludex B plus pegylated IFN-α 2a, HDV RNA became negative in 5 patients, and HBV DNA decreased significantly only in this group. Adverse events related to Myrcludex B included thrombocytopenia, lymphopenia, eosinophilia, and neutropenia; all were transient and mild, and none required dose modification. Antibodies against this drug were detected in 6 patients who received the treatment combined with pegylated IFN-α 2a; although the efficacy of the treatment was not impaired, the significance of the antibodies needs to be further investigated. Taurine-conjugated and glycine-conjugated bile acids were increased in both Myrcludex B treatment groups.

The results of a multicenter, open-label, phase 2b, clinical trial were recently presented in abstract form. A total of 120 patients with chronic hepatitis D were divided into 4 arms to receive 2, 5, or 10 mg of Myrcludex B daily for 24 weeks in combination with tenofovir, or tenofovir alone. Tenofovir was maintained for 24 weeks after interruption of Myrcludex B. The primary endpoint was HDV RNA reduction of 2 log or negativity. At the end of treatment, the median HDV RNA declined by -1.75 log, -1.60 log, and -2.70 log from lower to higher
Myrcludex B doses, respectively, and by -0.18 log with tenofovir alone. ALT normalization was achieved in 42.8%, 50.0%, 40.0%, and 6.6% of patients, respectively, in the 4 groups. A 12-week follow-up, an interim analysis of HDV RNA was available only in 41 patients. A relapse in HDV RNA occurred in 60%, 80%, and 83% of HDV RNA responders in the 3 Myrcludex B groups and was associated with a moderate increase in ALT levels. These preliminary data show a transient efficacy of Myrcludex B in chronic hepatitis D, which suggests that a longer treatment duration may be necessary for the treatment of this disease.

**Perspectives and Open Questions** Myrcludex B induced a significant reduction in HDV RNA, but this effect was transient and did not outlast the termination of therapy, as viremia reappeared in all patients. The results were significantly better in patients treated with this drug and pegylated IFN-α 2a, a result that does not support the use of Myrcludex B as monotherapy in chronic hepatitis D. Surprisingly, the levels of HBsAg were unchanged during Myrcludex B treatment, although the primary endpoint was HBsAg response. The authors hypothesized that HBsAg can be produced from integrated HBV DNA, independent of cccDNA, and that HBsAg declines with different kinetics from those of HBV DNA. The effects of a longer duration of Myrcludex B monotherapy or combination therapy with pegylated IFN-α 2a and tenofovir, and/or of higher doses are being assessed in ongoing studies.
Lonafarnib: A Virus Assembly Inhibitor

The antigenomic HDV RNA strand encoding the S-HDAg is edited by a cellular enzyme, ADAR1, which modifies the S-HDAg amber termination codon. This posttranscriptional RNA editing results in the production of the L-HDAg, which undergoes farnesylation, an essential modification to anchor the HDV RNP to the HBsAg during the assembly of HDV infectious particles. Thus, FTIs interfere with HDV virion assembly and release of infectious particles from infected hepatocytes, as shown both in vitro and in vivo in a mouse model where 2 farnesylation inhibitors (FTI-277 and FTI-2153) were effective at clearing HDV viremia. Blockade of the farnesylation process by FTIs leads to the accumulation of HDV replicative intermediates inside hepatocytes. Thus, the reduction of serum HDV RNA is not due to a reduction in the number of infected hepatocytes, but rather to a decrease in HDV assembly. The FTI lonafarnib (Eiger BioPharmaceuticals) was initially proposed as an anticancer drug because farnesyltransferase is an important cellular enzyme involved in several cellular functions, comprising farnesylation of several cytoplasmic proteins of the RAS family, which are involved in cell growth, differentiation, and survival, as well as in T-cell activation and cytokine production. Although the antitumor efficacy of this drug was not validated, studies in oncology provided important safety data and showed that the drug has mostly gastrointestinal side effects.

Clinical Trials

In a short-term placebo-controlled study, 14 patients who were HBeAg-negative and infected by HDV genotype 1 were assigned to receive either 100 mg (group 1) or 200 mg (group 2) of lonafarnib given orally twice daily for 28 days, and then were followed for 6 months. By the end of therapy, HDV RNA was significantly decreased compared to baseline (by 0.73 and 1.54 log in groups 1 and 2, respectively), whereas HDV RNA decreased by only 0.13 log in the placebo group. The decline in HDV RNA correlated with the drug levels. Serum ALT levels and HBsAg remained unchanged during treatment, whereas HDV RNA returned to baseline levels in all treated patients after cessation of treatment. A virologic HDV RNA relapse greater than 0.5 log over baseline was observed in 5 patients between weeks 4 and 8 of follow-up. At the time of virologic rebound, the ALT increase did not exceed 2.5 times the baseline values. In addition, the drug was not well tolerated, and all subjects receiving the higher dose experienced nausea, diarrhea, abdominal bloating, and weight loss (mean of 4 kg).

In the LOWR HDV-1 study (Lonafarnib With and Without Ritonavir for HDV), 15 patients were divided into 5 groups (3 per group) to explore the optimal dose for treatment. Lonafarnib was administered at doses of 200 and 300 mg twice daily or at 100 mg 3 times daily, either alone or combined with ritonavir or pegylated IFN-α for 8 to 12 weeks. Because ritonavir is an inhibitor of cytochrome P450-3A4, which is the principal mediator of lonafarnib metabolism, this combination was expected to maximize intrahepatic drug levels. After 4 weeks of treatment, HDV viremia significantly declined whether lonafarnib was given as monotherapy or in combination, and this was associated with a significant decline in ALT levels; however, no changes were observed in HBsAg levels. The addition of ritonavir at 100 mg to lonafarnib 100 mg twice daily induced a better antiviral response and fewer gastrointestinal side effects, but even with this association, HBsAg levels were not affected. By the end of treatment, both serum HDV RNA and ALT returned to baseline levels in all but 2 patients. Of note, 5 patients who received lonafarnib at doses of 200 and 300 mg twice daily with pegylated IFN-α discontinued treatment within 4 weeks due to intolerance.

Three other studies under the same acronym (LOWR HDV-2, -3, and -4) are currently ongoing, and the preliminary results have been published in abstract form. In the LOWR HDV-2 study, to identify the lowest effective dose of lonafarnib in combination therapy, 48 patients received lower doses of lonafarnib (75, 50, or 25 mg twice daily) plus ritonavir with or without pegylated IFN-α. The triple regimen with 25 or 50 mg of lonafarnib, ritonavir 100 mg twice daily, and pegylated IFN-α 180 mcg combined the best efficacy and tolerability.

In the LOWR HDV-3 study, 21 patients received single daily doses of lonafarnib (50, 75, or 100 mg) with ritonavir at 100 mg for either 12 or 24 weeks. Patients were placed on anti-HBV nucleoside analogue therapy prior to starting lonafarnib. After 12 weeks of therapy, the mean log HDV RNA decline from baseline ranged from 0.83 IU/mL (for lonafarnib 100 mg) to 1.6 IU/mL (for lonafarnib 50 mg). The combination of ritonavir with lonafarnib in patients treated for 6 months was safe and effective in lowering HDV viremia.

Finally, the strategy of dose escalation and tolerance achievement was evaluated in the LOWR HDV-4 study, which enrolled 15 patients. All patients were started with lonafarnib at 50 mg plus ritonavir at 100 mg, and then lonafarnib was increased to 75 mg and subsequently to 100 mg. Ritonavir was maintained at 100 mg twice daily. At the end of treatment, the decline of serum HDV RNA from baseline was -1.58 ± 1.38 log IU/mL, and ALT levels normalized in 53% of patients. The decrease in HDV RNA, as well as the ALT normalization, did not outlast the cessation of therapy; in addition, lonafarnib did not show any effect on HBsAg levels both during and after treatment. The emergence of resistant mutations was not observed.
Perspectives and Open Questions  The mechanism whereby lonafarnib lowers HDV RNA in serum remains to be fully elucidated. Of major concern, the effects of intracellular accumulation of RNP particles are not known. In particular, whether the accumulation of HDV-replicative intermediates in liver cells can induce a cytotoxic effect or enhance the immune-mediated killing of the cell remains to be established.37 Understanding the consequences of the RNP complex accumulation in hepatocytes is critical, especially in the perspective of using lonafarnib in long-term treatment or in cirrhotic patients. Moreover, farnesyltransferase is an important cellular enzyme; therefore, it will be essential to fully elucidate the effects of its blockade on various intracellular pathways.

REP 2139: A Nucleic Acid Polymer  Mechanism and Clinical Trials  Phosphorothioate NAPs are negatively charged oligonucleotides with broad-spectrum inhibitory activity against several viruses (eg, HIV, herpes simplex virus, lymphocytic choriomeningitis virus). Their activity is sequence-independent, but both size- and amphipathicity-dependent, and their mechanism of action was initially limited to interference with viral attachment and entry.65 However, in a duck HBV model, NAPs were shown to interfere both with viral entry and with the synthesis and/or release of duck HBsAg from hepatocytes.66,67 Based on results obtained in the duck model, the safety and efficacy of REP 2055 (Replicor) and REP 2139 (Replicor) were evaluated in the first proof-of-concept study in HBV-infected HBeAg-positive patients. In both studies, NAP monotherapy was accompanied by a reduction in serum HBsAg and HBV DNA by 2 to 7 logs and 3 to 9 logs, respectively, accompanied by the appearance of serum anti-HBs (10-1712 mIU/mL). Side effects during treatment included fever, headache, and chills.68

Based on the significant effects seen on serum HBsAg, REP 2139 was selected for a study of safety and efficacy in combination with pegylated IFN-α in chronic hepatitis D. A proof-of-concept trial was conducted in Moldova on 12 treatment-naive patients who were age 18 to 55 years, antibody to hepatitis B e antigen–positive, and HBV RNA–positive, and who had serum HBsAg concentrations over 1000 IU/mL and low levels of HBV DNA (<10 to 726 IU/mL).69 None of the patients had cirrhosis. Patients received 500 mg of REP 2139 intravenously once a week for 15 weeks, followed by 15 weeks of combined therapy with 250 mg of REP 2139 and 180 µg of pegylated IFN-α 2a, and then monotherapy with 180 µg of pegylated IFN-α 2a for 33 weeks. The patients were then followed for 1 year after cessation of treatment. During REP 2139 monotherapy, HDV viremia decreased rapidly in all patients, and 11 became HDV RNA–negative, with 9 patients remaining negative at the end of treatment and 7 remaining negative at the end of 1 year of follow-up. Six patients had HBsAg levels less than 50 IU/mL at the end of therapy, which remained stable after 1 year in 5 patients. In addition, 6 patients had anti-HBs–positive results with titers over 10 mIU at the end of treatment, which persisted in 5 patients at the end of the 1-year follow-up. HBV DNA in serum was suppressed at the end of treatment in 9 patients and remained less than 10 IU/mL in 7 patients during follow-up. A marked increase in ALT levels was observed after the introduction of pegylated IFN-α 2a in 5 patients (42%), but the patients remained asymptomatic and the elevation resolved without discontinuation of therapy. All patients experienced at least 1 adverse event, including anemia, neutropenia, or thrombocytopenia. Serious side effects were reported in 33% of patients but mostly referred to pegylated IFN-α 2a.

Perspectives and Open Questions  The results obtained in this study have not been reported with other HDV treatments; however, these findings are limited by the study’s size. By the end of 1 year of follow-up, combined therapy of REP 2139 with pegylated IFN-α 2a was associated with clearance of HBsAg and HDV RNA, high titers of anti-HBs, and HBV DNA suppression in nearly 50% of patients. REP 2139 appears to inhibit HBsAg production from both cccDNA and integrated HBV DNA.69

Despite these results, several questions remain to be addressed regarding treatment with NAPs. None of the patients included in this trial were cirrhotic, and it is well established that chronic hepatitis without cirrhosis responds better to IFN-α therapy. The ALT and aspartate aminotransferase flares observed during treatment, although clinically irrelevant in this cohort, need further investigation, especially if treatment is extended to cirrhotic patients, whose labile equilibrium may be unsettled by a disease flare-up with important clinical consequences. It will also be important to characterize the nature and function of early and high titers of anti-HBs, as these antibodies are common findings with NAP treatment. Finally, the molecular mechanisms underlying the inhibition of the release of HBsAg subviral particles are still unknown. As a consequence, it is unclear if and how HBsAg is prevented from accumulating inside hepatocytes, which may result in liver damage and increased risk of HCC.70

Summary  Although more than 30 years have elapsed since the introduction of IFN-α for the treatment of chronic hepatitis
D, this drug remains the only option currently available of proven benefit. However, treatment is unsatisfactory. The rate of virologic response to pegylated IFN-α rarely exceeds 25%, highlighting the need for more effective drugs against HDV, a defective RNA virus that, in contrast to HBV and HCV, lacks viral enzymes that can be targeted by specific inhibitors. The serious nature of hepatitis D and the uniqueness of HDV make this disease a difficult target for antiviral therapy. Over the past several years, new insights from the HDV life cycle have paved the way for the development of novel antiviral agents. Three new drugs are currently under clinical evaluation for the treatment of chronic hepatitis D (Myrcludex B, lonafarnib, and REP 2139), all of which act on HDV by interfering with HBsAg, the critical helper function provided by HBV. However, despite a significant reduction of HDV and HBV replication by all 3 inhibitors, a fast and sharp reduction of HBsAg levels was only observed in patients treated with NAPs. Several aspects of the mechanisms of action of these drugs remain unclear, and, as a consequence, concerns regarding safety remain. Major concerns are related to bile-acid transport interference and to the possible accumulation of HBV or HDV proteins inside hepatocytes. Long-term treatment is likely required, preferably in combination with other inhibitors. Ongoing studies are testing the combination of these novel drugs with pegylated IFN-α, although the side effects of IFN-α limit its use in HDV cirrhosis.

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