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Optimizing Lonafarnib Treatment for the Management of Chronic Delta Hepatitis: The LOWR HDV-1 Study

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In a proof-of-concept (POC) study, the oral prenylation inhibitor, lonafarnib (LNF), decreased hepatitis D virus (HDV) RNA during 4 weeks of treatment. Here, we explored optimal LNF regimens. Fifteen patients (five groups; 3 per group) completed dosing as follows: (1) LNF 200 mg twice-daily (BID; 12 weeks); (2) LNF 300 mg BID (12 weeks); (3) LNF 100 mg thrice-daily (5 weeks); (4) LNF 100 mg BID + pegylated interferon alfa (PEG-IFNα) 180 μg once-weekly (QW; 8 weeks); and (5) LNF 100 mg BID + ritonavir (RTV) 100 mg once-daily (QD; 8 weeks). Tolerability and efficacy were assessed. Higher LNF monotherapy doses had greater decreases in HDV viral load than achieved in the original POC study. However, this was associated with increased gastrointestinal adverse events. Addition of RTV 100 mg QD to a LNF 100 mg BID regimen yielded better antiviral responses than LNF 300 mg BID monotherapy and with less side effects. A similar improvement was observed with LNF 100 mg BID + PEG-IFNα 180 μg QW. Two of 6 patients who received 12 weeks of LNF experienced transient posttreatment alanine aminotransferase (ALT) increases resulting in HDV-RNA negativity and ALT normalization. *Conclusion*: The cytochrome P450 3A4 inhibitor, RTV, allows a lower LNF dose to be used while achieving higher levels of postabsorption LNF, yielding better antiviral responses and tolerability. In addition, combining LNF with PEG-IFNα achieved more substantial and rapid HDV-RNA reduction, compared to historical responses with PEG-IFNα alone. Twelve weeks of LNF can result in posttreatment HDV-RNA negativity in some patients, which we speculate results from restoring favorable immune responses. These results support further development of LNF with RTV boosting and exploration of the combination of LNF with PEG-IFN. (HEPATOLOGY 2018;67:1224-1236)

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Chronic delta hepatitis (CDH) infection leads to the most severe form of chronic viral hepatitis.[1] The only treatment with proven efficacy consists of the use of conventional or pegylated interferon (IFN) alpha (PEG-IFNα).[2] Treatment response is observed in around 25%-40% after 1 year of treatment[3-6] and extending treatment to 2 years does not appear to increase response rates.[7-9] Still, there are data to suggest that IFN may need to be given for an extended duration of time,[10,11] which is consistent with *in vitro* studies that appear to lend support for longer treatment

*Abbreviations: AE, adverse event; ALT, alanine aminotransferase; anti-HBe, HBe antibody; BID, twice-daily; CDH, chronic delta hepatitis; CTCAE, common terminology criteria for adverse events; CYP3A4, cytochrome P450 3A4; GC-MS, gas chromotography/mass spectroscopy; GI, gastrointestinal; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; IFN, interferon; LC-MS/MS, liquid chromatography/tandem mass spectroscopy; LLOQ, lower limit of quantification; LNF, lonafarnib; LOWR HDV-1, **LO**nafarnib **W**ith and without **R**itonavir in **HDV – 1**; NA, nucleos(t)ide analog; PEG-IFNα, pegylated interferon alpha; PO, per oral; POC, proof-of-concept; QD, once-daily; QW, once-weekly; RTV, ritonavir; TID, thrice-daily.*

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duration.[12,13] Viral kinetic studies also support the concept that CDH responds slower to IFN compared to hepatitis B (HBV) or hepatitis C virus (HCV).[14]

IFNα must be given as subcutaneous injections and is associated with a plethora of side effects. For patients not responding to IFNα, an alternative treatment does not exist. Hence, new treatment options are an urgent need in CDH. In this context, drugs targeting the hepatitis D virus (HDV) life cycle need to be explored. One such target is the virion assembly step in the hepatocyte cytoplasm, where the nascent HDV nucleoprotein complex is enveloped by hepatitis B surface antigen (HBsAg). This step involves the attachment of a 15-carbon prenyl group, farnesyl, to the large delta antigen, a reaction catalyzed by farnesyl transferase.[15] Prenylation inhibitors have been shown to specifically abolish HDV-like particle production *in vitro* and *in vivo*.[16,17] Recently, the first human data have been reported.[18] In that proof-of-concept (POC) study, the prenylation inhibitor, lonafarnib (LNF), dose dependently decreased HDV-RNA levels during 4 weeks of treatment, achieving 0.74 and 1.60 log reductions in HDV RNA with LNF 100 mg per oral (PO) twice-daily (BID) and LNF 200 mg PO BID, respectively. The aim of the current study was to explore additional dosing regimens capable of increasing the reduction in HDV viral load with LNF-based treatment, and assessing the safety and tolerability of LNF for up to 12 weeks.

Patients and Methods

STUDY DESIGN

This was a single-center, phase 2 pilot study called LOWR HDV-1 (**LO**nafarnib **W**ith and without **R**itonavir in **HDV – 1**) performed in the Department of Gastroenterology of the University of Ankara Medical School (Ankara, Turkey). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and the study methods, procedures, and materials were approved by the Ankara University Ethics Committee. Written informed consent was obtained from all patients. LOWR HDV-1 was a seven-arm, parallel, open-label clinical trial designed to dose 21 patients across seven groups (3 patients per group) as follows: group 1: LNF 200 mg BID for 12 weeks; group 2: LNF 300 mg BID for 12 weeks; group 3: LNF 100 mg thrice-daily (TID) for 8 weeks; group 4: LNF 100 mg BID + ritonavir (RTV) 100 mg PO once-daily (QD) for 8 weeks; group 5: LNF 100 mg BID + PEG-IFNα 180 μg once-weekly (QW) for 8 weeks; group 6: LNF 200 mg BID + PEG-IFNα 180 μg QW for 8 weeks; and group 7: LNF 300 mg BID + PEG-IFNα 180 μg QW for 8 weeks. The main reasons for selecting these treatment regimens are summarized below. We first wanted to assess if higher or more frequent dosing of LNF would be more efficacious, and if extending treatment duration to 12 weeks would lead to further HDV-RNA declines than previously observed with 4 weeks of dosing. Second, because LNF and PEG-IFNα have different mechanisms of action, we wished to test the hypothesis that addition of PEG-IFNα to LNF would increase efficacy over that previously observed with the same dose of LNF monotherapy. Finally, because already in the POC study LNF was associated with gastrointestinal (GI) adverse events (AEs), we sought to test the hypothesis that inhibiting the metabolism of postabsorbed LNF would enable greater LNF serum concentrations and efficacy while exposing the GI tract to lower LNF doses, resulting in better GI tolerability. We thus treated a cohort of patients with the lower LNF dose used in the POC study in combination with RTV—a potent inhibitor of cytochrome P450 3A4 (CYP3A4), which is the predominant mediator of

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LNF's metabolism.[19] The main objective of the study was to assess tolerability and viral response of different doses of LNF either as monotherapy or in combination therapy with RTV or PEG-IFNα. Viral response was defined as HDV-RNA decline between baseline and end of treatment. Blood sampling was done on days 1, 2, 3, 7, 14, and 28 and then every 4 weeks on-treatment for assessment of biochemical and virological parameters. Posttreatment follow-up consisted of one visit 1 month after treatment discontinuation, but patients continued to be followed at 1- to 3-month intervals thereafter. AEs were recorded at every visit and assessed for severity using the common terminology criteria for adverse events (CTCAE) 4.0. A medical monitor provided by the sponsor of the study was responsible for monitoring safety events. The study is registered at Clinicaltrials.gov under NCT02430181.

PATIENT POPULATION

Patients aged 18-65 years with documented HBsAg, antidelta positivity for at least 6 months, and compensated liver disease were eligible after evaluation for other forms of chronic liver disease. Patients were required to have detectable HDV-RNA levels at screening, platelet counts $\geq$100,000/mm^3, absolute neutrophil count $\geq$1,500 /mm^3, and international normalized ratio $<$1.5. All patients had an imaging study at screening, and patients with hepatocellular carcinoma or any significant disease that may have affected the conduct of the study were excluded. Furthermore, patients with a body mass index of $>$30 kg/m^2, patients coinfected with human immunodeficiency virus or HCV as documented by hepatitis C viremia by PCR and patients reporting substance abuse in the last 6 months were excluded. Patients with a history of excessive alcohol intake ($>$20 g/day for females or $>$30 g/day for males) in the last 2 years were also excluded.

Detailed inclusion and exclusion criteria are provided in Supporting Table S1.

VIRAL LOAD DETERMINATIONS

Quantitative HDV RNA was measured as described.[18] This assay has a lower limit of quantification (LLOQ) of 70 IU/mL and lower limit of detection of 50 IU/mL, and the assay was standardized against the World Health Organization HDV-RNA standard. Serum HBV-DNA level was quantified by the CobasTaqMan HBV test (Roche Molecular Systems, Inc, Mannheim, Germany). Quantitative HDV-RNA viral load determinations for the long-term follow-up of the 2 patients who experienced transient posttreatment alanine aminotransferase (ALT) increases resulting in HDV-RNA negativity and ALT normalization were performed locally with an in-house PCR assay as described.[20] This assay has an LLOQ of 6,170 IU/mL. HBsAg was quantified by the Architect HBsAg assay (Abbott Diagnostics, Germany) according to the manufacturer's instructions. Qualitative hepatitis serologies, including HBsAg, HBs antibody, hepatitis B e antigen (HBeAg), and HBe antibody (anti-HBe) were determined by a microparticle enzyme immunoassay method (Abbott Laboratories, North Chicago, IL), and anti-HDV was determined by an enzyme immunoassay (Abbott Laboratories).

MEASUREMENT OF DRUG CONCENTRATIONS

The concentrations of LNF and RTV in human serum were determined using liquid chromatography/tandem mass spectroscopy (LC-MS/MS) methods. LNF (LNF-D_6 as internal standard) + RRV (RTV-D_6 as internal standard) were extracted from the samples using protein precipitation. The assay range used for analysis of LNF and RTV was 1-2,500 ng/mL. For the extraction of controls, quality-control standards, and study samples, protein precipitation of sample aliquots (25 μL) was initiated by adding internal standard in acetonitrile (150 μL containing RTV-D_6-IS [10 ng/mL] for sample analysis). After vortexing for 2 minutes, the samples were centrifuged at 3,000 rpm for 10 minutes. A TomTec Quadra4 was used to simultaneously transfer 125 μL of the resulting supernatant from each well into a clean 96-well plate, and the plate was centrifuged for 1 minute at 3,000 rpm. The processed samples were then directly injected (10 μL) onto the LC-MS/MS for analysis.

The LC-MS/MS system consisted of a Triple Quadrupole MS (API 4000) mass spectrometer equipped with a Shimadzu Nexera UPLC system. Analytes were eluted from an Acquity UPLC CSH C18 column (2.10 × 50 mm, 1.7 μm; Waters) using gradient LC conditions consisting of water/formic acid (100:0.1, v/v) as mobile phase A and methanol/formic acid (100:0.1, v/v) as mobile phase B. LNF and RTV (RTV-D_6 as internal standard) were ionized using a TIS (Turbo Ion Spray) ion source in the positive mode, and data from multiple-reaction monitoring of mass transition pairs were acquired. Peak area ratios of LNF and RTV to internal standard were used to

quantify samples. The LNF and RTV calibration curves were linear with $1/x^2$ weighting over the assay range of 1-2,500 ng/mL. Samples outside of the linear range were diluted appropriately and reassayed.

RESISTANCE TESTING OF PATIENT HDV ISOLATES

RNA was extracted, reverse-transcribed followed by RT-PCR, and subjected to sequencing, as described.[18] As before, phylogenetic analysis was performed using Neighbor-Joining trees to verify within-patient sequence identity and to exclude PCR contamination or sample mix-up. Sequences from each time point from each patient were aligned to a reference Delta antigen sequence. Differences from reference between time points of each patient were compared to assess the presence of any amino acid changes that occurred during treatment.

STATISTICAL ANALYSIS

Data were analyzed using SPSS software (version 21; SPSS, Inc., Chicago, IL).

Continuous variables are presented by their mean values ± SD or as median values and range. Comparisons were made using the paired or unpaired *t* test or by Mann-Whitney U tests for categorical variables, where appropriate. Correlation analysis between serum HDV-RNA levels and serum LNF concentrations was performed using Spearman's correlation analysis. A *P* value of less than 0.05 was considered statistically significant.

Results

PATIENT CHARACTERISTICS

Twenty patients (14 male/6 female) were enrolled in the study, with 1 patient from group 3 re-enrolling in group 7 following a 6-month washout period (see Fig. 1 for study flow chart). Baseline characteristics of patients are presented in Table 1. Five patients who received higher doses of LNF (200 and 300 mg BID) with PEG-IFNα (groups 6 and 7) discontinued treatment within 4 weeks because of poor tolerance (see below section on safety and tolerability for details). Baseline characteristics of groups 6 and 7 were similar to the baseline characteristics of groups 1-5. Only 2 patients from groups 6 and 7 finished 4 of the planned 8 weeks of therapy.

Patients in these groups stopped treatment before their viral load and pharmacokinetics values could be tested; therefore, the latter data are not available. Of the 20 patients, 7 (35%) had cirrhosis at baseline. Patients were classified as having cirrhosis based on liver biopsy or on clinical grounds such as imaging studies displaying irregular liver margins or a nodular liver with splenomegaly or esophageal varices on endoscopy. All 7 patients were Child-Pugh class A, and 6 were among those in groups 1 to 5.

Of the 15 patients who completed dosing in groups 1 through 5, 3 patients had HBeAg-positive CDH, and the remaining 12 displayed the typical HBeAg negative anti-HBe-positive serology. All patients had compensated liver disease, had detectable HDV-RNA levels, and the majority had received IFN treatment in the past; there were only 3 patients who were treatment-naïve. Quantitative HBsAg levels ranged from 2.75 to 4.36 $\log_{10}$ IU/mL. Although HBV-DNA levels ranged from 1.3 to 5.77 $\log_{10}$ IU/mL, there was only 1 patient with an HBV-DNA level exceeding 5 $\log_{10}$ IU/mL, and this patient's HDV RNA was above 6 $\log_{10}$ IU/mL. Hence, HDV was the dominant virus in all patients. None of the patients had concomitant nucleos(t)ide analog use during the study.

AGGREGATE RESPONSES TO LONAFARNIB THERAPY

Lonafarnib, whether as monotherapy or as combination treatment, led to HDV-RNA viral load decline in every patient. After 4 weeks of treatment, mean HDV viral loads declined from the baseline value of 6.51 ± 1.22 $\log_{10}$IU/mL to 4.70 ± 1.22 ($n = 15$; $P < 0.0001$). This was associated with a decline in mean ALT levels from 107 ± 72 U/L at baseline to 56 ± 32 at week 4 ($n = 15$; $P = 0.0058$). HBV-DNA levels increased slightly: 2.65 ± 1.26 $\log_{10}$ IU/mL versus 3.12 ± 1.54 ($n = 14$; $P = 0.029$). ALT, log-transformed HDV RNA, and HBV-DNA levels displayed a homogenous distribution, and results would not have changed if we had used median instead of mean levels. HBsAg levels were not affected (data not shown). Treatment responses in detail are provided below in separate sections.

SAFETY AND TOLERABILITY

All patients in different treatment regimens reported GI AEs consisting of anorexia, nausea with or without vomiting, diarrhea, and weight loss. Grade of these

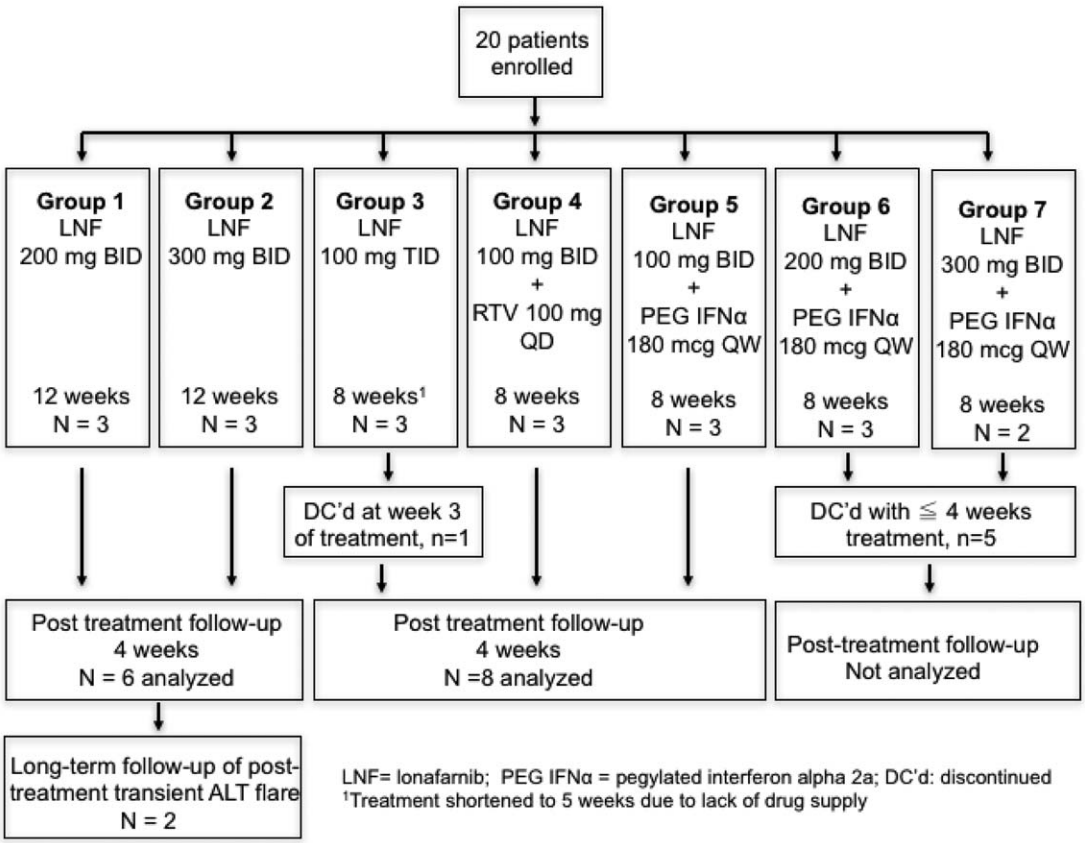

FIG. 1. LOWR HDV-1 study flow diagram.

side effects were dependent on the treatment regimen and have been provided in Table 2. Reported AEs were based on the highest toxicity grade observed at least once during treatment. Most of the AEs were GI AEs at the level of grade 1 or 2 according to the CTCAE. LNF monotherapy with 200 mg BID and 300 mg BID for 12 weeks was associated with mostly grade 2 AEs, including weight loss. After 12 weeks of treatment, patients lost a mean of 8.3 kg (range, 4-10). Overall, in the 12 patients who received 8 weeks of treatment as monotherapy with LNF or LNF in combination with either RTN or PEG-IFNα, median weight loss was 5 kg (range, 3-10). In contrast, LNF 100 mg BID in combination with RTV 100 mg QD for 8 weeks was tolerated and mostly associated with grade 1 toxicity.

LNF in combination with PEG-IFNα was tested at three different doses of LNF, namely, 100, 200, and 300 mg BID, respectively. Whereas LNF 100 mg BID in combination with PEG-IFNα for 8 weeks was reasonably well tolerated (Table 2), the higher LNF doses with PEG-IFNα were not tolerated. They were associated mostly with grade 2 and even with grade 3 toxicities. In addition, the frequency of these AEs was greater in these higher dose LNF/PEG-IFNα groups. More importantly, of the 5 patients in these high-dose LNF/PEG-IFN groups, 2 discontinued treatment within 4 weeks of treatment because of AEs. The other 3 patients discontinued treatment even earlier, 1 after 3 weeks, 1 after 1 week, and 1 after 3 days on treatment (Table 2). Besides the above-mentioned AEs, 1 patient reported headache and the same patient also developed renal colic attributed to passing urinary stones during treatment. These AEs were not considered causally related to treatment whereas all GI AEs were considered AEs secondary to treatment with LNF. RTV may have contributed to nausea and vomiting.

Overall, adherence to treatment appeared to be very good based on the report we gathered from patients at every visit and on the pill counts after each 4 weeks of treatment.

TABLE 1. Patient Characteristics at Time of Enrollment

Parameter	All N = 20	Group 1 LNF 200 mg BID N = 3	Group 2 LNF 300 mg BID N = 3	Group 3 LNF 100 mg TID N = 3	Group 4 LNF 100 mg BID + RTV 100 mg QD N = 3	Group 5 LNF 100 mg BID + PEG IFNα 180 μg QW N = 3	Group 6 LNF 200 mg BID + PEG IFNα 180 μg QW N = 3	Group 7 LNF 300 mg BID + PEG IFNα 180 μg QW N = 2
Age	49 (22-63)	59 (57, 63, 58)	89 (36, 38, 46)	46 (40, 48, 51)	51 (43, 49, 61)	41 (22, 52, 49)	46 (35, 53, 50)	49 (49, 48)
Sex	13 male/7 female	F, M, F	M, M, F	F, F, M	M, M, M	M, F, M	M, M, M	M, F
HDV RNA ($\log_{10}$ IU/mL)	6.05 (3.94-7.07)	5.85 (6.91, 4.20, 6.44)	5.19 (3.94, 5.84, 5.80)	5.90 (6.02, 6.96, 4.73)	6.56 (6.08, 6.54, 7.07)	5.36 (5.91, 5.79, 4.37)	6.51 (6.74, 6.78, 6.01)	6.53 (6.09, 6.96)
HBV DNA ($\log_{10}$ IU/mL)	2.42 (1.30-5.77)	2.89 (1.81, 4.48, 2.38)	3.82 (2.18, 5.77, 3.52)	2.04 (0, 3.23, 2.90)	2.86 (2.45, 4.23, 1.91)	0.8 (2.40, 0, 0)	2.88 (5.58, 1.75, 1.30)	3.22 (3.20, 3.23)
Quantitative HBsAg ($\log_{10}$ IU/mL)	3.87 (2.75-4.36)	3.81 (3.65, 3.90, 3.87)	4.46 (2.75, 4.10, 4.02)	3.97 (4.36, 4.00, 3.54)	4.05 (3.79, 4.07, 4.28)	3.46 (3.84, 2.80, 3.73)	ND	ND
ALT (U/L)	72 (23-258)	130 (49, 84, 258)	73 (50, 50, 119)	43 (23, 39, 67)	125 (222, 72, 82)	166 (180, 168, 150)	82 (39, 135, 72)	47 (33, 60)
Albumin (g/dL)	4.0 (3.1-4.6)	3.5 (3.1, 4.1, 3.3)	3.8 (4.2, 3.9, 3.3)	3.9 (3.6, 4.1, 3.9)	4.1 (4.4, 4.0, 4.0)	3.9 (4.0, 3.8, 4.0)	4.1 (4.3, 3.5, 4.6)	4.4 (4.2, 4.5)
GGT (U/L)	26.5 (11-154)	46 (19, 23, 96)	28 (27, 33, 24)	20 (11, 23, 25)	119 (52, 152, 154)	48 (47, 31, 65)	47 (18, 97, 26)	24 (24, 23)
Platelets ($\times 10^3$/mm^3)	138 (80-292)	93 (126, N/A, 59)	199 (145, 292, 159)	171 (76, 233, 205)	104 (80, 102, 129)	98 (90, 136, 67)	163 (160, 130, 198)	194 (140, 247)
Previous IFN treatment	14/20 patients	2/3	2/3	2/3	2/3	2/3	2/3	2/2

Mean and individual patient values for each parameter are listed. For the All patient column, data are given as median and range.
Abbreviations: GGT, gamma-glutamyl transferase; ND, not determined.

TABLE 2. Gastrointestinal AEs Observed With Different Treatment Regimens

	Group 1 N = 3				Group 2 N = 3				Group 3 N = 3				Group 4 N = 3				Group 5 N = 3				Group 6 N = 3				Group 7 N = 2			
	LNF 200 mg BID				LNF 300 mg BID				LNF 100 mg TID				LNF 100 mg BID + RTV 100 mg QD				LNF 100 mg BID + PEG IFN α 180 mcg QW				LNF 200 mg BID + PEG IFN α 180 mcg QW				LNF 300 mg BID + PEG IFN α 180 mcg QW			
Grade	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Nausea	1	2			1	2				2			1	2			2	1			1	3				1		
Diarrhea	1	2			1	2			1		1		2	1			2	1				1	1		1			
Fatigue	1	1	1		1	1				1	1		1	1				1					3				2	
Wt Loss		3			1	2			2					2				1				1				1		
Anorexia						1			1	1				1			1	1				1				2		
Vomiting	1	1			3					2				1								2	1					
Discontinuations		0				0				1				0				0				3				2		

Highest level of each indicated adverse event experienced in the respective treatment groups is recorded according to common terminology criteria for adverse events (CTCAE). The number of patients experiencing each adverse event is indicated.

TABLE 3. Patient Data During the Course of Treatment

Parameter		Group 1 LNF 200 mg BID	Group 2 LNF 300 mg BID	Group 3 LNF 100 mg TID	Group 4 LNF 100 mg BID + RTV 100 mg QD	Group 5 LNF 100 mg BID + PEG IFNα a 180 μg QW
Treatment duration		12 Weeks N = 3	12 Weeks N = 3	5 Weeks N = 3	8 Weeks N = 3	8 Weeks N = 3
Log HDV RNA (IU/mL)	Baseline	7.68 (8.78, 6.06, 8.19)	7.05 (5.80, 7.70, 7.66)	5.90 (6.02, 6.96, 4.73)	6.56 (6.08, 6.54, 7.07)	5.36 (5.91, 5.79, 4.37)
	Week 4	6.05 (6.93, 5.00, 6.21)	5.06 (3.85, 5.73, 5.59)	4.59 (5.50, 5.35, 3.38)	4.14 (3.55, 4.10, 4.74)	3.54 (3.84, 4.48, 2.31)
	Week 8	6.69 (7.66, 6.07, 6.35)	5.1 (4.06, 5.75, 5.49)	N/A	3.37 (2.51, 3.25, 4.34)	2.39 (2.26, 3.92, < 1)
	Week 12	7.71 (8.21, 7.89, 7.02)	5.27 (4.20, 5.42, 6.20)	N/A	5.36 (4.67, 5.24, 6.16)	3.51 (3.51, 3.98, 3.04)
Log HBV DNA (IU/mL)	Baseline	2.89 (1.81, 4.48, 2.38)	3.82 (2.18, 5.77, 3.52)	2.07 (0.10, 3.20, 2.90)	2.86 (2.45, 4.23, 1.91)	0.87 (2.40, 0.10, 0.10)
	Week 4	1.86 (0.10, N/A, 3.61)	4.79 (3.82, 6.15, 4.40)	2.61 (1.48, 3.45, 2.91)	3.81 (3.53, 5.53, 2.38)	1.30 (2.04, 0.10, 1.76)
	Week 8	4.55 (1.54, 6.68, 5.43)	5.55 (4.87, 6.53, 5.26)	N/A	4.23 (4.90, 5.38, 2.40)	1.19 (1.85, 0.10, 1.63)
	Week 12	5.62 (2.0, 7.93, 6.92)	5.95 (5.57, 6.34, 5.95)	N/A	3.76 (3.15, 5.85, 2.18)	0.56 (1.38, 0.10, 0.10)
Log HBsAg (IU/mL)	Baseline	3.71 (3.65, 3.90, 3.57)	3.61 (2.72, 4.11, 4.02)	3.97 (4.36, 4.00, 3.54)	4.05 (3.79, 4.07, 4.28)	3.46 (3.84, 2.80, 3.73)
	Week 4	3.73 (N/A, 3.91, 3.54)	3.69 (2.80, 4.13, 4.13)	3.95 (4.36, 3.95, 3.54)	3.97 (3.64, 3.95, 4.32)	3.62 (4.01, 2.99, 3.86)
	Week 8	3.83 (4.11, 3.89, 3.48)	3.72 (2.84, 4.16, 4.16)	N/A	3.91 (3.57, 3.88, 4.29)	3.59 (3.97, 2.88, 3.91)
	Week 12	3.82 (4.07, 3.99, 3.41)	3.48 (2.80, 4.15, N/A)	N/A	3.99 (3.65, 4.04, 4.26)	3.56 (3.92, 2.84, 3.93)

Mean and individual values for the indicated parameters are provided for each treatment group.
Abbreviation: N/A, not available.

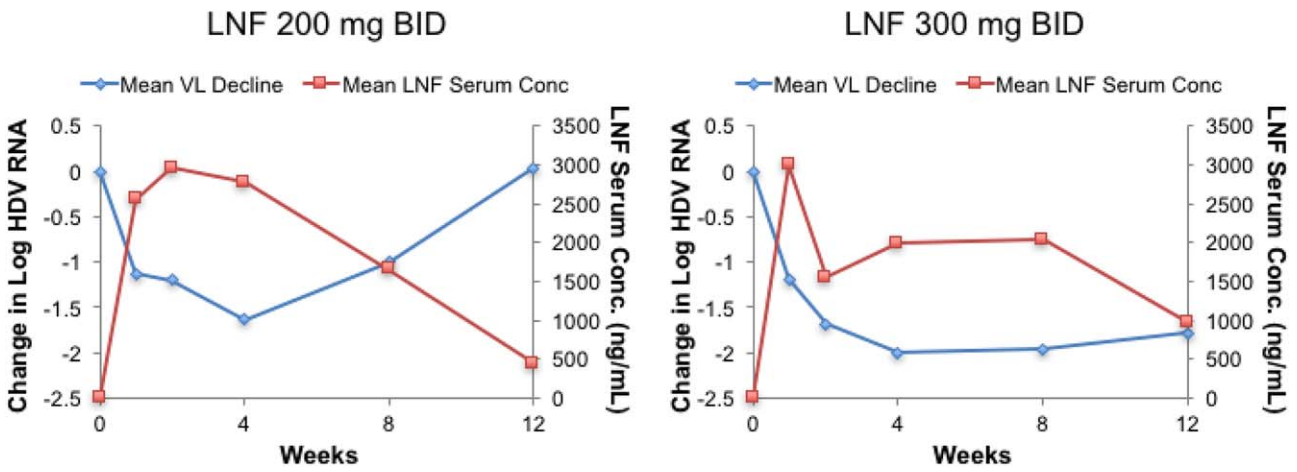

FIG. 2. Correlation of mean antiviral response with serum LNF levels. Left panel, group 1 (LNF 200 mg PO BID) patients; right panel, group 2 (LNF 300 mg PO BID) patients.

LNF MONOTHERAPY REGIMENS (GROUPS 1-3)

We hypothesized that more frequent or higher doses of LNF as well as longer dosing durations could achieve greater reductions in HDV RNA than previously observed.[18] To test this hypothesis, we enrolled 3 patients each into the following dosing groups: group 1, LNF 200 mg PO BID for 12 weeks; group 2, LNF 300 mg PO BID for 12 weeks; and group 3, LNF 100 mg PO TID for 8 weeks. In this latter group, however, treatment duration was limited to 5 weeks because of unforeseen circumstances related to drug supply.

After four weeks of treatment, group 1 patients experienced a 1.6 log reduction in HDV viral load and group 2 patients exhibited a 2.0 log reduction in HDV viral load. Group 3 patients had a 1.2 log reduction at 4 weeks, a response that did not appear to offer a significant additional benefit compared to group 1 (Table 3; for complete data sets on all patients, see Supporting Table S2).

Antiviral responses to longer LNF treatment in group 1 subjects revealed mean log viral load declines of –1.6, –1.0, and 0, at weeks 4, 8, and 12, respectively. Mean log viral load declines in group 2 subjects were –2.0, –2.0, and –1.8, at weeks 4, 8, and 12, respectively. The corresponding AEs included anorexia, nausea, diarrhea, and weight loss of grade 1 and 2 according to the CTCAE criteria (Table 2), and LNF levels generally declined after 4 weeks (see Discussion for possible explanation). In general, the decline in LNF serum concentrations inversely correlated with the HDV viral loads (Fig. 2). Individual patient graphs of HDV-RNA and LNF serum levels are shown in Supporting Fig. S1.

Although the rise in HDV viral load in individuals on LNF treatment may be explained by the above observed decreases in LNF serum concentration, it was important to rule out the appearance of any candidate viral resistance mutations. As such, HDV viral RNA was extracted from baseline, end of treatment, and 4 weeks posttreatment from each patient completing 12 weeks of therapy (groups 1 and 2) and subjected to sequencing. No changes in HDV sequence from baseline were observed in any patient at any time point (Supporting Table S3).

LNF COMBINATION REGIMENS (GROUPS 4-7)

The correlation between increased LNF serum concentration and viral load reduction observed in a past study[18] suggested that achieving higher postabsorption levels of LNF should result in still greater antiviral activity. Achieving such higher doses by simply increasing the dose of LNF monotherapy, however, appeared to be limited by tolerability. We hypothesized that inhibiting the metabolism of postabsorbed LNF could lead to greater LNF exposures with lower LNF doses delivered to the GI tract and hence

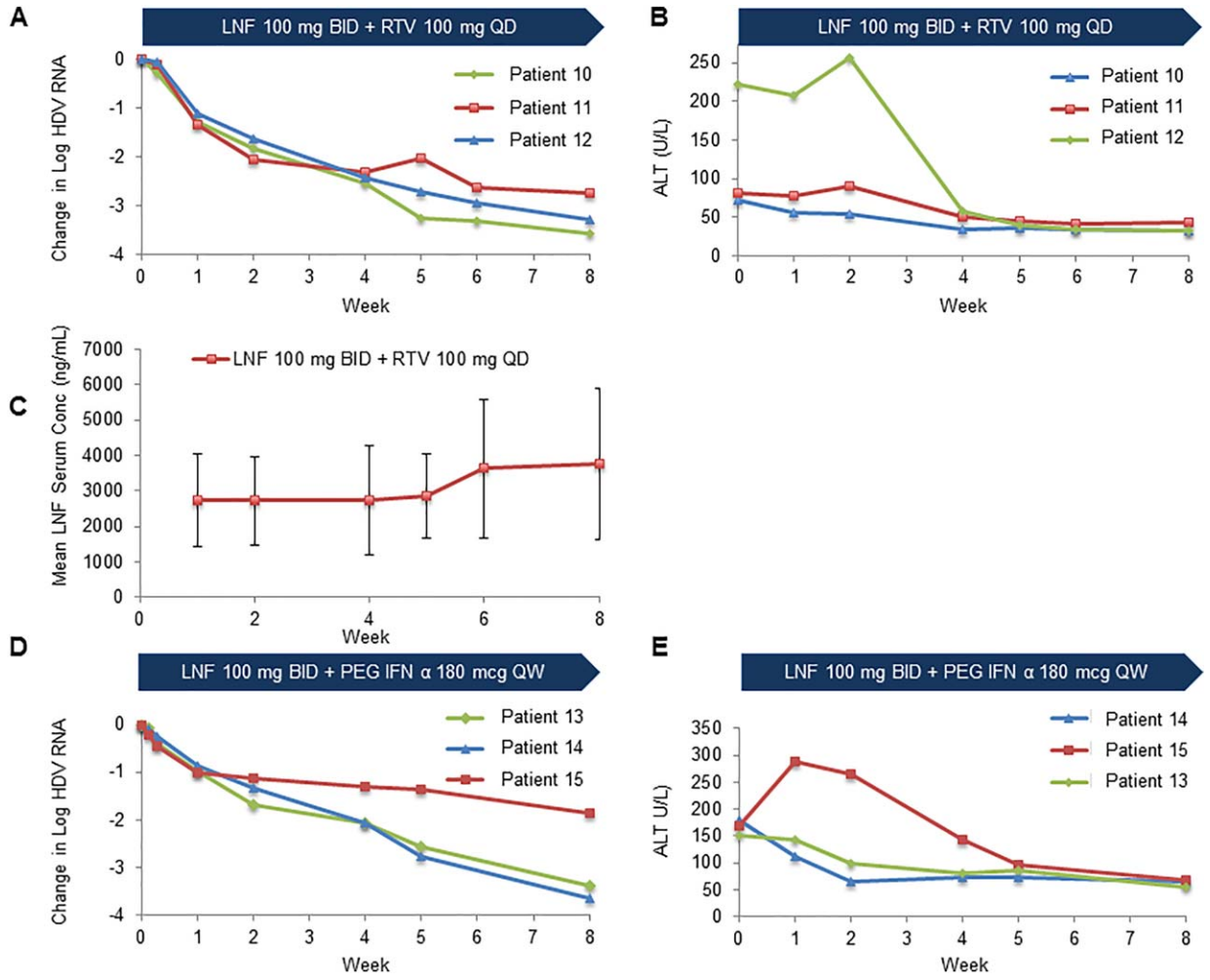

FIG. 3. Inhibiting LNF's metabolism with RTV is associated with greater efficacy attributed to the resulting higher serum LNF levels. (A) HDV viral load (VL) reductions observed with LNF 100 mg BID + RTV 100 mg QD. (B) Corresponding improvement in ALT levels. (C) Mean weekly LNF serum concentrations measured in samples from patients receiving LNF 100 mg BID + RTV 100 mg QD. (D) Addition of LNF to PEG-IFNα is associated with improved efficacy. Effect of LNF 10 0mg BID and PEG-IFNα combination treatment on serum HDV VL. (E) Corresponding ALT levels.

maximize antiviral efficacy with better tolerability. To test this hypothesis, we treated a cohort of patients with LNF 100 mg BID in combination with RTV—a potent inhibitor of CYP3A4, which is the predominant mediator of LNF's metabolism.[20] Addition of RTV 100 mg QD to LNF 100 mg BID resulted in substantial suppression of HDV RNA (Fig. 3A). Indeed, a 2.4 log reduction in HDV RNA was observed after just 4 weeks of treatment. Extending treatment to 8 weeks led to a mean reduction in HDV RNA of 3.2 logs (Fig. 3A). Importantly, these reductions in viral load were accompanied by normalization of ALT levels (Fig. 3B). LNF serum concentrations showed a linear correlation with HDV-RNA declines for all regimens during the first 4 weeks of treatment (Supporting Fig. S2; r = 0.685; $P = 0.006$). The measured mean trough serum LNF levels during the 8 weeks of RTV-boosted LNF 100 mg PO BID treatment were between 2,800 and 3,800 ng/mL (Fig. 3C). The higher efficacy of the RTV-boosted regimen was attributed to this predicted higher level in postabsorption LNF levels.

Similar increases in antiviral efficacy were observed when LNF 100 mg PO BID was combined with standard doses of PEG-IFNα (Fig. 3D). This was also associated with normalization of ALT levels (Fig. 3E). When compared to the monotherapy regimens, both

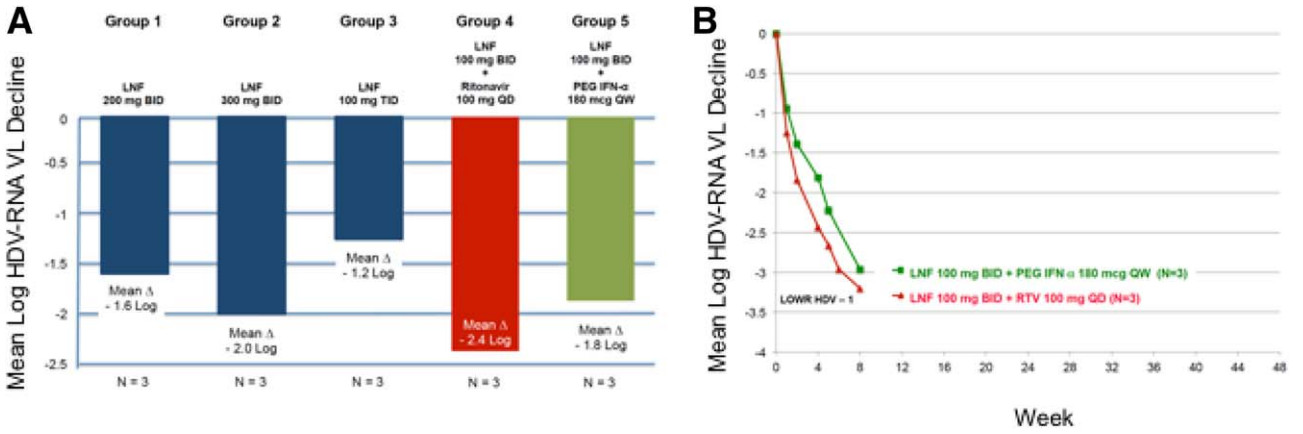

FIG. 4. HDV viral load declines on LNF monotherapy and combination regimens of LOWR HDV-1. (A) Four-week HDV viral load (VL) declines observed with the indicated regimens of LNF + RTV and LNF + PEG-IFNα versus LNF monotherapy regimens in the current LOWR HDV-1 study. (B) Rates of decline and extent of HDV-RNA reduction observed in patients receiving the indicated combination regimens of LNF + RTV and LNF + PEG-IFNα.

100 mg LNF-based combination regimens exhibited the greatest drops in HDV RNA after 4 weeks of therapy (Fig. 4A). As mentioned above, however, combining higher doses of LNF monotherapy (e.g., 200 and 300 mg PO BID) with standard doses of PEG-IFNα (groups 6 and 7) was not well tolerated, resulting in discontinuations in all patients. Remarkably, the viral kinetics on both 100 mg LNF-based combination regimens—with QD 100 mg RTV or QW PEG-IFNα—exhibited rapid declines in HDV-RNA serum levels of ~3 logs by week 8 of treatment (Fig. 4B).

Both 100 mg LNF-based combination regimens—with QD 100 mg RTV or QW PEG-IFNα—were better tolerated than the LNF monotherapy regimens, and GI side effects were mostly at grade 1 level according to CTCAE criteria, although the PEG-IFNα combination patients had fatigue of grade 2 toxicity, which may have been related to PEG-IFNα therapy.

POSTTREATMENT FOLLOW-UP

Except for 2 patients, HDV-RNA, ALT and HBV-DNA levels returned to pretreatment levels within 4-24 weeks posttreatment, occurring within 12 weeks for the majority of these patients. Two of the 6 patients that received 12 weeks of LNF demonstrated a different course. One patient had received LNF 200 mg BID and the other had received LNF 300 mg LNF BID. In these 2 patients, ALT levels at posttreatment weeks 4 and 8 were 10.5 and 2.2 × the baseline ALT, respectively. During the 12 weeks of LNF treatment, these 2 patients' HDV-RNA levels had initially rapidly declined, followed by subsequent increases, and serum HBV DNA increased by more than 3 logs over baseline levels (from 2.18 to 5.57 and from 4.48 to 7.93 $\log_{10}$ IU/mL). In both patients, this posttreatment rise in ALT was closely associated with a decline of HDV RNA to below the level of detection. HDV levels then fluctuated between undetectable and around the limit of quantification. ALT levels also displayed a gradual decrease to ultimately normal levels. Although the posttreatment reduction in HDV RNA was more profound, HBV-DNA levels also decreased posttreatment and remained at or below pretreatment levels without administration of a NA, nucleos(t)ide analog (NA; Fig. 5A,B). Thus, in 2 patients, HDV RNA and ALT returned to undetectable and normal levels, respectively, posttreatment, after this posttherapy flare. In both patients, this posttherapy flare did not lead to hepatic decompensation. Serum bilirubin levels and prothrombin time did not change, although in 1 of the patients serum albumin dropped from 4.0 to 3.4 and recovered back within 2 months.

Discussion

In this article, we describe our initial efforts to explore optimal LNF treatment regimens. Despite the low number of patients and different patient populations, a remarkably consistent result was obtained with

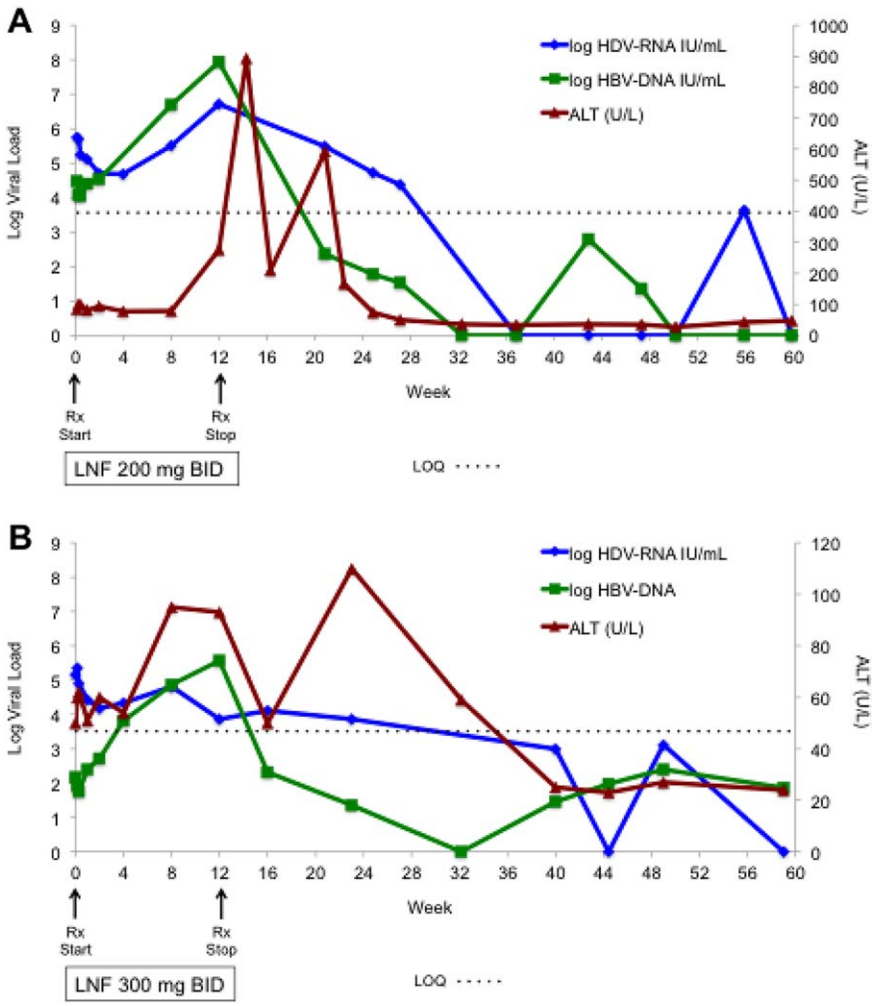

FIG. 5. Transient post-treatment ALT increases resulting in HDV RNA negativity and ALT normalization. Two patients developed a posttreatment biochemical flare with subsequent immune control of HBV and HDV after 12 weeks of LNF 200 mg BID (A), or 12 weeks of LNF 300 mg BID (B).

the same regimen (e.g., group 1, LNF 200 mg PO BID) when used here and in the original NIH POC study.[18] It appears that higher doses of LNF monotherapy had greater initial decreases in HDV viral load, yet this came at the cost of increased GI AEs. Indeed, excessive diarrhea associated with higher monotherapy doses might be responsible for decreasing the amount of absorbed LNF, resulting in suboptimal antiviral responses, as was observed with treatment beyond 4 weeks.

Addition of RTV to the lower LNF 100 mg PO BID dosing regimen, however, yielded better antiviral responses than LNF 300 mg PO BID (Fig. 4A) and with significantly less GI side effects. Thus, RTV most likely allowed a lower LNF dose to be in contact with the GI tract with a significantly higher sustained level of postabsorption LNF, yielding better antiviral responses. Indeed, measured serum LNF levels (Fig. 3C) were 4- to 5-fold higher than what was previously observed[18] with the same dose of LNF without RTV. Having established the value of adding RTV to LNF, larger-scale studies will be needed to determine the optimal combination doses to maximize antiviral efficacy and tolerability. This is the focus of the LOWR HDV-2 study.[21]

Although the first clinical study of LNF in HDV demonstrated important POC for the *in vivo* efficacy of prenylation inhibitors against HDV, the mean log reduction in HDV viral load for LNF 100 mg BID in that short 4-week treatment course was 0.74.[18] With addition of RTV to LNF in this study, however, the mean log reduction in HDV viral load for LNF 100 mg BID + RTV 100 mg QD at week 4 was 2.4 logs (Fig. 4A) and reached 3.2 logs at week 8 (Fig. 4B). In addition, combining this low-dose LNF with PEG-IFNα also exhibited impressive early viral load

declines (Fig. 4A,B). The results from this study (Fig. 4B) suggest that LNF 100 mg BID with either RTV or PEG-IFNα would result in a faster and greater HDV-RNA reduction than a published study where patients received PEG-IFNα 180 μg QW with or without tenofovir and only experienced a mean 2.78 log reduction after 48 weeks of therapy.[9]

In contrast to classical antiviral approaches that target virus-specific functions, LNF inhibits a host cell function upon which HDV depends. Thus, the targeted locus is not under genetic control of the virus, and this has long been postulated to have a higher barrier to the development of resistance.[22] The results of the current study now add to the increasing empirical data in support of this concept, which was first documented in patients receiving 28 days of LNF monotherapy.[18] Indeed, in spite of effectively treating with an antiviral monotherapy for up to 12 weeks in the current study, there was no evidence for the development of viral resistance, including in patients who experienced rises in viral load associated with drops in LNF serum concentration (Supporting Table S3). We believe that similar approaches can be contemplated for a wide range of viruses.

A most interesting phenomenon was observed in a subset of patients who were treated with the longer 12-week LNF regimens. In particular, 2 of 6 of these patients experienced transient posttreatment ALT elevations that were associated with HDV-RNA levels becoming undetectable followed by ALT normalization. Following achievement of undetectable HDV-RNA levels, the latter fluctuated for a period between negativity and very low levels near the limit of quantitation, with 1 patient going on to a sustained period of HDV-RNA negativity. However, sustained HDV-RNA negativity should not be seen as viral clearance. Late viral relapse has been well described in CDH,[23] and patients need to be on long-term close follow-up. Importantly, in both cases ALT levels normalized, highlighting that these appear to be beneficial, therapeutic flares. Interestingly, this was not observed in patients who were treated with <12 weeks of LNF, suggesting that there may be a certain treatment period required to induce this phenomenon.

As discussed further below, the precise mechanism of these LNF-induced therapeutic flares is, at present, uncertain. The possibility of an HBV-induced viral flare or HBV reactivation appears to be rather unlikely given that in both patients serum HBV DNA decreased to pretreatment levels within 4-8 weeks after discontinuation of LNF treatment without administration of NAs to patients. A more likely explanation is that in these patients, LNF resulted in a resetting or reactivation of the immune system such that upon cessation of LNF therapy the subsequent rise in HDV RNA was recognized more akin to an acute hepatitis, resulting in an apparent LNF-induced immunological control of HDV. Interestingly, this improved immune response was not limited to HDV. Indeed, posttreatment HBV-DNA levels were at or below baseline levels. Whereas low pretreatment levels of HBV could be explained by HDV viral dominance resulting in suppression of HBV, the low posttreatment HBV levels occurred without concomitant use of NAs and in the presence of low or undetectable HDV-RNA levels. This strongly suggests that the latter is unlikely to mediate the posttreatment suppression of HBV; rather, this most likely reflects improved posttreatment immunological control of HBV.

Although this approach may only work after years of NA treatment in HBeAg-negative chronic hepatitis B,[24,25] it is remarkable that such an immune reactivation may occur after only 12 weeks of LNF treatment in CDH, which may be attributed to LNF affecting a host function.

Although purposeful induction of flares has become a goal of many new treatment strategies for HBV, this requires caution and close monitoring, especially for patients with advanced fibrosis and critically limited hepatic reserve, who could be at risk of dangerous decompensation and possible need for liver transplantation. Similar caution should be observed in HDV. Although the remarkable outcomes of these LNF-associated posttreatment flares are unprecedented in hepatitis D, and were clearly of benefit to the patients described in this study, until the precise mechanism and outcomes in greater numbers of patients are better understood, patients with or suspected of having suboptimal hepatic reserve should probably be excluded from such treatment regimens. Analysis of peripheral blood mononuclear cell subsets and cytokine profiles before, during and after these LNF-associated posttreatment flares, may help better interpret the latter's precise nature and may lead to the prospective identification of patients likely to experience this dramatic pathway to HDV-RNA negativity.

Because of its pilot nature, this study involved a relatively small number of patients, had no placebo group, and in group 3 treatment duration had to be shortened to 5 weeks because of unexpected limitations to drug supply. Nevertheless, we conclude that these results support the further development of LNF with RTV

boosting. Combination of LNF with pegylated interferon should also be explored. Although our studies to date have used interferon alfa, the combination with interferon lambda may be particularly attractive, given the significantly improved safety profile associated with interferon lambda over alfa.[26,27]

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