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ORIGINAL ARTICLE

A phase 2 dose-finding study of lonafarnib and ritonavir with or without interferon alpha for chronic delta hepatitis

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Abstract

Background and Aims: Proof-of-concept studies demonstrated lonafarnib (LNF), a first-in-class oral prenylation inhibitor, efficacy in patients infected with HDV. The lonafarnib with ritonavir for HDV-2 (LOWR-2) study's aim was to identify optimal combination regimens of LNF + ritonavir (RTV) ± pegylated interferon alpha (PEG-IFNα) with efficacy and tolerability for longer-term dosing. Here we report the safety and efficacy at end of treatment for up to 24 weeks.

Approach and Results: Fifty-five patients with chronic HDV were consecutively enrolled in an open-label, single-center, phase 2 dose-finding study. There were three main treatment groups: high-dose LNF (LNF ≥ 75 mg by mouth [po] twice daily [bid] + RTV) (*n* = 19, 12 weeks); all-oral low-dose LNF (LNF 25 or 50 mg po bid + RTV) (*n* = 24, 24 weeks), and combination low-dose LNF with PEG-IFNα (LNF 25 or 50 mg po bid + RTV + PEG-IFNα) (*n* = 12, 24 weeks). The primary endpoint, ≥2 $\log_{10}$ decline or < lower limit of quantification of HDV-RNA from baseline at end of treatment, was reached in 46% (6 of 13) and 89% (8 of 9) of patients receiving the all-oral regimen of LNF 50 mg bid + RTV, and combination regimens of LNF (25 or 50 mg bid) + RTV + PEG-IFNα, respectively. In addition, multiple patients experienced well-tolerated transient posttreatment alanine aminotransferase increases, resulting in HDV-RNA negativity and alanine aminotransferase normalization. The proportions of grade 2 and 3 gastrointestinal adverse events in the high-dose versus low-dose groups were 49% (37 of 76) and only 22% (18 of 81), respectively.

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Abbreviations: AE, adverse event; ALT, alanine aminotransferase; bid, twice daily; CDH, chronic delta hepatitis; EOT, end of treatment; GI, gastrointestinal; GT, genotype; LLOQ, lower limit of quantification; LNF, lonafarnib; LOWR-2, lonafarnib with ritonavir for HDV-2 study; PEG-IFNα, pegylated interferon alpha; po, by mouth; qd, once daily; qw, once weekly; RTV, ritonavir; SAE, severe adverse event; VL, viral load.

The study is registered at clinicaltrials.gov under NCT02430194.

Conclusions: LNF, boosted with low-dose RTV, is a promising all-oral therapy, and maximal efficacy is achieved with PEG-IFNα addition. The identified optimal regimens support a phase 3 study of LNF for the treatment of HDV.

INTRODUCTION

Chronic delta hepatitis (CDH) leads to the most severe form of human viral hepatitis. CDH is always found as a co-infection with HBV, requiring HBsAg to complete HDV virion assembly. However, HDV-HBV co-infection leads to more rapid disease progression than HBV mono-infection alone.[1] There is currently no Food and Drug Administration (FDA)–approved treatment for HDV, although the entry inhibitor Bulevirtide has recently received conditional approval by the European Medicines Agency. Pegylated interferon alpha (PEG-IFNα) has demonstrated limited efficacy in off-label use, in which 1 year of treatment with PEG-IFNα is effective in approximately only a quarter of the patients.[2] With prolonged treatment durations for up to 10 years, response rates may increase,[3] but this remains a difficult strategy for patients and physicians alike due to management of side effects. New therapies are urgently needed.

The farnesyl transferase inhibitor lonafarnib (LNF) targets prenylation of HDV large delta antigen, a host function that is essential for HDV virion morphogenesis.[4] By targeting a host function, LNF can subvert the ability of the virus to develop resistance, as the drug-targeted locus is not under genetic control of the virus. A proof-of-concept clinical study of LNF monotherapy demonstrated a dose-dependent correlation of increased LNF serum levels and HDV viral load (VL) decline at 4 weeks.[5] This was followed by the LOnafarnib With and without Ritonavir (RTV) in HDV (LOWR-1) study, which evaluated higher doses of LNF monotherapy as well as lower LNF doses boosted with RTV for up to 12 weeks' duration.[6] Although higher doses were associated with better short-term declines in VL, this was accompanied by increased gastrointestinal (GI) side effects. Higher LNF serum levels and antiviral responses were observed with lower LNF doses when combined with an inhibitor of its postabsorption metabolism, RTV. This enabled lower LNF doses in the GI tract to achieve higher efficacy with concomitant better GI tolerability. The present LOWR-2 study was designed to extend these findings to identify optimal combination regimens of LNF + RTV ± PEG-IFNα with efficacy and tolerability for longer-term dosing, to induce clinically meaningful reductions in HDV RNA and alanine aminotransferase (ALT) normalization, and to provide the support for the phase 3 study for patients infected with HDV.

PATIENTS AND METHODS

Study design and participants

The study was a single-center, open-label, nonrandomized, uncontrolled, phase 2 pilot study. Safety and tolerability of EBP-994 (LNF) was a primary objective of the study. From a safety, pharmacokinetic, and pharmacodynamic perspective, no *a priori* assumptions were made as to the expected treatment effect and associated variability. All patients were enrolled in the Department of Gastroenterology of the University of Ankara Medical School. The study protocol and three amendments to the original protocol were approved by the University of Ankara Medical School Ethics Committee. Briefly, 18-year-old to 65-year-old patients with CDH infection, documented by a positive anti-HDV test of at least 6 months' duration and detectable HDV RNA by PCR within 3 months to study entry, were included. All patients were required to be HDV RNA–positive at baseline and have compensated liver disease. Platelet and white blood cell counts had to be ≥100,000 ($\times 10^9$/L) and 3000 ($\times 10^9$/L), respectively. Detailed inclusion and exclusion criteria are listed in Table S1 and the Supporting Materials. The study was approved by the University of Ankara Medical School and the Ministry of Health ethics committees. All patients gave written, informed consent.

Procedures

Enrollment into LOWR-2 began on December 2, 2014. The original open-label, randomized study protocol was modified to a nonrandomized dose finding study with amendment 1 of the original protocol, in which patients were consecutively enrolled into various treatment regimens. The prespecified number of patients per treatment regimen was at least 3 patients, with the number allowed to increase based on agreement with the principal investigator (CY) and the sponsor. Of the four groups of treatment regimens considered in the protocol (Group 1: LNF up to 600 mg daily dose as monotherapy; Group 2: LNF up to 200 mg daily dose in combination with RTV; Group 3: LNF up to 200 mg daily dose in combination with Peg-IFNα; and Group 4: LNF up to 200 mg daily dose in combination with RTV and Peg-IFNα), Groups 1 and 3 had been assessed in the LOWR-1 study and have now been published.[6] In the LOWR-1 study, combination of LNF 100 mg twice daily

(bid) with RTV 100 mg once daily (qd) demonstrated over 40-times-greater antiviral activity compared to that observed with LNF 100 mg bid alone at 4 weeks (−2.4 $\log_{10}$ vs. −0.73 $\log_{10}$), encouraging further dose optimization of LNF with RTV in the LOWR-2 study. The current LOWR-2 study further assessed Groups 2 and 4 by enrolling patients consecutively into 10 different treatment regimens, which were divided into three groups (Table 1 and Figure S1): Group 1 (high-dose LNF ≥ 75 mg bid), which included Regimen 1 (LNF 100 mg bid + RTV 100 mg qd for 12 weeks), Regimen 2 (LNF 100 mg bid + RTV 50 mg bid for 12 weeks), Regimen 3 (LNF 100 mg qd + RTV 100 mg qd for 12 weeks), Regimen 4 (LNF 150 mg qd + RTV 100 mg qd for 12 weeks), and Regimen 5 (LNF 75 mg bid + RTV 100 mg bid for 12 weeks followed by combination with PEG-IFNα 180 μg subcutaneously once weekly [qw] for 12 weeks); Group 2 (low-dose LNF ≤ 50 mg bid), which included Regimen 6 (LNF 25 mg bid + RTV 100 mg bid for 24 weeks), Regimen 7 (LNF 50 mg bid + RTV 100 mg bid for 24 weeks), and Regimen 8 (LNF 50 mg bid + RTV 100 mg bid for 12 weeks followed by combination with PEG-IFNα 180 μg qw for 12 weeks); Group 3 (low-dose LNF ≤ 50 mg bid combined with PEG-IFNα), which included Regimen 9 (LNF 25 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw for 24 weeks) and Regimen 10 (LNF 50 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw for 24 weeks). A subset of patients from Regimens 6, 7, 9, and 10 had their treatment extended in an exploratory fashion to 48 weeks (Table 1), which will be reported elsewhere.

Adverse events (AEs) of the treatment regimens were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.

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 virologic parameters. A brief physical examination was done at screening, at day 1, and at every visit starting from day 3. AEs were recorded at every visit. Posttreatment follow-up consisted of one visit 4 weeks after treatment discontinuation.

Virologic determinations

Serum HDV RNA was measured using the RoboGene assay according to instructions provided (Sonic Laboratory Manual, 2016). The assay uses real-time quantitative PCR of HDV RNA in human serum samples. The assay is designed to detect HDV genotype (GT)-1, GT-2, GT-3, GT-4, GT-5, GT-6, GT-7, and GT-8, applying probes and primers specific for a subsequence of the HDV delta antigen. The assay has a lower limit of quantitation (LLOQ) of 14 IU/ml based on calibrated standards using a reference HDV GT-1 positive serum. The analytical sensitivity of the kit

TABLE 1 Treatment regimens used in the LOWR-2 study

	Regimen		Duration (weeks)	Patients (*n*)
Group 1: high-dose regimens	1	LNF 100 mg bid + RTV 100 mg qd	12	4[a]
	2	LNF 100 mg bid + RTV 50 mg bid	12	4[a]
	3	LNF 100 mg qd + RTV 100 mg qd	12	5[b]
	4	LNF 150 mg qd + RTV 100 mg qd	12	3
	5	LNF 75 mg bid + RTV 100 mg bid (+ PEG-IFNα 180 μg qw starting Week 13)	24	3
Group 2: low-dose all-oral regimens	6	LNF 25 mg bid + RTV 100 mg bid	24	6[d]
	7	LNF 50 mg bid + RTV 100 mg bid	24	13[b]
	8	LNF 50 mg bid + RTV 100 mg bid (+ PEG-IFNα 180 μg qw starting Week 13)	24	5[a]
Group 3: low-dose combination regimens	9	LNF 25 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw	24	7[c,f]
	10	LNF 50 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw	24	5[e]
Total				55

Note: Patients were treated with 10 different treatment regimens (Regimens 1–10). These treatment regimens were allocated into three overall groups: high dose group (Group 1), low-dose all-oral group (Group 2), and low-dose triple combination group (Group 3) (Figure S1).

Abbreviations: qd, once daily; and qw, once weekly.

[a]One patient discontinued due to AEs. All patients were included in the intention-to-treat efficacy and safety assessments.

[b]Two patients discontinued due to AEs.

[c]Three patients discontinued due to AEs, and 1 patient was lost to follow-up.

[d]Five patients continued treatment to 48 weeks.

[e]Two patients continued treatment to 48 weeks.

[f]Three patients continued treatment to 48 weeks.

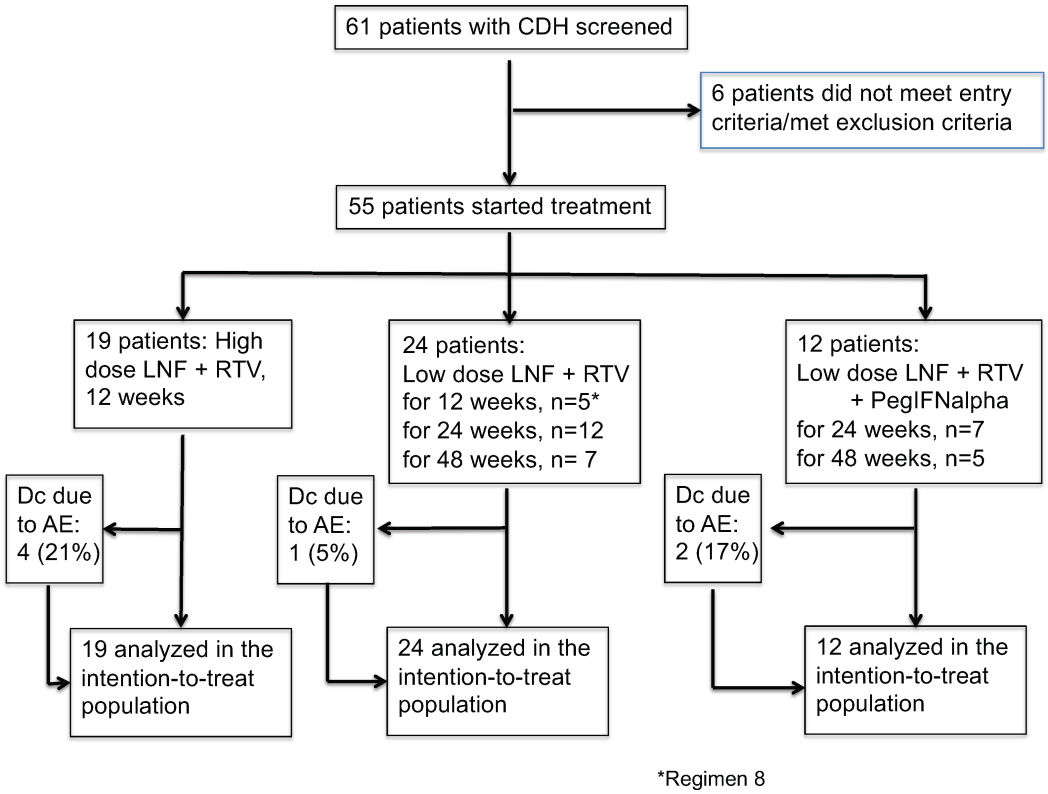


FIGURE 1 Lonafarnib with ritonavir for HDV-2 study (LOWR-2) patient flow (CONSORT diagram). Abbreviations: AE, adverse event; CDH, chronic delta hepatitis; LNF, lonafarnib; PEG-IFNα, pegylated interferon alpha; and RTV, ritonavir

TABLE 2 Baseline characteristics of patients

	All patients	High-dose regimens		
Baseline characteristics	***n* = 55**	**1 (*n* = 4)**	**2 (*n* = 4)**	**3 (*n* = 5)**
Median age, years (range)	50 (23–70)	31 (23–40)	51 (37–54)	61 (53–66)
% Male	65% (36 of 55)	100% (4 of 4)	100% (4 of 4)	40% (2 of 5)
% HBeAg (+)	14.8% (8 of 54)	0%[a] (0 of 3)	50% (2 of 4)	0% (0 of 5)
% Prior IFN therapy	52.7% (29 of 55)	25% (1 of 4)	75% (3 of 4)	40% (2 of 5)
Median log HDV RNA, IU/ml (range)	4.56 (0.88–7.26)	4.86 (4.59–6.15)	3.16 (0.88–6.36)	5.40 (3.42–6.15)
Median log HBV DNA, IU/ml (range)	1.75 (0–7.76)	1.73 (0–2.04)	1.77 (1.43–1.82)	1.40 (0–2.18)
Proportion of patients with detectable HBV DNA	70% (37 of 53)	67%[a] (2 of 3)	100% (4 of 4)	75%[a] (3 of 4)
% Prior nucleotide therapy	29% (16 of 55)	50% (2 of 4)	50% (2 of 4)	40% (2 of 5)
Median ALT, U/ml (range)	67 (24–651)	318 (40–651)	69 (42–94)	42 (29–264)
Median platelet, x 10^9/l (range)	154 (55–263)	208 (179–252)	122 (55–155)	142 (114–263)
Median BUN, mg/dl (range)	14 (6–24)	13 (6–17)	13 (10–15)	10 (9–14)
Median creatinine, mg/dl (range)	0.77 (0.17–1.11)	0.80 (0.77–0.93)	0.79 (0.69–0.82)	0.61 (0.61–0.91)
Median albumin, g/dL (range)	4.10 (2.90–4.86)	4.20 (3.40–4.40)	3.90 (3.30–4.00)	3.60 (3.60–4.00)
Median prothrombin, seconds (range)	11.6 (8.5–19.8)	11.7 (11.2–12.2)	11.7 (10.9–12.5)	11.8 (10.9–12.6)
% Cirrhosis	25% (14/55)	0% (0 of 4)	50% (2 of 4)	40% (2 of 5)
Median Child-Pugh (range)	5 (5–6)	5[a] (5–6)	5 (5–5)	5 (5–5)

Note: Baseline characteristics for all 55 LOWR2 study patients are broken down by individual treatment regimens.

Abbreviations: ALT, alanine aminotransferase; BUN, blood urea nitrogen.

[a]Data unavailable for 1 patient.

was determined by analyzing dilutions of the first World Health Organization International Standard for HDV RNA, GT-1 (#7657/12, provided by Paul Ehrlich-Institut). Imputed values of 7.5 IU/ml (i.e., the average of 14 IU/ml and 1 IU/ml) and 1 IU/ml were used for the assay measurements, which were below LLOQ and PCR-negative, respectively. Serum HBV-DNA level was quantified by the Cobas TaqMan HBV test (Roche Molecular Systems, Inc., Mannheim, Germany). HBsAg was quantified by the Architect HBsAg assay (Abbott Diagnostics, Germany) according to the manufacturer's instructions. Qualitative hepatitis serologies including HBsAg, hepatitis B surface antibody, HBeAg, and hepatitis B e antibody were determined by a microparticle enzyme immunoassay method (Abbott Laboratories, North Chicago, IL), and anti-HDV was determined by an enzyme immunoassay (Abbott Laboratories).

Outcomes

The primary endpoint of this study was defined as a ≥2 $\log_{10}$ decline or < LLOQ of HDV RNA from baseline at end of treatment (EOT), as specified in Table 1 for each regimen. A ≥2 $\log_{10}$ decline was considered as a surrogate marker for initial treatment efficacy, which mirrors the recent recommendation of a group of experts on the management of CDH[7] and was associated with significantly improved long-term clinical benefit.[8] Undetectable HDV RNA at EOT and normalization of ALT as well as quantitative decline of HDV RNA at EOT compared with baseline were assessed as secondary end points.

Statistical analysis

As noted in the protocol, a formal sample size calculation was not performed. Safety data was assessed on all patients who received at least one dose of lonafarnib (intention to treat analysis). Efficacy data was summarized for all patients who completed intended dosing. Patients who did not reach the intended treatment duration were thus not assessed by the per protocol analysis. Intent-to-treat analysis was used when comparing the number of patients receiving high vs. low dose of lonafarnib based regimens in achieving a ≥ 2 log10 decline at end of treatment.' Further details is provided in the Supporting Material Section.

RESULTS

Patient population

The study recruitment started December 2, 2014. The last patient was enrolled on October 25, 2016. Last per-protocol follow-up visit of a patient was on June 15, 2017.

		Low-dose regimens				
4 (*n* = 3)	**5 (*n* = 3)**	**6 (*n* = 6)**	**7 (*n* = 13)**	**8 (*n* = 5)**	**9 (*n* = 7)**	**10 (*n* = 5)**
56 (42–60)	59 (5–61)	49 (31–57)	41 (27–70)	39 (25–52)	50 (41–59)	39 (29–58)
100% (3 of 3)	67% (2 of 3)	50% (3 of 6)	54% (7 of 13)	100% (5 of 5)	43% (3 of 7)	60% (3 of 5)
0% (0 of 3)	0% (0 of 3)	17% (1 of 6)	8% (1 of 13)	20% (1 of 5)	14% (1 of 7)	20% (1 of 5)
67% (2 of 3)	33% (1 of 3)	67% (4 of 6)	46% (6 of 13)	80% (4 of 5)	0% (0 of 7)	0% (0 of 5)
4.86 (4.44–6.36)	4.59 (2.34–4.98)	4.14 (1.66–5.15)	3.87 (1.66–5.54)	5.15 (3.30–6.32)	5.52 (2.58–7.18)	5.29 (4.36–6.97)
1.83 (0–2.56)	1.89 (0–2.04)	1.73 (0–3.08)	1.63 (0–3.32)	1.54 (0–7.76)	3.30 (0–7.26)	1.79 (0–3.76)
67% (2 of 3)	67% (2 of 3)	67% (4 of 6)	62% (8 of 13)	50% (2 of 5)	86% (6 of 7)	80% (4 of 5)
33% (1 of 3)	0% (0 of 3)	17% (1 of 6)	15% (2 of 13)	40% (2 of 5)	0% (0 of 7)	0% (0 of 5)
67 (66–69)	37 (32–57)	68 (44–175)	69 (24–229)	87 (42–241)	60 (33–129)	78 (28–115)
205 (148–222)	94 (67–117)	133 (97–213)	156 (72–235)	159 (114–209)	144 (109–174)	191 (111–254)
13 (9–13)	13 (10–14)	14 (11–23)	15 (9–23)	14 (10–18)	14 (10–24)	15 (13–22)
0.81 (0.77–0.93)	0.76 (0.67–1.01)	0.68 (0.59–0.94)	0.81 (0.46–1.10)	0.99 (0.74–1.11)	0.66 (0.17–1.06)	0.83 (0.66–1.10)
3.80 (3.60–4.20)	4.10 (4.00–4.10)	4.05 (3.50–4.40)	4.30 (3.80–4.86)	4.50 (2.90–4.80)	4.20 (3.90–4.40)	4.10 (3.82–4.60)
13.0 (11.7–13.5)	11.5 (11.1–12.0)	14 (11–23)	15 (9–23)	14 (10–14)	15 (13–24)	15 (13–22)
33% (1 of 3)	67% (2 of 3)	33% (2 of 6)	15% (2 of 13)	20% (1 of 5)	14% (1 of 7)	20% (1 of 5)
6 (6)	5 (5–5)	5 (5–5)	5 (5)	5 (5)	5 (5)	5 (5)

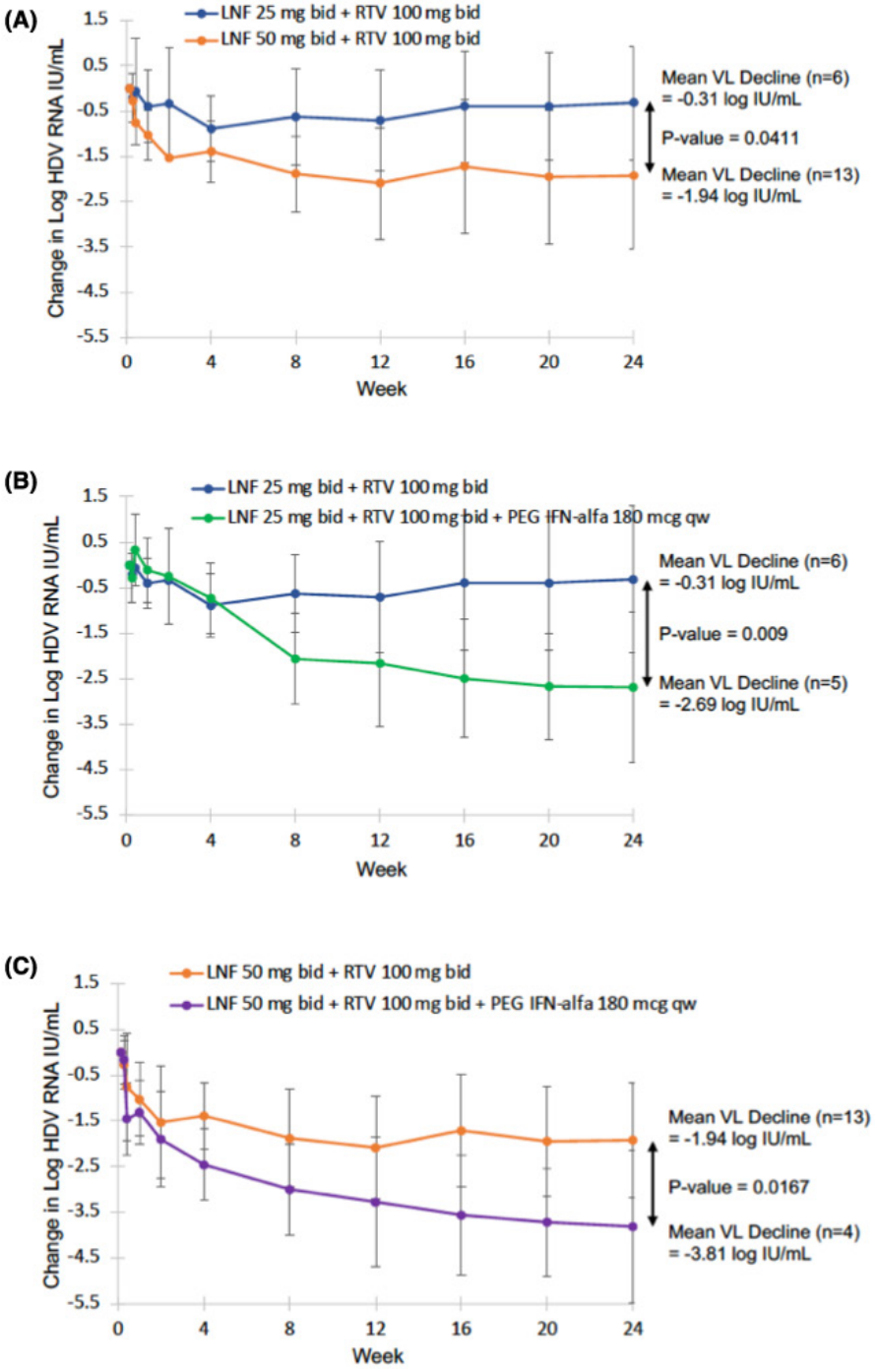


FIGURE 2 End of treatment (EOT) Week 24 antiviral efficacy of key regimens. (A) Comparison of the antiviral efficacy of LNF 25 mg twice daily (bid) + RTV 100 mg bid to LNF 50 mg bid + RTV 100 mg bid. (B) Comparison of LNF 25 mg bid + RTV 100 mg bid to LNF 25 mg bid + RTV 100 mg bid and PEG-IFNα. (C) Comparison of LNF 50 mg bid + RTV 100 mg bid to LNF 50 mg bid + RTV 100 mg bid and PEG-IFNα. LNF exhibits a dose-dependent increase in antiviral efficacy; addition of PEG-IFNα in the combination therapy regimens was associated with the best antiviral efficacy data. Mean declines and SEM are indicated. Abbreviation: mcg, microgram

The study was closed after this date, as the planned dose finding had been achieved. A total of 55 patients were enrolled in the study (Figure 1). Of the 55 enrolled patients, 3 patients with platelet counts below the inclusion criteria threshold were enrolled as protocol deviation based on mutual agreement of the principal investigator (CY) and sponsor. There were four additional patients who were not protocol deviations, as their platelet counts were above 100,000 at screening but below the threshold at baseline (Regimen 5: 94,000; Regimen 6: 97,000 and 98,000; and Regimen 7: 72,000). Baseline patient characteristics are shown in Table 2. Briefly, most of the

TABLE 3 Efficacy assessments at end of treatment for the indicated number of weeks (primary endpoint)

	Regimen	Treatment duration (Weeks)	Patients (*n*)	Mean baseline HDV RNA	End of treatment						
					Per protocol				Intention to treat		
					Mean Δ $\log_{10}$	≥2 $\log_{10}$ decline in HDV RNA	<LLOQ in HDV-	ALT nl[a]	≥2 $\log_{10}$ decline in HDV RNA	<LLOQ in HDV RNA	ALT nl[a]
1	LNF 100 mg bid + RTV 100 mg qd	12	4	5.12	−1.39 ± 1.27	1 of 3 (33.3%)	0 of 3 (0%)	3 of 3 (100%)	1 of 4 (25%)	0 of 4 (0%)	3 of 4 (75%)
2	LNF 100 mg bid + RTV 50 mg bid	12	4	3.39	+0.33 ± 1.08	0 of 3 (0%)	1 of 3 (33%)	1 of 3 (33.3%)	0 of 4 (0%)	1 of 4 (25%)	1 of 4 (25%)
3	LNF 100 mg qd + RTV 100 mg qd	12	5	5.10	−1.11 ± 3.10	1 of 3 (33.3%)	1 of 3 (33.3%)	1 of 2 (50%)	1 of 5 (20%)	1 of 5 (20%)	1 of 5 (20%)
4	LNF 150 mg qd + RTV 100 mg qd	12	3	5.22	−0.67 ± 0.24	0 of 3 (0%)	0 of 3 (0%)	2 of 3 (67%)	0 of 3 (0%)	0 of 3 (0%)	2 of 3 (67%)
5	LNF 75 mg bid + RTV 100 mg bid (+ PEG-IFNα 180 µg qw starting Week 13)	24	3	3.97	−1.96 ± 1.60[b]	1 of 3 (33.3%)	1 of 3 (33.3%)	0 of 1 (0%)	1 of 3 (33.3%)	1 of 3 (33.3%)	0 of 3 (0%)
6	LNF 25 mg bid + RTV 100 mg bid	24	6	3.75	−0.31 ± 1.61	1 of 6 (16.7%)	0 of 6 (0%)	2 of 5 (40%)	1 of 6 (16.7%)	0 of 6 (0%)	2 of 5 (40%)
7	LNF 50 mg bid + RTV 100 mg bid	24	13	4.00	−1.94 ± 1.30	5 of 13 (38.5%)	6 of 13 (46.2%)	6 of 11 (54.5%)	5 of 13 (38.5%)	6 of 13 (46.2%)	6 of 11 (54.5%)
8	LNF 50 mg bid + RTV 100 mg bid (+ PEG-IFNα 180 µg qw starting Week 13)	24	5	5.18	−2.85 ± 0.49[c]	4 of 4 (80%)	1 of 4 (20%)	0 of 4 (0%)	4 of 5 (80%)	1 of 5 (20%)	0 of 4 (0%)
9	LNF 25 mg bid + RTV 100 mg bid + PEG-IFNα 180 µg qw	24	7	5.29	−2.69 ± 1.66	3 of 5 (80%)	3 of 5 (60%)	5 of 5 (100%)	3 of 7 (43%)	3 of 7 (43%)	5 of 5 (100%)

(Continues)

TABLE 3 (Continued)

	Regimen	Treatment duration (Weeks)	Patients (*n*)	Mean baseline HDV RNA	End of treatment				Intention to treat		
					Per protocol						
					Mean Δ $\log_{10}$	≥2 $\log_{10}$ decline in HDV RNA	<LLOQ in HDV-	ALT nl[a]	≥2 $\log_{10}$ decline in HDV RNA	<LLOQ in HDV RNA	ALT nl[a]
10	LNF 50 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw	24	5	5.48	−3.81 ± 0.94	4 of 4 (100%)	2 of 4 (50%)	2 of 3 (66.7%)	4 of 5 (80%)	2 of 5 (40%)	2 of 3 (66.7%)

Note: The proportion of patients reaching the primary endpoint of the study of a ≥2 $\log_{10}$ decline or <lower limit of quantitation (LLOQ) in serum HDV RNA after 12 (Regimens 1–4) or 24 weeks of treatment, and the proportion of patients who normalized their ALT at end of treatment with different low-dose LNF regimens. Key regimens (6, 7, 9, and 10) are highlighted.

[a]Only patients with elevated ALT at baseline are included in this analysis.

[b]HDV-RNA decline at Week 12 = −2.63 ± 1.28.

[c]HDV-RNA decline at Week 12 = −2.07 ± 0.70.

patients were HBeAg-negative, as is typically seen in HDV/HBV co-infection. Twenty-nine (53%) patients had historically received IFN treatment, and 26 (47%) patients were treatment-naïve. Median HDV-RNA levels were 4.56 $\log_{10}$ IU/ml (0.88–7.26). Median HBV-DNA levels were 1.71 $\log_{10}$ (range 0–7.76). There were 2 patients with HBV-DNA levels exceeding 4 $\log_{10}$ IU/ml; however, neither of these patients had viral loads suggesting HBV dominance. Sixteen (29%) patients had received prior nucleoside analog (NA) therapy. No patients received NA treatment during the study. Median ALT level was 67 U/l (24–651). Median platelet level was 154 (55–263). Of the 55 patients in the study, 14 (25%) had cirrhosis, 36 (65%) had chronic hepatitis without cirrhosis, and 5 (9%) could not be classified. Of the 14 patients with cirrhosis, all were Child-Pugh class A, and all but 1 had a Child Pugh score of 5 (Table 2).

Low-dose LNF (LNF ≤ 50 mg bid + ritonavir) has comparable efficacy with less side effects than high dose (LNF ≥ 75 mg bid + RTV)

Initially, higher doses of LNF were explored in Regimens 1–5 (LNF 100 mg bid + RTV 100 mg qd, LNF 100 mg qd + RTV 100 mg qd, LNF 100 mg bid + RTV 50 mg bid, LNF 150 mg qd + RTV 100 mg bid, and LNF 75 mg bid + RTV 100 mg bid). Because a lack of resistance was confirmed through detailed sequence analysis of patients treated with LNF monotherapy in previous studies,[5,6] lower doses of LNF were explored in Regimens 6–8 (LNF 50 mg bid + RTV 100 mg bid and LNF 25 mg bid + RTV 100 mg bid).

Efficacy results for patients completing 12 weeks per protocol treatments on these regimens are presented in Figure S2, Table S2, and Table 3. Corresponding AEs for patients receiving at least one dose (intention to treat) on these regimens are presented in Table 4, Table S3, and Table S4.

Week 12 observations from all oral LOWR-2 treatments indicated that high-dose LNF Group 1 patients (LNF ≥ 75 mg bid + RTV; Regimens 1–5; *n* = 19) had a mean viral load decline at Week 12 of −1.21 $\log_{10}$ IU/ml (Table S5A) with four discontinuations (21.1%), 11 dose reductions (57.9%), and GI AEs, 49% of which were Grade 2 or 3 in severity (Table S5B). Low-dose LNF Group 2 (LNF ≤ 50 mg bid + RTV; Regimens 6–8; *n* = 24) had a mean viral load decline at Week 12 of −1.54 $\log_{10}$ IU/ml (Table S5) with discontinuation due to AEs in 1 patient and no dose reductions. In addition, GI AEs were predominantly Grade 1 (Table S5B). Baseline characteristics were comparable between these two groups (Table S6). No significant on-treatment elevations of liver enzymes, blood urea nitrogen, or creatinine were observed. With respect to severe AEs (SAEs), 3 occurred in the high-dose groups, probably related to LNF, and 5 in the low-dose groups—2 unrelated to study

TABLE 4 AEs for Regimens 1–5 and 8 (A) and low-dose LNF-based regimens 6, 7, 9, and 10 (B)

(A)

	Regimen 1 LNF 100 mg bid + RTV 100 mg qd (*n* = 4)			**Regimen 2 LNF 100 mg bid + RTV 50 mg bid (*n* = 4)**			**Regimen 3 LNF 100 mg qd + RTV 100 mg qd (*n* = 5)**		
	12 weeks of treatment								
	Grade								
GI AE	1	2	3	1	2	3	1	2	3
Diarrhea	0	1 (25%)	2 (50%)	0	1 (25%)	1 (25%)	2 (40%)	0	1 (20%)
Nausea	1 (25%)	1 (25%)	0	1 (25%)	1 (25%)	0	1 (20%)	1 (20%)	1 (20%)
Vomiting	0	0	0	0	1 (25%)	0	1 (20%)	1 (20%)	1 (20%)
Fatigue	0	2 (50%)	1 (25%)	2 (50%	1 (25%)	0	0	2 (40%)	2 (40%)
Anorexia	0	2 (50%)	1 (25%)	1 (25%)	3 (75%)	0	0	2 (40%)	0
Weight loss	1 (25%	2 (50%)	0	1 (25%)	1 (25%)	0	1 (20%)	1 (20%)	0
Discontinuation due to AE	1 (25%)			1 (25%)			2 (40%)		
SAE							3 (60%) diarrhea (1), fatigue (2)		
	Regimen 4 LNF 150 mg qd + RTV 100 mg qd (*n* = 3)			**Regimen 5 LNF 75 mg bid + RTV 100 mg bid (+ PEG-IFNα 180 μg qw starting Week 13) (*n* = 3)**			**Regimen 8 LNF 50 mg bid + RTV 100 mg bid (+ PEG-IFNα 180 μg qw starting Week 13) (*n* = 5)**		
	12 weeks of treatment								
	Grade								
GI AE	1	2	3	1	2	3	1	2	3
Diarrhea	0	0	2 (67%)	0	2 (67%)	1 (33%)	4 (80%)	0	1 (20%)
Nausea	2 (67%)	0	0	3 (100%)	0	0	2 (40%)	1 (20%)	1 (20%)
Vomiting	1 (33%)	0	0	0	0	0	1 (20%)	0	0
Fatigue	2 (67%)	0	1 (33%)	3 (100%)	0	1 (33%)	3 (60%)	2 (40%)	0
Anorexia	0	2 (67%)	1 (33%)	3 (100%)	0	0	3 (60%)	1 (20%)	0
Weight loss	1 (33%)	2 (67%)	0	1 (33%)	1 (33%)	0	1 (20%)	1 (20%)	1 (20%)
Discontinuation due to AE	0			0			1 (20%)		
SAEs							• 1 (20%) hepatic decompensation after starting PEG-IFNα		

(B)

	Regimen 6 LNF 25 mg bid + RTV (*n* = 6)		**Regimen 7 LNF 50 mg bid + RTV (*n* = 13)**			**Regimen 9 LNF 25 mg bid + RTV + PEG-IFNα (*n* = 7)**			**Regimen 10 LNF 50 mg bid + RTV + PEG-IFNα (*n* = 5)**		
	24 weeks of treatment										
	Grade										
GI AE	1	2	3	1	2	3	1	2	3	1	2	3
Diarrhea	2 (33%)	0	1 (17%)	4 (31%)	3 (23%)	2 (15%)	2 (29%)	1 (14%)	0	3 (60%)	2 (40%)	0
Nausea	3 (50%)	1 (17%)	0	6 (46%)	2 (15%)	0	3 (43%)	1 (14%)	0	1	2 (40%)	0
Vomiting	1 (17%)	0	1 (17%)	5 (38%)	2 (15%)	0	1 (14%)	0	0	0	1 (20%)	0
Fatigue	3 (50%)	0	1 (17%)	10 (77%)	0	0	4 (57%)	0	0	5 (100%)	0	0
Anorexia	2 (33%)	0	0	8 (62%)	0	0	2 (29%)	0	0	4 (80%)	0	0
Weight loss	2 (34%)	0	1 (17%)	5 (38%)	2 (15%)	0	3 (43%)	2 (29%)	0	2 (40%)	1 (20%)	0
Discontinuation due to AE	0			0			1 (14%)			1 (20%)		
SAEs				• 1 (7.7%) anemia (unrelated to study medication) • 1 (7.7%) vomiting (first day before study medication)			• 1 (14.3%) neuropathy and vasculitis (probably related to PEG-IFNα)			• 1 (20%) anemia and seizure (probably related to PEG-IFNα)		

Note: Data for Groups 1–4 are for 12 weeks of treatment, and data for Groups 5–10 are for 24 weeks of treatment.

Abbreviations: GI, gastrointestinal; SAE, severe AE.

drug, 1 possibly related to PEG-IFNα, and 2 probably related to PEG-IFNα, with 1 patient experiencing hepatic decompensation after starting PEG-IFNα. No treatment-related or unrelated death occurred during the conduct of the study. Moreover, per-protocol analysis revealed that the number of patients with ≥2 log decline in HDV RNA after 12 weeks was 4 of 15 (27%) in the high-dose group and 14 of 24 (58%) in the low-dose group (Table S5A). (See Table S2 and Figure S2 for individual patient group data for patients reaching 12 weeks of treatment).

Given the comparable, if not better, efficacy, and fewer and less severe GI AEs with low-dose LNF versus high-dose LNF at Week 12 (Table S5), with comparable baseline characteristics between the two groups (Table S6), dosing durations of low-dose LNF regimens using LNF 25 and 50 mg bid were extended to 24 weeks, and the addition of PEG-IFNα was explored (i.e., Regimens 9 and 10 were added). Further analysis focused on these cohorts (Regimens 6, 7, 9, and 10) (Table 1).

Better antiviral efficacy was observed for the all-oral LNF 50 mg bid + RTV than the all-oral LNF 25 mg bid + RTV

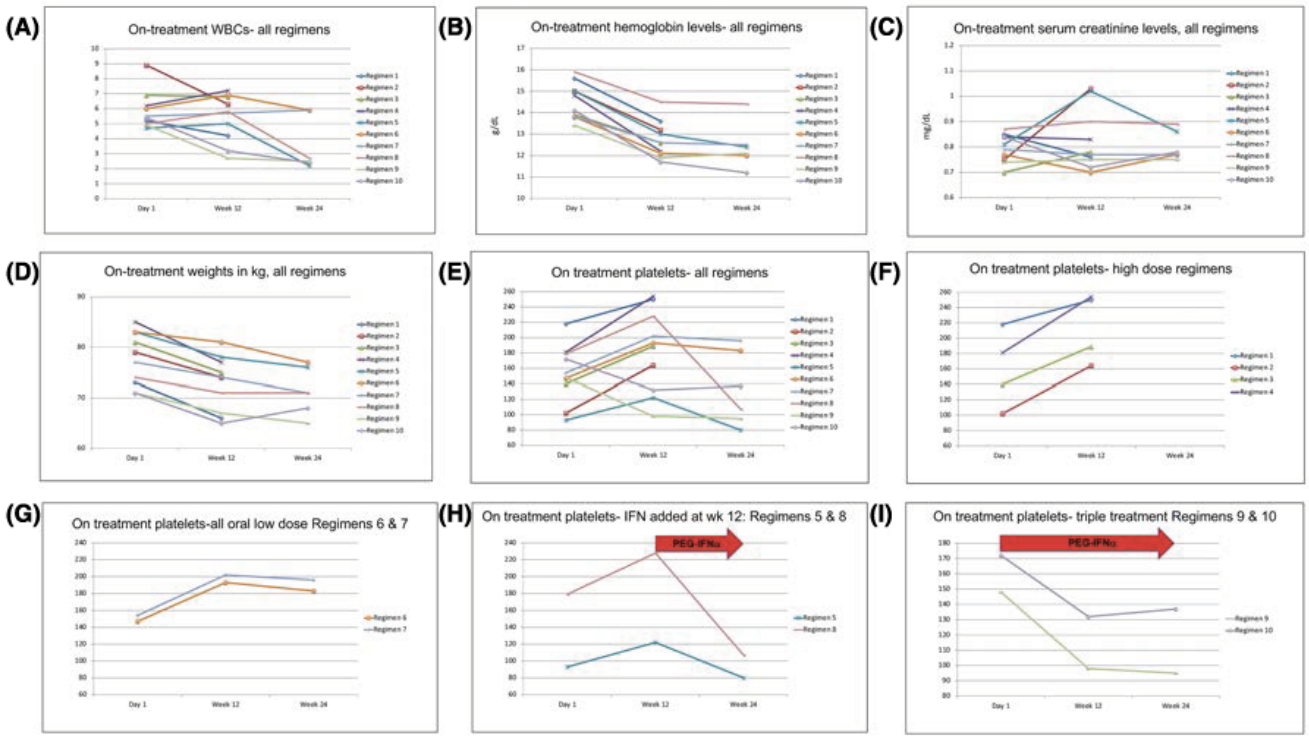
Assessment of the primary efficacy at 24 weeks of treatment revealed that LNF 50 mg bid + RTV 100 mg bid showed better antiviral efficacy compared with LNF 25 mg bid + RTV 100 mg bid. The latter regimen led to a mean HDV-RNA decline of −0.31 $\log_{10}$ IU/ml versus −1.94$_{10}$ log IU/ml for the LNF 50 mg + RTV, an improvement of −1.63 $\log_{10}$ IU/ml (Figure 2A). Both regimens were generally well tolerated, with most side effects reported at the Grade 0–1 levels (Table 4). These AEs were generally more pronounced in the beginning of therapy and managed with pro-re-nata administration of proton pump inhibitors, ondansetron, and in some patients metaclopramide for nausea, and loperamide for diarrhea. The primary endpoint of ≥2 $\log_{10}$ decline (or < LLOQ) at 24 weeks EOT was reached in 1 of 6 (17%) patients in the lonafarnib 25 mg bid + RTV 100 mg bid group compared with 6 of 13 (46%) patients in the LNF 50 mg bid + RTV 100 mg bid group (Table 3). Of the patients with baseline elevated ALT, 2 of 5 (40%) patients receiving the LNF 25 mg bid regimen normalized their ALT at 24 weeks of therapy, whereas this was observed in 6 of 11 (55%) patients receiving the LNF 50 mg bid regimen (Table 3). Individual patient responses are indicated in Figures S3A,B, and S4A,B.

Combination LNF 25 or 50 mg + RTV + PEG-IFNα demonstrates the most robust antiviral efficacy

Combination therapy, consisting of the addition of PEG-IFNα to the LNF 25 and 50 mg bid RTV -boosted regimens, resulted in the most robust antiviral responses, with increased efficacy at 24 weeks compared with the respective all-oral regimens. In particular, combination therapy with LNF 25 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw was associated with a −2.69 $\log_{10}$ IU/mL mean decline in HDV RNA, an additional decline of −2.38 $\log_{10}$ IU/mL over the LNF 25 mg + RTV 100 mg regimen (Figure 2B, Table 3). Addition of PEG-IFNα to LNF 50 mg bid + RTV 100 mg bid resulted in a −3.81 $\log_{10}$ IU/ml mean decline in HDV RNA, a further −1.87 $\log_{10}$ IU/ml decrease over the LNF 50 mg bid + RTV 100 mg bid regimen (Figure 2C, Table 3). Three of 5 (60%) patients on LNF 25 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw achieved a ≥2 $\log_{10}$ IU/ml drop (or < LLOQ) after 24 weeks of therapy, and 5 of 5 (100%) patients with elevated baseline ALT normalized their ALT at 24 weeks of therapy (Table 3). Four of 4 (100%) patients on LNF 50 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw achieved a ≥2 $\log_{10}$ IU/ml drop (or < LLOQ) after 24 weeks of therapy. All 3 patients with baseline elevated ALT normalized their ALT at EOT. Individual patient responses are indicated in Figures S3C,D and S4C,D. As expected, regimens with shorter durations of combination therapy (i.e., regimens 5 and 8; Table 1) had lower responses (Table 3). GI-related AEs, discontinuations due to AEs, and SAEs are summarized in Table 4 for all regimens. Effects on key hematologic and biochemical parameters are summarized in Table S3 and Figure 3. Effects on weight, dose modifications, and cumulative LNF dose are also included in Table S3. The most surprising finding from these analyses was a previously unrecognized effect of LNF on platelets (see Figure 3J). Indeed, lonafarnib exhibited an average 38% ($p < 0.01$) increase in platelet counts in all LNF-containing regimens (except those combining PEG-IFNα, which is known to be associated with cytopenias). This increase in platelets appears to be associated with a decrease in hemoglobin levels (Day 1: 14.4 ± 1.67 [x ± SD] g/dl vs. Week 12: 12.8 ± 1.86 [$p < 0.0001$]). An inverse correlation between the increase in platelet counts and decrease in hemoglobin levels, however, has not been observed (r: −0.289, $p < 0.088$ with Pearson correlation).

High response rates to all-oral LNF 50 mg bid + RTV in patients with low baseline viral loads

Patients with baseline viral loads ≤ 4 $\log_{10}$ demonstrated high response rates when treated with all-oral LNF 50 mg bid + RTV 100 mg bid, with 6 of 7 (86%) patients with ≥2 $\log_{10}$ IU/ml decline (or < LLOQ) after 24 weeks of treatment (Table 5). Only 1 patient with baseline low viral load received combination treatment with PEG-IFNα, making it difficult to draw any formal conclusion on the efficacy of triple combination treatment in low-viral patients, although this patient had a ≥2 $\log_{10}$ IU/ml decline (or < LLOQ) of HDV RNA after 24



(J) Quantified lonafarnib-induced increases in platelet counts.

Regimen	Mean change in platelets at week 12	
1. LNF 100 mg bid+ RTV 100 mg qd	+15%	
2. LNF 100 mg bid + RTV 50 mg bid	+89%	
3. LNF 100 mg qd + RTV 100 mg qd	+33%	
4. LNF 150 mg qd + RTV 100 mg qd	+42%	
5. LNF 75 mg bid + RTV 100 mg bid	+39%	
6. LNF 25 mg bid + RTV 100 mg bid	+35%	
7. LNF 50 mg bid + RTV 100 mg bid	+32%	
8. LNF 50 mg bid + RTV 100 mg bid	+15%	
9. LNF 25 mg bid+ RTV 100 mg bid + PEG-IFNα		-33%
10. LNF50 mg bid+ RTV 100 mg bid + PEG-IFNα		-19%
Mean for all lonafarnib regimens (without PEG-IFNα)	**+38%**	

FIGURE 3 Effects of LNF on key hematologic and biochemical parameters and weight, as a function of treatment regimen. (A) White blood count (WBC), all regimens. (B) Hemoglobin, all regimens. (C) Creatinine, all regimens. (D) Weight, all regimens. (E) Platelets, all regimens. (F) Platelets, high-dose regimens. (G) Platelets, all-oral low-dose regimens. (H) Platelets, IFN added at 12-week regimens (5 and 8). (I) Platelets, IFN-containing triple therapy regimens (9 and 10). (J) Quantified LNF-induced increases in platelet counts

weeks. Patients with baseline viral loads >4 $\log_{10}$, however, demonstrated high response rates when treated with LNF + RTV combined with PEG-IFNα, with 3 of 4 (75%) patients demonstrating ≥2 $\log_{10}$ IU/ml decline (or < LLOQ) with LNF 25 mg bid + RTV + PEG-IFNα, and 4 of 4 (100%) patients demonstrating ≥2 $\log_{10}$ IU/ml decline (or < LLOQ) with LNF 50 mg bid + RTV + PEG-IFNα after 24 weeks of treatment (Table 5).

Effect of extending treatment duration to 48 weeks

Because of the improved tolerability of the low-dose all-oral and combination with PEG-IFNα regimens, the effect of prolonging therapy to 48 weeks was explored in a small cohort of patients, and the results will be reported elsewhere.

TABLE 5 Efficacy of all-oral LNF 50 mg or combination therapy–based regimens in patients by baseline viral load

Regimen	Patients dosed 24 weeks (*n*)	Baseline VL ≤ 4 log_{10}		Baseline VL > 4 log_{10}	
		≥2 log_{10} decline (%)	<LLOQ (%)	≥2 log_{10} decline (%)	<LLOQ (%)
LNF 50 mg BID + RTV 100 mg BID	13	4 of 7 (57.1%)	6 of 7 (85.5%)	1 of 6 (16.7%)	0 of 6 (0%)
LNF 25 mg BID + RTV 100 mg BID + PEG-IFNα	5	0 of 1 (0%)	1 of 1 (100%)	3 of 4 (75%)	2 of 4 (50%)
LNF 50 mg BID + RTV 100 mg BID + PEG-IFNα	4	0 of 0 (0%)	0 of 0 (0%)	4 of 4 (100%)	2 of 4 (50%)

Abbreviation: VL, viral load.

Posttreatment follow-up

Our study's primary endpoint was EOT, and the protocol only contemplated 4-week follow-up data for safety assessments. Although not a feature of the original protocol, ≥24-week posttreatment data were available for some patients from the key regimens[6,7,9,10] (Table S7).

Three patients who did not have HDV-RNA responses on therapy experienced transient posttreatment beneficial ALT flares (>2-times baseline ALT) with no signs or symptoms of clinical hepatic decompensation, and also fortuitously had corresponding liver biopsies (Table S8). Peak flare ALTs were associated with declines in HDV RNA, and flare resolutions led to HDV-RNA negativity with ALT normalization at 24 weeks following treatment. One patient had been treated with the LNF 50 mg bid + RTV 100 mg bid regimen; the two others were in the higher lonafarnib group (LNF 200 and 300 mg bid, respectively) (Table S8). The former patient had a baseline liver biopsy revealing an Ishak fibrosis score of 6. A follow-up liver biopsy, performed after 6 months of sustained ALT normalization after the ALT flare, revealed a decrease of the Ishak fibrosis score to 4. The detailed kinetics of HDV RNA and ALT as a function of time for these 3 patients is displayed in Figure S5.

DISCUSSION

In this study, three groups of LNF-based treatment regimens were assessed: high-dose Group 1 (LNF ≥ 75 mg bid + RTV), the low-dose all-oral Group 2 (LNF 25 or 50 mg bid + RTV), and the low dose combination Group 3 (LNF 25 or 50 mg bid + RTV + PEG-IFNα). The main findings of the LOWR-2 study can be summarized as follows: (1) All-oral therapy with LNF and RTV appears to be effective, and nearly half of patients on LNF 50 mg bid + RTV 100 mg bid reached the primary endpoint of the study; LNF 25 mg bid + RTV is well-tolerated but less effective; (2) combination therapy, consisting of the addition of PEG-IFNα 180 ug qw to the low-dose LNF treatment groups, displayed the most robust antiviral efficacy indicating synergy; (3) ALT normalization typically follows antiviral response; (4) most patients treated with LNF 25 or 50 mg bid + RTV ± PEG-IFNα were treated for a duration of 24 weeks; (5) for patients with low baseline viral loads, excellent responses are observed with all oral LNF 50 mg bid + RTV 100 mg bid; and (6) a subset of patients displayed a posttreatment ALT flare followed by HDV-RNA negativity and ALT normalization. Because a relatively small number of patients was subdivided into 10 treatment regimens, firm statistical conclusions were not, nor were they expected to be, reached. Nevertheless, the primary purpose of this study—to identify regimens with acceptable tolerability and efficacy to take into larger and longer studies—was accomplished.

Most patients treated with LNF in LOWR-2 demonstrated a decline in HDV-RNA levels at Week 24. Seven of 19 (36.8%) and 8 of 9 (88.9%) patients on all-oral low LNF + RTV and combination LNF + RTV + PEG-IFNα regimens, respectively, achieved ≥2 log_{10} decline (or < LLOQ) at end of treatment. The proportion of patients demonstrating HDV RNA with ≥2 log_{10} decline (or < LLOQ) at Week 24 increased with the LNF 50 mg bid dose (vs. 25 mg) and with addition of PEG-IFNα. One of 6 (16.7%) of patients treated with LNF 25 mg bid + RTV 100 mg bid achieved ≥2 log_{10} decline (or < LLOQ) at Week 24, which increased to 46.2% (6 of 13) with LNF 50 mg bid + RTV 100 mg bid. Addition of PEG-IFNα provided the most robust data to date, where 80% and 100% of patients (4 of 5 and 4 of 4) achieved ≥2 log_{10} decline (or < LLOQ) at Week 24 with LNF 25 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw, and LNF 50 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw, respectively. Overall, there was a better viral load response of the LNF 50 mg bid + RTV 100 mg bid group as compared with the LNF 25 mg bid + RTV 100 mg bid group. Addition of PEG-IFNα to either LNF 25 mg bid + RTV 100 mg bid or LNF 50 mg bid + RTV 100 mg bid therapy resulted in an improved virologic response as compared with the corresponding all-oral therapies.

Achieving the above ≥2 log_{10} decline (or < LLOQ) at EOT is important because such EOT responses have

been associated with long-term survival benefit.[8] This latter study was based on treatment of CDH with conventional treatment for 1 year, whereas in the current study treatment duration was no more than 24 weeks, and a comparison with a study of longer treatment duration may not be appropriate. In addition, a ≥2 $\log_{10}$ decline (or < LLOQ) at EOT has been endorsed as the most appropriate surrogate for initial treatment efficacy for future registration studies in HDV by a panel of experts.[7]

Although combination treatment with PEG-IFNα demonstrated the most robust antiviral response, an all-oral regimen is an important option for patients who cannot tolerate PEG-IFNα or are treatment-refractory to PEG-IFNα In addition, all-oral LNF 50 mg bid + RTV has demonstrated good antiviral activity in the approximately 30%–40% patients who present with low baseline viral loads of ≤ 4 $\log_{10}$. Indeed, 6 of 7 (86%) patients with low baseline viral load achieved ≥2 $\log_{10}$ IU/ml decline (or < LLOQ) after 24 weeks of treatment. Moreover, at 24 weeks following treatment follow-up, 33% (2 of 6) of patients demonstrated a durable virologic response of ≥2 $\log_{10}$ decline (or < LLOQ) and 50% (3 of 6) of patients with elevated ALT at baseline had normal ALT. There are at least three mechanisms that appear to contribute to the antiviral efficacy of LNF. By directly inhibiting farnesylation of large delta antigen, LNF blocks the assembly and release of HDV virus particles.[4] As a consequence of not being secreted in the form of nascent HDV particles, large delta antigen can exert its transdominant effect on HDV-RNA genome replication.[9] Finally, the intracellular retention of large delta antigen that is associated with LNF treatment has been shown to induce innate immune responses in HDV-infected cultured cells.[10,11]

Patients with low baseline viral loads had higher rates of HDV RNA ≥ 2 $\log_{10}$ decline (or < LLOQ) at end of treatment. Therefore, patients with low viral load at baseline may be considered for all-oral treatment with 50 mg LNF in combination with RTV.

Most patients with HDV with ALT > upper limit of normal who were treated with low-dose LNF + RTV ± PEG-IFNα normalized their ALT after only 24 weeks of treatment. Spontaneous normalization of ALT is rare in untreated patients with HDV. ALT is a highly useful peripheral surrogate biochemical marker for the extent of inflammation in the liver. Numerous data sets from studies in patients with chronic HBV demonstrate the strong association of treatment-induced normalization of ALT with improved necro-inflammation based on liver histology using validated central pathology assessments.[12,13]

Three patients who did not achieve HDV responses at EOT experienced posttreatment flares and resulting HDV-RNA negativity with ALT normalization. Importantly, all of these episodes were well-tolerated without any signs of clinical decompensation. One of these patients had a baseline liver biopsy, allowing for assessment of the effect of this ALT normalization on liver histology, which demonstrated regression of fibrosis from baseline. This, along with similar posttreatment flare results,[6] represents data on regression of fibrosis with new compounds in patients with HDV, although we do realize that the number of patients is small. The fact that HDV is associated with rapid progression of fibrosis might explain why such regressions of fibrosis have been observed in relatively short periods of time. Importantly, this suggests that the high rates of ALT normalization observed with LNF 50 mg bid regimens (either as all-oral with RTV, or as combination therapy with PEG-IFNα) are also likely to result in improvements of liver histology. This would be in line with other studies that have demonstrated that removal of the underlying major trigger of inflammation with resulting prolonged ALT normalization is associated with histologic improvement and regression of fibrosis.[14]

Limitations of this study include its open-label, single-center, and nonrandomized nature. Furthermore, no quantitative HBsAg kinetics or pharmacokinetic data were available to evaluate in this study, including in patients with a posttreatment flare. In addition, a large number of dosing groups were explored to identify optimal dosing regimens, and these dosing regimens had relatively small numbers of patients, which led to lack of statistical power associated with the findings of this study. Future clinical studies with larger numbers of patients focusing on the key dosing regimens will enable more firm statistical evidence. In particular, the effect of extending the key low-dose regimens (i.e., low-dose LNF all oral, or in combination with PEG-IFNα) to 48 weeks of treatment is currently being rigorously evaluated in a randomized, placebo-controlled, phase 3 study of 400 patients, which should provide definitive and statistically sound data (NCT03719313).

Several approaches targeting different steps of the HDV life cycle are in clinical development for the treatment of HDV.[15] LNF is the only oral HDV treatment in phase 3 development and inhibits the critical step of large delta antigen prenylation, which is essential for HDV particle assembly.[4,16] LNF has now been studied in over 120 patients, most of whom were in the LOWR-2 study described herein, as well as in the LOWR-3 and LOWR-4 studies.[17,18] We previously reported that LNF was not associated with resistance development in patients treated with LNF for 12 weeks.[6] The absolute barrier to the development of resistance to a given host-targeting strategy can vary with each specific host target–virus pair.

The main aim of the current study using 10 subgroups was to create a regimen or regimens that combines efficacy with tolerability. Gastrointestinal side effects to LNF such as anorexia, nausea, diarrhea, and weight loss were clearly more common and

intense with the all-oral high-dose regimens, leading to our decision not to consider high doses for future studies in CDH. Weight loss, an objective tool to assess gastrointestinal side effects, of Grade 2 toxicity according to CTCAE criteria within the duration of 12 weeks, during which high-dose all-oral LNF was given in Regimens 1 to 5, was observed in one-third of patients (5 of 15). Within the same duration, weight loss of Grade 2 toxicity was seen in 21% of patients (5 of 24) with the low-dose group. However, while discontinuations of the treatment regimen due to AEs was seen in 21% (4 of 19) of patients in the high-dose group, none of the patients (0 of 24 patients) in the low-dose all-oral regimens discontinued treatment. In the context of weight loss, Grade 2 toxicity is defined as weight loss of 10% to <20% of baseline weight. Treatment duration in regimens 5 to 10 was 24 weeks, which allowed us to assess trends in weight loss over time as a reflection of gastrointestinal AEs. There were 9 patients, including regimens 9 and 10, with weight loss of toxicity Grade 2 at Week 12. Of these 9 patients, 3 patients discontinued treatment after Week 12; in 1 patient, toxicity grade further increased to Grade 3. In another patient, toxicity grade did not change, whereas in 4 patients toxicity grade decreased to Grade 1. In 1 patient, weight determinations at Week 24 was unfortunately missing. Thus, Grade 2 toxicity within 12 weeks was—in the current study—either associated with continuation of the AE intensity, leading to premature discontinuation of treatment or further worsening of the AE in some patients, whereas in others, AEs either improved or did not change with treatment continuation, suggesting adaptation of these patients to the treatment regimen.

An interesting observation was seen in all patients receiving LNF with RTV. LNF led to an asymptomatic increase in platelet counts. The mechanism of this increase in platelets is unknown, but could be potentially very interesting, especially in a patient population in whom thrombocytopenia is common. Secondary or reactive thrombocytosis as a consequence of acute blood loss, iron deficiency anemia, hemolytic anemia, acute or chronic infection or inflammation, and drug reactions has been well described.[19] In the current study, the increase in platelet count was associated with a decrease in hemoglobin levels in some patients but not in others. Although the mechanism for a decrease in hemoglobin levels needs to be clarified, a potential inverse correlation between a decrease of hemoglobin levels and an increase in platelet counts was not observed. Another consideration is that the increase in platelet count is a direct effect of LNF.

The mechanism of a decrease in hemoglobin levels needs to be clarified, which was not done in the current study, partly because most patients were asymptomatic, and in patients with symptoms such as fatigue, there were other confounders such as diarrhea or concomitant peg-IFN use. Hemoglobin levels in some patients fell below 10g/dl, although at Week 24 such patients constituted a minority (4 of 36 patients [11%]). Hemolysis could not be excluded as a cause of anemia with certainty, as several hemolysis markers were not available to us, but there was no increase in indirect bilirubinemia.

The lack of a placebo group and the rather small number of patients necessitates avoiding overinterpretation. The currently ongoing phase 3 study will enable a more conclusive approach. It needs to be said, however, that myelosuppression has been reported as a common AE of farnesyl transferase inhibitors in patients with leukemia.[20] An effect in this context was not observed on white blood cells and obviously not on platelets, at least in the doses used in the current study.

Identifying therapeutic regimens capable of achieving on-treatment HDV-RNA declines of ≥2 $\log_{10}$, such as those described here, represents an important advancement for CDH. Thus, identifying candidate regimens that are sufficiently well-tolerated, and capable of achieving on-treatment HDV-RNA declines of ≥2 $\log_{10}$, are important findings of this study. The all-oral LNF 50 mg bid + RTV 100 mg bid appears to represent one such regimen. Similarly, the combination regimen of low-dose LNF + RTV + PEG-IFNα appears promising, demonstrating both apparent synergy and maximal antiviral efficacy. Because of its comparable antiviral efficacy but significantly improved tolerability,[21] replacing PEG-IFNλ for PEG-IFNα in the LNF + RTV + IFN combination regimen may therefore allow comparable efficacy with maximal tolerability—a hypothesis that has recently been successfully tested (clinicaltrials.gov NCT03600714).

Most importantly, the results of the current study have led to the identification of well-tolerated and efficacious regimens (e.g., all-oral LNF 50 mg bid + RTV 100 mg bid; LNF 50 mg bid + RTV 100 mg bid + PEG-IFNα 180 μg qw) that have now entered the pivotal phase 3 study (NCT03719313) designed to seek FDA registration for the treatment of HDV.

ACKNOWLEDGMENTS

We wish to dedicate this manuscript to the memory of Dr. Hugo Rosen, a tireless researcher, academic leader, and editor, dedicated to advancing the field of hepatology, valuable mentor, and most of all dear friend. [Correction added on January 4, 2022, after first online publication: Acknowledgment was included for Dr. Hugo Rosen]

CONFLICT OF INTEREST

Dr. Apelian advises, is employed, and owns stock in Eiger. Dr. Yurdaydin advises and is on the speakers' bureau for Gilead. He is on the speakers' bureau and received grants from AbbVie and Eiger. Dr. Choong is employed, owns stock, and holds intellectual property

rights with Eiger. Dr. Glenn is the founder and a director of, holds intellectual property rights with, owns stock and royalty rights from Eiger.

AUTHOR CONTRIBUTIONS

Cihan Yurdaydin and Jeffrey S. Glenn contributed to the study design. Cihan Yurdaydin, Onur Keskin, Esra Yurdcu, Aysun Çalişkan, Soner Önem, Fatih Karakaya, Çağdaş Kalkan, Ersin Karatayli, Senem Karatayli, Ramazan Idilman, and A. Mithat Bozdayi contributed to the performance of the study. Cihan Yurdaydin, Onur Keskin, Ingrid Choong, David Apelian, Christopher Koh, Theo Heller, and Jeffrey S. Glenn contributed to the analysis and manuscript writing.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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