Oral Prenylation Inhibition with Lonafarnib in Chronic Hepatitis D Infection: a randomized, double-blinded, placebo controlled phase 2a trial.

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Abbreviations: HDV, delta hepatitis; LNF, lonaFarnib; RNA, ribonucleic acid; HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; FDA, food and drug administration; AASLD, American Association for the Study of Liver Diseases; EASL, European Association for the Study of the Liver; LDHAg, large delta antigen; NIH, National Institutes of Health; qPCR, quantitative polymerase chain reaction; ALT, alanine aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; BID, twice daily; DNA, deoxyribonucleic acid; SD, standard deviation; SE, standard error;

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Abstract

Background: Therapies for chronic delta hepatitis (HDV) infection are unsatisfactory. Prenylation is essential for HDV and inhibition abrogates HDV production in experimental models. In a proof-of-concept study in chronic HDV, antiviral effects and safety of the oral prenylation inhibitor, lonafarnib (LNF), were evaluated.

Methods: In a phase 2a double-blinded, randomized, placebo-controlled study, subjects received LNF 100 mg (Group 1) or LNF 200 mg (Group 2) twice daily for 28 days with 6 months follow-up. Both groups enrolled 6 treatment and 2 placebo subjects. Group 1 placebo patients received open-label LNF as Group 2 participants; ClinicalTrials.gov number, NCT01495585

Findings: 14 patients were enrolled; 71% male, median age of 38, without significant baseline differences between groups. At day 28, compared to placebo, mean log HDV RNA declines from baseline were -0.73 log IU/mL in Group 1 ($p=0.03$) and -1.54 log IU/mL in Group 2 ($p<0.0001$). LNF serum concentrations correlated with HDV RNA change ($r^2=0.78$, $p<0.0001$). Model fits show HBsAg remained stable after a short pharmacological delay (0.75[standard error, $se=0.24$] days), LNF effectiveness in blocking HDV production was greater in Group 2 compared to Group 1 (0.952[$se=0.06$] vs. 0.739[$se=0.05$], $p<0.001$), and HDV half-life was 1.62[$se=0.07$] days. There was no evidence of virologic resistance. Adverse events were mild-moderate (mainly gastrointestinal) and prompted no treatment discontinuations.

Interpretation: Treatment of chronic HDV with LNF significantly reduces virus levels. The decline in virus levels significantly correlated with serum drug levels, providing further evidence for the efficacy of prenylation inhibition in chronic HDV.

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Introduction

The hepatitis delta virus (HDV) is an incomplete ribonucleic acid (RNA) virus composed of a 1.7 kb single-stranded circular genomic RNA, virally encoded small and large delta antigens, and a surrounding lipid envelope.(1, 2) It is the smallest pathogenic animal virus known to infect humans, and it is estimated that up to 20 million people are chronically infected worldwide.(3) HDV infection has been described to be endemic across most of the world. Although HDV shares epidemiological patterns with hepatitis B virus (HBV), HDV in its own right continues to be a major global health problem.(4, 5) The virus was first identified in 1977 and is known only to propagate in individuals infected with HBV, either via superinfection or co-infection.(4, 6) Eight HDV genotypes have been described and genotype 1, which is most prevalent in North America, Europe, North Africa and the Middle East has been associated with more severe disease.(7, 8) Like other forms of viral hepatitis, HDV progresses to cirrhosis, but is the most severe form of human viral hepatitis. (5, 9-14) [ENREF 9](#)

Currently, therapy for HDV is unsatisfactory and no FDA approved therapy exists for HDV infection. The American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) have recommended treatment with interferon-alfa for chronic HDV infection.(15, 16) However, therapy with interferon-alfa is generally unsatisfactory with high relapse rates even with therapy extended out to 5 years.(17, 18)

Prenylation is a post-translational lipid modification that involves the covalent addition of prenyl lipids to proteins resulting in promotion of membrane association and protein-protein interactions.(1, 19) Prenylation plays a vital role in

the life cycle of HDV, and disruption of prenylation of the large delta antigen (LDHAg) prevents its ability to interact with, and form secreted particles with the hepatitis B surface antigen.(19, 20) Use of prenylation inhibitors has been evaluated with success in vitro as well as in vivo in a mouse model of HDV replication.(1, 19-21) In this proof-of-concept study, we examined the utility of the prenylation inhibitor lonaFarnib (LNF) in patients with chronic delta hepatitis and evaluated its effect on HDV RNA, safety and tolerability.

Methods

Patients

Patients ≥ 18 years of age with chronic HDV infection were enrolled between January 19, 2012 and April 28, 2014 at the National Institutes of Health (NIH) Clinical Center. Full eligibility criteria are provided in the supplementary methods section and the study protocol which is available on-line. All patients provided written informed consent.

Study Design

In this single-center, double-blinded, randomized, placebo-controlled clinical trial, patients received orally administered LNF or placebo twice daily. Fourteen enrolled patients were sequentially assigned into one of two dosing groups which consisted of LNF 100 mg twice daily in Group 1 and LNF 200 mg twice daily in Group 2, with placebo controls in each group (Figure 1). Group 1 participation was completed before starting of Group 2. Patients were randomized by the NIH Pharmaceutical Development Services (PDS) with random-number tables blocked in groups of 4 without stratification. Patients received treatment for 28 days, followed

by post-treatment monitoring for six months. Each group consisted of 8 patients (6 treatment and 2 placebo), and the 2 patients who received placebo in Group 1 were offered treatment as Group 2 participants with open-label LNF (200 mg twice daily). Additional details of the study design are described in the supplementary methods section.

Study Assessments

The primary therapeutic endpoint of the study was defined as a decrease in HDV RNA viral titer in serum, as measured by quantitative polymerase chain reaction (qPCR). The primary safety endpoint of the study was the ability to tolerate the drug at the prescribed dose for the full 4-week duration. The secondary endpoints included change in: ALT levels; symptom scales.

Assessments throughout the study period included standard laboratory testing, measurement of quantitative HDV RNA, HBV DNA and HBsAg by qPCR, HDV viral mutations associated with LNF nonresponse (at baseline, end of therapy and end of follow-up), vital signs, electrocardiography (while on therapy and at weeks 1 and 2 post-therapy), and symptom-directed physical examinations. All adverse events were recorded and graded according to the CTCAE scale (see study protocol). All patients were infected with HDV Genotype 1 strains and the assay used in this study for HDV RNA qPCR was designed to correctly evaluate HDV viral load values by targeting highly conserved regions of the viral genome. Details of the HDV RNA qPCR, HBV DNA qPCR, HBsAg ELISA and HDV viral mutation testing are described in the supplementary methods section.

Safety

Safety was assessed by the study investigators at each time-point based on laboratory analysis and study participant reports. Throughout the study period, patients also underwent monitoring for electroretinographic and electrocardiographic changes and reproductive toxicity. Additional details are described in the supplementary methods section, study protocol and supplemental table 1.

Study Oversight

This investigator-initiated clinical trial was performed at the NIH Clinical Center and was designed and conducted by the principal and associate investigators who collected the data, monitored the study conduct, performed the statistical analysis and prepared the manuscript. The study was registered in ClinicalTrials.gov (#NCT01495585) and the all study materials were approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) at the NIH Clinical Center. LNF was provided by Eiger Biopharmaceuticals, Inc under a clinical trial agreement with the NIDDK. Administration of LNF was conducted under an Investigational New Drug Application (IND# 113,137) held by the NIDDK.

Kinetic analysis and mathematical modeling

HDV kinetic characterization during LNF therapy was performed using a segmented linear regression approach. To study HDV dynamics and estimate LNF effectiveness, a dual HDV and HBsAg model (See Supplemental Modeling Data, Eq. S1 and Supplemental Figure 1) that was recently developed for understanding HDV-host dynamics during pegIFN therapy was employed.(22) The model was fitted to the log-scaled HDV viral loads and HBsAg levels, using a nonlinear mixed effect

modeling approach. Further details are provided in the supplementary modeling data section.

Statistical Analysis

The primary statistical endpoint of the study was the change in serum HDV RNA after 28 days of therapy with LNF. This study was intended as a proof-of-concept pilot study evaluating viral decline and safety of LNF. Based on a 1 log decline of quantitative HDV RNA with lonafarnib therapy, with a sigma of 0.5 log of normal HDV viral fluctuations over time and an alpha of 0.5 with a power of 0.80, the sample size required to show a difference is 3 subjects. Given that 6 patients received therapy in each group and that there were 4 placebo subjects, we feel that this study was adequately powered to show a difference in viral decline and the presence of significant safety signals. Comparison of HDV RNA and HBV DNA changes (after logarithmic transformation) between groups was performed via student's t-test. Other continuous variables were analyzed via a Mann-Whitney. Association between HDV RNA change (after logarithmic transformation) and serum LNF concentration was analyzed using linear regression. Viral-host parameters estimated by fitting the dual HDV and HBsAg model (Supplemental Modeling Data Section Eq. S1) with the experimental data were tested with a likelihood ratio test. All statistical tests and confidence intervals were two-sided and a p value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed with JMP 11.0 (SAS Inc. Cary, NC) and SAS 9.3 (SAS, Inc., Cary, NC).

Role of the funding source

Eiger Biopharmaceuticals, Inc. did not play any role in the study design, data collection, decision to publish, or writing of the manuscript. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Baseline Characteristics

Of the 22 screened patients, 14 were enrolled. Of the 8 patients not enrolled, 4 had undetectable HDV RNA, 3 did not complete the pre-enrollment evaluations and 1 was diagnosed with hepatocellular carcinoma (Figure 1). The demographic and baseline clinical characteristics of the patients that were enrolled are described by their randomization group in table 1.

Overall, the median age was 38 years, 10 (71%) were male, all with HBeAg negative status and HDV genotype 1 infection; 7 (50%) were Asian, 4 (43%) were Caucasian and 1 (7%) were African. 12 (86%) of the 14 patients originated from endemic regions and 2 (14%) had traditional risk factors for blood and body fluid transmission. 5 (36%) of the patients were on nucleos(t)ide analogues (tenofovir or entecavir) at least 6 months prior to screening and 3 (21%) had failed interferon therapy at least 6 months prior to screening. The median Ishak fibrosis score was 3, HAI necroinflammation was 10 and baseline platelets were 190 K/ul. The median baseline serum HDV RNA was 9.27×10^5 IU/mL and in patients not taking nucleos(t)ide analogues, the median baseline HBV DNA was 22.5 IU/mL. All but one patient had low level HBV DNA replication <2500 IU/mL.

Virologic Response to Therapy with LonaFarnib

During 28 days of therapy, patients randomized to LNF (100 mg BID or 200 mg BID) experienced a decline in serum HDV RNA levels at all weeks of therapy compared to baseline and placebo (Figure 2). By the end of therapy, the mean HDV RNA decline in serum from baseline was 0.13 log (95%CI -0.14, 0.34) in the placebo group, 0.73 log (95% CI 0.17, 1.31) in Group 1 and 1.54 log (95% CI 1.21, 1.93) in Group 2 (Table 2 and Figure 3a). The viral declines seen in Group 1 and Group 2 were significantly different when compared to placebo ($p=0.03$ and <0.0001 , respectively). Mean serum LNF concentrations strongly correlated with mean serum log HDV RNA change from baseline to end of therapy ($r^2=0.78$, $p<0.0001$) (Figure 3b).

As expected, since LNF inhibits host cell farnesyltransferase, a high barrier to the development of resistance was observed. Indeed, population-based sequencing for viral mutations of the large delta antigen (LDHAg) in serum at baseline, end of therapy and 24 weeks after completion of therapy did not identify viral mutations associated with LNF nonresponse (Supplemental Tables 2, 3 and 4).

During therapy, excluding for patients on nucleos(t)ide analogues, serum HBV DNA levels did not significantly change in Group 1 patients (Table 2). Interestingly, there was a trend towards increasing HBV DNA at the end of therapy in Group 2 patients with an increase in HBV DNA of 1.12 log ($p=0.05$). Baseline HBsAg levels did not significantly differ between groups at baseline (Table 1) nor did they change during or post therapy (Table 2).

Biochemical Response to Therapy with Lonafarnib

During therapy, no significant changes in liver associated enzymes were found on comparison between either dosing group with placebo (Table 2). In group

1, the median baseline ALT was 125 U/L (IQR: 86-168) and the median end of therapy ALT was 117 U/L (IQR: 102-168), and no patients had normalization of ALT at the end of therapy. In group 2, the median ALT at baseline was 63 U/L (IQR: 52-162) and the median end of therapy ALT was 48 (IQR: 37-96). Two patients (not on nucleoside analogues) had normalization of ALT (35 and 31 U/L), however their baseline ALT value was 47 U/L.

Virological and Biochemical Profiles during Post-Therapy Follow-up

On post-therapy follow-up, HDV RNA levels returned to baseline in all Group 1 patients by week 4 (range week 1-4) (supplemental table 5). A single patient (not on nucleos(t)ide analogue) experienced a virological flare of HDV RNA greater than 0.5 log over baseline at week 4 of post-therapy follow-up which was accompanied by an ALT increase of 1.5 times the patient's baseline level.

In Group 2, HDV RNA levels returned to baseline in all patients by week 4 of post-therapy follow-up (range week 1-4). Four patients (2 on nucleos(t)ide analogues) experienced a flare of HDV RNA greater than 0.5 log over baseline between week 4 and 8 (range 0.79-1.48 log IU/mL) of post-therapy follow-up. In these 4 patients, at the time of virologic rebound, ALT was increased no more than 2.5 times (mean 1.81, SD:0.6) the patients' baseline levels.

Kinetic Analysis & Mathematical Modeling with Lonafarnib Therapy

A biphasic decline during therapy was characterized in all LNF-treated patients, with a first rapid decline phase followed by a second slower (or plateau) phase (Supplemental Tables 6 and 7). The first phase duration was longer in Group 2 compared to Group 1 (12.3 (standard error, se=2.1) vs. 8.72 (se=2.5) days) and the decline was faster in Group 2 (-0.143 (se=0.02) vs. -0.098 (se=0.03) log IU/mL/day),

leading to a significantly greater 1st-phase decline (1.59 vs. 0.78 log IU/mL, $p=0.015$) in the group 2 patients. The second slope phase was not statistically different between Groups 2 and 1 (-0.06 log IU/mL/day, $p=0.94$) (Supplemental Tables 6 and 7).

Fitting the HDV and HBsAg dual model (Supplemental Modeling Data Eq. S1 and Supplementary Figure 1) with measured HDV and HBsAg kinetics yielded excellent fit curves (Supplementary Figure 2). The pretreatment serum HDV RNA and HBsAg levels were estimated as 5.97($se=0.13$) log IU/mL and 4.17($se=0.12$) log ng/mL. A short pharmacological delay of $t_0=0.73$ ($se=0.24$) day, in which HDV remained at baseline levels, was not associated with LNF dose. The HDV clearance rate in blood, c , was estimated as 0.426 ($se=0.04$) d⁻¹, corresponding to an HDV half-life in blood of 1.63 ($se=0.15$) days. LNF effectiveness in blocking HDV production was significantly ($p<0.001$) higher in Group 2($\epsilon=0.952$ ($se=0.057$)) than Group 1 ($\epsilon=0.739$ ($se=0.05$)) (Supplementary Table 9). Individual parameters are shown in Supplementary Table 8.

Safety

No patients prematurely discontinued LNF due to grade 3 or 4 adverse events or a serious adverse event. There were no significant changes from baseline on digitized electrocardiography, ophthalmologic examination with retinal photography or reproductive toxicity testing performed throughout the study (supplemental table 1). The major side-effects in Group 1 patients included mild diarrhea (3 of 6 patients, 50%), nausea (2 of 6, 33%), anorexia (1 of 6, 17%), fatigue (1 of 6, 17%), abdominal bloating/dyspepsia (1 of 6, 17%) and weight loss >2kg (1 of 6, 17%). In Group 2 patients, major side-effects included nausea, diarrhea,

abdominal bloating and weight loss >2kg (mean of 4 kg) in all patients (6 of 6 patients, 100%) and anorexia in 5 of 6 (83%) patients followed by intermittent vomiting (3 of 6, 50%), headaches and fatigue (1 of 6, 17%). While the symptoms in Group 1 could not be distinguished from placebo the symptoms described by Group 2 participants, while not affecting daily activities, could be distinguished from placebo patients.

Discussion

In this first-in-humans, proof-of-concept study, we demonstrate that lonafarnib, can decrease serum hepatitis delta virus levels in a dose dependent manner during 28 days of therapy. This study has value in that it demonstrates that an oral small molecule prenylation inhibitor can affect HDV and thereby warrants further study in a field with unmet medical needs. Moreover, half of the patients in the higher dosing group achieved a decrease from baseline to nadir of greater than -2 log IU/mL. As further evidence supporting the antiviral efficacy of prenylation inhibition against HDV, two-thirds of the patients in the higher dosing group experienced HDV virologic rebound with accompanied ALT flare after stopping therapy. Although this is the first demonstration of virologic rebound after stopping prenylation inhibitor therapy, the concept of HDV rebound after treatment cessation has been demonstrated in interferon-based studies for HDV.(23) As expected, there was no evidence of HDV viral mutations associated with LNF nonresponse throughout the study, which is consistent with the mechanism of action of prenylation inhibitors that target host enzymes. Additionally, while this study consisted solely of HDV genotype 1 patients, in vitro studies with farnesyltransferase inhibitors suggest that comparable results would be expected with other HDV genotypes.(20) However a

challenge across HDV genotypes might be to adequately monitor and compare viral levels based on the various qPCR techniques available.

Although small in patient numbers and short in duration, this study provides evidence in humans of a novel mechanism for potential therapeutic eradication of HDV. In the higher dosing group of patients not taking nucleos(t)ide analogues, there was a nearly significant trend of increased HBV DNA levels at the end of therapy ($p=0.05$). Previous studies using interferon have described HBV reactivation when HDV is suppressed or eliminated.(3, 24) However, unlike interferon-based studies, which may treat both HDV and HBV, prenylation inhibitors only inhibit HDV, this allows for the study of the effect of HDV on HBV. With extended therapy, HBV monitoring and nucleos(t)ide therapy should be considered.

In this study, there were no premature discontinuations or serious adverse events during 28 days of therapy with LNF. Gastrointestinal side effects (nausea, diarrhea, abdominal bloating and decreased appetite) were the most common, and are similar to studies evaluating LNF for other disease states.(25-27) The exploration of antiemetics, antidiarrheal and acid suppressive therapies may improve tolerability in future studies. The average weight loss of 4 kg in group 2 suggests that prolonged dosing may be challenging especially in higher doses and would warrant close monitoring. One potential route of further exploration may include the fact that LNF is metabolized by CYP3A4, allowing for enhanced post absorption hepatic concentrations. By blocking this pathway and using lower doses of LNF, it may be possible to minimize side effects while potentiating the relationship between HDV decline and drug levels. Alternatively, future exploration

as a combination therapy with other novel therapies could be considered as they become available.

Analysis of viral kinetics demonstrated additional promising benefits of LNF. The modeling analysis indicated a dose dependent effect of LNF in blocking HDV release. Moreover, the 95% efficacy of the 200 mg LNF dose was similar to recent estimates in patients treated with peg-IFN ($\varepsilon=96\%$) and was achieved much earlier with LNF (median 12.5 days) compared to pegIFN (median 25.2 days). Frequent measurements during LNF therapy allowed for a refined estimate of HDV $t_{1/2}=1.6$ day that was about 2-fold shorter than during peg-IFN ($t_{1/2}=2.9$ day), and suggests a higher HDV production rate than recently estimated.(22) Finally, additional viral kinetic analyses based on these results and future studies should determine the strategy for proper eradication of those infected with HDV by LNF. Unlike interferon-based therapies, where only a percentage of patients respond, the mechanism of action of LNF and our study results suggests that a treatment response in all patients may be possible.

In conclusion, despite the small numbers, this study demonstrates that treatment of chronic HDV with the prenylation inhibitor lonafarnib significantly reduces virus levels in patients. The drug was well tolerated by all subjects. The decline in virus levels significantly correlated with increased serum drug levels, providing further evidence for the efficacy of prenylation inhibition in chronic HDV. These results provide a compelling rationale for next studies aimed at achieving eradication of HDV in humans.

Research in context

Evidence before this study:

We performed a review of the advances in the treatment of hepatitis D virus (HDV) to identify therapies that have been studied for chronic HDV infection by consulting published systematic reviews and searching PubMed with the terms “HDV treatment” and “Delta hepatitis treatment” between 1984 and 2015. To date, most therapeutic HDV trials have evaluated the utility of interferon, peginterferon and nucleos(t)ide analogues either as mono or combination therapies for differing durations. Systematic reviews have also described these studies and their lack of success, but have suggested that prenylation inhibition may be the future of HDV therapy given the success seen in two decades worth of preclinical HDV studies. To date, there have been no published human studies evaluating oral prenylation inhibitors for chronic HDV infection. This study reports the results from our first-in-humans study testing the hypothesis that a host-targeting small molecule prenylation inhibitor has authentic anti-HDV activity in humans.

Added value of this study:

This short-term proof-of-concept study shows that HDV viral load decreases on prenylation inhibitor therapy, and rebounds upon withdrawal of the drug—thereby demonstrating *in vivo* specific antiviral activity in humans of this novel mechanism of action. Additionally, this study describes a clear dose-dependent antiviral effect with dose response curves indicating that there is no inherent limit on the ability to drive viral loads down with increased drug levels. Finally, this study shows that the kinetics of response to prenylation inhibition is superior to those observed with interferon without evidence of viral resistance in spite of 28 days of monotherapy treatment with lonafarnib.

Implications of all the available evidence:

In this phase 2a double-blinded, randomized, placebo-controlled study, the prenylation inhibitor lonafarnib significantly reduces virus levels providing the first-in-humans evidence for the efficacy of prenylation inhibition in chronic HDV. Although this 28-day study did not result in HDV eradication, it provides the underpinnings that HDV may be susceptible to eradication through an alternate mechanism other than interferon and that this represents the beginning of a new era of therapy free of interferon for HDV.

Conflicts of Interest:

J.S.G: Equity interest in Eiger Biopharmaceuticals, Inc.

H.D.: Received partial travel support from Eiger Biopharmaceuticals, Inc to attend scientific meetings.

C.Y.: Advisory board of Merck, AbbVie, Janssen and Gilead Pharma. Speaker's bureau of Roche, Gilead, BMS and Merck Pharma and has received a research grant from BMS.

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Analysis and interpretation of data: Koh, Canini, Dahari, Zhao, Uprichard, Winters, Subramanya, Pinto, Wolff, Bishop, Han, Idilman, Keskin, Yurdaydin, Cotler, Kleiner, Glenn, Heller.

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Obtained funding: Heller

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Figure Legends

Figure 1. Study flow diagram. Of the 22 patients with positive HDV antibodies screened, 14 were enrolled into this study. This figure shows the flow of the study.

Figure 2. Mean serum HDV RNA (with standard deviation) decline during therapy with lonafarnib.

Figure 3. (A) HDV RNA decline after 28 days of therapy with lonafarnib.

Compared to placebo, HDV RNA significantly declined at the end of therapy in Group 1 (0.73 log IU/mL, $p=0.03$) and Group 2 (1.54 log IU/mL, $p<0.0001$). **(B)**

Correlation of the mean serum lonafarnib concentration with the mean change in serum HDV RNA from baseline to day 28. Mean serum lonafarnib concentrations strongly correlated with mean serum HDV RNA change from baseline to the end of therapy ($r^2=0.78$, $p<0.0001$). Black represents placebo, blue represents Group 1 and orange represents Group 2.

Supplementary Figure 1. Schematic representation of the HDV and HBsAg

dual model. HDV (small circles), V , infects HBsAg-infected cells, T , with rate constant β , generating infected cells, I , which produce new HDV virions at rate p per infected cell. Infected cells are lost at a rate δ per infected cell and virions are assumed to be cleared from blood at rate c per virion. HBsAg (triangles), H , is produced at rate p_H by infected cells, I , and cleared from serum at rate c_H .

Supplementary Figure 2. HDV and HBsAg kinetics and modelling during 100

(A) or 200 (B) mg bid of lonafarnib (LNF). HDV viral load and HBsAg levels

observations are shown with blue circles and green squares, respectively. The best-fit curves are shown in blue and green for HDV viral load and HBsAg levels, respectively. TNF, tenofovir; ENT, entecavir. Individual and per group viral kinetic parameters are shown in Tables S7 and S8.

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.

Patient Demographics	All (N=14)	Placebo (N=4)	Group 1 (N=6)	Group 2 (N=6)
Males sex – no. (%)	10 (71)	2(50)	5(83)	4(67)
Median Age-Yr (IQR)	38(33-48)	34(33-39)	36(30-42)	45(38-55)
Median BMI (IQR)	24.4(21.9-27.3)	26.5(24.1-28.9)	22.8(21.9-23.5)	26.1(25.5-27.3)
Pre-Tx Nucleoside Analogues (%)	5(36)	1(25)	2(33)	2(33)
Race - no. (%)				
Asian	7(50)	2(50)	3(50)	3(50)
Caucasian	6(43)	1(25)	3(50)	3(50)
African	1(7)	1(25)	0	0
Disease Characteristics				
Median Ishak Fibrosis (IQR)	3(1)	3(1)	3(1)	3(0)
Median HAI inflammation (IQR)	10(10-13.25)	12.5(11.75-13.25)	10(10-14)	11(10-11)
Median ALT U/L (IQR)	89(67-176)	101(80-162)	125(86-168)	63(52-162)
Median AST U/L (IQR)	61(42-80)	72(56-88)	61(50-75)	42(41-60)
Median Platelet K/uL (IQR)	190(175-242)	231(136-218)	174(170-183)	197(180-229)
Median Total Bilirubin mg/dL(IQR)	0.38 (0.30-0.55)	0.4 (0.30-0.55)	0.35 (0.30-0.48)	0.4 (0.40-0.60)
Median HDV RNA IU/mL (IQR)	9.27x10 ⁵ (3.20x10 ⁵ - 2.85x10 ⁶)	1.72x10 ⁶ (4.28x10 ⁵ - 4.43x10 ⁶)	1.29x10 ⁶ (4.74x10 ⁵ - 4.84x10 ⁶)	8.68x10 ⁵ (4.60x10 ⁵ - 1.94x10 ⁶)
Median log HDV RNA IU/mL (IQR)	5.94(5.50-6.43)	6.06(5.60-6.51)	6.06(5.56-6.60)	5.92(5.62-6.26)
Median HBV DNA IU/mL (IQR)*	22.5(<21-684)	<21(<21-26.5)	463 (<21-1239)	635(<21-947.5)
Median HBsAg ng/mL (IQR)	13216	15718 (10520-	17707 (11268-	11417 (8970-

	(8966-21792)	23534)	32085)	13103)
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* Analysis of patients not on nucleoside analogues. IQR, Interquartile range Q1-Q3.

Table 2: Laboratory Changes at End of Lonafarnib Therapy (Day 28).

Finding	Placebo*	Group 1*	Placebo vs. Group 1, p Value	Group 2*	Placebo vs. Group 2, p Value
Log HDV RNA IU/mL (SD)	-0.13 (0.14)	-0.73 (0.54)	0.03 ^δ	-1.54 (0.36)	<0.0001 ^δ
ALT U/L (SD)	18 (13)	4 (93)	0.96 [^]	29 (85)	0.6 [^]
AST U/L (SD)	2 (9)	12 (58)	0.7 [^]	-3 (14)	0.6 [^]
Total Bilirubin mg/dL(SD)	-0.05 (0.06)	-0.05 (0.18)	1.0 [^]	-0.08 (0.19)	0.1 [^]
Log HBV DNA IU/mL (SD)	-0.60 (0.72)	0.08 (0.13)	0.1 ^δ	1.12 (1.03)	0.05 ^δ
HBsAg ng/mL (SD)	-1448 (3784)	3702 (7904)	0.2 [^]	3104 (3800)	0.1 [^]

*Values are presented in means with standard deviation.

[^] P-values calculated using Mann-Whitney

^δ P-values calculated using student's t-test

Table 3: Treatment Discontinuations and Adverse Events of lonafarnib

Variable- no. of patients (%)	Placebo (N=4)	Group 1 (N=6)	Group 2 (N=6)
Discontinuation of therapy due to an adverse event	0(0)	0 (0)	0 (0)
Serious Adverse Events	0(0)	0 (0)	0 (0)
Common Adverse Events			
Nausea	1(25)	2 (33)	6 (100)
Diarrhea	0(0)	3 (50)	6 (100)
Decreased Appetite	0(0)	1 (17)	5 (83)
Abdominal Bloating	1(25)	1 (17)	6 (100)
Vomiting	0(0)	0 (0)	3 (50)
Weight Loss >2kg	0(0)	1 (17)	6 (100)
Headaches	1(25)	1(17)	1(17)
Testicular Pain	0(0)	1(17)	0(0)
Lightheadedness	1(25)	0(0)	0(0)
Fatigue	0(0)	1(17)	1(17)

Figure 1

Figure 2

Mean (SD) Change in HDV RNA Per Week

Figure 3a

Figure 3b

Clinical Research Protocol

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

DATE: September 16, 2011

CLINICAL PROTOCOL NO.:
IND NO.: 113,137

TITLE: Treatment of Chronic Delta Hepatitis with Lonafarnib.

SHORT TITLE: Lonafarnib for Delta Hepatitis

IDENTIFYING WORDS: Antiviral agents, Viral Hepatitis, Hepatitis D Virus, Delta Hepatitis, Chronic Hepatitis, Cirrhosis, Farnesyltransferase inhibitors, Lonafarnib, Prenylation inhibitors

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Vanessa Haynes-Williams, RN, LDB, CRC, 4-5722, 451-7007

ESTIMATED DURATION OF STUDY: 5 YEARS

NUMBER AND TYPE OF PATIENTS: 10 to 12 patients with chronic delta hepatitis, ages greater than or equal to 18 years, both male and female.

SUBJECTS OF STUDY:	Number	Sex	Age Range
Patients	12-14	Male & Female	≥ 18 years
Volunteers	None		

PROJECT USES IONIZING RADIATION: Yes, for medically indicated reasons only.
PROJECT USES "DURABLE POWER OF ATTORNEY": No

OFF-SITE PROJECT: No

MULTI-INSTITUTIONAL PROJECT: No

PRECIS

Chronic delta hepatitis is a serious form of chronic liver disease caused by infection with the hepatitis D virus (HDV), a small RNA virus that requires farnesylation of its major structural protein (HDV antigen) for replication. We propose to treat between 12 and 14 patients with chronic delta hepatitis using the farnesyltransferase inhibitor (FTI) lonafarnib for a duration of twenty-eight days. Farnesyltransferase inhibitors have not been used in the therapy of delta hepatitis. Patients with HBsAg and HDV RNA in serum, elevated aminotransferases, or moderate-to-severe chronic hepatitis and HDV antigen on liver biopsy will be enrolled. Before receiving therapy, patients will be monitored for at least three months with regular testing for alanine aminotransferase (ALT) levels and will undergo Clinical Center admission for medical evaluation and percutaneous liver biopsy. Two dosing groups of lonafarnib will be assessed, with a placebo cohort in each group. At each clinic visit, patients will be questioned about side effects and symptoms, undergo focused physical examination, and have blood drawn for complete blood counts, HDV RNA, and routine liver tests (including ALT, AST, alkaline phosphatase, direct and total bilirubin, and albumin). At two-week intervals, for a period of 28 days, patients will also be tested for HBsAg, anti-HBs, HBV DNA, and prothrombin time. At the end of 28 days of treatment, patients will undergo repeat physical examination, assessment of symptoms (using a symptom scale questionnaire), complete blood counts, routine liver tests, and hepatitis B and D viral markers. The primary therapeutic endpoint will be an improvement in quantitative serum HDV RNA levels after 28 days of lonafarnib therapy. The primary safety endpoint will be the ability to tolerate the drug at the prescribed dose for the 4 week duration. Several secondary endpoints will be measured, including side effects, ALT levels, and symptoms. Therapy will be stopped for intolerance to lonafarnib (which will be carefully defined). This study is designed as a phase 2a study assessing the safety, tolerance and antiviral activity of two dose levels of lonafarnib, a farnesyltransferase inhibitor.

Background

A. Chronic Delta Hepatitis

The hepatitis D virus (HDV) is an incomplete RNA virus, which is composed of a 1.7 kb single-stranded circular genomic RNA, virally encoded small and large delta antigens, and a surrounding lipid envelope.¹ HDV only infects persons who are also infected with hepatitis B virus (HBV), because its lipid envelope is embedded with HBV surface antigen (HBsAg).²⁻⁵ The HBsAg envelope protein provided by HBV protects the HDV nucleocapsid antigen and provides a means for the virus to enter and exit the hepatocyte. HBV and its antigens are not necessary for the replication of HDV once it has entered the cell, but they are necessary for spread of the infection to other cells, the development of acute and chronic hepatitis D and transmission of delta hepatitis to others.⁶

Delta hepatitis is the most severe and dreaded form of human viral hepatitis. HDV infection leads to chronic hepatitis in a high proportion of persons and is associated with progression to cirrhosis in 5 to 10 years in up to 80% of cases. Because of the unique nature of HDV and its requirement for the helper function of HBV infection, delta hepatitis occurs only in persons who also have hepatitis B. HDV infection is found worldwide² but most commonly in Central Africa, the Amazon Basin of South America, Mongolia, and in Eastern European countries. In the United States, delta hepatitis is most common among immigrants from areas of the world where HDV infection is endemic and in persons who have multiple exposures to hepatitis, particularly injection drug users and persons with hemophilia who received blood products before 1986. Delta hepatitis occurs either as co-infection with acute hepatitis B or as super-infection in patients with pre-existing chronic HBV infection.⁷ Hepatocellular carcinoma may develop as a complication of cirrhosis in some cases.⁸ The complications associated with hepatitis B and D are identical except that the progression to cirrhosis is more rapid in hepatitis D. Thus, delta hepatitis shares epidemiological patterns and clinical features with hepatitis B, but tends to be more severe and more rapidly progressive. The seriousness of chronic hepatitis D has led to attempts to develop therapies for this chronic viral infection.

Currently, there is no satisfactory therapy for HDV infection, nor is there any FDA approved therapy. The American Association for the Study of Liver Diseases (AASLD) guidelines state that the only suggested therapy for chronic HDV is alpha interferon.^{3,4,9-11} Therapy has been quite unsuccessful and generally results in normalization of serum aminotransferase activity in only 40-70% of patients. Additionally, these improvements are not always sustained. Thus, in follow up studies, 60-97% of patients who initially responded to alpha interferon relapsed. Long-term treatment with alpha interferon has been reported to be beneficial in few cases.¹² Instances of a complete response to therapy, defined as a loss of HDV RNA and HBsAg and development of anti-HBs, have been reported, although rarely. Thus, standard preparations of alpha interferon are an unsatisfactory therapy for chronic hepatitis D and are limited by a relatively low long-term response rate. For these reasons, alternatives to standard interferon therapy for chronic delta hepatitis have been sought.

B. Prenylation Inhibitors

Prenylation is a post-translational lipid modification that involves the covalent addition of either farnesyl or geranylgeranyl prenyl lipids derived from mevalonic acid to conserved cysteine residues at or near the C-terminus of proteins.¹ These reactions are catalyzed by farnesyltransferase (FTase) or the geranylgeranyltransferases (GGTases).^{6,13,14} The substrate for FTase or GGTase I is a characteristic tetrapeptide found in the amino acid sequence of the protein referred to as a CXXX box motif (where C is a cysteine and X is one of the last three amino acids at the C-terminus of the protein).¹³ The effect of prenylation is to promote membrane association of the modified protein. Prenylation also plays a major role in protein-protein interactions.^{1,13}

Prenylation plays a vital role in the viral life cycle of HDV, which may allow for therapeutic opportunities for eradication. The HDV genome has a single open-reading frame that can encode two proteins: a small or a large delta antigen. The large delta antigen differs from the small one by having an additional 19 amino acids on its C-terminus. The production of small and large delta antigens is dependent upon editing of the HDV RNA to change the stop codon which ends the production of small delta antigen and allows “read through” to the next stop codon which produces the large delta antigen.

The small delta antigen is essential for genome replication, whereas the large delta antigen (LHDAg) performs other functions such as transactivation of various genes and mediation of assembly and release of HBsAg-enveloped particles⁶. In both cell-free translation reactions and in intact cells, it has been shown that the LHDAg is subject to prenylation.^{1,14} Genetic disruption of the CXXX box prevents prenylation of LDHAg and its ability to interact with, and form secreted particles with, HBsAg.¹³ Thus, prevention of prenylation is a reasonable approach to blocking the HDV life cycle. Use of prenylation inhibitors has been evaluated with success in vitro in cell culture as well as in vivo in a mouse model of HDV replication.^{1,6,13,14}

The novel idea of prenylation inhibition was originally developed as a therapeutic approach for cancer, as exemplified by the farnesyltransferase inhibitor (FTI) BZA-5B to prevent prenylation of the farnesylated oncogene RAS.¹ Various phase I/II/III trials in oncology with FTIs have revealed a favorable side effect profile, with good safety and relative lack of toxicity, and ease of administration as they are taken orally.^{6,14} Initial studies in mice with the FTI BZA-5B in HDV have shown it to be a potent inhibitor of large delta antigen prenylation and able to specifically inhibit the prenylation-dependent production of HDV virus-like particles in a dose-dependent manner.¹ Continued evaluation with both BZA-5B and FTI-277 revealed no effect on general protein synthesis; however, there was significant prenylation inhibition on the production of complete, infectious HDV virions.¹⁴ More recently, in an in vivo mouse model of HDV, these compounds were able to completely clear HDV viremia to below the limit of detection.⁶ These results demonstrate that prenylation inhibitors can indeed effectively inhibit HDV viremia and may prove to be a novel treatment in patients with hepatitis D infection. **Lornafarnib is an orally bioavailable tricyclic farnesyltransferase inhibitor that has been shown to have anti-tumor activity in various phase I, II and III trials and anti-HDV properties in various preclinical models.**

C. Interaction between HBV and HDV during Therapy

Patients with chronic delta hepatitis have both HDV and HBV infection, yet most patients with delta hepatitis have detectable serum HBsAg with negative HBeAg, and have low levels of HBV DNA in serum. It seems that HDV replication competes with,

and inhibits HBV replication. An important consideration, however, is that therapeutic inhibition of one virus may be accompanied by an increase in replication of the other.

At the present time, a 4 to 6 month course of alpha interferon therapy is an approved therapy of chronic hepatitis B.^{15,16} Therapy is recommended only for patients with elevated serum aminotransferase activity chronic hepatitis on liver biopsy and either HBeAg or high levels of HBV DNA in serum. Pegylated interferon alfa-2a has also been approved for treatment of chronic HBV in the United States and has been suggested to have similar or slightly improved efficacy with more convenient administration than standard alpha interferon.¹⁶ The recommended treatment duration with peginterferon alfa-2a in patients with chronic HBV is for 48 weeks.¹⁶ The Liver Disease Branch has recently studied the effects of peginterferon alfa-2a in the treatment of patients with chronic delta hepatitis (01-DK-0247). This study is still ongoing and acquiring data but although preliminary results appear promising, not all patients have responded to therapy. Additionally, in many patients, continued administration of peginterferon is required to maintain a response.

Other approved therapies for chronic hepatitis B include oral nucleoside/nucleotide analogues: lamivudine (Epivir-HBV, 3TC), adefovir disproxil (bis-POM PMEA, Hepsera), tenofovir disoproxil fumarate (Viread), entecavir (Baraclude), and L-deoxythymidine (Telbivudine/LdT, Tyzeka).¹⁶ These agents are potent inhibitors of the HBV polymerase and reduce serum levels of HBV DNA, which is usually followed by improvements in serum aminotransferase levels and liver histology.

Lamivudine monotherapy has been evaluated in chronic hepatitis D by the Liver Diseases Branch (95-DK-199)¹⁷ and by investigators in Europe¹⁸ and shown to be without beneficial effect. Lamivudine resulted in rapid decreases in HBV DNA levels (which were low to begin with) but had no effect on HDV RNA levels, serum aminotransferase levels, or histology. Similarly, short-term combination therapy of alpha interferon and lamivudine has been found to be largely ineffective.¹⁹ A possible use of lamivudine in chronic delta hepatitis, however, is the uncommon patient with high levels of HBV DNA. The pattern of high HBV DNA levels despite chronic delta hepatitis is found in approximately 20% of patients with this disease seen at the Clinical Center of the NIH. In these patients, inhibition of HBV may be important particularly because

suppression of HDV RNA may be followed by rises in HBV DNA levels. In this current study, clinically approved nucleoside analogues for chronic HBV infection will only be used if patients also qualify to be treated for their hepatitis B, having high levels of HBV DNA (above 20,000 IU/mL).¹⁶ Thus, the standard of care for chronic HBV infection will not be started in these patients unless HBV DNA levels remain high with persistently elevated ALT.

Study Alternatives

Alternatives to the study are routine therapy with interferon for 12 months, being monitored on no therapy, or awaiting results of this or other studies of therapy of chronic delta hepatitis.

Hypothesis

Therapy with lonafarnib will lead to a decrease in hepatitis D virus levels.

Protocol

We propose to treat between 12 and 14 patients with chronic delta hepatitis with lonafarnib or placebo for twenty-eight days monitoring HDV RNA and HBV DNA levels as well as routine safety measures and liver function tests.

A. Entry Criteria

Inclusion Criteria:

1. Age 18 years or above, male or female.
2. Serum alanine or aspartate aminotransferase activities above the upper limit of normal (ALT >41 or AST >31 U/L) on an average of three determinations taken

during the previous 6 months. The mean of the three determinations will be defined as “baseline” levels.

3. Presence of anti-HDV in serum.
4. Evidence of chronic hepatitis on liver biopsy done within the previous 12 months with a necroinflammatory score in histology activity index of at least 5 (out of a maximum of 18) and at least 1 for hepatic fibrosis (out of a maximum of 6).
5. Presence of HDV antigen in liver tissue or HDV RNA in serum.
6. Written informed consent.

Exclusion Criteria:

1. Decompensated liver disease, defined by bilirubin >4mg/dL, albumin <3.0 gm/dL, prothrombin time >2 sec prolonged, or history of bleeding esophageal varices, ascites or hepatic encephalopathy. Laboratory abnormalities that are not thought to be due to liver disease may not necessarily require exclusion. Patients with ALT levels greater than 1000 U/L (>25 times ULN) will not be enrolled but may be followed until three determinations are below this level.
2. Pregnancy or inability to practice adequate contraception, in women of childbearing potential or in spouses of such women. Adequate contraception is defined as vasectomy in men, tubal ligation in women, or use of two barrier methods such as condoms and spermicide combination, birth control pills, an intrauterine device, Depo-Provera, or Norplant.
3. Significant systemic or major illnesses other than liver disease, including, but not limited to, congestive heart failure, renal failure (eGFR <50 ml/min), organ transplantation, serious psychiatric disease or depression (only if felt to be at high risk by the NIH psychiatric consultation service), and active coronary artery disease.
4. Systemic immunosuppressive therapy within the previous 2 months.
5. Evidence of another form of liver disease in addition to viral hepatitis (for example autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, alcoholic liver disease, nonalcoholic steatohepatitis (but not steatosis), hemochromatosis, or alpha-1-antitrypsin deficiency).

6. Active substance abuse, such as alcohol, inhaled or injection drugs within the previous year.
7. Evidence of hepatocellular carcinoma.
8. Evidence of concurrent hepatitis C infection with positive serum HCV RNA.
9. Any experimental therapy apart from pegylated interferon within 6 months prior to enrollment.
10. Diagnosis of malignancy in the five years prior to the enrollment with exception granted to superficial dermatologic malignancies.
11. Evidence of HIV co-infection; HIV 1/2 antibody positivity on serum testing.
12. Concurrent usage of statins as these drugs inhibit mevalonate synthesis which reduces protein prenylation.
13. Concurrent usage of moderate and strong CYP3A inhibitors and inducers.
14. Inability to understand or sign informed consent.
15. Any other condition, which in the opinion of the investigators would impede the patient's participation or compliance in the study.

B. Initial Evaluation:

Patients will be admitted to the NIH Clinical Center and undergo the following evaluation shortly before starting therapy:

1. History and physical examination.
2. Concomitant medication query (including dose and indication for each).
Documentation of concomitant medication which may be CYP3A, 2C9, and P-gp substrates will be performed.
3. A standard symptom questionnaire [which focuses on fatigue and abdominal pain], which provide information on categorical presence of symptoms as well as their severity, pattern and frequency (Appendix 1).
4. Blood tests. These include complete blood count (CBC with differential and platelet count), prothrombin time (PT), partial thromboplastin time (PTT), sedimentation rate (ESR), reticulocyte count, plasma haptoglobin level, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct and total serum bilirubin, albumin, total protein, lactate dehydrogenase (LDH), creatine

phosphokinase (CK), sodium, chloride, bicarbonate, potassium, blood urea nitrogen, creatinine, glucose, magnesium, uric acid, calcium, phosphorus, cholesterol, triglycerides, iron and iron-binding capacity (transferrin), ferritin, immunoglobulin levels, thyroid stimulating hormone (TSH), antinuclear antibody (ANA), alpha fetoprotein (AFP), rheumatoid factor, cryoglobulins, hepatitis B surface antigen (HBsAg), antibody to HBsAg (Anti HBs), antibody to hepatitis B core antigen (anti HBc), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti HBe), hepatitis B virus DNA (HBV DNA), antibody to hepatitis D antigen (anti HDV), hepatitis D virus RNA (HDV RNA), anti-HCV, HCV RNA, and anti-HAV. HBV DNA, HCV RNA and HDV RNA will be tested by polymerase chain reaction (PCR). All HDV RNA testing for this protocol will be performed by the Eiger Biopharmaceuticals. This test is not commercially available. An additional 50 ml of whole blood and 3 mm of liver tissue (if a liver biopsy is performed) will be collected for immunological studies and 7 ml of serum will be collected and stored at -20° C. In addition, if a liver biopsy is performed, and adequate tissue is obtained, some will be stored. Women of childbearing potential will have a pregnancy test.

5. In [all](#) males [enrolled in this study](#), AM levels of inhibin B, luteinizing hormone (LH), follicle stimulating hormone (FSH) and total testosterone will be obtained.
6. A reproductive endocrinology or urological consultation for evaluation of current and future reproductive potential, the potential of reversibility in the event that reproductive toxicity occurs, and guidance on potential barrier methods will be obtained. Sperm samples will be obtained, wherever possible, based on the recommendations of the consult. Female subjects of reproductive potential and female partners of male subjects must use two reliable forms of contraception (e.g. oral contraceptive plus barrier method) from the start of the study until 60 days from the end of lonafarnib dosing.
7. Pharmacokinetic drug levels will be obtained at time 0, 30 minutes, 1 hour, and 2 hours after the initial dose has been administered. A pre-dose drug level, prior to [scheduled](#) dosing, will also be obtained at 48 hours and at 72 hours.

8. Routine urinalysis and a 24-hour urine for creatinine clearance and protein excretion.
9. An abdominal ultrasound will be performed.
10. An electrocardiogram (EKG) in digital format will be performed.
11. A detailed ophthalmology exam including retinal photography will be performed.
12. A percutaneous liver biopsy will be obtained and the tissue examined by light microscopy and evaluated using standard criteria for disease grade and stage using the histological activity index and for hepatic fibrosis using the Ishak score.^{18,19} A liver biopsy need not be done if the patient had a liver biopsy within the previous year and adequate tissue is available for analysis. If adequate tissue is available, the specimen will also be tested for quantitative iron levels and in vitro assays of the immune response to HDV.

C. Treatment

The treatment design of this study entails two dosing groups (I and II) of the FTI lonafarnib, with a placebo control in each group (Figure 1). Lonafarnib will be supplied by Eiger BioPharmaceuticals, Inc. Placebo pills of identical color, shape and size to that of the study drug will be provided by the Eiger BioPharmaceuticals, Inc. Lonafarnib is intended to be administered with food. The difference between groups I and II is the dose of lonafarnib given (100 mg twice daily and 200 mg twice daily), which have proven safety in various phase II oncologic trials.²⁰⁻²⁴ Enrolled patients will first be randomized to complete group I (100 mg twice daily) for a total of 8 patients (6 treatment and 2 placebo). Once group I has completed 28 days of therapy and a preliminary safety data analysis has been performed, group II (200 mg twice daily) will begin enrollment. Individuals who were enrolled in group I as placebo patients will be promised therapy and enrolled in group II as treatment patients should they choose to be treated. Therefore, these two individuals will not be blinded, once enrolled in group II. Similar to group I, group II will comprise of up to 8 patients (6 treatment and 2 placebo). These two placebo patients in group II will not be offered therapy in this trial. After completion of 28 days of treatment, all patients will be monitored for six months off therapy as

described in Table 1. As the normal spermatogenesis cycle is 90 days, a repeat sperm sample (if available) will be evaluated at 120 days after therapy has been completed.

During the preliminary evaluation (table 1), through a multilevel consent process, this study will be explained to the patient with multiple opportunities to entertain protocol specific questions. This will be documented in the patient's chart during the preliminary evaluation visits. Additionally, a copy of the consent will be provided to the patient week -12 or -8 for review prior to signing at week -4. After preliminary evaluation, as mentioned in Table 1, patients will be started on lonafarnib. Patients will be admitted to the Clinical Center, for a duration of 72 hours, for induction of therapy, observation for side effects, administration of the medication, and timed blood draws to facilitate analysis of virological response kinetics. Additionally, patients will have an electrolyte panel drawn on day 2.

After induction of therapy, as mentioned above, patients will be treated as outpatients and followed on a regular basis in the outpatient clinic at the NIH (See below and Table 1). Compliance will be monitored through patient diaries. Therapy will be stopped for intolerance to lonafarnib (which will be carefully defined).

At the 28-day point, patients will undergo a repeat outpatient evaluation. This evaluation will include all of the elements listed above under "initial evaluation" except the liver biopsy and will include multiple blood tests, urine tests, symptom questionnaires, eye exam, retinal photography, reproductive endocrinology consultation and virological testing.

After 28 days of therapy, patients will be monitored and followed off therapy for HDV without treatment at various intervals (Table 1) for the next 6 months and thereafter patients will be followed in the usual fashion by the Liver Diseases Branch. If therapy for HBV was started, this will be maintained. As interferon is the only approved therapy for HDV, if a patient has been previously treated with interferon, there is no additional therapy to be offered. It is not planned to routinely offer interferon to all patients after participating in this study. However, if a prolonged HDV flare with significant liver damage is demonstrated, rescue therapy with interferon will be offered. This information has also been provided to the patient in this study's consent form. Although it is not clear

if delaying interferon therapy for a year will have adverse events, patients will be made aware of the option of obtaining standard of care therapy prior to enrollment.

Those patients who have active chronic hepatitis B infection (patients with positive serum HBeAg or HBV DNA above 20,000 IU per ml) concurrent with chronic hepatitis D infection will be treated for their hepatitis B infection by the Liver Diseases Branch according to standard of care¹⁶. Therapy for HBV will not be delayed unless it is in the best interests of the patient in terms of clinical care. The currently approved therapeutic regimens for hepatitis B include: alpha interferon, peginterferon, lamivudine, adefovir, tenofovir, entecavir and telbivudine.¹⁶

Patient who choose to enroll in this clinical trial, if not previously treated, may delay therapy with interferon for up to 7 months. For this reason, if a patient has advanced disease, the study investigators may choose to recommend treatment with interferon before enrollment (either by the Liver Diseases Branch or outside of the NIH).

Figure 1: Study Design

- 2 dosing groups:
 - Group I: Lonafarnib 100 mg, twice daily
 - Group II: Lonafarnib 200 mg, twice daily
- 6 drug, 2 placebo / group
- 1 mo Rx / 6 mo off drug

D. Randomization

After initial evaluation and determination of eligibility, patients will be asked to provide written informed consent. Patients will also be provided the opportunity to store sperm or eggs at an outside facility of their own choosing and at their own cost prior to starting therapy. All patients who consent will then be randomized by a set of random numbers held in the Pharmaceutical Development Service (PDS) into either the treatment group or placebo group. Both the investigators and the patient will be blinded as to specific results of this randomization (treatment or placebo) until the interim analysis

before the randomization for group II. Group I will complete enrollment and 28 days of therapy, with an interim evaluation of safety data, prior to starting enrollment in Group II. The randomization code will be blocked in groups of 4 so that equal numbers of patients will be randomized to each group of eight patients that have been enrolled.

Randomization will not be stratified due to the small sample size. The two individuals who were placebo patients in group I will not be randomized nor blinded in group II. If they elect to enroll in group II, they will both be treated.

After completion of enrollment and treatment of all individuals in Group I (enrollment of the final patient plus 28 days of therapy), the code for this group will be broken. HDV RNA results and safety data will be evaluated in an interim analysis prior to enrollment of Group II. As previously mentioned, those individuals that were randomized to the placebo arm in Group I will be preferentially offered a spot in treatment arm of Group II. Of the remaining six spots (4 treatment and 2 placebo), the PDS will randomize these patients in a similar blinded fashion.

E. Monitoring during therapy:

Patients will be seen, interviewed and have blood tests taken at regular intervals before, during and after therapy (Table 1). Patients will be seen and have blood drawn at least 3 times during the 6 months before treatment. Patients will be admitted and have frequent blood samples during the first 72 hours of therapy (0,6,12,18,24,36,48 and 72 hours), to assess initial viral kinetics in response to lonafarnib, to have baseline immunological analysis, an eye exam and retinal photography, and a reproductive endocrinology consultation. After 72 hours, patients will be discharged and seen in the outpatient clinic for blood testing at 7, 14, 21 and 28 days. At each time point, an acceptable deviation would include +/-1 day. After stopping therapy patients will be seen for blood testing at 1, 2, 4, 8, 16, and 24 weeks. At each time point, an acceptable deviation would include +/-1 day. The timing of blood specimens, physical examinations and special evaluations is provided in Table 1. Routine blood tests will include complete blood count and differential, serum Chem-20 (ALT, AST, alkaline phosphatase, LDH, CPK, direct and total bilirubin, albumin, total protein, electrolytes, calcium, phosphate, glucose, blood urea nitrogen, magnesium, creatinine and uric acid) and HDV

RNA (a stored serum sample will be kept and viral RNA levels done in batches). Extended blood tests will also include prothrombin time, HBsAg, anti-HBs, HBeAg, anti-HBe and HBV DNA levels. In non-vasectomized males, AM reproductive hormone levels of inhibin B, LH, FSH and total testosterone will be collected as shown in Table 1. Pharmacokinetic (PK) drug levels (pre-dose) will be drawn on days 7, 21 and 28 as shown in Table 1. On day 14, intensive PK monitoring will be performed for 12 hours; pre-dose, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, and 12 hrs post dose. Blood will be drawn for immunological analysis while on therapy on days 7, 14, 28 and at weeks 8 and 24 after the end of therapy. When appropriate, blood will be drawn in pediatric tubes and the amount of blood that will be drawn from adult patients (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period. Patients will also undergo electrocardiogram (EKG) evaluations collected in digital format on days 0, 7, 14, 21 and 28, due to the rare instances of asymptomatic bradycardia and QT/QTc interval prolongation described in oncologic trials.²¹ At specified outpatient visits during therapy (Table 1), patients will be asked about their symptoms, fill out a symptom questionnaire and will undergo assessment of vital signs and medication diaries. At extended outpatient visits, patients will undergo a brief history and physical examination.

Patients in whom therapy is stopped early or who discontinue treatment on their own will initially be followed in the same manner as those who do not respond as defined in the protocol. After treatment is discontinued, patients will be followed for at least 6 months with visits to the outpatient clinic as specified in table 1. Thereafter, patients will be followed on an informal basis as is typical for patients who have participated in studies done by the Liver Diseases Branch.

HBV DNA will be tested at the NIH Clinical Center utilizing the COBAS Ampliprep/COBAS Taqman HBV v2.0 test, which is an FDA approved assay intended for the use as an aid in the management of chronic HBV infection. HDV RNA testing will be done by qualitative PCR. Samples will be collected at baseline and at each sampling time point to enable resistance monitoring and analysis and viral kinetics. The latter will include determination of baseline isolate sequence analysis to enable identification and monitoring of changes in viral genome sequences as a function of

treatment duration. Analysis of both drug-treated and placebo-treated patients will enable an assessment of background genomic variability. Any clinical evidence of breakthrough or rebound (defined as a greater than 1 log increase above an observed viral load nadir) will prompt a more in-depth analysis that will focus first on the region encoding the CXXX box, as changes in the prenylation substrate sequence represent a logical potential mechanism of escape from prenylation-inhibiting drugs such as lonafarnib. Because other unanticipated mechanisms of resistance could theoretically be operative, however, we would anticipate also performing full genome sequencing to enable maximally unbiased assessment for candidate resistance mutations. We will also have the ability to engineer any identified candidate resistance mutation back into an HDV vector competent for in vitro replication, in order to confirm that the mutation is indeed causative of any suspected decreased sensitivity to lonafarnib. 70 ml of serum will also be obtained at the initial evaluation and end of the study for immunologic studies. The immunologic studies will be done in the Liver Diseases Branch of the NIH by Barbara Rehermann MD and at the Stanford University School of Medicine by Jeffrey S. Glenn MD.

Table 1: Design of Study

Time	Therapy	Physician Visit	Extended Visit I	Extended Visit II	Immunological Evaluation	HDV RNA & HBV DNA Tests	Comments
-12 wk	None	*	*			*	
-8 wk	None	*			†	*	
-4 wk	None	*				*	Symptom Questionnaire, Consent Form
Day 0-3	Start Therapy	*	*	*	§	*	Admission, EKG, Eye Exam & Retinal Photography, Reproductive Endocrinology Consultation, Start Therapy, Symptom Questionnaire
Day 7	lonafarnib	*		*	‡	*	Symptom Questionnaire, EKG
Day 14	lonafarnib	*	*	*	†	*	EKG, Symptom Questionnaire, Intensive pharmacokinetic drug level monitoring
Day 21	lonafarnib	*		*		*	Symptom Questionnaire, EKG
Day 28	Stop therapy	*	*	*	†	*	EKG, Eye Exam & Retinal Photography, Reproductive Endocrinology Consultation, Symptom Questionnaire
Post Therapy Follow-up							
Week 1	None	*	*	*		*	Symptom Questionnaire
Week 2	None	*	*	*		*	Symptom Questionnaire

Week 4	None	*	*	*		*	Symptom Questionnaire
Week 8	None	*	*		†	*	Symptom Questionnaire
Week 12	None	*	*			*	Symptom Questionnaire
Week 16	None	*	*		†	*	Symptom Questionnaire, Sperm sample if possible
Week 20	None	*	*			*	Symptom Questionnaire
Week 24	None	*	*	*	§	*	Symptom Questionnaire, Reproductive Endocrinology Consultation

Physician visit includes review of diary, vital signs, review of symptoms, interim medical history, urinalysis, and routine blood tests for ALT, AST, alkaline phosphatase, direct and total bilirubin, albumin, total protein, BUN and creatinine, magnesium, hematocrit, white blood cell count and differential, platelet count.

Nursing Visit includes review of diary, vital signs, and review of symptoms.

Extended Visit I includes symptom questionnaire, HBV markers, prothrombin time, and physical examination. Women of childbearing age will have a pregnancy test.

Extended Visit II includes pharmacokinetic drug levels (Pre-dose) and AM reproductive levels of inhibin B, LH, FSH, total testosterone.

Immunological evaluation includes blood to be drawn (30-50 ml) for laboratory assessment (§ denotes 40 ml, † denotes 60 ml, § denotes 70ml).

HDV RNA and HBV DNA tests are qualitative polymerase chain reaction assay (PCR) or a quantitative PCR (qPCR) for viral titer.

Symptom questionnaire will be administered on several occasions, before and after 28 days of treatment and in subsequent off treatment follow-up visits for 6 months.

Start therapy includes blood drawn immediately before and then 6, 12, 18, 24, 48, 72 hours after the first dose of lonafarnib for measurement of HDV RNA.

F. Assessment of Response

The primary therapeutic endpoint will be the decrease of HDV RNA viral titer in serum, as measured by quantitative PCR (qPCR). Secondary therapeutic end-point will be change in ALT levels.

The primary safety end-point will be ability to tolerate the drug at the prescribed dose for the full 4-week duration. Discontinuation of the medication will be considered failure to tolerate.

Secondary safety endpoints will include changes in specific values in the symptom scales, in the safety questionnaire or worsening of liver disease. Lonafarnib has already undergone various phase II & III studies and has been used in various oncologic treatments and an ongoing progeria study with good safety and tolerability. Although we cannot be certain, we do not expect to have differing safety and tolerability issues in comparison to these studies.

G. Immunologic Analysis

Chronic delta hepatitis is characterized by a lymphomononuclear infiltrate of the liver. The relative composition of this cellular infiltrate (HDV-specific T cells versus HBV-specific T cells versus nonspecific bystander cells), the antigenic targets within HDV that are recognized by these T cells and the contribution of the HDV-specific T cell response to disease progression are not known.

We aim to define the HDV-specific T cell response in paired blood and liver biopsy samples of HDV/HBV-coinfected patients in the chronic stage of disease prior to treatment (when available). Peripheral blood mononuclear cells (PBMC) and liver-infiltrating lymphocytes will be isolated from a paired blood and biopsy samples. The HDV-specific CD4 and CD8 T cell response to recombinant delta antigen will be assessed by measuring cytokines (IFN γ , TNF α , IL-2) by multicolor flow cytometry and enzyme-linked immunospot assays. Individual responses will be mapped with 41 overlapping peptides spanning the delta antigen and minimal optimal epitopes will be defined. The quality and strength of the HDV-specific T cell response will be compared to the quality and strength of the HBV core and surface antigen-specific response.

We also plan to take advantage of the fact that the prenylation inhibitor is a potent antiviral drug with no direct effect on the immune system. We will also evaluate whether a reduction of HDV viral or antigen load determined by treatment with the

prenylation inhibitor causes a qualitative or quantitative change of the T cell response in the peripheral blood, and specifically, the emergence of HDV-specific memory T cells that we have identified in a rare case of a patient who spontaneously recovered from HDV infection (Heller, Rehermann, unpublished).

Additionally, Dr. Jeffrey S. Glenn will measure changes in prenylation in peripheral blood mononuclear cells (PBMCs). This will act as a control for HDV prenylation inhibition.

H. Design of the Trial/Statistical considerations.

This study is designed as a proof-of-concept treatment trial to determine the ability of lonaFarnib to suppress HDV at tolerable doses. Neither the effective dose nor the tolerance at different doses is known. Furthermore, chronic delta hepatitis is a rare disease and has been found to be inexorably progressive and likely to result in cirrhosis in 80% of patients. For these reasons, individuals that were enrolled in group I as placebo patients, will be promised therapy and enrolled in group II as treatment patients. This trial should show if HDV suppression is possible, help suggest the optimal dose for viral suppression, and how well each dose is tolerated. There is therefore no planned statistical analysis between two groups, rather an assessment of response and tolerance in each open label group as well as a direct comparison to a total of 4 placebo patients. In addition, two patients will serve as their own controls, initially undergoing therapy with a placebo and then switching to the treatment arm in group II.

Measurement of HDV RNA levels will be performed in batch and the results will not necessarily be known to the treating physician or the investigator until a patient may have finished treatment.

I. Hazards and Discomforts

The hazards associated with this study are the following.

1. *The risks of percutaneous liver biopsy.* Patients will undergo one liver biopsy prior to participation in this study. The major side effects of liver biopsy are pain, bacteremia and bleeding. Local pain and discomfort at the liver biopsy site occurs in about 20% of persons undergoing percutaneous liver biopsy. This is transient (lasting one to twelve hours) and is usually mild, rarely requiring analgesics.

Bacteremia occurs in 1-2% of persons undergoing liver biopsy. In the absence of bile duct obstruction, this is almost always self-limited and is rarely symptomatic. Significant bleeding after liver biopsy is the most serious side effect of this procedure. In the absence of a blood coagulation defect or hepatic malignancy, significant bleeding is rare, occurring in less than one in a thousand cases of liver biopsy. Death due to bleeding after liver biopsy has been reported to occur in less than 1/10,000 cases. At the NIH Clinical Center, the Liver Diseases Branch has performed approximately 150 liver biopsies each year for the last ten years. During this time, only two patients developed a serious, life threatening complication of biopsy. These patients had cirrhosis and advanced hepatocellular carcinoma and a coagulation disorder respectively, bled from the liver biopsy and died after surgical attempts to stop the bleeding were unsuccessful.

2. *The risks and discomforts of frequent phlebotomy.* To document stable levels of biochemical and serologic markers of chronic hepatitis and to monitor the effects and toxicities of the therapy, frequent blood sampling will be required. Patients will have between 15-20 venipunctures during their course of therapy. Each venipuncture will be for 15 to 70 ml of blood. The amount of blood that will be drawn from adult patients (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.
3. *The risks and hazards of lonafarnib therapy.* Lonafarnib is an orally bio-available tricyclic farnesyl transferase inhibitor that has been shown to have anti-HDV properties in various preclinical models and anti-tumor activity in various phase I and II trials.²⁴ To date, thirty-one phase 1 and 2 and two phase 3 studies have been completed with lonafarnib alone or in combination with a variety of chemotherapeutic regimens. This has amounted to the administration of lonafarnib to approximately 1645 subjects.

In initial animal studies, lonafarnib was well tolerated and had no major end-organ toxicities at repeated doses of 15 mg/kg up to 6 months in rats and 10 mg/kg up to 1 year in monkeys; these doses result in systemic exposures that are less than those attained at a clinical dose of 200 mg twice per day (BID). At

higher doses, representing animal to human exposure multiples similar to that seen in humans at 200 mg BID, the key side effects included bone marrow suppression and testicular toxicity in rats and monkeys, lymphoid and kidney changes in rats, and diarrhea and electroretinographic changes in monkeys. The no-effect doses for reproductive effects on fertility (10 mg/kg in rats) and embryo-fetal development (15 mg/kg in rats and <10 mg/kg in rabbits) result in systemic exposures less than those attained at a clinical dose of 200 mg BID. The no-effect dose for peri- and post natal development in rats was >20 mg/kg, the highest dose evaluated. Lonafarnib was not mutagenic or clastogenic.

Metabolism studies conducted in vitro have shown that cytochrome P450 enzymes, CYP3A4, and CYP3A5, were mainly responsible for the oxidative metabolism of lonafarnib. This metabolism was studied extensively in vivo and the principle metabolic pathways were similar in rats, monkeys and humans and primarily involved oxidation or dehydrogenation, and combinations of these two processes. No metabolites specific to humans were detected in vitro or in vivo. The main route of excretion was fecal, with urinary excretion representing <2% of the administered dose. Lonafarnib also causes mixed induction/inhibition of hepatic drug-metabolizing enzymes in rats and monkeys when administered once daily for 3 months in toxicology studies. In vitro studies in human liver microsomes showed that lonafarnib inhibited the activity of CYP3A4 and, to a lesser extent CYP2C9. Neither of these systems is used in the metabolism of currently approved drugs to treat HBV. Multiple co-administered doses of the P450 inhibitor ketoconazole resulted in a 5-fold increase in exposure to lonafarnib.

In clinical studies, phase 1 dose-finding studies have shown that doses ranging from 25 mg to 200 mg twice daily (BID) are safe and well tolerated. Doses of 100, 200 and 300 mg BID have been employed in various phase II studies involving solid tumors and hematologic malignancies.²⁰⁻²² In the initial dose-finding phase 1 trial, minor hematologic and non-hematologic side effects were seen.²¹ The hematologic side effects included transient reversible neutropenia and transient reversible thrombocytopenia. At doses of less than 200 mg twice daily,

these grade 1 toxicities (NCI common toxicity criteria) were seen in 3 of 12 patients.²¹ At the 200 mg twice daily dose level, no toxicities were seen. At higher doses, 300 mg BID and 400 mg BID, 2 of 6 individuals experienced grade 4 dose limiting toxicities of neutropenia and 1 of 6 experienced grade 4 dose limiting toxicity of thrombocytopenia during a median treatment duration of 40 days.²¹

The major non-hematologic side effects that were observed were gastrointestinal and consisted of diarrhea, nausea, vomiting and anorexia which were mild and did not require intervention (grade 1 and 2) within the 200 mg twice daily group. Other toxicities that were observed included grade 1 or 2 elevation of liver enzymes, reversible grade 1 or 2 elevated plasma creatinine levels. At the higher doses of 300-400 mg twice daily, toxicities observed included; transient fever (grade 2), asymptomatic sinus bradycardia (55 beats/min) resolving at the end of therapy, reversible (grade 3) neurocortical toxicity consisting of disorientation and confusion.²¹ During the above mentioned phase 1 study, no toxicity greater than grade 1 in both hematologic and non-hematologic categories was recorded at the 200 mg twice daily administration level.²¹

Subsequent phase II studies, at doses of 200 mg twice daily, have replicated similar grade 1 and 2 side effects of; diarrhea, nausea, vomiting, dyspepsia, anorexia, asthenia, fever/infection, hemorrhage, increase of serum creatinine, increased liver function tests and rash.^{22,23} In the first study, patients were treated for a median duration of 185 days^{22,23} Currently, there are various phase I, II and III investigational studies ongoing (www.clinicaltrials.gov) with no significant safety alerts (www.fda.gov).

Although it is not specified if patients with pre-existing advanced liver disease were excluded in clinical trials with lonafarnib, specific data on dosing in liver patients and side-effect profile in them are not available. Patients with chronic delta hepatitis tend to have advanced disease at presentation. For example, in our previous trial for HDV (01-DK-0247), all 13 patients had at least bridging fibrosis (Ishak score ≥ 3) on their enrollment biopsy. It is possible that the safety profile in these patients may be different than previously reported. Furthermore, these

patients tend to have pre-existing low blood counts due to hypersplenism, and this may increase the risk of the hematologic toxicity described above. It should be noted that these considerations are applicable to all new treatments attempted for this rare, severe disease.

As there are no HDV-specific therapies, lonafarnib could be the first direct acting anti-HDV agent by compromising the RNA replication complex through inhibition of prenylation at the level of the farnesyl transferase. A risk associated with the use of direct acting antiviral agents would be the development of resistance by the virus. However, because prenylation is a host function not coded for by the HDV genome, the risk of resistance development could be mitigated. Viral surveillance will be conducted throughout the study to assess the risk of viral resistance.

An additional risk that may be encountered with the therapy of HDV includes an increase in liver function tests during and after therapy, the risk of a flare in HBV (≥ 2 log increase and a total HBV DNA over 2,000 IU/mL) as the HDV viral load goes down, and an increase in HDV viral load after therapy is ended. Flares in liver function tests are not uncommon with the start of treatment of HBV disease and necessitate a careful and frequent review of liver function tests. We will follow the same approach in this study unless there is a concomitant flare in HBV viral load. A flare of HBV viral load while on lonafarnib will be treated by starting specific anti-HBV therapy using one of the 5 approved drugs, the selection being made by the investigator based on the subject's condition and history of prior anti-HBV therapy. It should also be noted, that lonafarnib has undergone extensive analysis and has exhibited neither synergy nor antagonism in combination with 5 available HBV antiviral medications. A flare in HDV viral load after termination of lonafarnib will be monitored closely and interferon therapy may be started as determined by the investigator. It should be noted that when interferon is used for HDV it is typically given for a year, and cessation of therapy may be associated with flares of HDV.

J. Adverse Events and Modification of Dose of Prenylation Inhibitor.

Patients will be monitored for side effects. The use of statins are prohibited during the course of the study as are the use of moderate and strong CYP3A inhibitor and inducers. Discontinuation of lonafarnib will be based upon the scoring of adverse events as shown in table II. The scoring of toxicity will be performed from the CTCAE Version 3.0 with modifications for leukocytes, platelets, prothrombin time, partial thromboplastin time, ALT, AST and bilirubin. These variables have been modified because the original version was designed by the National Cancer Institute (NCI) with use for cancer trials and not for clinical trials in liver disease. The modified version to be utilized for this clinical trial accounts for accepted variations of liver tests used in various other liver disease clinical trials by the Liver Diseases Branch. Factors that will lead to discontinuation of lonafarnib include pregnancy, any grade 3 (except leukocyte and platelet categories) or any grade 4 adverse events or any adverse event, which, in the opinion of the investigator, places the patient at increased risk. [Drug discontinuation may also be based on individual clinical presentations of each subject. For example, AST and ALT values will be evaluated in the context of each individual's baseline values. Rather than utilizing specific cutoff values, which may not be in the best interest of each subject, if the investigator identifies a significant elevation in liver related laboratory tests which may jeopardize the patient's safety, the drug may be discontinued.](#) If the female becomes pregnant, the patient's obstetrician will be provided with the standard of care guidelines to prevent HBV transmission. Lonafarnib will not be restarted unless another cause for the abnormality or symptom is found.

Table II. Scoring of toxicity for dose modification. Scoring of to toxicity from the CTCAE Version 3.0, with modifications for leukocytes, platelets, prothrombin time, partial thromboplastin time, ALT, AST and bilirubin. Normal ranges for values at the NIH Clinical center are used.

Scoring of toxicity for dose modification						
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Allergic reaction/ hypersensitivity (including drug fever)	Allergic reaction	Transient flushing or rash; drug fever <38 C (<100.4 F)	Rash; flushing; urticaria; dyspnea; drug fever >38 C (>100.4 F)	Symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy- related edema/angioedema; hypotension	Anaphylaxis	Death
Anorexia	Anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); IV fluids, tube feedings or TPN indicated	Life threatening consequences	Death
Nausea	Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated <24 hrs	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated >24 hrs	Life threatening consequences	Death
Fatigue (asthenia, lethargy, malaise)	Fatigue	Mild fatigue over baseline	Moderate or causing difficulty performing some ADL	Severe fatigue interfering with ADL	Disabling	-----
Diarrhea	Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output	Increase of 4- 6 stools per day over baseline; IV fluids	Increase of >6 stools per day over baseline; incontinence; IV fluids >24 hrs;	Life threatening consequences (e.g. hemodynamic	Death

		compared to baseline	indicated <24 hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL	hospitalization; sever increase in ostomy output compared to baseline; interfering with ADL	collapse)	
Distention/bloating, Abdominal	Distention	Asymptomatic	Symptomatic, but not interfering with GI function	Symptomatic interfering with GI function	-----	-----
Vomiting	Vomiting	1 episode in 24 hrs	2-5 episodes in 24 hrs; IV fluids indicated <24 hrs	>5 episodes in 24 hrs; IV fluids, or TPN indicated >24 hrs	Life threatening consequences	Death
Creatinine	Creatinine	>ULN – 1.5 X ULN	> 1.5 – 2.0 X ULN	> 2.0 – 6.0 X ULN	>4.0 X ULN	Death
Glomerular Filtration Rate	GFR	<75-60% LLN	<60-40% LLN	<40% LLN, chronic dialysis not indicated	Chronic dialysis or renal transplantation indicated	Death
Glucose, serum-low (hypoglycemia)	Hypoglycemia	<LLN – 55 mg/dL <LLN – 3.0 mmol/L	<55 – 40 mg/dL <3.0 – 2.2 mmol/L	<40-30 mg/dL <2.2-1.7 mmol/L	<30 mg/dL <1.7 mmol/L	Death
Triglyceride, serum-high (hypertriglyceridemia)	Hypertriglyceridemia	>ULN – 2.5 X ULN	>2.5 – 5.0 X ULN	>5.0 – 10 X ULN	>10 X ULN	Death
Pain - Headache	Pain - Headache	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering with ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling	-----
Pain - Abdominal	Pain - Abdominal	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering	Severe pain; pain or analgesics severely interfering with ADL	Disabling	-----

			with ADL			
Infection- Upper Airway NOS	Infection – Upper Airway NOS	Mild	Moderate	Severe	Life – threatening; disabling	Death
Infection - Nasopharyngitis	Infection - Nasopharyngitis	Mild	Moderate	Severe	Life-threatening; disabling	Death
Hemoglobin	Hemoglobin	<LLN – 10.0 g/dL <LLN – 6.2 mmol/L <LLN – 100 g/L	< 10.0 – 8.0 g/dL <6.2 – 4.9 mmol/L <100 – 80 g/L	<8.0 – 6.5 g/dL <4.9 – 4.0 mmol/L <80 – 65 g/L	<6.5 g/dL <4.0 mmol/L <65 g/L	Death
Leukocytes (total WBC)	Leukocytes	<2000/mm ³ <2.0 X 10 ⁹ /L	<1500 – 1000/mm ³ <1.5 – 1.0 X 10 ⁹ /L	<1000 – 500/mm ³ 1.0 – 0.5 X 10 ⁹ /L	<500/mm ³ <0.5 X 10 ⁹ /L	Death
Platelets	Platelets	<70,000/mm ³ -60,000/mm ³ <70.0 – 60.0 X 10 ⁹ /L	<60,000 – 40,000/mm ³ <60.0 – 40.0 X 10 ⁹ /L	<40,000 – 25,000/mm ³ <40.0 – 25.0 X 10 ⁹ /L	<25,000/mm ³ <25.0 X 10 ⁹ /L	Death
INR (International Normalized Ratio of prothrombin time)	INR	>1 – 1.5 X ULN	>1.5 – 2 X ULN	>2 X ULN	-----	-----
PTT Partial Thromboplastin time)	PTT	>1 – 1.5 X ULN	>1.5 – 2 X ULN	>2 X ULN	-----	-----
Bicarbonate, serum-low	Bicarbonate, serum-low	<LLN – 18 mmol/L	<18 – 15 mmol/L	<15-11 mmol/L	<11 mmol/L	Death
Acidosis (metabolic or respiratory)	Acidosis	pH < normal, but >7.3	-----	pH <7.3	pH <7.3 with life threatening consequences	Death
Alkaline Phosphatase (U/L)	Alkaline Phosphatase	>ULN – 2.5 X ULN	>2.5 0 5.0 X ULN	>5.0 – 20.0 X ULN	>20.0 X ULN	-----
Bilirubin (hyperbilirubinemia)	Bilirubin	>ULN – 1.5 X ULN	>1.5 – 3.0 X ULN	>3.0 – 10.0 X ULN	>10.0 X ULN	-----
Albumin, serum-low (hypoalbuminemia)	Hypoalbuminemia	<LLN – 3 g/dL <LLN – 30 g/L	<3 - 2 g/dL <30 – 20 g/L	<2 g/dL <20 g/L	-----	Death
AST, SGOT (serum glutamic oxaloacetic transaminase)	AST	>3 - 5 X patient's baseline values	>5-10 X patient's baseline values	>10 – 20 X patient's baseline values	>20 X patient's baseline values	Death
ALT, SGPT (serum glutamic pyruvic transaminase)	ALT	>3-5 X patient's baseline values	>5-10 X patient's baseline values	>10 – 20 X patient's baseline values	>20 X patient's baseline values	Death

K. Data and Safety Monitoring

Data and safety will be monitored by the principal and associate investigators in this protocol. Data and safety are reviewed weekly in clinical research rounds by the Liver Diseases Branch, NIDDK. These rounds are separate from regular clinical rounds and consist of review of all study patients including flow sheets of major safety and efficacy measurements. The rationale for not using an outside data and safety monitoring committee is that this is a small, single center study using a medication with defined side effects. All measurements and tests are well established in clinical medicine. After completion of group I (enrollment and 28 days of therapy), an interim analysis of safety data will be performed with review by the principal and associate investigators. Once adequate safety has been determined, group II enrollment will proceed. Yearly reports are made to the NIDDK/NIAMS IRB regarding safety and efficacy.

L. Adverse Event Reporting

Summary of adverse events and efficacy data will be provided yearly in annual reports to the IRB. All serious adverse events will be reported to the clinical director within 7 days for death or life threatening adverse events and within 15 days for all other that are serious, unexpected and possibly, probably or definitely related to Lonafarnib. Reports will be sent to the FDA, MEDWATCH program (telephone 1-800-FDA-1088; or via the internet at www.fda.gov/medwatch/index.html) and the Eiger Pharmaceuticals, the company that produces the prenylation inhibitor.

M. Regulatory Requirements

Documents and data generated by the investigator will be reviewed on a regular schedule by independent monitors and auditors to ensure that the study is conducted according to standards consistent with the International Conference on Harmonization Good Clinical Practice Guidelines and applicable FDA guidelines. During review by independent monitors and auditors, all patient identifiers and patient identifying data will be removed to protect the privacy and confidentiality of the patients in the study.

N. Recruitment strategy

We will advertise on the NIH web site and through local physicians and clinics for patients.

O. Recruitment of Women, Minority Individuals and Children.

Delta hepatitis is a rare disease in the general population. The major risk factors are injection drug use and hemophilia. It is rare to find the disease in childhood, the majority of children with delta hepatitis being immigrants from areas of the world where it is common (Eastern Europe, Amazon Basin). In addition, hepatitis is often mild in children. Finally, FTIs have not been adequately evaluated for safety and efficacy in children. We therefore do not plan to recruit children below the age of 18.

Women are less likely to have HDV, as one of the risk factors, hemophilia is a disease of males. Notwithstanding this, every effort will be made to find both women and members of minorities with HDV. To increase the representation of both women and minority individuals in this trial, we plan to advertise widely.

O. Research Use, Storage and Disposition of Human Subject's Samples and Data.

Patients will have serum stored from selected time points during this study. These specimens will be used for repeat virological testing and special tests as needed. Samples may be used to assess factors associated with response or non-response to therapy. Liver biopsy tissue may be stored if residual tissue is available after routine samples are taken for routine histological staining and evaluation. These samples will be tested in the Liver Diseases Branch or the routine clinical and surgical pathology services of the Clinical Center. If residual samples are evaluated by outside collaborators in the future, this will be done so only after all identifying data have been removed from all samples. Samples will be numbered and a key to the number system will be stored and backed up by the principal investigator of this study. Research records and data as well as liver biopsy slides, biopsy reports, liver tissue and sera will be stored indefinitely in our locked offices and freezers, the medical record department and the pathology department. These materials will be protected and tracked by standard operating procedures in the medical record and pathology departments as well as a compulsive filing system in our locked

offices and freezers. There will be redundant storage of clinical information in the medical record department and our offices. Likewise, there will be redundant storage of biopsy information and materials in the pathology department and our offices. Computer files will be maintained on password-protected computers and servers. Serum samples will be processed and stored by the NIDDK core laboratory facility. These samples will be stored in locked freezers inside locked rooms. Access to these samples will require written approval from the Liver Diseases Branch chief, and will be recorded by the LDB and by the core laboratory. This should minimize the risk of loss or destruction of information and specimens. If that were to occur we would report it to the IRB. We do not plan to destroy this personal medical information or the liver biopsy specimens or research subject sera after completion of the study because it may be critically important for physicians (here or elsewhere) to have access to this information when caring for these patients in the future. Eiger Biopharmaceuticals (who will be providing the Lonafarnib), will have access to the clinical data only after all personal identifiers have been removed. They will also be included in all serious adverse event notifications.

P. Remuneration/Compensation

No compensation is offered to study participants.

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FARNESYL TRANSFERASE INHIBITORS FOR CHRONIC HEPATITIS D

Patient Name _____

Enrollment Criteria

No	Criteria	Yes/No	Comment
1	Is patient 18 years or above?		Age = _____ years
2	Is mean ALT level above 41 U/L? Or mean AST level above 31 U/L?		Mean ALT = (_____ + _____ + _____)=_____ Mean AST = (_____ + _____ + _____)=_____
3	Are anti-HDV and HDV RNA positive?		Date of sample: ____/____/____
4	Is HAI score above 5?		HAI score ____+____+____+____ = _____ Biopsy date ____/____/____
5	Is consent form signed?		Date of signature ____/____/____
6	Is HDV antigen present in liver tissue		HDV Ag score:_____ Biopsy date ____/____/____
	The answers to these questions should all be yes for the patient to be enrolled in this study.		

The answers to the above questions are all yes.

Signature and Date

PRENYLATION INHIBITORS FOR CHRONIC HEPATITIS D

Patient Name _____

Exclusion Criteria

No	Criteria	Yes/No	Comment
1	Is ALT > 1000U/L?		
2	Is serum bilirubin > 4 mg%?		Bilirubin =
3	Is serum albumin < 3 gm%?		Albumin =
4	Is protime > 2 seconds prolonged or INR > 1.7?		Protime = INR
5	Is there a history of bleeding varices, ascites or PSE?		
6	Is patient pregnant or unable to practice birth control?		Not applicable ____ or Method = _____
7	Are there significant other medical illnesses such as CHF, renal failure, transplant, psychiatric dx, and angina?		
8	Is hematocrit below 36% men or 34% women? Or a history of hemolysis?		Hematocrit = _____%
9	Has patient received antiviral therapy within last 6 months is currently on therapy?		Should be no for group I.
10	Does patient have evidence of another liver disease?		Ceruloplasmin _____, AIAT level _____
11	Does patient have a history of coronary or cerebral vascular dx?		Exercise stress test ____ (Y/N) Cholesterol _____, BP _____ Smoking _____ (Y/N)
12	Has patient had active substance abuse in the last one year?		Alcohol, inhaled or injection drugs
13	Is ANA level > 1:160		ANA = _____
14	Is AFP > 200 ng/ml and dose ultrasound show evidence of a mass in the liver?		AFP = _____ US date __/__/__

The answer to all of the above questions (except 9 for group II) is no:_____
Signature and Date

SYMPTOM SCALE: Delta Hepatitis

Name: _____ Date:(M/D/Yr)____/____/____

Mark with an "x" the place on the lines below that best describes how you have felt during the past week.

None	Worst ever
Fatigue	

Nausea	

Diarrhea	

Confusion	

Loss of Appetite	

Indigestion	

In general, how do you feel overall?

Very Good	Awful

Are you having side effects with Prenylation Inhibitor therapy?

Yes ____ No ____

If yes, how much do these side effects interfere with your ordinary..

	Not at all	A little	A lot	Extremely
<i>Responsibilities at home</i>	☐	☐	☐	☐
<i>Ability to work</i>	☐	☐	☐	☐
<i>Enjoyment of life</i>	☐	☐	☐	☐

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	5-6
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	7-8
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8 and supplementary
Participants	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	8-9-none
	4a	Eligibility criteria for participants	8 and supplementary
Interventions	4b	Settings and locations where the data were collected	8 and 10
	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8-9 and supplementary
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9-10 and supplementary
Sample size	6b	Any changes to trial outcomes after the trial commenced, with reasons	9-none
	7a	How sample size was determined	8-9 and supplementary
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9 and supplementary

			y
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8-9 and supplementary
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8-9 and supplementary
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8-9 and supplementary
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8-9 and supplementary
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	8 and supplementary
	11b	If relevant, description of the similarity of interventions	n/a
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10-11 and supplementary
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10-11 and supplementary
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11-12
	13b	For each group, losses and exclusions after randomisation, together with reasons	11-12 and supplementary figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11-12 and supplementary figure 1

	14b	Why the trial ended or was stopped	11-12
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11-12 and table 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11-15
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	11-15
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	11-15 and supplementary results
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	14-15 and table 3
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15-17
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15-17
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15-17
Other information			
Registration	23	Registration number and name of trial registry	10
Protocol	24	Where the full trial protocol can be accessed, if available	8
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	10

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

CONSORT

TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram