NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

VERSION DATE: 05/11/2020

PROTOCOL#: 18DK0123

TITLE: **Treatment of Chronic Delta Hepatitis with Lonafarnib, Ritonavir and Lambda Interferon.**

SHORT TITLE: **Lonafarnib, Ritonavir and Lambda Interferon for Delta Hepatitis**

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

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Investigational Agents *(if applicable)***:**

Drug Name:	Lonafarnib Lambda interferon
IND Number:	113,137
Sponsor:	NIDDK
Manufacturer:	Eiger Pharmaceuticals

ESTIMATED DURATION OF STUDY: 2 years

NUMBER AND TYPE OF PATIENTS:

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	32	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. Based on previous and ongoing clinical trials demonstrating effectiveness against HDV, we propose to treat 26 adult patients with chronic delta hepatitis using the combination of the farnesyltransferase inhibitor (FTI) lonafarnib (LNF), the protease inhibitor ritonavir (RTV) and peginterferon lambda-1a(lambda). In this phase 2a open label study, the safety and antiviral effects of triple therapy with LNF, RTV and lambda for a period of 6 months. After dosing, all patients will be monitored for 24 weeks off therapy. Nucleos(t)ide analogue therapy will be instituted/continued during this study to prevent the possibility of HBV reactivation/flare for the duration of participation in this clinical trial. Patients with quantifiable HDV RNA in serum will be enrolled. At each clinic visit, patients will be questioned about side effects, symptoms and quality of life, 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 the end of the treatment, patients will be admitted to the clinical center and will undergo repeat liver biopsy and HVPG measurements, 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 a decline of HDV RNA viral titer of 2 logs at the end of therapy. The primary safety endpoint will be the ability to tolerate the drugs at the prescribed dose for the full course of therapy. This clinical trial is designed as a phase 2a study assessing the antiviral activity, safety and tolerance of fixed doses of lonafarnib, ritonavir and peginterferon lambda.

BACKGROUND

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).[2–4]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.[5]

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, is associated with progression to cirrhosis in 5 to 10 years in up to 80% of cases and poor outcomes.[6] 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 worldwide[2] 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]epatocellular carcinoma may develop as a complication of cirrhosis in some cases[6]. 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. Several studies have shown a lack of efficacy of nucleos(t)ide analogues used in chronic HBV infection for chronic HDV. [8–10]The American Association for the Study of Liver Diseases (AASLD) guidelines suggest therapy with alpha interferon for chronic HDV infection, however recent results have identified the need for alternative therapies. In a large multicenter randomized clinical trial, the Hep-Net/International Delta Hepatitis Intervention Trial (HIDIT-1), sustained HDV RNA clearance with interferon based therapy with or without adefovir for 48 weeks was successful in only about a quarter of patients with chronic HDV infection.[11] In a follow-up study, HIDIT-2, which increased treatment duration up to 96 weeks with peginterferon plus tenofovir or placebo, more than one third of patients, experienced a post-treatment relapse.[11] Additionally, the Liver Diseases Branch of the NIDDK has recently published its long-term peginterferon HDV study, which evaluated increasing the doses of peginterferon (up to 360 mcg/wk) for up to 5 years. In this study, only 39% achieved the primary endpoint of histological improvement or loss of serum HDV and HBsAg at 3

years and at the end of the study, only 23% seroconverted HBsAg.[12] Thus, given the unsatisfactory results of interferon-based therapies against chronic HDV infection, alternative therapies have been sought.[13]

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.[14,15] 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 then standard alpha interferon.[15] The recommended treatment duration with peginterferon alfa- 2a in patients with chronic HBV is for 48 weeks.

Other approved therapies for chronic hepatitis B include oral nucleoside/nucleotide analogues: lamivudine (Epivir-HBV, 3TC), adefovir dispivoxil (bis-POM PMEA, Hepsera), tenofovir disoproxil fumarate (Viread), entecavir (Baraclude), and L-deoxythymidine (Telbivudine/LdT, Tyzeka). Currently, Entecavir or Tenofovir are considered first line therapies for chronic hepatitis B. [15]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) and by investigators in Europe [16] 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.[17] 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 19 patients with HIV/HDV[18] long term exposure to nucleotide analog tenofovir lead to significantly reduced HDV RNA in addition to completely suppressing HBV DNA[13]. In this current study, clinically approved nucleoside analogues for chronic HBV infection will be used to prevent the possibility of on-therapy and post-therapy HBV flares.

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).[19,20] 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).[20] The effect of prenylation is to promote membrane association of the modified protein. Prenylation also plays a major role in protein-protein interactions.[20]

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.[19] 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.[19]

Prenylation Inhibitors in Chronic Delta Hepatitis

Prenylation plays a vital role in the viral life cycle of HDV, which allows 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 particles.[5] In both cell-free translation reactions and in intact cells, it has been shown that the LHDAg is subject to prenylation.[19] Genetic disruption of the CXXX box prevents prenylation of LDHAg and its ability to interact with, and form secreted particles with, HBsAg.[20] 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.[1,19,20]

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.[5] 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.[5]These results demonstrate that prenylation inhibitors can indeed effectively inhibit HDV viremia and may prove to be an important therapeutic target in patients with hepatitis D infection**.** Lonafarnib is an orally bioavailable tricyclic farnesyltransferase inhibitor that is metabolized by the cytochrome P-450 (3A4) system and has been shown to have anti-tumor activity in various phase I, II and III trials and anti-HDV properties in preclinical models.

In a first-in-humans for HDV proof-of-concept (POC) study, the Liver Diseases Branch of the NIDDK completed a phase 2a double-blinded, randomized, placebo-controlled, evaluating the safety and utility of the prenylation inhibitor, Lonafarnib (LNF) dosed for 4 weeks, in patients with chronic HDV (12-DK-0046). In this POC study, compared to patients who received placebo, patients receiving lonafarnib 100 mg orally twice daily for 4 weeks experienced mean HDV RNA declines of 0.73 log IU/ml declines in serum (p=0.04) and patients who received lonafarnib 200 mg orally twice daily for 4 weeks experienced a 1.54 log IU/ml decline of HDV RNA in serum (p=0.002) (Figure 1). Mean lonafarnib serum concentrations significantly correlated with mean change HDV RNA from baseline to the end of therapy (R2=0.76, p<0.0001) (Figure 2). As expected, since lonafarnib works on host machinery, there was no evidence of viral resistance. In the lonafarnib 100 mg BID group, side effects included mild nausea (25%), diarrhea (37.5%), anorexia (12.5%), and abdominal bloating (12.5%). In the lonafarnib 200 mg BID group, side effects included nausea (75%), diarrhea (75%), anorexia (62.5%), dyspepsia (75%), vomiting (37.5%), mean weight loss of 4kg (75%). There were no grade 3 or 4 adverse events nor serious adverse events.

Figure 1 HDV RNA decline after 28 days of therapy

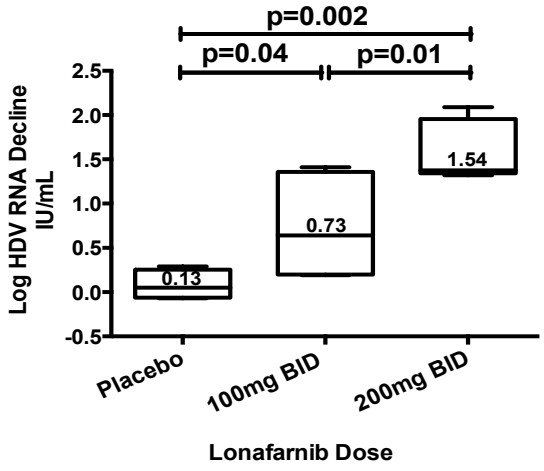
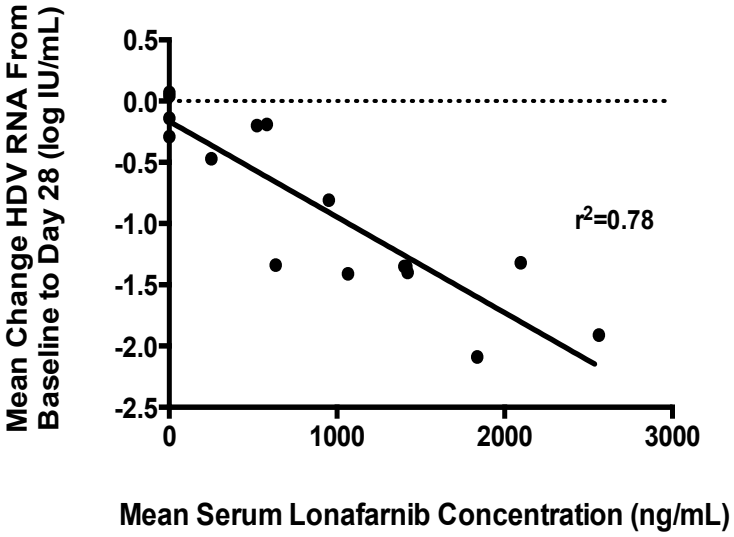


Figure 2 Correlation between serum lonafarnib levels with change in serum HDV RNA.



After the completion of this study, colleagues from the University of Ankara Medical School performed a single center, phase 2 pilot study called LOWR HDV-1 (**LO**nafarnib **W**ith and without **R**itonavir in HDV-**1**) to further explore the utility of LNF in chronic HDV infected patients (NCT02430181). This study was designed as a 7-arm, parallel, open-label clinical trial designed to dose 21 patients across 7 groups (3 patients per group) as follows: Group 1: LNF 200 mg BID for 12 weeks; Group 2: LNF 300 mg BID for 12 weeks; Group 3: LNF 100 mg TID for 8 weeks; Group 4: LNF 100 mg BID + ritonavir (RTV) 100 mg daily for 8 weeks; Group 5: LNF 100 mg BID + Pegylated interferon (PEG-IFN) alpha 2a 180 mcg weekly for 8 weeks; Group 6: LNF 200 mg BID + PEG-IFN alpha 180 mcg weekly for 8 weeks; and Group 7: LNF 300 mg BID + PEG-IFN alpha 180 mcg weekly for 8 weeks.

In this study, all subjects receiving LNF experienced an HDV RNA viral load decline. In the LNF monotherapy groups (Group 1, 2 and 3), subjects in group 1 experienced mean viral load declines of -1.6, -1.0, and 0 at weeks 4, 8 and 12, respectively. Subjects in group 2 experienced mean viral load declines of -2.0, -2.0, and -1.8, at weeks 4, 8 and 12 respectively. All subjects in groups 1 and 2 did not demonstrate evidence of viral resistance no changes in HDV viral sequences were seen at end of treatment and 4 weeks post-treatment compared to baseline. Group 3 subjects were discontinued at week 5 due to unforeseen circumstances related to drug supply, however demonstrated mean declines of 1.4 logs of HDV RNA at week 4. The adverse events in the monotherapy groups (groups 1-3) were noted to be similar to what was demonstrated in the NIDDK POC study and were predominantly GI related (anorexia, nausea, diarrhea and weight loss). Adverse events were grade 1 and 2 (CTCAE), without any grade 3 or 4, and severity of AEs worsened with higher doses.

In subjects that received LNF combination regimens, group 4 subjects (LNF 100 mg BID + RTV 100 mg daily for 8 weeks) demonstrated 2.4 log reduction in HDV RNA after 4 weeks of therapy and 3.2 log reduction after 8 weeks of therapy (Figure 3). In addition to

this viral decline, all subjects demonstrated normalization of alanine aminotransferase (ALT) levels while on therapy (Figure 4). Compared to the same LNF dosing in the NIDDK POC study (group 1 – LNF 100 mg twice daily), subjects receiving the same dose with ritonavir boosting demonstrated higher mean serum LNF concentrations with resultant greater HDV RNA declines by week 4 of therapy (Figure 5).

Figure 3: HDV RNA viral load decline in subjects receiving LNF 100 mg BID + RTV 100 mg daily for 8 weeks

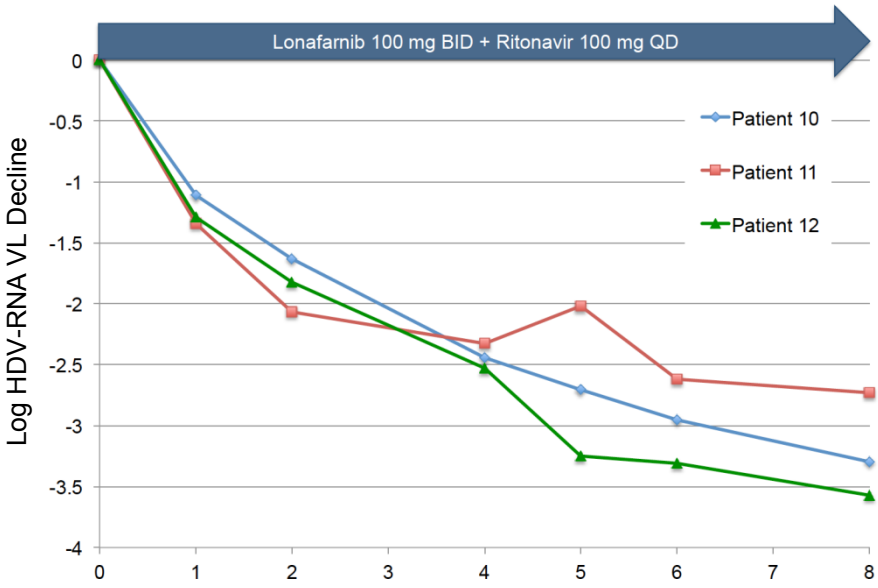


Figure 4: Alanine aminotransferase patterns of subjects receiving LNF 100 mg BID + RTV 100 mg daily for 8 weeks. All subjects normalized ALT levels during therapy

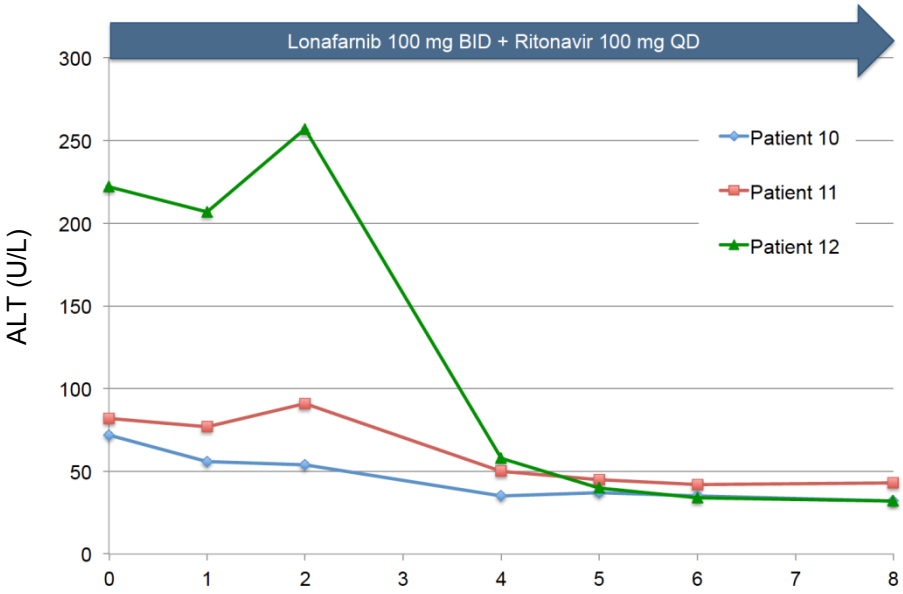
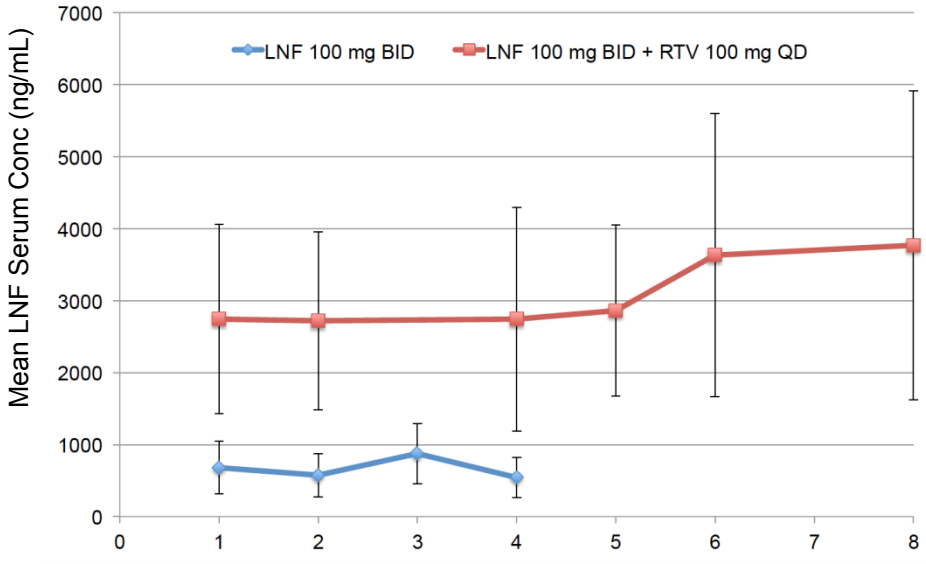


Figure 5: Mean serum lonfarnib concentrations in subjects receiving LNF 100 mg + RTV 100 mg twice daily for 8 weeks (LOWR-1, Group 4) in red and LNF 100 mg twice daily for 4 weeks (NIDDK POC, Group 1) in blue



The remaining combination groups in this study (groups 5-7) received varying doses of LNF (100 mg, 200 mg, 300 mg daily with PEG-IFN alpha). Group 5 subjects receiving LNF 100 mg + PEG-IFN alpha 180 mcg weekly demonstrated a mean HDV RNA decline of 3 logs along with declines in ALT. Unfortunately, higher doses of LNF (200 and 300 mg daily) with PEG-IFN alpha in groups 6 and 7 were poorly tolerated and resulted in discontinuations in all subjects within 4 weeks of initiation. Subjects receiving the lower dose of LNF in group 5 experienced grade 1 and 2 adverse events which was less severe than those in group 6 and 7 (Figure 6)

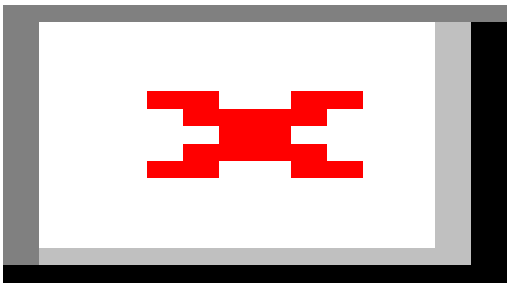
Figure 6: GI adverse events observed with different treatment regimens in the LOWR-1 study.

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

As a follow-up to the LOWR-1 study, the Ankara University School of Medicine initiated the LOWR-2 study which was a phase 2 dose-optimization study in hopes of identifying optimal combination regimens of LNF and RTV+/- PEG-IFN alpha. This single center study evaluated dosing combinations of LNF + RTV with and without PEG-IFN alpha for 12, 24 or 48 weeks. In this study, aside from the varying doses of LNF ranging from

25 mg BID to 100 BID plus ritonavir, 20 subjects received LNF 25 mg (low dose) or 50 mg (high dose) BID with RTV 100 mg BID for 24 or 48 weeks and 14 subjects received the same dosing (25 mg or 50 mg LNF BID + RTV 100 mg BID) with PEG-IFN alpha 180 mcg weekly for 24 or 48 weeks. In subjects that received therapy for 24 weeks, both low and high doses of LNF with RTV resulted in 5 of 14 subjects (36%) achieving an HDV RNA level below the lower limit of quantification (LLOQ) and one subject achieving HDV RNA PCR negativity at week 24. However, the response rates were notably improved with the addition of PEG-IFN alpha to the low and high doses of LNF with RTV (Figure 7), with 4 of 5 (80%) of patients achieving <LLOQ at week 24 and 3 of 5 (60%) of patients becoming HDV RNA PCR negative at week 24. Additionally, 60-78% of subjects normalized their ALT levels at week 24. Adverse events were predominantly grade 1 and 2 with most common side effects being nausea, diarrhea, fatigue, weight loss, anorexia and vomiting, similar to what was seen in the NIDDK POC and LOWR-1 studies.

Figure 7: Comparison of HDV RNA responses in serum in the LOWR-2 study based 25mg or 50 mg of LNF with RTV with and without PEG-IFN alpha



In conjunction with the clinical trials performed at Ankara University, the Liver Diseases Branch, NIDDK recently completed the all-oral combination study (LOWR-3) which evaluated the safety and utility of once-daily ritonavir-boosted lonafarnib at doses of 50, 75 and 100 mg once daily for 12 and 24 weeks (15-DK-0170). This phase 2a double-blinded, placebo controlled, clinical trial randomized 21 patients into one of six groups: LNF 50, 75, or 100mg + RTV 100mg once daily for 24 weeks (12 patients) or 12 weeks of placebo followed by LNF 50, 75, or 100mg + RTV 100mg once daily for 12 weeks (9 patients). Additionally, all subjects were maintained or initiated on hepatitis B nucleoside analogue therapy to prevent the possibility of HBV related flares. After 12 weeks of therapy, median HDV RNA decline from baseline was 1.60 log IU/mL (LNF 50 mg), 1.33 (LNF 75 mg) and 0.83 (LNF 100 mg) (p=0.001). In subjects treated for 24 weeks, HDV RNA levels significantly differed from placebo. During the course of the study 6 subjects achieved HDV RNA levels below 125 IU/mL where 3 were below the lower

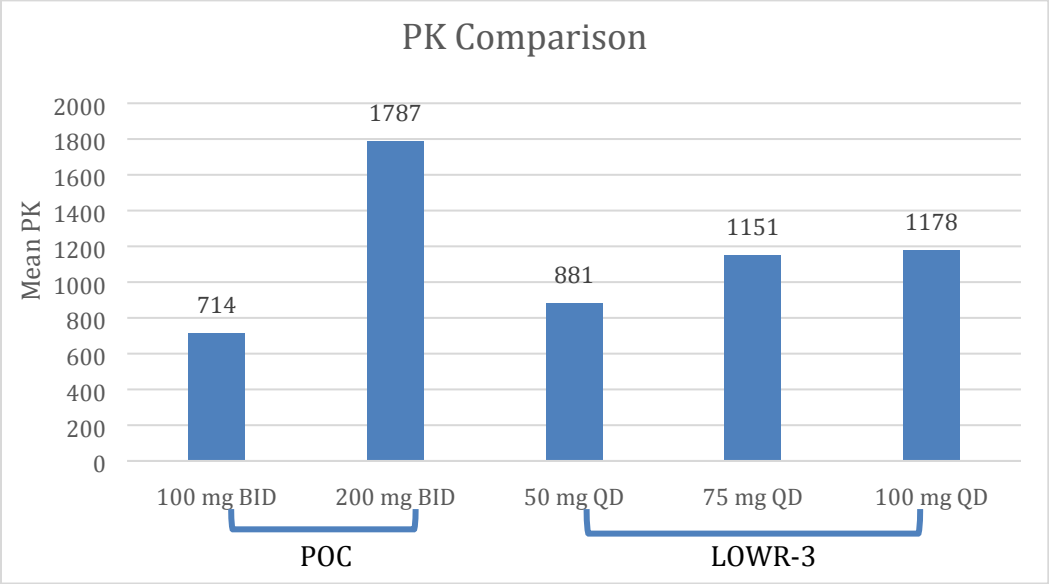
limit of quantification (<14 IU/mL) and 1 achieved HDV RNA negativity. LNF serum concentrations correlated with ritonavir levels (R=0.45, p<0.0001). Adverse events were mild to moderate and included nausea, vomiting, dyspepsia, anorexia, diarrhea, and weight loss and were all CTCAE grade 1 or 2. There were no treatment discontinuations for adverse events. (unpublished data- manuscript in process).

Ritonavir boosting of Lonafarnib

Through inhibition of metabolic enzymes including cytochrome P-450 CYP3A4 and drug transporters, ritonavir has been utilized as a booster mechanism to increase the bioavailability of concomitant drugs.[21] Ritonavir has historically been used in HIV as a booster to enhance the bioavailability and efficacy of other protease inhibitors to yield increased and improved penetration into HIV reservoirs. This effect is achieved through ritonavir's ability to inhibit the cytochrome P450 CYP3A4 enzyme, thereby reducing the metabolism of concomitant administered protease inhibitors leading to changes their pharmacokinetic parameters, including area under the curve (AUC), maximum concentration, minimum concentration and half-life.[22] The use of ritonavir as a therapeutic booster in patients with chronic liver disease and chronic hepatitis B and/or C have been evaluated successfully in multiple previous studies.[23,24] In hepatitis C, one such successful example of the use of ritonavir with Danoprevir, which successfully enhanced the pharmacokinetic parameters of Danoprevir, thus allowing a lower dose of danoprevir and enabling higher plasma trough concentrations with lesser exposure to Danoprevir.[25–27] Ritonavir significantly inhibited Danoprevir metabolism, including the production of reactive metabolites, which reduced the risk of alanine aminotransferase elevation that was observed with high dose unboosted Danoprevir.[26]

In HDV infection, lonafarnib metabolism is also mediated via the cytochrome P450 CYP3A4 system. The ability of ritonavir to boost lonafarnib levels has been demonstrated in the already completed HDV LOWR-1 (Figure 5 above), LOWR-2 and LOWR-3 (Figure 8 below) studies performed in Ankara, Turkey and the NIDDK. The use of ritonavir boosted LNF has demonstrated the ability to substantially increase serum drug levels of LNF while minimizing the drug exposure to the gastrointestinal tract. In the LOWR-3 study performed by at the NIH Clinical Center, the combination of LNF and RTV were well tolerated and safe for 24 weeks without electrocardiographic changes or pancreatitis.

Figure 8: Pharmacokinetic comparison of the NIH POC study doses (Lonafarnib 100 mg BID and 200 mg BID) and the LOWR-3 study doses (Lonafarnib 50/75/100 mg daily with ritonavir 100 mg daily)

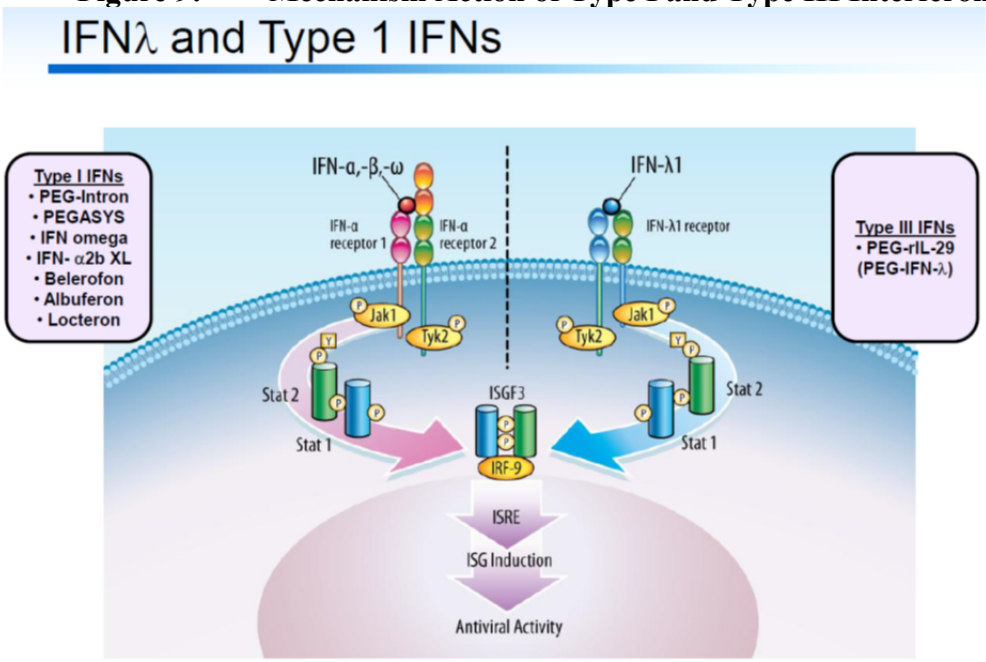


In the LOWR-HDV-2 study in Ankara, Turkey, which evaluated differing doses of lonafarnib and ritonavir (LNF/RTV 100/50 mg twice daily, LNF/RTV 100/100 mg once daily, LNF/RTV 150/100 mg once daily and LNF/RTV 100/100 mg twice daily) for 3 months, a mean HDV RNA decline >1.5 log IU/ml was demonstrated in 8 patients after 1 month of therapy. Notably, the dosing of LNF/RTV 100/100 mg once daily showed a continuous HDV RNA decline with a mean decline of >3log HDV RNA IU/ml . Therefore, ritonavir-has demonstrated success as a pharmacokinetic booster of lonafarnib as it optimizes the pharmacokinetic profile of lonafarnib while allowing for a lower dose (with less side effects) and still maintaining the antiviral activity of lonafarnib.

Peginterferon lambda therapy in chronic viral hepatitis

IFN-lambda is a type III of IFN with different structural features, receptor characteristics, and biological activities than IFN-α (Type I). IFN-lambda was first reported in the early 2000s[28]. IFN-lambdaand IFN-α share the common interferon-stimulated gene (ISG) induction pathway that leads to broad-spectrum antiviral activities (Figure 9). The biological activity of Lambda is derived from its recombinant human IFN λ1 moiety. Lambda binds to the human Type III IFN receptor leading to receptor dimerization. Receptor dimerization activates multiple intracellular signal transduction pathways initially mediated by the JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway.

Figure 9: Mechanism Action of Type I and Type III Interferons



Lambda is a covalent conjugate of human recombinant non-pegylated IFN lambda (IFN λ) and a 20-kDa linear PEG chain. Lambda has been investigated extensively in the treatment of CHB and CHC, and is now being investigated in CHD.

As of March 2017, over 3,700 subjects (including 237 healthy subjects; 197 subjects with HBV; 3,276 subjects with HCV; and 12 subjects with HDV) have received Lambda or comparator in 19 Phase 1, 2, or 3 clinical trials. These studies that have evaluated the antiviral activity of lambda IFN and chronic hepatitis B and hepatitis C infection.

In chronic HBV infection, lambda interferon was evaluated as monotherapy in a dose finding study in interferon naïve patients (LIRA-B)[22]. Subjects were randomized to receive weekly lambda 180 or 240 µg or interferon alpha at 180 µg for 48 weeks. The 240 µg dose was discontinued for development during the study and only 13 patients received this dose. Hepatitis B e antigen seroconversion rates were comparable for interferon lambda and alpha groups at week 48 (17.5% versus 16.9%, respectively) however lambda noninferiority was not met at week 24 post-treatment. (13.8% versus 30.1%, respectively; lambda versus alpha 80% CI). Lambda was well tolerated at single subcutaneous doses of up to 5 mcg/kg and multiple subcutaneous doses of up to 180 µg administered weekly for 48 weeks. Interestingly, known side-effects of interferon alpha (constitutional symptoms, neurologic events, flulike symptoms, musculoskeletal symptoms, and psychiatric events) were lower in the interferon lambda 180 ug group (50%) compared to the IFN alpha group (72.3%).

The antiviral activity of lambda interferon against HCV has had the most extensive patient experience amounting to >3700 subjects in various phase 2 and 3 studies. In HCV, lambda interferon has been administered with various oral combination therapies including Ribavarin plus daclatasvir and Ribavarin plus asunaprevir. [29,30] Treatment durations have ranged from 24-48 weeks and lambda interferon dosing has ranged from 80, 120, 184 to 240 µg per week[23,24]. In patients with HCV genotype 1 and 4 infection,

there was a significant relationship between lambda exposure (based on AUC or C Max) and undetectable HCV RNA at week 4, with the largest difference between ages and dose levels between the 120 and 180 µg exposure ranges. Episodes of anemia and neutropenia were significantly less frequent in subjects receiving lambda interferon compared to alpha interferon across all doses and exposure duration. Based on promising phase 2 HCV studies, phase 3 studies in chronic HCV infection were performed evaluating fixed 180 µg doses of lambda in combination with Ribavirin and daclatasvir and telaprevir for 24–48 weeks in genotype is ¼ infection and 12–24 weeks in genotype 2/3 infection[31].

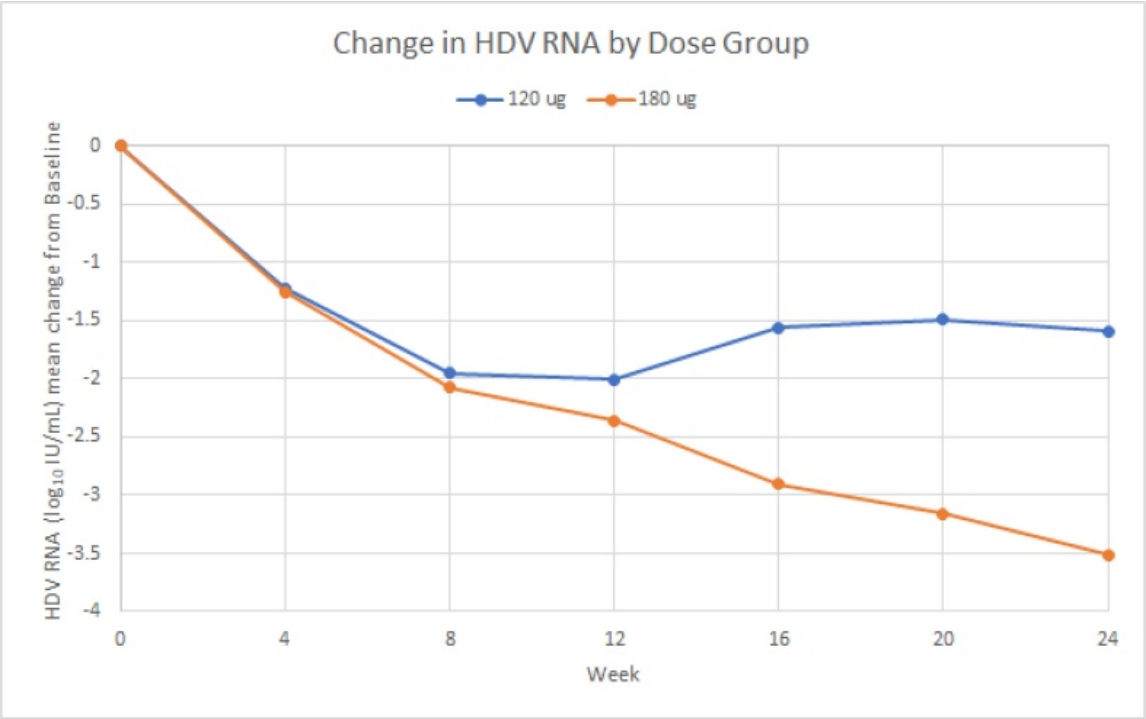
In general, lambda interferon has been generally well tolerated by patients in clinical studies. Compared to alpha interferon, there is a lower frequency of musculoskeletal (myalgia, arthralgia, and back pain) and flu-like symptoms (chills, pyrexia, and pain) and a notable lack of hematologic toxicity in the WBC or platelet lineages in subjects receiving Lambda interferon based regimens. Laboratory abnormalities have generally been of low grade and self-limited. When study regimens included concomitant RBV administration, decreases in hemoglobin levels were observed as expected; however, anemia was less frequent and milder with Lambda/RBV than with alfa/RBV. Hematologic toxicity was also reported less frequently in groups treated with Lambda compared with alfa.

Lambda Inteferon in Chronic HDV Infection

Lambda has demonstrated in vivo activity against hepatitis D and hepatitis B viruses. In an HBV/HDV-infected human liver chimeric mouse model, Lambda reduced HDV and HBV viremia by 1.2-log and serum HBsAg level by 2.5-fold after 4 weeks of treatment.[24] The 4 weeks of Lambda treatment also decreased intrahepatic genomic/antigenomic HDV RNA by 6.1/2.2-fold and intrahepatic HBV RNA by 8.6-fold in the mice.

In a first-in-humans for HDV, multicenter, phase 2 clinical trial, Lambda interferon monotherapy is currently being explored (NCT:02765802) in New Zealand, Pakistan and Israel. The Lambda Interferon MonoTherapy study (LIMT) study, has completed enrollment and initiation of dosing in 33 patients that are randomized to receive either 120 µg or 180 µg of weekly administered lambda interferon for 48 weeks followed by 24 weeks of post-therapy follow-up. At the AASLD liver meeting in October 2017, the study investigators reported interim results [32] describing that 10 of 33 patients had reached 24 weeks of therapy. 6 of 10 patients were responders, achieving >/= 2 log decline or below the lower limit of quantitation in HDV RNA at week 24 (Figure 9). Preliminary results also demonstrated that lambda interferon had comparable anti-HDV activity to historical peginterferon alpha at 24 weeeks of therapy. These preliminary results appear comparable to peginterferon alpha therapy at similar time points where 15 of 91 subjects (16%) achieved HDV RNA negativity by week 8 and 19 of 91 subjects (21%) achieved HDV RNA negativity by week 12 of therapy in the HIDIT-2 clinical trial[12]. Adverse effects have included grade 3 elevations in ALT in 3 patients and jaundice in 3 patients, two of which required permanent drug discontinuation and 1 required temporary interruption and resumption at a reduced dose. Constitutional symptoms were much milder and less frequent than historical data with IFN alfa.

Figure 9: Mean viral load change from baseline to week 24 for peginterferon lambda.



Role of intestinal microbiome in chronic liver diseases

With increasing appreciation of the gut-liver axis, the role of the intestinal microbiome and bacterial translocation on the pathogenesis of liver disease and its progression including inflammation and fibrosis has been a recent focus of investigators.

In patients with chronic hepatitis B, investigators have described extensive differences between patients with and without cirrhosis regarding the fecal microbiota community and altered composition of intestinal Bifidobacterium with a shift from beneficial species to opportunistic pathogens in patients with cirrhosis.[33] Further studies have described that the existence of cirrhosis (regardless of etiology) is associated with microbial dysbiosis and changes in intestinal microbial composition. Specifically, in patients with cirrhosis, pathogenic species appear to be more likely to translocate across the gut wall[34].

In addition to the data in cirrhotic patients, emerging data suggests bacterial translocation (BT) may occur at earlier stages of compensated liver disease and can also contribute to progression of liver disease. Kupffer cells (KC) express Toll-like Receptors (TLRs) that are highly sensitive to lipopolysacharide LPS-triggered TLR activation. Additionally, KC, are also potent activators of hepatic stellate cells (HSC) which play a vital role in hepatic fibrogenesis. [35] In an *in vivo* mouse model, *Seki et al* reported a mechanism that promotes the pro-fibrogenic response where LPS binds to TLR-4 on the HSC which, in turn, leads to KC chemotaxis as well as to sensitization of HSC to TGF-β signaling.[36] *Henao-Mejia and Elinav* in another landmark study described that bacterial translocation through TLR-4 and TLR-9 dependent pathways drove non-alcoholic fatty liver disease

(NAFLD) and its progression to steatohepatitis[37]. These and other studies outline the role of microbial products in activation of key intracellular events involved in liver inflammation and fibrosis, thus conceptually establishing a link between BT to the portal system and liver disease progression. Other studies have also suggested that BT to the systemic circulation may occur in earlier stages of liver fibrosis. [38,39] In the serum of chronic hepatitis B and C patients in different stages of liver fibrosis, elevated levels of soluble CD14 (sCD14), a macrophage-derived marker of LPS bioreactivity were identified. [45] In addition, evidence of portosystemic shunting and decreased Kupffer cell function was described in pre-cirrhotic HCV patients using highly sensitive quantitative liver function tests and SPIO-MRI imaging respectively[27,40].

Microbiome diversity and bacterial translocation in chronic HDV patients has not been described in the literature. We plan to assess the fecal microbiota diversity and its bacterial translocation in chronic hepatic D (HDV) and their changes during treatment with lonafarnib, ritonavir and lambda. The initial focus would be to recapitulate the findings of dysbiosis seen in other liver diseases in patients with HDV.

HYPOTHESIS AND AIMS

Therapy with lonafarnib plus ritonavir and lambda interferon will lead to a significant decline in hepatitis D virus levels.

ASSESSMENT OF RESPONSE TO THERAPY

Primary Therapeutic End Point:

Decrease of HDV RNA by >2 log from baseline at 24 weeks of therapy with lonafarnib plus ritonavir and lambda interferon.

Primary Safety End Point:

The ability to tolerate lonafarnib plus ritonavir with lambda interferon therapy for 24 weeks of therapy. Discontinuation of the medication by the clinical team or patient will be considered a failure to tolerate the medicine.

Secondary End Points:.

1. Sustained undetectable HDV RNA in serum at weeks 12 and 24 post treatment follow up.
2. Reduction in histologic inflammatory scores (modified HAI) by at least two points with no progression in histologic fibrosis (Ishak) at week 24 post-treatment follow-up.
3. Normalization of serum ALT (ALT <20 in females and ALT <31 in males) at the end of therapy, at week 12 of post-therapy follow-up and at week 24 of post-therapy follow-up, OR reduction in serum ALT by >50% of baseline at week 12 of post therapy follow up and week 24 of post therapy follow up.
4. Reduction in hepatic venous pressure gradient (HVPG) measurements by >25% of baseline OR normalization of HVPG (<5 mm Hg) at end of therapy.
5. Reduction in Fibroscan® transient elastography values by >25% of baseline at end of therapy.

6. Loss of HBsAg from the serum at the end of therapy, at week 12 of post-therapy follow-up or at week 24 of post-therapy follow-up.
7. Changes in quantitative HBsAg levels at the end of therapy and at week 24 of post therapy follow up, compared to baseline.
8. Changes in symptom scale measurements and quality of life before, during and after therapy.
9. Changes in the fecal microbiome before and after treatment.

STUDY PROCEDURES AND SCHEDULE

This is an open-label clinical trial treating 26 adult patients with chronic delta hepatitis with lonafarnib plus ritonavir and lambda interferon for 24 weeks. Patients will undergo baseline testing including blood draws, imaging, safety consults, and liver biopsy with portal pressure measurements prior to starting therapy. During therapy, all patients will be monitored with quantitative HDV RNA and HBV DNA levels as well as routine safety measures and liver function tests. After 24 weeks, therapy will be stopped and patients will again undergo repeated testing including blood draws, imaging, and safety consults. After stopping therapy, patients will be followed for an additional 24 weeks. At the end of the 24 weeks of post-therapy follow-up, patients will be re-admitted to the NIH Clinical Center and will undergo repeat testing including blood draws, imaging, as well as a transjugular liver biopsy with hepatic venous pressure gradient measurements.

STUDY ENTRY CRITERIA

Inclusion Criteria:

1. Age 18 years or above, male or female.
2. Presence of anti-HDV in serum.
3. Presence of quantifiable HDV RNA in serum at three time pre-treatment points with a mean HDV RNA level >2 $\log_{10}$ above the lower limit of quantification (LLOQ) of the HDV RNA assay.
4. Demonstration of chronicity as evidenced by the presence of HDV RNA in serum for >/= 6 months, or presence of Anti-HDV antibody >/= 6months.

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. Patients with an absolute neutrophil count <1000/dL and platelets <75,000/dL will be excluded from the study as well.
2. Pregnancy, active breast-feeding, or inability to practice adequate contraception, in women of childbearing potential or in partners of such women. Adequate

contraception is defined as vasectomy in male sexual partners of female participants, tubal ligation in women, or use of two contraceptive methods such as condoms and spermicide combination with an intrauterine device or 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), or active coronary artery disease.
4. Systemic immunosuppressive therapy within the previous 2 months before enrollment.
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, ongoing drug induced 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. This will be determined on the basis of imaging with ultrasound/ CT scan or MRI performed a maximum of 6 months prior to enrollment. Elevated AFP levels will be evaluated clinically and further imaging may be performed if felt necessary.
8. Evidence of concurrent hepatitis C infection with positive serum HCV RNA.
9. Any experimental therapy or pegylated interferon therapy within 6 months prior to enrollment.
10. Active, serious autoimmune disease such as systemic lupus erythematosus, ulcerative colitis, Crohn's disease or rheumatoid arthritis, that is in the opinion of the investigators might be exacerbated by therapy with lambda interferon. This will be evaluated at baseline and during follow-up laboratory testing (including blood and urine studies) in addition to described symptoms at each outpatient visit.
11. Diagnosis of malignancy in the five years prior to the enrollment with exception granted to superficial dermatologic malignancies.
12. Evidence of HIV co-infection; HIV 1/2 antibody positivity on serum testing.
13. Concurrent usage of statins as these drugs inhibits mevalonate synthesis, which reduces protein prenylation.
14. Concurrent usage of moderate and strong CYP3A inhibitors and inducers.
15. Concurrent usage of alpha 1 adrenoreceptor antagonist, antiarrhythmic, pimozide, sildenafil, sedative and hypnotics, ergot and St. John's Wort due to possible effect of ritonavir on hepatic metabolism of these drugs resulting in potentially life-threatening side effects.

16. Clinically significant baseline EKG abnormalities such as QT_c interval >450 ms and/or prolonged PR interval.
17. Uncontrolled elevated triglycerides (>500 mg/dL). Patients on lipid lowering therapy other than statins will be eligible for this study.
18. History of pancreatitis from causes other than gallstone pancreatitis. Subjects with a baseline amylase and/or lipase level >3 ULN will be excluded from the study.
19. Inability to understand or sign informed consent.
20. Any other condition, which in the opinion of the investigators would impede the patient's participation or compliance in the study.

STUDY PROCEDURES/EVALUATIONS

Study Procedures

- **Physician Visit –** history and physical, vital signs, review of symptoms, concomitant medications, symptom questionnaire and review of medication compliance while on treatment.
- **History and Physical –**
    - **Full H&P :** A full history and physical examination will be performed at the screening visit as well as inpatient visits.
    - **Interim H&P :** An interim history and physical covering liver and medication related symptoms as well as a limited physical exam will be performed during all other visits.
- **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.
- **Hepatitis B Treatment** To prevent the possibility of a hepatitis B flare during the study, all patients will be treated with nucleos(t)ide analogues while on the study (24 weeks of entacavir or tenofovir + lonafarnib/ritonavir/lambda and 24 weeks of entacavir or tenofovir during the post-therapy monitoring phase). Patients who are not on nucleos(t)ide analogues prior to starting lonafarnib/ritonavir/lambda will be started on either entecavir or tenofovir (which are first-line nucleos(t)ide analogues recommended by the AASLD and EASL for the treatment of HBV) prior to instituting HDV therapy (to reduce the risk of a hepatitis B flare). HBV viral loads will be monitored after starting nucleoside analogue therapy at each outpatient visit during the entire course of study participation. Adequate HBV suppression (serum HBV DNA level <20 IU/mL or undetectable) must be achieved on nucleoside analogue therapy prior to starting HDV therapy which will allow for improved determination of response to experimental HDV therapy. Patients will be informed about the possible adverse effects of these medications during their pretreatment clinic visits and via the package insert that is provided with the medication. Patients will also be offered multiple opportunities to ask questions about these medications during their clinic visits. For patients started on

nucleos(t)ide analogue therapy as a result of their participation in this protocol, the medication will be administered under this protocol after the consent has been signed and eligibility is determined. If the patient has been taking a nucleos(t)ide analogue prior to enrolling in this study, the specific nucleos(t)ide(s) analogue will be continued. The currently approved nucleoside analogues for hepatitis B include: lamivudine, adefovir, tenofovir, entecavir and telbivudine.[20] At the conclusion of participation in this study, subjects that were prescribed nucleoside analogue therapy by their outside practitioners will be continued on therapy (if clinically indicated) and they will receive this therapy from their outside physicians. For subjects that are started on nucleoside analogue therapies for the purposes of participation in this study, nucleoside analogue therapy may be continued after completion of this study if clinically indicated. If this is the case, therapy will be continued under the 91-DK-0214 protocol, with encouragement that therapy should be received from their outside physicians. Otherwise, if not clinically indicated, nucleoside therapy will be discontinued at the conclusion of participation in this study.

- **Symptom Questionnaire** provides information on categorical presence of symptoms as well as their severity, pattern and frequency (Appendix 1)
- **EKG –** Patients will undergo electrocardiogram (EKG) evaluations collected in digital format due to the rare instances of asymptomatic bradycardia, PR prolongation[48] and QT/QTc interval prolongation described in oncologic trials with use of lonafarnib.[48, 49]
- **Imaging**
    - **Ultrasound and Fibroscan -** An abdominal ultrasound with sheer wave elastography and vibration controlled transient elastography (Fibroscan ®) will be performed for clinical staging of liver disease and to evaluate for hepatocellular carcinoma, masses or any other findings that may preclude a safe transjugular liver biopsy with hepatic venous pressure gradient measurements.
    - **MRI** A structural examination of the liver will be performed via magnetic resonance imaging with gadolinium. An MRI without contrast will be performed in the event that contrast cannot be administered.
- **Liver Biopsy with Hepatic Venous Pressure Gradient (HVPG) Measurements**
    - **Transjugular Liver Biopsy with HVPG :** A transjugular liver biopsy will be performed in the interventional radiology suite with measurement of hepatic venous pressure gradient. If size of core during one pass permits, a small piece of liver will be flash frozen at -80 C for further analysis.
    - **Percutaneous Liver Biopsy –** in the event that the transjugular liver biopsy cannot be performed (ie: technical difficulty), a percutaneous liver biopsy will be performed.

- **Consults**
    - **Reproductive** - 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 contraceptive methods will be obtained. Sperm samples will be obtained, wherever possible, based on the recommendations of the consult. Sperm sampling will be offered and if obtained, given that the normal spermatogenesis cycle is 90 days, a repeat sperm sample will be evaluated at 90 days after therapy has been completed. 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.
    - **Opthalmologic** – an ophthalmogic evaluation including vision testing as well as retinal exams will be performed.
- **Medication Compliance Assessment** Compliance with drug dosing will be monitored through patient diaries and pill counts at outpatient visits. Patients will be asked to complete drug diaries as they take their study medication including the date and time. They will be asked to bring unused medication with them and the residual pills will be counted and retained. If compliance falls below 90%, the patient will be counseled about the importance of complying with drug therapy and made aware that if compliance falls below 80% their participation in the study may end. If compliance falls eblow 80%, the patient may be withdrawn from the study. Reasons for non-compliance will be assessed and documented in the patients record.

LABORATORY PROCEDURES/EVALUATIONS

- **Baseline Labs** - prothrombin time (PT/INR), partial thromboplastin time (PTT), reticulocyte count, plasma haptoglobin level, anti-HBc.
- **Routine Labs** - complete blood count (CBC with differential), acute care panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, urea nitrogen, eGFR), mineral panel (albumin, calcium, magnesium, phosphorus), hepatic panel (alkaline phosphatase, ALT, AST, total bilirubin, direct bilirubin), lipid panel (total cholesterol, triglycerides, HDL, LDL), LDH, CK, serum uric acid and total protein, urinalysis.
- **Monthly Labs** - PT, thyroid panel, pregnancy test
- **Serology Labs** - HBsAg, anti-HBs, HBeAg, and anti-HBe, anti-HDV, quantitative HBsAg levels.
- **Pancreatitis Monitoring:** amylase, lipase
- **Viral Kinetic and Pharmacokinetic Assessment (VK and PK)**: includes HDV RNA and HBV DNA qualitative tests via polymerase chain reaction assay (PCR) or a quantitative PCR (qPCR) for viral titer. One red top tube (10 mL) will be obtained and stored in -80 for pharmacokinetic (PK) analysis of lonafarnib, ritonavir and lambda.

- **Reproductive Panel -** In males, AM levels of inhibin B, luteinizing hormone (LH), total and free testosterone, and follicle stimulating hormone (FSH) will be obtained. In females, AM levels of luteinizing hormone (LH), estradiol, progesterone, antimullerian hormone, follicle-stimulating hormone (FSH), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone, sex hormone binding globulin (SHBG) and 17-hydroxyprogesterone (17-OHP) will be obtained.
- **Immunological Studies** - 30 - 50 ml of whole blood will be collected for immunological studies at specified time points described in table 1. 5 mm of liver tissue at baseline and end of therapy will also be collected for immunological studies, provided sufficient liver tissue is available after the required amount is obtained for histopathology. An additional pass will not be performed to obtain additional tissue for immunological studies.
- **Research Blood for Storage** - 7 ml of serum will be collected and stored at -80° C in the NIDDK clinical core lab.

Fecal Microbiome and Bacterial Translocation (BT) analysis - we plan to utilize pyro-sequencing techniques to survey microbial diversity in patients' fecal samples. Patients may refuse the microbiome portion of the study and still participate in other portions of this study. Prior to stool sample collection, all the patients will continue their routine diets. At each visit, a specific medication history (including prescription and OTC medications) and dietary recordings (appendix 2) will be taken. Samples (stool and serum) will be elicited at the time points shown in Table 2 and stored in -80C for future analysis.

SPECIMEN PREPARATION, HANDLING AND STORAGE

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 NIH Clinical Center rules over any eight-week period.

- **HDV RNA** testing will be done by quantitative PCR. Quantification will be performed at The Doctors Laboratories (TDL). TDL has performed the HDV RNA quantification for previous lonafarnib studies including LOWR-1 through LOWR-4 and the LIMT (lambda interferon for HDV) study. There currently is a lack of standardization across available HDV RNA quantitative assays and no commercially approved assay exists in the United States. The lower limit of quantification (LLOQ) for this test is 14 IU/mL and the limit of detection (LOD) is 2 IU/mL . If the test does not detect the virus, the result will be reported as "<LLOQ, target not detected." If the test detects the presence of the virus but was not able to accurately quantify the number of copies, the result will be reported as "<LLOQ, target detected.".
- **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.

- **Resistance monitoring** 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 drug-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 or HDV RNA that becomes quantifiable following a reduction to <LLOQ, target not detected) 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. Samples for analysis of potential host polymorphisms in the farnesyl transferase gene wil be collected from all subjects. 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.
- **Immunological Studies** –
    - 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 blood samples of HDV/HBV-coinfected patients in the chronic stage of disease prior to treatment. Peripheral blood mononuclear cells (PBMC) will be isolated from blood samples. The HDV-specific CD4 and CD8 T cell response to recombinant delta antigen will be assessed by measuring cytokines (IFNγ, TNFα, 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).

- **Fecal Microbiome and Bacterial Translocation (BT)** Bacterial DNA will be extracted from patient serum and stool using QIAamp DNA mini kit (Qiagen) according to the manufacturer's protocol. Patients' dietary history of the day prior to stool sample collection and any changes made in medication use since screening visit will be recorded. Polymerase chain reaction (PCR) for a conserved region of the 16S rRNA gene will be performed with two universal primers, forward primers (27F-Bac, 5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primers (543R-Bac, 5'-ATTACCGCGGCTGCTGGC-3') to detect and identify the bacterial DNA. For each sample, uniquely barcoded universal primers will be used for multiplexing. Amplified PCR products will be analyzed by agarose gel electrophoresis and then be pooled for pyrosequencing. Amplicons will undergo deep sequencing to be performed at SAIC-Frederick Inc. Sequences with a minimum length of 200 bp will be analyzed using mothur (version 1.21.0) for 16S rRNA gene sequence analysis. Taxonomic classification will be done using the RDP Classifier and phylogenetic tree will be generated using the Clearcut program. Blood will be drawn and used to measure microbial cell wall products, (LPS, peptidoglycan and beta-D-glucan), host response markers and selected cytokines.

Specimen Shipment

Specimens will be shipped using Federal Express in insulated and refrigerated specimen boxes.

STUDY SCHEDULE

Screening

Before admission to the NIH Clinical Center to start therapy, patients will undergo an evaluation for eligibility in this protocol. During the eligibility evaluation, patients will undergo a thorough explanation of this protocol, be provided this protocol's consent for review, and be given multiple opportunities to ask questions about this study. This will be documented in the patient's chart. Female subjects of reproductive potential and male subjects with female partners of reproductive potential will be informed and educated that two reliable forms of contraception as described in the exclusion criteria from the start of the study until 6 months from the end of lonafarnib, ritonavir and lambda dosing must be utilized. Women will be instructed that they may not breastfeed during their participation in this study.

After signing consent, patients will begin screening for the study. Results for labs drawn within the prior six months may be used to determine eligibility. Screening will include:

A. History and physical

B. Review of concomitant medications

C. Blood draw to include:

    1. Hepatic panel

    2. Mineral panel

3. PT/INR
4. AFP
5. Anti-HDV
6. HDV RNA
7. HDV and HBV genotyping
8. HCV RNA
9. Pregnancy test
10. ANA, ASMA, AMA, ceruloplasmin, ferritin, alpha 1 antitrypsin.
11. Ultrasound and Fibroscan
12. EKG
13. Anti-HIV 1/2
14. Lipid panel

Baseline Evaluation and Initiation of Treatment (within 6 months of screening)

If found to be eligible, the patient will be admitted to the NIH Clinical Center and undergo the following evaluation shortly before starting therapy.

If the patient was on HBV therapy prior to consenting to the study, they will continue on treatment for HBV. If the patient was not on HBV therapy, they will start on approved nucleos(t)ide treatment for a sufficient period of time prior to starting study medication in order for to demonstrate adequate suppression of HBV (serum HBV DNA of <2000 IU/mL).

Upon admission the patient will undergo the following:

1. Continuation of HBV therapy
2. MD Visit
3. Routine labs
4. Baseline labs
5. Pancreatic labs
6. Monthly labs
7. Reproductive labs
8. Serology panel
9. Immunological studies
10. Viral kinetics, pharmacokinetics
11. Microbiome studies
12. EKG

13. Ophthalmology, reproductive endocrine/urology, and nutrition consults (can occur prior to admission per patient's preference)
14. MRI
15. Fibroscan
16. Liver biopsy with hepatic venous pressure gradient measurements prior to starting therapy

Initiation of Treatment

All patients will be initiated on lonafarnib 50 mg orally twice daily, ritonavir 100 mg orally twice daily and lambda 180 mcg subcutaneously weekly. Eiger BioPharmaceuticals, Inc will supply the lonafarnib, ritonavir and lambda interferon under a clinical trials agreement. Patients will be initiated on therapy one or two days after the liver biopsy and will remain in the clinical center for 72 hours after induction of therapy for observation of side effects, administration of medication, and timed blood draws to facilitate analysis of virological response kinetics and pharmacokinetic analysis. During the admission, frequent blood sampling will be performed (0, 6, 12, 18, 24, 36, 48 and 72 hours after, the first dose) for viral kinetics, pharmacokinetics and storage.

Monitoring on Therapy

After induction of therapy, and completion of the inpatient stay as described above, patients will be treated as outpatients and followed on a regular basis in the outpatient clinic at the NIH Clinical Center.

Treatment Weeks 1, 2 and 3 (+/- 3 days)

Patients will be scheduled as outpatients to undergo the following:

1. Continuation of HBV therapy
2. MD visit
3. Monitoring of vital signs and weight (>10% weight loss from baseline would result in a follow-up nutrition consult).
4. Routine labs
5. Pancreatic labs
6. Serology
7. Viral kinetics, pharmacokinetics and storage
8. EKG

Treatment Weeks 4, 8, 12, 16 and 20 (+/- 3 days)

Patients will undergo the following as outpatients:

1. Continuation of HBV therapy
2. Continuation of study medication

3. MD Visit
4. Monitoring of vital signs and weight (>10% weight loss from baseline would result in a follow-up nutrition consult).
5. Routine labs
6. Pancreatic labs
7. Serology
8. Monthly labs
9. Reproductive labs
10. Immunological testing
11. Viral kinetics, pharmacokinetics and blood draw for storage
12. Microbiome studies
13. EKG

End of Therapy

At the end of therapy (week 24), patients will be be seen at the NIH Clinical Center for an evaluation as follows:

1. Continuation of HBV therapy
2. Discontinuation of study therapy
3. MD Visit
4. Monitoring of vital signs and weight (>10% weight loss from baseline would result in a follow-up nutrition consult).
5. Routine labs
6. Pancreatic labs
7. Serology
8. Baseline labs
9. Monthly labs
10. Consults
11. Reproductive labs
12. Immunological testing
13. Viral kinetics, pharmacokinetics and blood for storage
14. Microbiome
15. Imaging
16. Fibroscan
17. EKG

Post Treatment Monitoring

Patients will return for outpatient visits per the following schedule. As interferon alpha is the only recommended 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 alpha to all patients after participating in this study. However, if a prolonged HDV flare with significant liver damage is demonstrated, rescue therapy with interferon alpha will be offered. Patients failing experimental therapy will not be excluded from therapy with standard interferon alpha.

Post Treatment Weeks 1 and 2 (+/- 3 Days)

After discontinuation of therapy at week 24, patients will be monitored on an outpatient basis and followed off-therapy for follow-up and blood testing:

1. Continuation of HBV therapy
2. MD Visit
3. Monitoring of vital signs and weight
4. Routine labs
5. Serology panel
6. Viral kinetics, pharmacokinetics and blood for storage

Post Treatment Weeks 4, 8, 12, 16, 20 (+/- 5 days)

1. Continuation of HBV therapy
2. MD Visit
3. Monitoring of vital signs and weight
4. Routine labs
5. Serology Panel
6. Monthly labs
7. Reproductive labs
8. Immunological testing (weeks 4, 8 and 16)
9. Viral kinetics, pharmacokinetics and blood for storage
10. Microbiome studies (window for microbiome is +/- 5 days)

Post Treatment Week 24

At the end of study followup (week 24 post treatment), patients will be readmitted to the NIH Clinical Center for an evaluation as follows:

1. Continuation of HBV therapy
2. MD Visit

3. Monitoring of vital signs and weight (>10% weight loss from baseline would result in a follow-up nutrition consult).
4. Routine labs
5. Pancreatic labs
6. Serology
7. Baseline labs
8. Monthly labs
9. Consults
10. Reproductive labs
11. Immunological testing
12. Viral kinetics, pharmacokinetics and blood for storage
13. Microbiome
14. Imaging
15. Fibroscan
16. EKG
17. Liver biopsy

Unscheduled Visits

Data from unscheduled visits may be used in the study analysis. Unscheduled visits may occur due to the development of unexpected symptoms, laboratory abnormalities, or side effects which may require clinical follow-up outside of the scheduled visits. Additional evaluations, including laboratory, radiologic and EKG evaluations may be performed based on presenting symptoms or clinical suspicion.

Remote Visits

In the event that circumstances beyond our control preclude the travel of one or more of the study participants to the NIH Clinical Center for a visit, or otherwise would put their health at greater risk (i.e. epidemic or pandemic), we will endeavor to establish capabilities for remote visits. Specifically, for safety visits, the study team will arrange for local laboratory studies to be done, which include comprehensive metabolic panel, Liver function tests (including Tbili, AST and ALT), CBC with diff, PT/INR, HBV DNA quantitative and HDV quantitative measurements and conduct a telehealth visit with the patient. This telehealth visit will adhere to HHS guidance (https://www.hhs.gov/hipaa/for-professionals/special-topics/emergency-preparedness/notification-enforcement-discretion-telehealth/index.html) and can use all telehealth modalities under the good faith provision during a pandemic/epidemic as allowed by HHS for care of study participants. Unscheduled visits, due to safety, may also be conducted remotely through telehealth and/or offsite laboratory and radiologic studies and/or in conjunction with a local physician with the supervision of the study PI. The laboratory studies will be performed through Quest or LabCorp or with the local physician, and results will be sent to the study team for safety monitoring and

oversight. Radiology studies, when needed, will be arranged through a local, non-study physician, or directly by the study team, at a site close to the participants home area if travel to the NIH is not possible. As per FDA guidance, The study team will attempt to collect research blood at these visits to be sent to the NIH Clinical Center when possible, to address study outcome measures. During a pandemic affecting patient travel, serial missed research collections will not be considered major study deviations and will be reported to the IRB at the time of continuing review.

Monitoring of Patients Discontinuing Therapy

Therapy will be stopped for intolerance to lonafarnib, ritonavir and/or lambda (which is carefully defined in table 4). Patients who stop therapy due to intolerance, withdraw because of an adverse event, self-discontinue therapy or drop out from the study will be considered as treatment failures. 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, the patient will be followed for at least 24 weeks with visits to the outpatient clinic as per the post-treatment monitoring schedule described above. Thereafter, patients may be followed on an unscheduled basis under protocol 91-DK-0214 as is typical for patients who have participated in studies done by the Liver Diseases Branch.

Table 1: Study Design									
			Day 0	Study Weeks (+/- 3 days)				Study Weeks (+/-5 days)	
	Study Week/Procedures	Baseline Evaluation	Start Treatment	1, 2, 3	4, 8, 12, 16, 20	24	25, 26	28, 32, 36, 40, 44	48
Lonafarnib, Ritonavir, Lambda	HDV Therapy		x	x	x	x			
HBV Flare Prophylaxis	HBV Therapy	x	x	x	x	x	x	x	X
H&P	MD Visit	x		x	x	x	x	x	X
Vital signs									
Review of symptoms									
Concommitant meds									
Symptom Questionnaire									
Review of medication compliance									
CBC + diff	Routine labs	x		x	x	x	x	x	X
acute									
mineral									
lipid									
hepatic									
LDH									
CK									
Serum uric acid									
total protein									
amylase	Pancreatic labs	x		x	x	x			X
lipase									
HBsAg	Serology Panel	x		x	x	x	x	x	X
anti-HBs									
HBeAg									
anti-Hbe									
PT/INR	Baseline Labs	x				x			X
PTT									
Reticulocyte count									
Plasma Haptoglobin									
anti HBc									
PT	Monthly labs	x			x	x		x	X
Thyroid panel									
Pregnancy test									
Ophthalmology exam	Consults	x				x			X
Reproductive consult									
LH	Reproductive labs - Females	x			x	x		28, 32, 36	X
FSH									
estradiol									
Progesterone									
AMH									
DHEA									
DHEAS									
Androstenedione									

Testosterone Free testosterone SHBG 17-OHP									
Inhibin B LH FSH Free testosterone total testosterone	Reproductive labs - Males	x			x	x		28, 32, 36	X
30-50 mL serum for immunology.	Immunological Testing	x	0, 24 hours		4, 8, 12	x		28, 32, 36	X
10 mL red top for PK HBV DNA HDV RNA 7 mL whole blood for storage	VK, PK and Storage		0, 6, 12, 18, 24, 36, 48, 72 hours	x	x	x	x	x	X
Blood Stool Dietary questionnaire	Microbiome Studies	x			x	x		x	X
MRI Ultrasound Fibroscan	Imaging	X				x			X
EKG	EKG	x		x	x	x			X
Transjugular liver biopsy with portal pressure measurements or percutaneous liver biopsy	Liver Biopsy	x							X

Fecal microbiome and Bacterial Translocation (BT) analysis

With the recent introduction of highly sensitive analytical methods, the ability to meticulously describe the intestinal microbiome in health and disease has allowed for identification of fecal microbial dysbiosis. More recently, investigators have begun evaluating associations between the fecal microbiome and bacterial translocation in chronic liver disease, advanced liver disease and its potential contribution in poor outcomes in liver disease.

In this study, for research related purposes, we plan to utilize pyro-sequencing techniques to survey microbial diversity in patients' fecal samples. Patients may refuse the microbiome portion of the study and still participate in other portions of this study. Prior to stool sample collection, all the patients will continue their routine diets. At each visit, a specific medication history (including prescription and OTC medications) and dietary recordings (appendix 2) will be taken. Samples (stool and serum) will be elicited at the time points shown in Table 2 and stored in -80C for future analysis.

At the time of analysis, we intend to evaluate the relationship between specific microbiome maps and the occurrence of BT. In addition, species homology between blood and fecal samples will be determined by deep sequencing.

Bacterial DNA will be extracted from patient serum and stool using QIAamp DNA mini kit (Qiagen) according to the manufacturer's protocol. Patients' dietary history of the day prior to stool sample collection and any changes made in medication use since screening visit will be recorded. Polymerase chain reaction (PCR) for a conserved region of the 16S rRNA gene will be performed with two universal primers, forward primers (27F-Bac, 5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primers (543R-Bac, 5'-ATTACCGCGGCTGCTGGC-3') to detect and identify the bacterial DNA. For each sample, uniquely barcoded universal primers will be used for multiplexing. Amplified PCR products will be analyzed by agarose gel electrophoresis and then be pooled for pyrosequencing. Amplicons will undergo deep sequencing to be performed at SAIC-Frederick Inc. Sequences with a minimum length of 200 bp will be analyzed using mothur (version 1.21.0) for 16S rRNA gene sequence analysis. Taxonomic classification will be done using the RDP Classifier and phylogenetic tree will be generated using the Clearcut program. Stool samples will be collected at the beginning of study, every 4 weeks, at the end of treatment week 24 and every 4 weeks of post treatment. (Table 2)

Blood will be drawn and used to measure microbial cell wall products, (LPS, peptidoglycan and beta-D-glucan), host response markers and selected cytokines. This blood samples will be collected whenever we collect the stool sample. (Table 2).

Table 2. Time points for collection of samples for Microbiome & Bacterial Translocation Analysis

Time	Therapy	Blood and stool sample for bacterial translocation and blood sample for cytokines
Baseline Time = -2 to 0	None	*
On Tx Week 4,8,12,16,20	Lonafarnib/Ritonavir and lambda IFN	*
End of Tx Week 24	Lonafarnib/Ritonavir and lambda IFN	*
Post Tx week 4, 8,12, 16, 20, 24	None	*

An acceptable deviation for collection of these samples is +/- 5 days.
All microbiome related testing is intended for research purposes.

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 blood samples of HDV/HBV-coinfected patients in the chronic stage of disease prior to treatment. Peripheral blood mononuclear cells (PBMC) will be isolated from blood samples. The HDV-specific CD4 and CD8 T cell response to recombinant delta antigen will be assessed by measuring cytokines (IFNγ, TNFα, 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).

Design of the Trial/Statistical considerations

This study is designed as a phase 2a FDA regulated clinical study evaluating the utility of combination triple therapy of lonafarnib, ritonavir and lambda interferon in patients with chronic HDV infection. This study should show if HDV suppression is possible; assist in suggesting if this dose is effective for viral suppression; and how well lonafarnib, ritonavir and lambda interferon are tolerated. Thus, assessment of virologic response is the primary therapeutic endpoint and safety/tolerability is the primary safety endpoint.

Measurement of HDV RNA levels will be performed in two batches, the first batch will be analysed after the last patient has completed treatment, and the second batch when the last patient completes post-therapy follow-up.

In order to achieve a primary virologic response goal of a 2-log decline in HDV RNA in serum at end of 24 weeks of therapy, a total of 26 patients will be enrolled. This designation was made based on a power calculation with a statistical power designated at 0.9, alpha at 0.05, and two tailed testing, the effect size was found to be -1.1.

$$effect\ size = \frac{2672.231 - 267223.1}{240500.8} = -1.1$$

Based off prior interferon based studies, we would also include a potential drop out rate of 30%. Thus, the sample size desired will be 26 subjects.

HAZARDS AND DISCOMFORTS

The hazards associated with this study are the following.

1. ***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 30-32 venipuncture during their course of participation. 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.

2. ***The risks and hazards of liver biopsy.*** Patients will undergo liver biopsy as part of this study (as described above). The major side effects of liver biopsy are pain, infection and bleeding.

    a. *Transjugular Liver Biopsy*: Localized neck pain, hematoma and bleeding are reported in 6.5% of patients that undergo transjugular liver biopsy.[50] This is transient (lasting one to twelve hours) and is usually mild, rarely requiring analgesics. Severe pain requiring prolongation of the hospitalization occurs in approximately 1 in 500 cases. Organs that have been reported to be punctured during routine liver biopsies include lung, colon, gall bladder, kidney, or adrenal gland. 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. A transjugular liver biopsy will not be performed if patients have evidence of coagulopathy unresponsive to vitamin K (INR >1.5) or low platelets (Platelets < 50,000 k/uL) which may place them at a potentially higher risk for bleeding. Death due to bleeding after liver biopsy has been reported to occur in less than 1 in 10,000 cases.[51]

    At the NIH Clinical center, over 150 transjugular liver biopsies have been performed in the past 7 years on various patient populations, and there have been 2 reported complications. One of the complications was bile leak that required endoscopic retrograde cholangiopancreatography (ERCP) and stenting to fix the leak. The patient recovered without long-term sequelae. After this, the technique of transjugular liver biopsy at the clinical center was changed to minimize the risk of this happening again. A second patient developed mild itching after intravenous contrast administration. This resolved with Benadryl administration and the procedure was completed without further complication. Patients will be monitored for 24 hours post procedure to observe clinical symptoms such as abdominal pain and bilious vomiting that may suggest presence of a leak. If this occurs, appropriate intervention will be performed.

    b. *Percutaneous Liver Biopsy:* In this protocol, it is intended that most patients will undergo a transjugular liver biopsy. However, there may be rare instances when a transjugular liver biopsy cannot be performed for technical reasons. An example of this would be due to rare anatomical variations of the hepatic vein that do not allow for advancement of the liver biopsy needle and catheter into a suitable position for acquisition of

liver tissue. In these instances, a percutaneous liver biopsy will be performed given the importance of acquisition of liver tissue per research protocol. This determination will be made by the interventional radiologist at the time of performing the liver biopsy and HVPG measurements. A percutaneous liver biopsy will not be performed if patients have evidence of coagulopathy unresponsive to vitamin K (INR >1.5) or low platelets (Platelets < 75,000 k/uL) which may place them at a potentially higher risk for bleeding. As such, the transjugular liver biopsy, percutaneous liver biopsy and HVPG measurements will be described in depth (along with the intended choice to perform either the transjugular liver biopsy or percutaneous liver biopsy) to the patient with an opportunity to ask questions prior to the intended schedule procedure. The required NIH Clinical Center procedural consents will also be completed for all procedures as well.

A thorough description of the percutaneous liver biopsy and its hazards and discomforts will also be included in the general consent form for patient reference as well. A percutaneous liver biopsy is performed by passing an aspiration needle through the skin and subcutaneous tissues over the lower right side of the chest, through the intercostal muscles and into the liver. The needle is quickly withdrawn applying suction pressure. Local anesthesia as well as sedation (usually with midazolam) is typically used. The biopsy is done with the patient lying on his/her back with the right arm above the head. Sterile technique is used and the optimal site for the biopsy is chosen on the basis of physical examination for maximal liver dullness as well as ultrasound to demonstrate the position of the liver, gall bladder, and kidneys. The biopsy itself is done using an in-and-out motion with the needle being in the liver for less than one second. However, the biopsy requires approximately 30 minutes of preparation and monitoring of blood pressure and pulse regularly over the next 12-24 hours. In clinical practice, liver biopsies are usually done as an outpatient procedure; in the Liver Diseases Branch, we prefer to keep the patient overnight after the biopsy. The major hazards and discomforts of percutaneous liver biopsy are pain, fainting, bacteremia, puncture of another internal organ, and bleeding. The discussion below is supported by the ongoing quality assurance analysis of complications of liver biopsies performed by the members of the Liver Diseases Branch of the NIH. At the NIH, Medical Staff Fellows or Senior Staff of the Liver Diseases Branch, all of who are fully trained and credentialed in performing liver biopsies, performs liver biopsies. Furthermore, all liver biopsies by the Hepatology or Gastroenterology fellows are physically supervised by a member of the Senior Staff. The staff are quite proficient at liver biopsy technique since an average of 2 to 10 liver biopsies are done each month. Two of the complications of liver biopsy can be considered common (pain and fainting), the remaining are quite rare (bacteremia, puncture of another organ, severe bleeding). (1) 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. (2) Fainting or a vasovagal attack occurs in about 2% of patients undergoing liver biopsy. Patients who faint after liver biopsy are typically those who faint after blood drawing or traumatic experiences. The vasovagal attack occurs within 1 to 2 minutes of the biopsy and can occur even in a completely sedated patient (who appears to be asleep). Serious complications of vasovagal attacks have not been reported but they can be quite distressing to the patient and staff. (3) Asymptomatic 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. Of 3357 biopies performed at the NIH over the past 36 years, omplications occurred in 135 (4%) biopsies with 33 (1%) resulting in major complications. Severe pain occurred in 78 (2.3%) subjects and bleeding occurred in 21 (0.6%) subjects. Biliary injury occurred in 8 (0.2%) biopsies. Three subjects died as a result of massive intraperitoneal bleeding.[41]

3. ***The risks of hepatic venous pressure gradient (HVPG) measurements***: The hepatic venous pressure gradient (HVPG) will be measured during transjugular liver biopsy as a combined procedure in patients that participate in this study. Portal pressure measurements will not be performed if the patient has evidence of coagulopathy unresponsive to vitamin K (INR >1.5) or low platelets (Platelets < 50,000 k/uL) which may place them at a potentially higher risk for bleeding. Portal pressure measurements will be repeated at the end of therapy (week 24). This will be done as a direct measure for portal hypertension and is expected to provide a more dynamic, functional assay of the direction of change in liver disease to follow the evolution of disease and the need for additional clinical intervention. Portal pressure measurements were initially used as a diagnostic test for portal hypertension and as part of the work up of patients with chronic liver diseases.[52] Normal gradients are less than 5mmHg. It is accepted that decreasing the gradient to 12 mmHg or less will prevent variceal bleeding. It has also become clear that portal pressures convey prognostic information, which is an independent predictor of mortality in patients with liver disease related to cirrhosis.[42–44] The commonest current use in North America for portal pressure measurements is related to placement of transjugular intrahepatic portosystemic shunts by interventional radiology.

   There are various ways to measure portal pressure. The simplest and safest way is by measuring the hepatic venous wedged pressure with a balloon catheter and subtracting the free hepatic vein pressure to obtain a gradient. The main drawback of this technique is that presinusoidal portal hypertension may be underestimated. The hepatic venous pressure gradient is the current standard for measuring portal pressures.

   Hepatic venous pressure gradient measurements are typically performed by interventional radiologists. They are normally performed as combined procedures along with a liver biopsy to further aid in staging of liver disease. Aside from the

inherent risks of each individual procedure (percutaneous liver biopsy or transjugular liver biopsy or portal pressure measurements), there is no published added risk (in adults or pediatrics) in performing the combined procedure of portal pressure measurements with a liver biopsy. We are not aware of any series describing complications of this technique in detail. It is a technique that the clinical center interventional radiologists have considerable experience with, as it is essentially cannulation of a central vein. Furthermore, the remainder of the procedure is very similar to venous sampling, a technique used to locate endocrine tumors, again a common procedure at the clinical center. At the clinical center interventional radiology practice involves central venous cannulation and is done under ultrasound guidance and not the Seldinger technique. As such it is expected that complications at the puncture site would be less common.

The most likely, although rare, risks of portal pressure measurement are bleeding, thrombosis, and arrhythmias. Other complications are far less likely. It is estimated that the most likely complications would, at most, occur in less than 5% of procedures and the less likely complications would, at most, occur in less than 1% of procedures.

a) **Bleeding** is most likely to occur at the site of catheterization and typically manifests as a hematoma. It can occur at the time of the procedure or a few hours later (rebleeding). Very rarely the measurement catheter can tear or rupture the vein internally and cause internal bleeding. This may necessitate surgical repair or transfusion.

b) **Thrombosis** occurs as a result of introducing a catheter into a vessel and is uncommon. Anticoagulants can be given during the procedure at the discretion of the radiologist, however this increases the risk of bleeding. Anticoagulants can be used to treat thrombosis, if necessary, and also rarely thrombolytics.

c) **Arrhythmias** are caused by cardiac irritation as the catheter is passed through the heart to the liver. Patients will be monitored throughout the procedure by continuous telemetry. Arrhythmias are usually aborted just by moving the catheter to a less sensitive area. Should abnormal rhythms persist they will be treated appropriately.

d) Highly unlikely risks include the introduction of infection, pain, pneumothorax, arterial puncture, vocal cord paralysis, compression of adjacent structures by a hematoma, pseudoaneurysim formation, cardiac rupture, cardiac tamponade, and death.

4. ***Risk of ionizing radiation****:* The only exposure to radiation in this study is from fluoroscopy which will be utilized during the transjugular liver biopsy and portal pressure measurements. The potential amount of radiation to be received in this study is 2.5 rem that is below the guideline of 5 rem per year allowed for adult research subjects by the NIH Radiation Safety Committee.

5. ***The risks and discomfort of vibration controlled transient elastography (Fibroscan®)***: The Fibroscan® procedure is very similar to a regular ultrasound

examination and both are considered to be procedures with less than minimal risk to patients. Fibroscan® measures how quickly the wave is transmitted through the liver, which gives a measurement of the liver stiffness. The vibration and measurement of liver stiffness is repeated a total of 10 times and takes 5 to 10 minutes to complete. In 5 to 10 percent of persons, the test is not successful. The most common causes for failure include a large amount of fat over the rib cage, or a narrow space between the ribs or fluid in the abdomen.

Although there is minimal risk to patients, rarely, patients may experience mild discomfort between the ribs from having the tip of the probe placed there. This discomfort resolves when the probe is taken off the skin. Fibroscan has been performed on thousands of patients and no adverse effects have been reported.

6. ***Magnetic Resonance Imaging Scan (MRI)****:* MRI scanning may cause anxiety in some patients since current equipment used at the Clinical Center uses a closed tube. Patients will be offered sedatives such as Valium if they express concern about being in a closed space. The MRI will be performed for clinical purposes will be used to screen for hepatocellular carcinoma.

7. ***Risk of Gadolinum*****: Gadolinum** can cause an allergic reaction and nephrogenic systemic fibrosis (NSF). NSF causes fibrosis of the skin and connective tissues throughout the body. Patients develop skin thickening that may prevent bending and extending joints, resulting in decreased mobility of joints. Patients at risk are those with acute or chronic severe renal (kidney) insufficiency (glomerular filtration rate < 30 mL/min/1.73m$^{2)}$. The eGFR will have to be greater than >30 ml/min/1.73 m^2 body surface area for MRI to be performed. In the event that an MRI cannot be performed with gadolinium, an MRI without contrast will be performed.

8. ***Risks related to clinical relevance:*** Standard clinical tests that are performed by the Clinical Center laboratories will be placed in the medical record and appropriately discussed with the subject. Clinical tests not performed at the NIH will be reviewed as part of the medical record review. Other non-CLIA certified tests will not be included in the medical record or routinely discussed with the patient. In view of the research nature of this protocol, the non-CLIA certified results can not be meaningfully interpreted outside of the narrow focus of this study.

9. ***Risks related to the use of questionnaires*****:** The quality of life questionnaires will be distributed to all subjects in this study. These questionnaires will not be analyzed in real time but will be analyzed in batch form at a later date. There are no risks to completing the questionnaires and we do not expect to uncover additional medical problems with these questionnaires that would not otherwise be evaluated during routine clinical visits during the course of participation in this study.

10. ***The risks and hazards of lonafarnib therapy*****.** Lonafarnib is an orally bio-available tricyclic farnesyl transferase inhibitor that has been shown to have anti-tumor activity in various phase I and II trials and anti-HDV properties in various preclinical models and five recently completed phase 2 clinical studies performed

at several centers, including the NIH clinical center.[45,46] Multiple phase 1 and 2 and 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 >7000 subjects. Specifically, the administration of lonafarnib to patients with known liver disease and chronic HDV infection is >120 subjects, to date.

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 postnatal 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 extend 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[47,48] In the initial dose-finding phase 1 trial, minor hematologic and non-hematologic side effects were seen.[48] 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.[47] 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.[47]

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. 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.

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.[49,50] In the first study, patients were treated for a median duration of 185 days. Currently, there are various phase I, II and III investigational studies ongoing (www.clinicaltrials.gov) with no significant safety alerts (www.fda.gov).

To date, lonafarnib has been administered to >120 subjects with chronic liver disease and chronic HDV infection at doses ranging from 50 mg once daily to 300 mg twice daily. In the NIDDK studies (proof-of-concept study (12-DK-0046) and the LOWR-3 (15-DK-0170)), patients underwent digitized electrocardiography (baseline, each study visit during therapy, and 1-2 weeks after stopping therapy), ophthalmologic examinations with retinal photography (baseline, 4 weeks, 12 weeks and 24 weeks while on therapy, and 24 weeks post-therapy) and evaluations for reproductive toxicity (specialist consultations, serological testing and transvaginal ultrasounds on day 3 of menstrual cycle for females (12-DK-0046 only)). No patients were found to have any significant changes in these parameters at any time during participation in these studies.

In our proof-of-concept, lonafarnib was administered at doses of 100 mg BID and 200 mg BID in a total of 12 patients. During this study, patients underwent digitized electrocardiography (at baseline, weekly while on therapy and weeks 1 and 2 after stopping therapy), ophthalmologic examination with retinal photography (at baseline, end of therapy and 24 weeks post-therapy) and evaluations (specialist consultations, serologic testing and transvaginal ultrasounds on day 3 of menstrual cycle for females) for reproductive toxicity (at baseline, end of therapy and 24 weeks post-therapy). No patients were found to have any significant changes in these parameters at any time during participation in this study. Regarding symptoms encountered in this study, side effects of lonafarnib included mild nausea (25%), diarrhea (37.5%), anorexia (12.5%), and abdominal bloating (12.5%) in the lonafarnib 100mg bid group. In the lonafarnib 200 mg BID group, nausea (75%), diarrhea (75%), anorexia (62.5%), dyspepsia

(75%), vomiting (37.5%) and mean weight loss of 4kg (75%) were found. There were no grade 3 or 4 adverse events or serious events.

As there are no HDV-specific therapies, lonafarnib appears to potentially 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. As proof of absence of viral resistance with prenylation inhibitors in HDV, population-based sequencing of LDAg (codons 115-215) at baseline, end of therapy and 24 weeks after end of therapy was performed in our recently completed study (12-DK-0046). Analysis at these time points revealed no changes in viral sequences thus confirming the absence 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 treat all the patients with nucleoside analogues for HBV, 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 the 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.

11. ***The risks and hazards of Ritonavir therapy***. Ritonavir is an orally bio-available protease inhibitor, which was used as a pharmacokinetic booster (coadministration of 100mg Ritonavir) of second protease inhibitor or other anti-retroviral agents. In this study, ritonavir will be used as a pharmacokinetic booster of Lonafarnib. The most common side effects (>10%) of ritonavir are nausea, diarrhea, vomiting, altered sense of taste (taste perversion), abdominal pain, arthralgia, back pain, cough and loss of appetite. If it is used more than 60 days, there is increase in cholesterol levels and triglycerides, as well as blood sugar, potentially resulting in the development of diabetes mellitus. The increase in cholesterol and triglycerides can occur within one week of taking this medication. Lipid profile should be monitored prior to initiating therapy and at periodic intervals during therapy. After the completion of the study, cholesterol and triglyceride levels should return to baseline.

    a. Elevation of AST/ALT can exceed 5 times the upper limit of normal, clinical hepatitis and jaundice in patients receiving Ritonavir alone or in combination with other antiretroviral drugs. [48] Dosage adjustment is not necessary in patients who have mild (Child- Pugh Class A) to moderate

(Child-Pugh Class B) hepatic impairment. There is no safety data available for severe (Child- Pugh Class C) hepatic impairment patients. Ritonavir is not recommended in patients who have severe hepatic impairment. As we are not going to enroll decompensated liver disease patients, it should not be a problem.

b. Pancreatitis has been observed in those who developed hypertriglyceridemia. If clinical symptoms or laboratory abnormalities (increased serum amylase or lipase), pancreatitis should be considered. Patients should be evaluated and Ritonavir should be discontinued if a diagnosis of pancreatitis is made.

c. Allergic reaction/Hypersensitivity including urticarial, mild skin eruptions, bronchospasm and angioedema have been reported. Cases of anaphylaxis, toxic epidermal necrolysis, Stevens-Johnson syndrome have also been reported. We will discontinue treatment if severe reactions develop.

d. Prolong PR interval in some patients. Post-marketing cases of second or third degree atrioventricular block have been reported in patients. The effect on PR interval of co-administrating with other drugs that prolong PR interval (calcium channel blockers, beta blockers, digoxin and atazanavir) has not been studied. But caution should be undertaken when co-administration of Ritonavir with these drugs, especially with those drugs metabolized by CYP3A.

e. Metabolism of Ritonavir is mainly through cytochrome P450 CYP3A4. [21]Concurrent usage of other moderate and strong CYP3A inhibitors and inducers will lead to change in plasma concentration of concomitant medications, resulting prolonged therapeutic or adverse effects. Patients who are taking these medications should be excluded.

12. The risks of the combination of Lonafarnib and Ritonavir. In the LOWR-2 study, the combination of lonafarnib (25 mg and 50 mg BID) and ritonavir, a total of 18 patients were treated, with 7 patients reporting grade 1 adverse events (GI adverse events namely nausea, diarrhea, fatigue, weight loss, anorexia and vomiting.) with lonafarnib 50 mg BID with ritonavir and 4 reporting grade 1 GI AEs with lonafarnib 20 mg BID. 2 patients on lonafarnib 50 mg BID and 1 patient on lonafarnib 25 mg BID suffered grade 2 GI AEs. Only 1 patient on the 25 mg BID group experienced grade 3 adverse events. In the LOWR-4 study, 5 patients had post-treatment ALT flares with normal liver function. AEs were mostly grade 1-2 intermittent diarrhea; 4 patients had grade 3 AE (2 diarrhea; 1 asthenia, 1 weight loss). There was 1 SAE (traumatic jaw fracture, unrelated to LNF).

13. The risks of the combination of Lonafarnib, Ritonavir and HBV therapy. During the NIDDK study 12-DK-0046, 5 of 14 patients were on nucleos(t)ide analogues while on lonafarnib monotherapy. During this 28-day study, there were no additional side effects that could be attributed to the use of a nucleos(t)ide analogue in combination with lonafarnib. Similarly, in the LOWR-2,3 and 4 studies, nucleos(t)ide analogues were used in combination with lonafarnib and ritonavir with no adverse events pertinent to the combination noted. Ritonavir has

been used in combination with nucleos(t)ide analogues in HIV approved therapies and is thought to be safe. Nucleos(t)ide analogues for hepatitis B function through a different mechanism of action and have different pharmacokinetics and side effect profile. As such, it is believed that there is no added risk of the combination of lonafarnib, ritonavir and a nucleos(t)ide analogue for hepatitis B.

14. The risks and hazards of lambda interferon therapy

Lambda has been generally well tolerated in clinical studies. A lower frequency of musculoskeletal (myalgia, arthralgia, and back pain) and flu-like symptoms (chills, pyrexia, and pain) was observed across Phase 2 studies in subjects receiving IFN regimens containing Lambda compared with alfa. In addition, there was a notable lack of hematologic toxicity in the WBC or platelet lineages in subjects receiving Lambda regimens.

Laboratory abnormalities have generally been of low grade and self-limited. When study regimens included concomitant RBV administration, decreases in hemoglobin levels were observed as expected; however, anemia was less frequent and milder with Lambda/RBV than with alfa/RBV. Hematologic toxicity was also reported less frequently in groups treated with Lambda compared with alfa.

The main safety finding associated with Lambda treatment has been the higher frequency of transaminase elevations, accompanied, in some cases, by increases in total and conjugated (direct) bilirubin, but without evidence for impaired liver synthetic function such as decreased albumin or increased INR. The hyperbilirubinemia cases resolved with no laboratory evidence of sustained hepatic dysfunction following dose reduction or discontinuation of study drug.

In one study, 3 cases of concurrent ALT and TBILI elevations in patients with HCV treated with Lambda/RBV/asunaprevir (ASV) met program-defined criteria for pDILI; this led to discontinuation of this regimen in future development plans. Frequencies of concurrent ALT/AST and TBILI elevations across studies were as follows: 18.2% for Lambda/RBV/ASV, 3.6% for Lambda/RBV, 2.3% for alfa-2a/RBV/ASV, and 3.5% for alfa-2a/RBV.

In another study in patients with HBV, 4 subjects (5.0%) in the Lambda 180-μg group and 1 subject (7.7%) in the 240 μg group, and 1 subject (1.2%) in the alfa group met the laboratory criteria for pDILI. In 5 of the 6 cases, an alternative explanation (early, on-treatment, host-mediated ALT flare) was identified.

In vitro data did not suggest a hypothesis-driven metabolism- or transporter-related rationale for the clinically observed differences in rates of hyperbilirubinemia between subjects treated with alfa-2a or Lambda, but retrospective analysis of safety data from the EMERGE study (Zwirtes 2016) found that hyperbilirubinemia events occurred within the first 12 weeks of treatment, and the temporal pattern of mean total bilirubin level in the Alfa/RBV group was similar to that observed in the Lambda 120 and 180 mg groups (although with lower quantitative values for Alfa), suggesting a similar underlying mechanism of hyperbilirubinemia, probably RBV-induced hemolysis, for Lambda and Alfa treatments during the first 4–6 weeks of therapy. For the

Lambda 240 mg group, however, a second peak occurred after week 12 suggesting additional reasons for increases in bilirubin levels, probably as a result of direct toxicity to hepatocytes with that dose.

Decompensation of cirrhosis has been observed with Lambda, as with alfa IFNs. A total of 4 cases have been reported. One subject with baseline cirrhosis and Gilbert's disease developed Grade 1 hepatic decompensation and ascites while receiving Lambda 120 μg in the EMERGE Phase 2b study. The events resolved with drug interruption, and the subject subsequently resumed therapy at a lower dose. In the Phase 3 study (AI452017) in GT-2, -3 HCV-infected subjects, 3 of approximately 70 randomized subjects with preexisting compensated cirrhosis developed decompensated cirrhosis while on treatment with Lambda. All 3 subjects were Child- Pugh Class A at entry and had evidence of portal hypertension. Two of these subjects subsequently recovered; however, 1 subject died of infectious complications. Based on these data, Lambda appears to be associated with decompensation of cirrhosis especially in the context of portal hypertension. Enrollment of cirrhotic subjects in current studies is restricted to compensated cirrhotic subjects (Child-Pugh Class A) without evidence of portal hypertension.

In analyses of the cardiac effects of Lambda, no evidence of a clinically meaningful impact on QT/QTc interval or other key ECG parameters was detected in 32 healthy subjects given a single dose of Lambda 80 to 240 μg or in 176 patients with HBV treated with Lambda 180 μg for 24 weeks. A slight increase in mean ΔQTcF was seen in the HBV patients. Lambda was not associated with AEs of ventricular arrhythmias or QT/QTc prolongation.

15. **The risks of the combination of Lonafarnib, Ritonavir, Lambda Interferon and HBV therapy**: The risk of combination therapy with lonafarnib, ritonavir and lambda interferon are not known Lonafarnib, in combination with ritonavir was administered along with alpha interferon to 11 patients in the LOWR-2 study in Turkey. In the LIRA-B study, where patient with mono-infection with chronic hepatitis B were randomized to treatment with either interferon alpha or lambda interferon, overall adverse events, serious adverse events and discontinuation rates were comparable between arms however the AE spectra differed, with more cytopenias, flu-like and musculoskeletal symptoms in the alpha group and more ALT flares and bilirubin elevations seen in lambda.

Adverse Events and Modification of Dose of Prenylation Inhibitor and Ritonavir

Patients will be monitored for side effects. The use of statins will be prohibited during the course of the study as are the use of moderate and strong CYP3A inhibitor and inducers. Discontinuation of lonafarnib, ritonavir and lambda interferon will be based upon the scoring of adverse events as shown in table 5. The scoring of toxicity will be performed from the CTCAE Version 4.03 with modifications for leukocytes, platelets, prothrombin time, partial thromboplastin time, ALT, AST and bilirubin. These variables have been modified because the National Cancer Institute (NCI) designed the original version 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, ritonavir and lambda interferon include pregnancy, any grade 3 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. This is further defined below. If the female becomes pregnant, the patient's obstetrician will be provided with the standard of care guidelines to prevent HBV transmission. Medications will be stopped if pregnancy occurs, with the exception of HBV therapy, given the risk of hepatitis B flare during pregnancy. Lonafarnib, ritonavir and lambda interferon will not be restarted unless another cause for the abnormality or symptom is found.

Table 3: Dose reduction plan for Lambda/Lonafarnib intolerability

Assigned Treatment	Starting Dose	Dose Reduction (DR1)
	Dose	Dose
Lambda	180 µg	120 µg
Lonafarnib	50 mg	25 mg

Table 4: Guidelines for Treatment Interruption or Discontinuation and Dose Modification

Toxicity	Management, Monitoring, and Dose Modification	Discontinuation of Drug
	Immediate Actions/Resolution	
Adverse Events		
Lambda AE ≥ Grade 3, considered at least possibly related to study drug and clinically significant, and no specific guidance given below	Interrupt dosing and monitor weekly Resolution: If event resolves to ≤ Grade 1 or baseline value, restart dosing at DR1	If event does not resolve in 2 weeks (missed 2 consecutive doses of drug) or If event recurs at DR1, discontinue drug
Lonfarnib AE ≥ Grade 3, considered at least possibly related to study drug and clinically significant, and no specific guidance given below	Interrupt dosing and monitor weekly Resolution: If event resolves to ≤ Grade 1 or baseline value, restart dosing at DR1	If event does not resolve in 2 weeks (missed 14 consecutive doses of drug) or If event recurs at DR1, discontinue drug

Toxicity	Management, Monitoring, and Dose Modification	Discontinuation of Drug
Lambda AE ≥ Grade 3, considered at least possibly related to study drug and not clinically significant, and no specific guidance given below	Discuss with medical monitor. Not all Grade 3 AEs will require dose modification.	
Lonafarnib AE ≥ Grade 3, considered at least possibly related to study drug and not clinically significant, and no specific guidance given below	Discuss with medical monitor. Not all Grade 3 AEs will require dose modification.	
Lambda Depression	See Table 5 and CTCAE neurologic definitions for mild/moderate/severe depression Interrup dosing. Obtain psychiatric consult	
Lambda New ocular symptoms: New decrease or loss of vision or other clinically significant ocular sign or symptom	Interrupt dosing. Obtain complete eye examination performed by an ophthalmologist. Discuss further management with the medical monitor prior to restarting therapy.	
Hematologic abnormalities		
Lambda		
ANC		
≥750/mm^3	Maintain dose	
< 750/mm^3	Interrupt dosing Resolution: If ANC > 500/mm^3, restart dosing at DR1	If event does not resolve in 4 weeks (missed 4 consecutive doses) or If event recurs (confirmed) at DR1, discontinue drug
Platelets		
< 40,000	Reduce dose to DR1	
< 25,000		If event is confirmed, discontinue drug

Toxicity	Management, Monitoring, and Dose Modification	Discontinuation of Drug
Hepatobiliary abnormalities **Lambda**		
ALT/AST/TBILI ≥ CTCAE grade 2	Repeat tests within 48-72 hours and monitor weekly. Monitor ALT, AST, TBili, DB, Albumin, PT/INR Involve medical monitor Resolution: CTCAE <grade 2, return to routine monitoring.	
ALT/AST ≥ CTCAE grade 3	Interrupt dosing Monitor ALT, AST, TBILI, DB, albumin, PT/INR weekly Involve medical monitor Resolution: If ALT/AST <CTCAE grade 3, restart dosing at DR1 if agreed upon with medical monitor with weekly monitoring for the first 4 weeks. If stable after 4 weeks of monitoring can resume regular per protocol monitoring.	If event does not resolve in 4 weeks (missed 4 consecutive doses) or If event recurs (confirmed) at DR1, discontinue drug
TBILI ≥ CTCAE grade 3	Interrupt dosing Monitor ALT, AST, AP, TBILI, DB, albumin, PT/INR weekly Involve medical monitor Resolution: If TBILI ≤CTCAE grade 3, restart dosing at DR1 if agreed upon with medical monitor with weekly monitoring for the first 4 weeks. If stable after 4 weeks of monitoring can resume regular per protocol monitoring.	If event does not resolve in 2 weeks (missed 2 consecutive doses) or If event recurs (confirmed) at DR1, discontinue drug
Lonafarnib		
ALT/AST/TBILI ≥ CTCAE grade 2	Repeat tests within 48-72 hours and monitor weekly. Monitor ALT, AST, TBili, DBili, Albumin, PT/INR Involve medical monitor Resolution: CTCAE <grade 2, return to routine monitoring.	
ALT/AST ≥ CTCAE grade 3	Interrupt dosing Monitor ALT, AST, TBILI, DB, albumin, PT/INR weekly Involve medical monitor Resolution: If ALT/AST <CTCAE grade 3, restart dosing at DR1 if agreed upon with medical monitor with weekly monitoring for the first 4 weeks. If stable after 4 weeks of monitoring can resume regular per protocol monitoring.	If event does not resolve in 2 weeks (missed 14 consecutive doses) or If event recurs (confirmed) at DR1, discontinue drug

Toxicity	Management, Monitoring, and Dose Modification	Discontinuation of Drug
TBILI ≥ CTCAE grade 3	Interrupt dosing Monitor ALT, AST, AP, TBILI, DB, albumin, PT/INR weekly Involve medical monitor Resolution: If TBILI <CTCAE grade 3, restart dosing at DR1 if agreed upon with medical monitor with weekly monitoring for the first 4 weeks. If stable after 4 weeks of monitoring can resume regular per protocol monitoring.	If event does not resolve in 2 weeks (missed 14 consecutive doses) or If event recurs (confirmed) at DR1, discontinue drug
Renal function abnormalities **(lonafarnib AND lambda)**		
Creatinine clearance < 50 mL/min		Discontinue drug

In addition to the above rules, all subjects who meet criteria for a treatment related liver event will undergo the following clinical work up:

- Ultrasound of the liver should be performed, including doppler, for subjects with a bilirubin level greater than 1.5 times baseline
- If clinically feasible, a liver biopsy should be performed.
    - o When a percutaneous biopsy is contraindicated, a transjugular biopsy may be discussed.
- Liver and chemistry labs should be performed weekly (minimally include ALT, AST, bilirubin, INR, alkaline phosphatase, albumin and gamma-GT) until the bilirubin returns to baseline value.
- HBV DNA and HDV RNA should be monitored weekly
- 5 ml of serum plus 5 ml of plasma should be collected for possible later biomarker analysis
- Autoimmune markers (antinuclear antibody [ANA], anti-smooth muscle antibody [anti-SMA], anti-LC1, anti-SLA liver kidney microsome type 1 and type III antibody [anti-LKM1,3]),
- _C3, C4 and CH50,
- _Acute viral hepatitis,
    - o Serologies for acute hepatitis A and E (IgM);
    - o PCR for HCV, hepatitis E (stool and blood),
    - o *Cytomegalovirus* (CMV), PCR
    - o *Epstein-Barr virus* (EBV), PCR
    - o Herpes simplex viruses 1 and 2 (HSV), PCR
- _Review of pre-existing hepatic disease (excluding HBV),

- Review of concomitant medication(s), or herbal medications and substances known to be hepatotoxic, tests for alcohol and acetaminophen and drugs of abuse, if indicated

Table 5. Scoring of toxicity for dose modification. Scoring of toxicity from the CTCAE Version 4.03, 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; urticarial; dyspnea; drug fever >38 C (>100.4 F)	Symptomatic bronchospasm, with or without urticarial; 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;	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated >24 hrs	Life threatening consequences	Death

			IV fluids indicated <24 hrs			
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 compared to baseline	Increase of 4-6 stools per day over baseline; IV fluids indicated <24 hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL	Increase of >6 stools per day over baseline; incontinence; IV fluids >24 hrs; hospitalization ; sever increase in ostomy output compared to baseline; interfering with ADL	Life threatening consequences (e.g. hemodynamic collapse)	Death
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
Depression	Depression	Mild depressive symptoms	Moderate depressive symptoms, limiting instrumental ADL	Severe depressive symptoms, limiting self care ADL, hospitalization not indicated	Life threatening consequnes, threats of harm to self or others, hospitalisation indicated	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 transplant ation indicated	Death
Glucose, serum-low (hypoglyce mia)	Hypoglyce mia	<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 (hypertrigly ceridemia)	Hypertrigly ceridemia	>ULN – 2.5 X ULN	>2.5 – 5.0 X ULN	>5.0 – 10 X ULN	>10 X ULN	Death
Cholesterol, serum high (Hyperchol esterolemia)	Hypercholesterolemia	>ULN - 300 mg/dL; >ULN - 7.75 mmol/L	>300 - 400 mg/dL; >7.75 - 10.34 mmol/L	>400 - 500 mg/dL; >10.34 - 12.92 mmol/L	>500 mg/dL; >12.92 mmol/L	-----
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 with ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling	-----
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	< 10.0 – 8.0 g/dL	<8.0 – 6.5 g/dL	<6.5 g/dL	Death

		<LLN – 6.2 mmol/L <LLN – 100 g/L	<6.2 – 4.9 mmol/L <100 – 80 g/L	<4.9 – 4.0 mmol/L <80 – 65 g/L	<4.0 mmol/L <65 g/L	
Granulocytes	Granulocytes					
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	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

oxaloacetic transaminase)						
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
Pancreatitis	Pancreatitis		Enzyme elevation or radiologic finding only	Severe pain; vomiting; medical intervention indicated (e.g., analgesia, nutritional support)	Life-threatening consequences; urgent intervention indicated	Death

DEFINITION AND MANAGEMENT OF ON-TREATMENT ALT FLARES

On treatment flares will be managed per guidelines defined in Table 4. Post treatment ALT flares are defined as ALT ≥ grade 2 CTCAE score, and participants will be followed every 4 weeks till ALT falls below grade 2 CTCAE score. Severe post-treatment flares are defined as ALT ≥ grade 3 CTCAE score. Participants with post treatment severe ALT flares will be evaluated every week with until ALT decreases below CTCAE grade 3, then will be seen every 4 weeks for ALT between CTCAE grades 2-3, after which scheduled visits will resume per study protocol.

Subjects who experience post-treatment flares may undergo an evaluation for other causes of liver disease, as well as may be initiated on Peg-interferon alpha per discretion of the study investigator.

Table 6: Post treatment ALT flare management schedule

ALT (CTCAE grade)	Intervention/Management	Follow up schedule
≥ 2-3	Labs per protocol 1. Liver panel 2. CBC 3. PT/INR	Every 4 weeks, till ALT < Grade 2 CTCAE, then resume visits per study protocol.
≥3	Labs per protocol 1. Liver panel 2. CBC 3. PT/INR	Every week, till ALT < Grade 3 CTCAE, then every 4 weeks till ALT < Grade 2 CTCAE, then resume visits per study protocol.

DATA AND SAFETY MONITORING

The Principal Investigator will function as the data and safety monitor and report any adverse events to the IRB. The Principal and Associate Investigators in this protocol will monitor data and safety. The Liver Diseases Branch, NIDDK, reviews data and safety weekly in clinical research rounds. 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. Additionally, an outside data/safety monitor will be utilized. Dr. Sasan Sakiani, MD from the Division of Gastroenterology and Hepatology at the University of Maryland Medical Center, Baltimore, MD will serve as the data/safety monitor and will review data every two weeks during the course of the study, or sooner based on clinical necessity. Yearly reports are made to the NIDDK/NIAMS IRB regarding safety and efficacy.

DATA MANAGEMENT PLAN

Data will be maintained electronically on electronic case report forms (eCRF). Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record. Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into CRIS, which is 21CFR Part 11 compliant, and into to REDCap, both of which are maintained at the NIH Clinical Center. The data systems include password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Information derived from patient interview will be entered to eCRFs and considered source documents.

REPORTING OF ADVERSE EVENTS, UNANTICIPATED PROBLEMS AND DEVIATIONS

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

FDA AND SPONSOR REPORTING REQUIREMENTS

The study investigator will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the

information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

REGULATORY REQUIREMENTS

The IND associated with this study (113137), in which the NIDDK is the sponsor, is associated with lonafarnib and lambda interferon. The FDA has not requested an IND for the use of ritonavir in combination with lonafarnib. Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan and applicable FDA guidelines. Audit and/or monitoring visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

RECRUITMENT STRATEGY

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

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.

CONSENT PROCESS

Informed consent begins with the initial approach of an investigator to a potential subject (for example through a flyer or advertisement) and continues (a) until the completion of the research study, (b) until the individual completes study participation, (c) the subject withdraws him/herself from the study or (d) the subject is withdrawn from the study by the investigator.

Written informed consent will be obtained from the participant prior to any screening visits, study procedures or treatments. The Principal Investigator or other designated qualified protocol investigators will explain the study in language understandable to the subject. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. The participant and the investigator will sign

the current IRB-approved informed consent document. A copy of the consent will be given to the subject for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the electronic medical record (CRIS).

RESEARCH USE, STORAGE AND DISPOSITION OF HUMAN SUBJECT'S SAMPLES AND DATA.

Patients will have serum and stool stored from selected time points during this study. The serum specimens will be used for repeat virological testing and special tests as needed and the stool tests will be used for microbiome analysis. Samples may be used to assess factors associated with response or non-response to therapy. These samples will be tested in the Liver Diseases Branch or the routine clinical services of the Clinical Center. Residual samples may be used for future research related to liver disease and its' associated conditions. 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 and all applicable tech transfer are initiated. ARUP and The Doctors Laboratory will perform all HDV RNA quantitative testing for this protocol. All samples sent to outside collaborators, ARUP and The Doctors Laboratory 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 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 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. 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 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. If requested, Eiger Biopharmaceuticals (who will be providing the Lonafarnib, Ritonavir and Lambda), 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.

Data Sharing:

After obtaining consent for data sharing, de-identified data collected from demographic, dietary, quality of life, and symptom questionnaires, biochemical testing of urine, serum, radiological and liver biopsy samples of study participants will be made available without cost to researchers and analysts through NIH/ NIDDK data repositories. Data will be submitted at the time of publication. Users must agree to the conditions of use governing access to the public release data, including limitation of research to investigations

consistent with the participants' consent, restrictions against attempting to identify study participants, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties, and proper acknowledgement of the data resource.

Study Alternatives:

Alternatives to the study may be offered at the NIH or at outside medical centers and 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. Patients who choose to enroll in this clinical trial, if not previously treated, may delay therapy with interferon for up to 48 weeks. 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 under protocol 91-DK-0214 or outside of the NIH).

REMUNERATION/COMPENSATION

No compensation is offered to study participants.

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Lonafarnib/Ritonavir/Lambda Interferon for Chronic Hepatitis Delta

Patient Name _____

Enrollment Criteria

No	Criteria	Yes/No	Comment
1	Is patient 18 years or above?		Age = _____ years
2	Is anti-HDV present in serum?		Date of sample: ___/ ___/ ___
3	Is HDV RNA positive at three time points?		Date of samples: ___/___/___ ___/ ___/ ___ ___/ ___/ ___
4	Is chronic HDV infection demonstrated by the present oc HDV RNA in serum >/= 6 months, or presence of anti-HDV antibody for >/= 6 months		
5	Is consent form signed?		Date of signature ___/___/___
	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	Are any of the last 3 ALT levels > 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	Are triglycerides >500 mg/dL		
6	Is PR or QT_c interval prolonged on EKG?		
6	**Is there a history of bleeding varices, ascites or encephalopathy?**		
7	For females with childbearing potential: Is the patient pregnant, actively breast-feeding, or unable to practice birth control?		Not applicable if patient has had a tubal ligation ____
8	For males: Is the patient able to practice birth control?		Not applicable if patient has had a vasectomy _____
9	Are there significant other medical illnesses such as CHF, renal failure, transplant, psychiatric dx, and angina?		
10	Has the patient been on systemic immunosuppressive therapy within the previous 2 months?		
11	Does the patient have another form of liver disease in addition to coinfection of HBV and HDV?		
12	Has the patient undergone any experimental therapy or pegylated interferon within 6 months prior to starting the study?		

13	Has the patient had a diagnosis of malignancy within 5 years prior to enrollment with the exception of superficial dermatologic malignancies?		
14	Has patient had active substance abuse in the last one year?		Alcohol, inhaled or injection drugs
15	Does the patient have evidence of HIV co-infection?		Date of test: date __/__/__
16	Is AFP > 200 ng/ml and does the ultrasound/MRI/CT show evidence of a mass in the liver?		AFP = _____ US/MRI/CT date __/__/__
17	Is the patient currently on statin medications?		
18	Is the patient currently on medications that would alter metabolism of ritonavir?		Alpha-1-adrenoreceptor antagonist Antiarrythmic medications Pimozide Sildinafil Sedative and hynotics Ergot St. John's Wort

The answer to all of the above questions is no:

_____

Signature and Date

Appendix 1

SYMPTOM SCALE: Delta Hepatitis

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

For the following questions, please respond by circling one of the numbers from 1 to 7. The numbers designate the following answer:

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

1. How much of the time since your last visit have you been troubled by a feeling of abdominal bloating?
2. How much of the time have you been tired or fatigued since your last visit?
3. How much of the time since your last visit have you experienced bodily pain?
4. How often since your last visit have you felt sleepy during the day?
5. How much of the time since your last visit have you experienced abdominal pain?
6. How much of the time since your last visit has shortness of breath been a problem for you in your daily activities?
7. How much of the time since your last visit have you not been able to eat as much as you would like?
8. How much of the since your last visit have you been bothered by having decreased strength?
9. How often since your last visit have you felt anxious?
10. How often since your last visit have you felt a decreased level of energy?
11. How much of the time since your last visit have you felt unhappy?
12. How often since your last visit have you felt drowsy?
13. How often since your last visit have you been irritable?
14. How much of the time since your last visit have you had difficulty sleeping at night?

15. How much of the time since your last visit have you been troubled by a feeling of abdominal discomfort?
16. How much of the time since your last visit have you had mood swings?
17. How much of the time since your last visit have you been unable to fall asleep at night?
18. How often since your last visit have you had muscle cramps?
19. How much of the time since your last visit have you felt depressed?
20. How much of the since your last visit have you had problems concentrating?
21. How much of the time have you been troubled by itching since your last visit?
22. Since your last visit have you had any excessive hair loss?
23. Since your last visit have you had any rashes?

Appendix 2

Microbiome Monthly Intake Questionnaire: Delta Hepatitis

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

1. Over past month, have you taken any new antibiotic medications prescribed by a doctor? Yes [ ] No [ ]

If yes, when was the last time you took the antibiotic? _____

Name of antibiotic _____

Duration of treatment _____

2. Over past month, have you taken any probiotics? Yes [ ] No [ ]

If yes, when was the last time you took probiotic? _____

Name of probiotic _____

How long have you been taking probiotics? _____

3. Over past month, have you eaten any yogurt? Yes [ ] No [ ]

If yes, when was the last time you ate yogurt? _____

Name of yogurt _____

Does yogurt have probiotics in it? Yes [ ] No [ ]