

**NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY  
DISEASES**

DATE: January 24, 2018

CLINICAL PROTOCOL NO.: 15-DK-0170

IND NO.: 113137

IND SPONSOR: NIDDK

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

SHORT TITLE: **Lonafarnib and Ritonavir for Delta Hepatitis**

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

PRINCIPAL INVESTIGATOR: **\*Theo Heller, M.D. LDB, 10/9B16 402-7147**

LEAD ASSOCIATE INVESTIGATOR: **\*Christopher Koh, M.D., LDB: CRC, 5-2740, 443-9402**

ASSOCIATE INVESTIGATORS:

Barbara Rehermann, M.D., NIDDK/LDB: 10/9B16, 4961721

ESTIMATED DURATION OF STUDY: 5 YEARS

NUMBER AND TYPE OF PATIENTS: 21 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	21	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

\* Designates investigators who can obtain consent.



## ***PRECIS***

Chronic delta hepatitis is a serious form of chronic liver disease caused by infection with the hepatitis D virus (HDV), a small RNA virus that requires farnesylation of its major structural protein (HDV antigen) for replication. We propose to treat 21 adult patients with chronic delta hepatitis using the combination of the farnesyltransferase inhibitor (FTI) lonafarnib (LNF) and the protease inhibitor ritonavir (RTV). LNF has been shown to decrease serum quantitative HDV RNA in patients with chronic delta hepatitis infection, but dosing is limited by its side effects. RTV inhibits one of the cytochrome P-450 systems that metabolizes LNF leading to higher serum levels of LNF with minimal side effects. In this randomized, double-blinded, placebo-controlled study, there will be six groups of patients; Group 1 (4 patients) will receive LNF/RTV 50/100 mg daily for 24 weeks, Group 2 (4 patients) will receive LNF/RTV 75/100mg daily for 24 weeks, Group 3 (4 patients) will receive LNF/RTV 100/100mg daily for 24 weeks, Group 4, 5 and 6 (3 patients for each group) will initially receive placebo for 12 weeks followed by either LNF/RTV 50/100 mg daily (3 patients) or LNF/RTV 75/100mg daily (3 patients) or LNF/RTV 100/100 mg daily (3 patients) for 12 weeks. After dosing, all patients will be monitored for 24 weeks off therapy. Nucleos(t)ide analogue therapy will be instituted during this study to prevent the possibility of HBV reactivation/flare; Patients on pre-existing nucleos(t)ide analogues will be continued and patients not on pre-existing therapy will receive either entecavir or tenofovir for 48 weeks. Patients with quantifiable HDV RNA in serum and elevated aminotransferases will be enrolled. Before receiving therapy, patients will be evaluated for at least 3 visits with regular testing for HDV RNA quantitation and alanine aminotransferase (ALT) levels and will undergo Clinical Center admission for medical evaluation, timed blood draws and to start therapy. 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 undergo repeat physical examination, assessment of symptoms (using a symptom scale questionnaire), complete blood counts, routine liver tests, and hepatitis B and D viral markers. The primary therapeutic endpoint will be 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. Several secondary endpoints will be measured, including side effects, ALT levels, maintained virological response, undetectable HDV RNA in the serum, loss of HBsAg and symptoms. Therapy will be stopped for intolerance to lonafarnib and/or ritonavir (which will be carefully defined). This clinical trial is designed as a phase 2a study assessing the antiviral activity, safety and tolerance of three different doses of lonafarnib and ritonavir.

## **Background**

### ***A. Chronic Delta Hepatitis***

The hepatitis D virus (HDV) is an incomplete RNA virus, which is composed of a 1.7 kb single-stranded circular genomic RNA, virally encoded small and large delta antigens, and a surrounding lipid envelope.<sup>1</sup> HDV only infects persons who are also infected with hepatitis B virus (HBV), because its lipid envelope is embedded with HBV surface antigen (HBsAg).<sup>2-5</sup> 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.<sup>6</sup>

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.<sup>7</sup> 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<sup>2</sup> 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.<sup>8</sup> Hepatocellular carcinoma may develop as a complication of cirrhosis in some cases.<sup>9</sup> 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.<sup>10, 11</sup> 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.<sup>3, 4, 12-14</sup> 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.<sup>15</sup> 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.<sup>16</sup> 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.<sup>17</sup> Thus, given the unsatisfactory results of interferon-based therapies against chronic HDV infection, alternative therapies have been sought.<sup>18</sup>

More recently, in a phase 2a double-blinded, randomized, placebo-controlled clinical trial, the Liver Diseases Branch of the NIDDK has evaluated the safety and utility of the prenylation inhibitor, Lonafarnib, in patients with chronic HDV (12-DK-0046). Compared to placebo, patients that received lonafarnib 100 mg BID for 28 days experienced a significant mean HDV RNA decline of 0.73 log (p=0.04) and patients who received lonafarnib 200 mg BID experienced a significant mean HDV RNA decline of 1.54 logs (p=0.002)(unpublished data, manuscript in preparation).

### ***B. Prenylation Inhibitors***

Prenylation is a post-translational lipid modification that involves the covalent addition of either farnesyl or geranylgeranyl prenyl lipids derived from mevalonic acid to

conserved cysteine residues at or near the C-terminus of proteins.<sup>1</sup> These reactions are catalyzed by farnesyltransferase (FTase) or the geranylgeranyltransferases (GGTases).<sup>6, 19, 20</sup> 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).<sup>19</sup> The effect of prenylation is to promote membrane association of the modified protein. Prenylation also plays a major role in protein-protein interactions.<sup>1, 19</sup>

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.<sup>1</sup> 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.<sup>6, 20</sup>

### ***C. Prenylation Inhibitors in Chronic Delta Hepatitis***

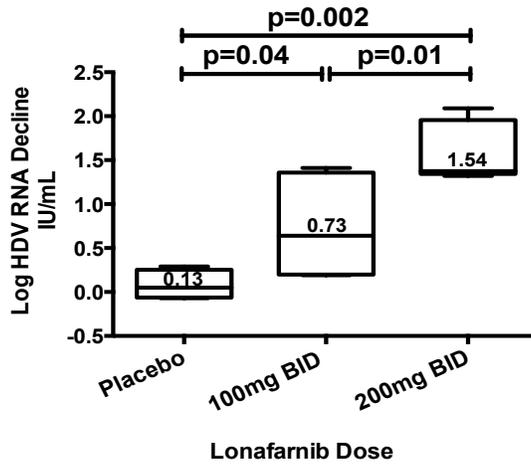
Prenylation plays a vital role in the viral life cycle of HDV, which allows for therapeutic opportunities for eradication. The HDV genome has a single open-reading frame that can encode two proteins: a small or a large delta antigen. The large delta antigen differs from the small one by having an additional 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<sup>6</sup>. In both cell-free translation reactions and in intact cells, it has been shown that the LHDAg is subject to prenylation.<sup>1, 20</sup> Genetic disruption of the CXXX box prevents prenylation of LDHAg and its ability to interact with, and form secreted particles with, HBsAg.<sup>19</sup> 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.<sup>1, 6, 19, 20</sup>

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.<sup>1</sup> 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.<sup>20</sup> 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.<sup>6</sup> 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.

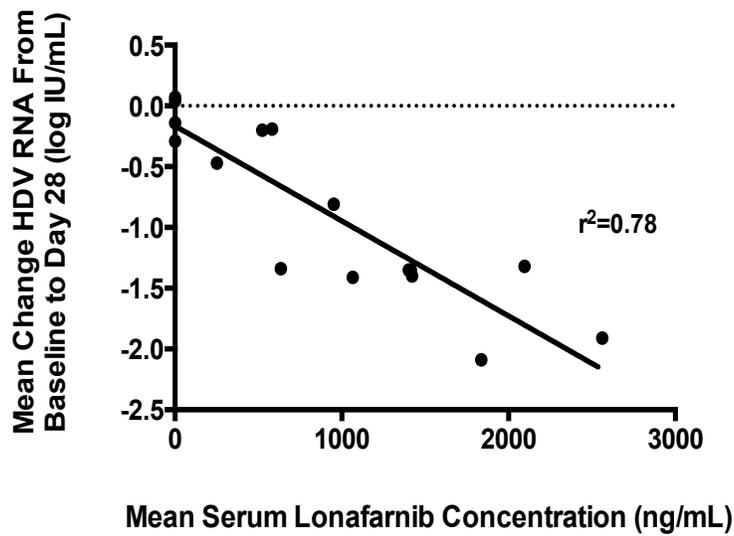
Recently, the Liver Diseases Branch of the NIDDK completed a phase 2A randomized, double-blinded placebo-controlled trial evaluating Lonafarnib for 28 days in the patients with chronic HDV (12-DK-0046). In this first-in-human study for patients with chronic HDV infection, compared to placebo, patients who received lonafarnib 100 mg orally twice daily experienced a 0.73 log IU/ml decline of HDV RNA in serum ( $p=0.04$ ) and patients who received lonafarnib 200 mg orally twice daily 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 ( $R^2=0.76$ ,  $p<0.0001$ ) (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 (unpublished data, manuscript in preparation). Because of weight loss, as well as other gastrointestinal side effects, in the patients who received the higher dose of lonafarnib, it is intended to use the lower dose of lonafarnib with ritonavir (to boost serum levels) and avoid the gastrointestinal side effects of the higher

dose.

**Fig.1 HDV RNA decline after 28 days of therapy**



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



#### **D. Concomitant use of Ritonavir (Booster effect)**

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.<sup>21</sup> 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.<sup>22</sup> 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.<sup>23-27</sup> 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.<sup>28-30</sup> 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.<sup>31</sup> In chronic HDV infection, there are no published studies to date evaluating ritonavir as a therapeutic booster. However, given that the metabolism of lonafarnib is mediated via CYP3A4 (the same enzyme that ritonavir inhibits), one would expected to see enhanced pharmacokinetic parameters at lower doses as compared to monotherapy with lonafarnib.<sup>32</sup> In a recently completed small proof-of-concept dose finding study in Ankara, Turkey (LOWR-HDV-1), the combination of lonafarnib 100 mg twice daily and ritonavir 100 mg daily has resulted in a mean HDV RNA decline of >2 log IU/mL (range, 2.37 to >5) with normalization of ALT after 5 weeks of therapy in 2 of 3 patients (unpublished results). The combination of lonafarnib and ritonavir was well tolerated and safe for 8 weeks and serum lonafarnib concentrations were greatly increased with ritonavir (approximately 3x) compared to lonafarnib monotherapy at the same dose.

In an ongoing follow-up study in Ankara, Turkey (LOWR-HDV-2) that is evaluating 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, preliminary results show a mean HDV RNA decline  $>1.5$  log IU/ml in 8 patients after 1 month of therapy. Notably, in this ongoing study, the dosing of LNF/RTV 100/100 mg once daily has shown a continuous HDV RNA decline with a mean decline of  $>3$ log HDV RNA IU/ml (unpublished results). This evidence suggests that ritonavir could be used as a pharmacokinetic booster of lonafarnib as it appears to optimize the pharmacokinetic profile of lonafarnib while allowing for a lower dose (with less side effects) and still maintaining the antiviral activity of lonafarnib.

#### ***E. 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.<sup>33,34</sup> Therapy is recommended only for patients with elevated serum aminotransferase activity chronic hepatitis on liver biopsy and either HBeAg or high levels of HBV DNA in serum. Pegylated interferon alfa-2a has also been approved for treatment of chronic HBV in the United States and has been suggested to have similar or slightly improved efficacy with more convenient administration than standard alpha interferon.<sup>34</sup> The recommended treatment duration with peginterferon alfa-2a in patients with chronic HBV is for 48 weeks.<sup>34</sup> The Liver Disease Branch of the NIDDK has studied the effects of long-term therapy of peginterferon alfa-2a in the treatment of patients with chronic delta hepatitis for a median of 140 weeks. The results showed only 39% of the patients responded to therapy with three seroconverting HBsAg.<sup>17</sup>

Other approved therapies for chronic hepatitis B include oral nucleoside/nucleotide analogues: lamivudine (Epivir-HBV, 3TC), adefovir disproxil (bis-POM PMEA, Hepsera), tenofovir disoproxil fumarate (Viread), entecavir

(Baraclude), and L-deoxythymidine (Telbivudine/LdT, Tyzeka). Currently, Entecavir or Tenofovir are considered first line therapies for chronic hepatitis B.<sup>34</sup> 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)<sup>35</sup> and by investigators in Europe<sup>36</sup> 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.<sup>37</sup> A possible use of lamivudine in chronic delta hepatitis, however, is the uncommon patient with high levels of HBV DNA. The pattern of high HBV DNA levels despite chronic delta hepatitis is found in approximately 20% of patients with this disease seen at the Clinical Center of the NIH. In these patients, inhibition of HBV may be important particularly because suppression of HDV RNA may be followed by rises in HBV DNA levels. In this current study, clinically approved nucleoside analogues for chronic HBV infection will be used.

#### **F. 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.<sup>38,39</sup> 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<sup>40</sup>.

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 lipopolysaccharide LPS-triggered TLR activation. Additionally, KC, are also potent activators of hepatic stellate cells (HSC) which play a vital role in hepatic fibrogenesis.<sup>41</sup> 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- $\beta$  signaling.<sup>42</sup> *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.<sup>43</sup> 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.<sup>44, 45</sup> 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.<sup>46</sup> 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<sup>47, 48</sup>.

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 and ritonavir. 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 and ritonavir will lead to a significant decline in hepatitis D virus levels.

**Assessment of Response To Therapy:**

**Primary Therapeutic End Point:**

Decline of HDV RNA quantitative measurements of >2 logs from baseline at 12 and 24 weeks of therapy with lonafarnib and ritonavir.

**Primary Safety End Point:**

The ability to tolerate lonafarnib and ritonavir at different doses for 12 and 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. Undetectable HDV RNA in serum by quantitative measurements at the end of therapy.
2. Sustained undetectable HDV RNA in serum at weeks 12 and 24 post treatment follow up.
3. Comparison of serologic HDV RNA decline between different doses of lonafarnib.
4. Comparison HDV RNA decline between 12 and 24 weeks of therapy at the differing doses.
5. Normalization of serum ALT (ALT <20 or AST <20 U/L in females and ALT <31 or AST <31 U/L in males) at the end of therapy, at week 12 of post-therapy follow-up and at week 24 of post-therapy follow-up.
6. Comparison of ALT changes between different doses of lonafarnib.
7. Loss of HBsAg from the serum at the end of therapy, at week 12 of post-therapy follow-up and at week 24 of post-therapy follow-up.
8. Changes in symptom scale measurements and quality of life before, during and after therapy.
9. Seroconversion of HBsAg at the end of therapy, at week 12 of post- therapy follow up and at week 24 of post-therapy follow-up.
10. Changes in the fecal microbiome before and after treatment.
11. Comparison of fecal microbiome analysis between different groups.

## **Protocol**

This is a randomized, double-blinded, placebo-controlled study treating 21 adult patients with chronic delta hepatitis with different doses of lonafarnib plus ritonavir for 12 weeks and 24 weeks. All patients will be monitored with quantitative HDV RNA and HBV DNA levels as well as routine safety measures and liver function tests.

### **A. Entry Criteria**

#### ***Inclusion Criteria:***

1. Age 18 years or above, male or female.
2. Serum alanine or aspartate aminotransferase activities above the upper limit of normal (ALT  $\geq$ 20 or AST  $\geq$ 20 U/L in females and ALT  $\geq$ 30 or AST  $\geq$ 30 U/L in males) on an average of three determinations taken during the previous 6 months at the NIH clinical center. The mean of the three determinations will be defined as “baseline” levels.
3. Presence of anti-HDV in serum.
4. Presence of quantifiable HDV RNA in serum.

#### ***Exclusion Criteria:***

1. Decompensated liver disease, defined by bilirubin  $>$ 4mg/dL, albumin  $<$ 3.0 gm/dL, prothrombin time  $>$ 2 sec prolonged, or history of bleeding esophageal varices, ascites or hepatic encephalopathy. Laboratory abnormalities that are not thought to be due to liver disease may not necessarily require exclusion. Patients with ALT levels greater than 1000 U/L ( $>$ 25 times ULN) will not be enrolled but may be followed until three determinations are below this level.
2. Pregnancy, active breast-feeding, or inability to practice adequate contraception, in women of childbearing potential or in spouses of such women. Adequate contraception is defined as vasectomy in men, tubal ligation in women, or use of two barrier methods such as condoms and spermicide combination, birth control pills, an intrauterine device, Depo-Provera, or Norplant.

3. Significant systemic or major illnesses other than liver disease, including, but not limited to, congestive heart failure, renal failure (eGFR <50 ml/min), organ transplantation, serious psychiatric disease or depression (only if felt to be at high risk by the NIH psychiatric consultation service), and active coronary artery disease.
4. Systemic immunosuppressive therapy within the previous 2 months.
5. Evidence of another form of liver disease in addition to viral hepatitis (for example autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, alcoholic liver disease, nonalcoholic steatohepatitis (but not steatosis), hemochromatosis, or alpha-1-antitrypsin deficiency).
6. Active substance abuse, such as alcohol, inhaled or injection drugs within the previous year.
7. Evidence of hepatocellular carcinoma.
8. Evidence of concurrent hepatitis C infection with positive serum HCV RNA.
9. Any experimental therapy or pegylated interferon therapy within 6 months prior to enrollment.
10. Diagnosis of malignancy in the five years prior to the enrollment with exception granted to superficial dermatologic malignancies.
11. Evidence of HIV co-infection; HIV 1/2 antibody positivity on serum testing.
12. Concurrent usage of statins as these drugs inhibits mevalonate synthesis, which reduces protein prenylation.
13. Concurrent usage of moderate and strong CYP3A inhibitors and inducers.
14. 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.
15. Clinically significant baseline EKG abnormalities.
16. Uncontrolled elevated triglycerides.
17. History of pancreatitis as a result of hypertriglyceridemia.
18. Inability to understand or sign informed consent.

19. Any other condition, which in the opinion of the investigators would impede the patient's participation or compliance in the study.

### **B. Initial Evaluation:**

Before admission to the NIH Clinical Center to start therapy, patients will undergo evaluations for eligibility in this protocol (Table 1). These evaluations will include at least 3 visits within 6 months of starting therapy with regular testing for HDV RNA and HBV DNA quantitation and alanine aminotransferase (ALT) levels. These eligibility evaluations (except reproductive hormone assessments and consultations) will be performed under the liver diseases branch omnibus protocol 91-DK-0214 (Evaluation of patients with liver disease). The reproductive hormone assessments and consultations will be performed under this protocol after informed consent has been obtained (Table 1). During the eligibility evaluations, patients will undergo a thorough explanation of this protocol, provided this protocol's consent for review, and provided multiple opportunities to ask questions about this study. This will be documented in the patient's chart. Testing performed for clinically related purposes or research related purposes are defined in Table 1 testing descriptions. If found to be eligible, the patient will be consented to this protocol and will be admitted to the NIH Clinical Center and undergo the following evaluation shortly before starting therapy:

- C. History and physical examination.
- D. 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.
- E. A standard symptom questionnaire [which focuses on fatigue and abdominal pain], which provides information on categorical presence of symptoms as well as their severity, pattern and frequency (Appendix 1).
- F. Blood tests. These include complete blood count (CBC with differential and platelet count), prothrombin time (PT), partial thromboplastin time (PTT), sedimentation rate (ESR), reticulocyte count, plasma haptoglobin level, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct and total serum bilirubin, albumin, total protein, lactate dehydrogenase (LDH), creatine

- phosphokinase (CK), sodium, chloride, bicarbonate, potassium, blood urea nitrogen, creatinine, glucose, magnesium, uric acid, calcium, phosphorus, cholesterol, triglycerides, iron and iron-binding capacity (transferrin), ferritin, immunoglobulin levels, thyroid stimulating hormone (TSH), antinuclear antibody (ANA), alpha fetoprotein (AFP), rheumatoid factor, cryoglobulins, hepatitis B surface antigen (HBsAg), antibody to HBsAg (Anti HBs), antibody to hepatitis B core antigen (anti HBc), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti HBe), hepatitis B virus DNA (HBV DNA), antibody to hepatitis D antigen (anti HDV), hepatitis D virus RNA (HDV RNA), anti-HCV, HCV RNA, and anti-HAV. HBV DNA, HCV RNA and HDV RNA will be tested by polymerase chain reaction (PCR). The research laboratory of Dr. Jeffrey S. Glenn at Stanford University will perform all HDV RNA testing for this protocol. This test is not commercially available. An additional 50 ml of whole blood will be collected for immunological studies and 7 ml of serum will be collected and stored at -20° C. Women of childbearing potential will have a pregnancy test.
- G. In males, AM levels of inhibin B, luteinizing hormone (LH), total and free testosterone, and follicle stimulating hormone (FSH) will be obtained. These can be obtained after informed consent has been obtained either during the pre-treatment visits or at the time of inpatient admission to start therapy.
- H. 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. These can be obtained after informed consent has been obtained either during the pre-treatment visits or at the time of inpatient admission to start therapy.
- I. A reproductive endocrinology or urological consultation for evaluation of current and future reproductive potential, the potential of reversibility in the event that reproductive toxicity occurs, and guidance on potential barrier methods will be obtained. Sperm samples will be obtained, wherever possible, based on the recommendations of the consult. These baseline examinations will be performed

- after informed consent has been obtained either during the pretreatment evaluation visits or during the inpatient admission to start therapy. Sperm sampling will be offered and if obtained, given that the normal spermatogenesis cycle is 90 days, a repeat sperm sample (if available) 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.
- J. Female subjects of reproductive potential and female partners of male subjects must use two reliable forms of contraception (e.g. oral contraceptive plus barrier method) from the start of the study until 6 months from the end of lonafarnib and ritonavir dosing.
  - K. Pharmacokinetic drug levels of lonafarnib and ritonavir will be obtained at time 0, 30 minutes, 1 hour, and 2 hours after the initial dose has been administered. A pre-dose drug level, prior to scheduled dosing, will also be obtained at 48 hours and at 72 hours. Pharmacokinetic drug levels of lonafarnib and ritonavir will also be obtained at each outpatient visit to correlate viral levels with drug levels.
  - L. An abdominal ultrasound will be performed.
  - M. An electrocardiogram (EKG) in digital format will be performed.
  - N. A detailed baseline ophthalmology exam including retinal photography will be performed either during the pretreatment evaluation visits or during the inpatient admission to start therapy.

### **C. Randomization**

All patients who are determined eligible and provide written informed consent will then be randomized by a set of random numbers held in the Pharmaceutical Development Service (PDS) into either one of six groups. Both the investigators and the patient will be blinded as to specific results of this randomization (groups 1, 2, 3, 4, 5 or 6) until the final patient has completed the 24-week treatment period. The randomization code will be blocked so that equal numbers of patients will be randomized to each group. Randomization will not be stratified due to the small sample size.

#### **D. Treatment**

The treatment design of this study entails 6 groups (1-6) of patients (Figure 3). A total of 21 patients will be enrolled and patients will be randomized into one of six groups. Group 1 will consist of 4 patients and will receive lonafarnib and ritonavir at a dose of 50/100 mg once daily orally for 24 weeks. Group 2 will consist of 4 patients and will receive lonafarnib and ritonavir at a dose of 75/100 mg once daily orally for 24 weeks. Group 3 will consist of 4 patients and will receive lonafarnib and ritonavir at a dose of 100/100mg once daily orally for 24 weeks. Group 4, 5 and 6 will consist of 3 patients in each group and will receive placebo for the initial 12 weeks followed by lonafarnib and ritonavir at either the 50/100 mg dose (total of 3 patients) or the 75/100 mg dose (total of 3 patients) or the 100/100 mg dose (total of 3 patients) for 12 weeks (Figure 3). In each group, after completing the first 24 weeks of the study, patients will be followed off anti-HDV therapy for 24 weeks as described in Table 1. Eiger BioPharmaceuticals, Inc will supply the lonafarnib and ritonavir.

After the eligibility evaluations completed under protocol 91-DK-0124 (as described above and in Table 1), eligible patients will be consented to this protocol and will be randomized to start therapy in one of six groups as described above and in figure 3. At the start of therapy, patients will be admitted to the Clinical Center, for duration of 72 hours, for induction of therapy, observation for side effects, administration of the medication, and timed blood draws to facilitate analysis of virological response kinetics. During the 72-hour admission, frequent blood sampling will be performed (0,6,12,18,24,36,48 and 72 hours) to assess initial viral kinetics in response to therapy, to have baseline immunological analysis, pharmacokinetic analysis and an electrocardiogram.

To prevent the possibility of a hepatitis B flare, all patients will be treated with either entecavir or tenofovir (which are first-line nucleos(t)ide analogues recommended by the AASLD and EASL) for a duration of 48 weeks (24 weeks of entecavir or tenofovir + lonafarnib/ritonavir for group 1-3, 12 weeks of entecavir or tenofovir+placebo followed by 12 weeks of entecavir or tenofovir + lonafarnib/ritonavir for 12 weeks for group 4-6 and 24 weeks of entecavir or tenofovir during the post-therapy monitoring phase for all groups). In patients who are not on nucleos(t)ide analogues prior to entering

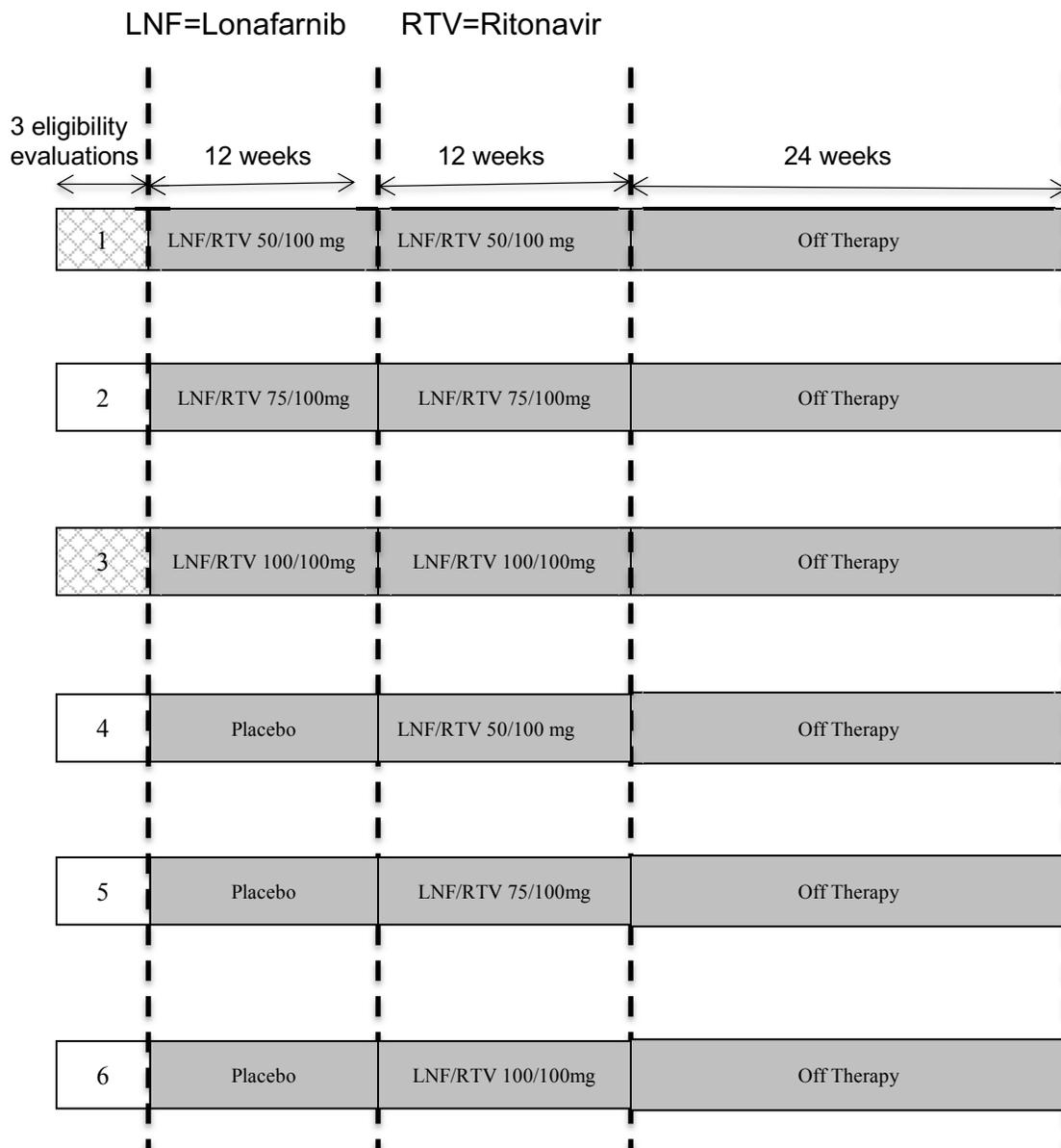
this study, they will be started on a nucleos(t)ide analogue at least 2 weeks before dosing of lonafarnib/ritonavir or placebo so that steady state has been reached prior to instituting HDV therapy (to reduce the risk of a hepatitis B flare). Patients will be informed about the possible adverse effects of these medications during their pretreatment clinic visits and via the package insert that is provided 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. If therapy for HBV has been started (a nucleos(t)ide analogue(s)) 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.<sup>34</sup>

After induction of therapy, as mentioned above, patients will be treated as outpatients and followed on a regular basis in the outpatient clinic at the NIH Clinical Center (See below and Table 1). Compliance with drug dosing will be monitored through either patient diaries or assessments noted in the medical record at outpatient visits. Therapy will be stopped for intolerance to lonafarnib and/or ritonavir (which is carefully defined in table 3). Patients who stop therapy due to intolerance, self-discontinue therapy or drop out from the study will be considered as treatment failures.

At 12 and 24 weeks of dosing (therapy or placebo+therapy), patients will undergo a thorough evaluation including an updated history and physical examination, multiple blood tests, urine tests, symptoms and quality of life questionnaires, EKG testing, virological testing, ophthalmologic exam with retinal photography and reproductive medicine consultation. After the final patient completes 24 weeks of dosing, all patients and physicians will be unblinded and patients will be told which group they participated in. Once the final patient has completed treatment and followed for at least 24 weeks off of therapy, the results of the overall study will be made to the participants.

Figure 3: Study Design

- Group 1: LNF/RTV 50/100mg once daily (4 patients).
- Group 2: LNF/RTV 75/100mg once daily (4 patients).
- Group 3: LNF/RTV 100/100mg once daily (4 patients).
- Group 4: Placebo for 12 weeks followed by LNF/RTV 50/100mg once daily for 12 weeks (3 patients).
- Group 5: Placebo for 12 weeks followed by LNF/RTV 75/100mg once daily (3 patients).
- Group 6: Placebo for 12 weeks followed by LNF/RTV 100/100mg once daily (3 patients).



### **E. Monitoring during therapy:**

After the 72-hour admission to start therapy, patients will be seen in the outpatient clinic, interviewed and have blood tests taken at regular intervals during and after therapy (Table 1). At each time point, an acceptable deviation would include +/-3 days.

Ophthalmologic examination with retinal photography and reproductive medicine consultation will be performed at week 12 and week 24 during therapy and end of the study. AM reproductive hormone evaluations (LH, FSH estradiol, progesterone, AMH, DHEA, DHEAS, Androstenedione, Testosterone and Free Testosterone, SHBG and 17-OHP for females and inhibin B, LH, FSH, Free and total testosterone levels for males) will be obtained monthly. At week 24, therapy will be stopped and patients will undergo an updated history and physical exam, viral kinetics, immunological analysis, pharmacokinetic analysis, and electrocardiogram. The timing of blood specimens, physical examinations and special evaluations is provided in Table 1. Routine blood tests will include complete blood count and differential, serum acute care panel, mineral panel, lipid panel, hepatic panel (ALT, AST, alkaline phosphatase, LDH, CPK, direct and total bilirubin, albumin, total protein, amylase, lipase, electrolytes, calcium, phosphate, glucose, blood urea nitrogen, magnesium, creatinine, and uric acid). Virologic assessments will include HDV RNA (a stored serum sample will be kept and viral RNA levels done in batches) HBsAg, anti-HBs, HBeAg, anti-HBe and HBV DNA levels. "Extended Visit I" blood tests will include prothrombin time, and a pregnancy test in females of reproductive age. "Extended visits II" blood tests will include ophthalmologic examination with retinal photography, reproductive medicine consultation, and AM reproductive hormone levels (LH, FSH, estradiol, progesterone, AMH, DHEA, DHEAS, Androstenedione, Testosterone, SHBG, 17-OHP for females and inhibin B, LH, FSH, free and total testosterone for males) will be collected as shown in Table 1.

Pharmacokinetic (PK) drug levels of lonfarnib and ritonavir (pre-dose) will be drawn on every visit while on therapy (weeks 1-24) and at weeks 1, 2, 4, 8, 12, 16, 20 and 24 after cessation of therapy as shown in Table 1. Blood will be drawn for immunological analysis while on therapy on as shown in Table 1. 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. Patients will also undergo electrocardiogram (EKG) evaluations collected in digital format on every visit while on therapy, due to the rare instances of asymptomatic bradycardia, PR prolongation<sup>49</sup> and QT/QTc interval prolongation described in oncologic trials with use of lonafarnib.<sup>49, 50</sup> In the rare event that diabetes mellitus should develop during participation in this study an endocrinology consultation will be obtained as needed. At specified outpatient visits during therapy (Table 1), patients will be asked about their symptoms, fill out a symptoms and quality of life questionnaire and will undergo assessment of vital signs and medication diaries.

HBV DNA will be tested at the NIH Clinical Center utilizing the COBAS Ampliprep/COBAS Taqman HBV v2.0 test, which is an FDA approved assay intended for the use as an aid in the management of chronic HBV infection. HDV RNA testing will be done by quantitative PCR. Samples will be collected at baseline and at each sampling time point to enable resistance monitoring and analysis and viral kinetics. The latter will include determination of baseline isolate sequence analysis to enable identification and monitoring of changes in viral genome sequences as a function of treatment duration. Analysis of 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) will prompt a more in-depth analysis that will focus first on the region encoding the CXXX box, as changes in the prenylation substrate sequence represent a logical potential mechanism of escape from prenylation-inhibiting drugs such as lonafarnib. Because other unanticipated mechanisms of resistance could theoretically be operative, however, we would anticipate also performing full genome sequencing to enable maximally unbiased assessment for candidate resistance mutations. We will also have the ability to engineer any identified candidate resistance mutation back into an HDV vector competent for in vitro replication, in order to confirm that the mutation is indeed causative of any suspected decreased sensitivity to lonafarnib. 30-50 ml of serum will also be obtained at the initial evaluation and end of the study for immunologic studies. The immunologic studies will be done in the Liver Diseases Branch of the NIH by Barbara Rehermann MD.

## **F. Post Treatment Monitoring**

After therapy at week 24, patients will be monitored on an outpatient basis and followed off-therapy for follow-up and blood testing at 1, 2, 4, 8, 12, 16, 20, and 24 weeks. At each time point, an acceptable deviation would include +/- 5 days. The timing of blood specimens, physical examinations and special evaluations is provided in Table 1. Routine blood tests will include complete blood count and differential, serum acute care panel, mineral panel, hepatic panel (ALT, AST, alkaline phosphatase, LDH, CPK, direct and total bilirubin, albumin, total protein, electrolytes, calcium, phosphate, glucose, blood urea nitrogen, magnesium, creatinine, and uric acid). Virologic assessments will include HDV RNA (a stored serum sample will be kept and viral RNA levels done in batches) HBsAg, anti-HBs, HBeAg, anti-HBe and HBV DNA levels. Thereafter, patients will be followed in the usual fashion by the Liver Diseases Branch under protocol 91-DK-0214. As interferon is the only approved therapy for HDV, if a patient has been previously treated with interferon, there is no additional therapy to be offered. It is not planned to routinely offer interferon to all patients after participating in this study. However, if a prolonged HDV flare with significant liver damage is demonstrated, rescue therapy with interferon will be offered. Although it is not clear if delaying interferon therapy for 9 months will have adverse events, patients will be made aware of the option of obtaining standard of care therapy prior to enrollment.

### **G. Monitoring of Subjects who Discontinue Therapy**

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 specific blind will be broken for the patient, and the patient will be followed for at least 24 weeks with visits to the outpatient clinic as specified in Table 1. Thereafter, patients will 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.

### **H. 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. Stool microbial analysis will be performed in the research laboratory of Dr. Jeffrey S. Glenn at Stanford University in addition to the primary investigator's research laboratory. 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. Those who have taken antibiotics, probiotics or have routine yogurt consumption for 30 days may be excluded from the microbiota analysis. 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 1: Design of Study**

Study Visit	HDV Therapy (RTV/LNF or Placebo)	HBV Therapy <sup>o</sup>	MD Visit	RN Visit	Extended Visit I	Extended Visit II	AM Reproductive Blood	Virologic & PK Assessment	Comments
<b>PreTx Visit 1<sup>^</sup></b>			*		*	* (To be completed once at one of these visits)		*	Extended visit II can be done any of pre visit or during first admission. Visit should be <6 months of starting therapy.
<b>PreTx Visit 2<sup>^</sup></b>			*				* (To be completed once at one of these visits after consent is signed)	*	Visit should be <6 months of starting therapy. Immunologic evaluation
<b>PreTx Visit 3<sup>^</sup></b>			*					*	Symptom & QOL Questionnaire, Consent Form, visit should be <6 months of starting therapy.
<b>Admit: Day 0-4</b>	*	*	*		*			*	Admission, EKG, Start Therapy, symptoms & QOL Questionnaire, immunologic evaluation
<b>Outpt Wks: 4, 8,12, 16,20*</b>	*	*	*		*		*	*	QOL Questionnaire, EKG, immunologic evaluation
<b>Outpt Wks: 1, 2, 3*</b>	*	*		*				*	EKG, symptoms & QOL Questionnaire
<b>Outpt Wk:</b>	*	*	*		*	*	*	*	QOL Questionnaire,

24*									EKG, immunologic evaluation
Actual visit may vary +/- 3 days.									

<b>Post Therapy Follow-up</b>									
<b>Study Visit</b>	<b>HDV Therapy (RTV/LNF or Placebo)</b>	<b>HBV Therapy</b>	<b>MD Visit</b>	<b>RN Visit</b>	<b>Extended Visit I</b>	<b>Extended Visit II</b>	<b>AM Reproductive blood work</b>	<b>Viologic &amp; PK Assessment</b>	<b>Comments</b>
Week 1*	None	*		*				*	Symptom & QOL Questionnaire
Week 2*	None	*		*				*	Symptom & QOL Questionnaire
Week 4*	None	*	*	*	*		*	*	Symptom & QOL Questionnaire, Immunologic evaluation
Week 8*	None	*	*	*	*		*	*	Symptom & QOL Questionnaire, immunologic evaluation
Week 12*	None	*	*	*	*		*	*	Symptom & QOL Questionnaire
Week 16*	None	*	*	*	*			*	Symptom & QOL Questionnaire, immunologic evaluation
Week 20*	None	*	*	*	*			*	Symptom & QOL Questionnaire
Week 24*	None	*	*	*	*	*	*	*	Symptom & QOL Questionnaire
Actual visit may vary +/-3 days on week 1 & 2 and +/-5 days between weeks 4 through 24.									

° Patients on preexisting nucleos(t)ide analogues will be maintained on therapy throughout the study. Those not on nucleos(t)ide analogues for HBV will be started two weeks before dosing of lonafarnib/ritonavir or placebo.

^ PreTx visits 1,2 & 3 will be performed under protocol 91-DK-0214. Reproductive endocrine testing will be performed under this protocol after informed consent has been obtained.

**Physician visit** includes review of diary, vital signs, review of symptoms, interim medical history, physical examination, symptom questionnaire, urinalysis, and routine blood tests for acute care panel (chem-7), mineral panel (albumin, magnesium), hepatic panel (ALT, AST, alkaline phosphatase, direct and total bilirubin), complete blood count with differential, and total protein. These tests are clinical blood tests utilized to monitor response to therapy and safety.

**Extended Visit I** includes prothrombin time and thyroid function tests. Women of childbearing age will have a pregnancy test. These tests are clinical blood tests utilized to monitor response to therapy and safety.

**Extended Visit II** includes ophthalmologic exam, retinal photography, reproductive medicine consultation. These examinations are clinical examinations utilized to monitor safety.

**AM reproductive blood work** AM hormone levels of (LH, FSH, estradiol, progesterone, AMH, DHEA, DHEAS, Androstenedione, Testosterone, Free testosterone, SHBG, 17\_OHP for females and inhibin B, LH, FSH, free and total testosterone for males). These tests are clinical blood tests utilized to monitor safety.

**Immunological evaluation** includes blood to be drawn (30-50 ml) for laboratory assessment. The blood obtained for testing in this evaluation is for research related testing.

**Virologic and PK Assessment** includes: HDV RNA and HBV DNA qualitative tests via polymerase chain reaction assay (PCR) or a quantitative PCR (qPCR) for viral titer. HBV virologic characterization including HBsAg, HBeAg and HBeAb will also be performed. This testing is for clinical purposes to monitor response to therapy and safety. One red top tube (10 mL) will be obtained and stored in -80 for pharmacokinetic (PK) analysis of lonafarnib and ritonavir. This blood obtained for PK testing is for research related testing.

**Symptom and Quality of Life questionnaire** will be administered on several occasions, before, during and after 12 weeks of treatment and in subsequent off treatment follow-up visits for 24 weeks. The data obtained from these questionnaires are for research related purposes.

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

**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 = 0	None	*
On Tx Week 4,8,12,16,20	Lonafarnib+Ritonavir	*
End of Tx Week 24	Lonafarnib+Ritonavir	*
Post Tx week 4, 8,12, 16, 20, 24	None	*

An acceptable deviation for collection of these samples is +/- 3 days. All microbiome related testing is intended for research purposes.

### I. 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 (IFN $\gamma$ , TNF $\alpha$ , 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, Rehmann, unpublished).

#### **J. Design of the Trial/Statistical considerations.**

This study is designed as a phase 2a FDA regulated clinical study evaluating the utility of combination therapy of lonafarnib and ritonavir in patients with chronic HDV infection. This study should show if HDV suppression is possible; assist in suggesting the lowest effective dose for viral suppression; and how well lonafarnib and ritonavir is 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 batch and the treating physician or the investigator will not necessarily know the results until a patient may have finished treatment.

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 21 patients will be enrolled (4 patient's per 24 week treatment group and 3 patient's per placebo + 12 week treatment group). This allows for pooling of week 12 data between all dosing groups while also allowing for statistical adequacy in assessing therapy out to 24 weeks with a statistical power of 80%, an alpha of .05 and accounting for a 20% dropout rate.

#### **K. 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 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 a recently completed phase 2a clinical study performed at the NIH clinical center.<sup>51-53</sup> 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 >1600 subjects.

In initial animal studies, lonafarnib was well tolerated and had no major end-organ toxicities at repeated doses of 15 mg/kg up to 6 months in rats and 10 mg/kg up to 1 year in monkeys; these doses result in systemic exposures that are less than those attained at a clinical dose of 200 mg twice per day (BID). At higher doses, representing animal to human exposure multiples similar to that seen in humans at 200 mg BID, the key side effects included bone marrow suppression and testicular toxicity in rats and monkeys, lymphoid and kidney changes in rats, and diarrhea and electroretinographic changes in monkeys. The no-effect doses for reproductive effects on fertility (10 mg/kg in rats) and embryo-fetal development (15 mg/kg in rats and <10 mg/kg in rabbits) result in systemic exposures less than those attained at a clinical dose of 200 mg BID. The no-effect dose for peri- and 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 extent CYP2C9. Neither of these systems is used in the metabolism of currently approved drugs to treat HBV. Multiple co-administered doses of the P450 inhibitor ketoconazole resulted in a 5-fold increase in exposure to lonafarnib.

In clinical studies, phase 1 dose-finding studies have shown that doses ranging from 25 mg to 200 mg twice daily (BID) are safe and well tolerated. Doses of 100, 200 and 300 mg BID have been employed in various phase II studies involving solid tumors and hematologic malignancies.<sup>50, 54, 55</sup> In the initial dose-finding phase 1 trial, minor hematologic and non-hematologic side effects were seen.<sup>50</sup> 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.<sup>50</sup> 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.<sup>50</sup>

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.<sup>50</sup> 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.<sup>50</sup>

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.<sup>55, 56</sup> In the first study, patients were treated for a median duration of 185 days<sup>55, 56</sup> Currently, there are various phase I, II and III investigational studies ongoing ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) with no significant safety alerts ([www.fda.gov](http://www.fda.gov)).

Although it is not specified if patients with pre-existing advanced liver disease were excluded in the above-mentioned clinical trials with lonafarnib, specific data on dosing in liver patients and side-effect profile in them are not available. In our recently completed, first-in-humans, proof-of-concept study evaluating lonafarnib in patients with chronic HDV infection, 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 ( $\geq 2$  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.

3. ***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 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 increased in cholesterol, triglycerides, and 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 monitor 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.<sup>49</sup> 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.<sup>49</sup>
- 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.<sup>49</sup>
- 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.

Metabolism of Ritonavir is mainly through cytochrome P450 CYP3A4.<sup>21</sup>

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.

4. ***The risks of the combination of Lonafarnib and Ritonavir.*** Aside from the small proof-of-concept Turkish study (mentioned above) evaluating three patients with this combination regimen for chronic HDV infection, this is the first study utilizing the combination of lonafarnib and ritonavir for 12 weeks therapy for chronic HDV infection. In the three patients that received this combination the regimen was reported to be safe and well tolerated. Thus, we do not anticipate additional significant side effects aside from each drug's individual side effect profile mentioned above.
5. ***The risks of the combination of Lonafarnib, Ritonavir and HBV therapy.*** The risks of the combination of lonafarnib, ritonavir and a nucleos(t)ide analogues are not known. In the small proof-of-concept Turkish study (mentioned above) a single patient on a nucleos(t)ide analogue was administered lonafarnib and ritonavir. This patient did not experience any additional side effects that could be attributed to the use of a nucleos(t)ide analogue in combination with lonafarnib and ritonavir. 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. 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.

#### **L. 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 or placebo will be based upon the scoring of adverse events as shown in table 3. After discontinuation, the patient's grouping will be unblinded so that the investigators are aware of the therapy and dose so that additional blinded patients can be closely monitored. 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 and ritonavir 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. If the female becomes pregnant, the patient's obstetrician will be provided with the standard of care guidelines to prevent HBV transmission. Lonafarnib and ritonavir will not be restarted unless another cause for the abnormality or symptom is found.

**Table 3. 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; IV fluids indicated <24 hrs	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated >24 hrs	Life threatening consequences	Death
Fatigue (asthenia, lethargy, malaise)	Fatigue	Mild fatigue over baseline	Moderate or causing difficulty performing some ADL	Severe fatigue interfering with ADL	Disabling	-----
Diarrhea	Diarrhea	Increase of <4 stools per day over baseline; mild increase	Increase of 4-6 stools per day over baseline; IV	Increase of >6 stools per day over baseline; incontinence; IV	Life threatening consequences (e.g.	Death

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

			interfering with function, but not interfering with ADL	severely interfering with ADL		
Infection- Upper Airway NOS	Infection – Upper Airway NOS	Mild	Moderate	Severe	Life – threatening; disabling	Death
Infection – Nasopharyngitis	Infection – Nasopharyngitis	Mild	Moderate	Severe	Life-threatening; disabling	Death
Hemoglobin	Hemoglobin	<LLN – 10.0 g/dL <LLN – 6.2 mmol/L <LLN – 100 g/L	< 10.0 – 8.0 g/dL <6.2 – 4.9 mmol/L <100 – 80 g/L	<8.0 – 6.5 g/dL <4.9 – 4.0 mmol/L <80 – 65 g/L	<6.5 g/dL <4.0 mmol/L <65 g/L	Death
Leukocytes (total WBC)	Leukocytes	<2000/mm <sup>3</sup> <2.0 X 10 <sup>9</sup> /L	<1500 – 1000/mm <sup>3</sup> <1.5 – 1.0 X 10 <sup>9</sup> /L	<1000 – 500/mm <sup>3</sup> 1.0 – 0.5 X 10 <sup>9</sup> /L	<500/mm <sup>3</sup> <0.5 X 10 <sup>9</sup> /L	Death
Platelets	Platelets	<70,000/mm <sup>3</sup> <60,000/mm <sup>3</sup> <70.0 – 60.0 X 10 <sup>9</sup> /L	<60,000 – 40,000/mm <sup>3</sup> <60.0 – 40.0 X 10 <sup>9</sup> /L	<40,000 – 25,000/mm <sup>3</sup> <40.0 – 25.0 X 10 <sup>9</sup> /L	<25,000/mm <sup>3</sup> <25.0 X 10 <sup>9</sup> /L	Death
INR (International Normalized Ratio of prothrombin time)	INR	>1 – 1.5 X ULN	>1.5 – 2 X ULN	>2 X ULN	-----	-----
PTT Partial Thromboplastin time)	PTT	>1 – 1.5 X ULN	>1.5 – 2 X ULN	>2 X ULN	-----	-----
Bicarbonate, serum-low	Bicarbonate, serum-low	<LLN – 18 mmol/L	<18 – 15 mmol/L	<15-11 mmol/L	<11 mmol/L	Death
Acidosis (metabolic or respiratory)	Acidosis	pH < normal, but >7.3	-----	pH <7.3	pH <7.3 with life threatening consequences	Death
Alkaline Phosphatase (U/L)	Alkaline Phosphatase	>ULN – 2.5 X ULN	>2.5 0 5.0 X ULN	>5.0 – 20.0 X ULN	>20.0 X ULN	-----
Bilirubin (hyperbilirubinemia)	Bilirubin	>ULN – 1.5 X ULN	>1.5 – 3.0 X ULN	>3.0 – 10.0 X ULN	>10.0 X ULN	-----
Albumin, serum-low (hypoalbuminemia)	Hypoalbuminemia	<LLN – 3 g/dL <LLN – 30 g/L	<3 – 2 g/dL <30 – 20 g/L	<2 g/dL <20 g/L	-----	Death
AST, SGOT (serum glutamic oxaloacetic transaminase)	AST	>3 – 5 X patient’s baseline values	>5-10 X patient’s baseline values	>10 – 20 X patient’s baseline values	>20 X patient’s baseline values	Death

ALT, SGPT (serum glutamic pyruvic transaminase)	ALT	>3-5 X patient's baseline values	>5-10 X patient's baseline values	>10 – 20 X patient's baseline values	>20 X patient's baseline values	Death
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

### **M. 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. The rationale for not using an outside data and safety monitoring committee is that this is a small, single center study using medications with defined side effects and known safety concerns. All measurements and tests are well established in clinical medicine. Yearly reports are made to the NIDDK/NIAMS IRB regarding safety and efficacy.

### **N. Reporting of Adverse Events, Unanticipated Problems and Deviations**

Adverse events, protocol deviations, unanticipated problems (UP), , serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 (“Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations.”). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, and serious protocol deviations, will be reported to the IRB, the Sponsor and CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB, the Sponsor and CD as soon as possible but not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director and IRB within 7 days after the PI first learns of the event.

The PI will immediately report Suspected Adverse Reactions (SARs) to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor.

Not serious protocol deviations will only be reported to the IRB and the Sponsor (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious

protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

Adverse events that are clearly not related to the study procedures, such as those that occur prior to initiation of study related procedures, adverse events that are expected and thought to be related to the natural history of HDV or comorbidities will not be reported to the IRB or the Sponsor. If it is determined from the review of the aggregate data that an adverse event is occurring at a greater frequency or level of severity than previously expected, it constitutes a UP, and the problem will be reported to the IRB, the Sponsor and CD expeditiously. All other study related adverse events, all unanticipated problems and all protocol deviations will be reported in aggregate to the IRB at the time of continuing review. ... A summary of all suspected adverse reactions and study efficacy data will be provided yearly in annual reports to the Sponsor. Reports will be sent to the FDA, MEDWATCH program (telephone 1-800-FDA-1088; or via the internet at [www.fda.gov/medwatch/index.html](http://www.fda.gov/medwatch/index.html)) and the Eiger Pharmaceuticals, the company that produces the prenylation inhibitor.

#### **O. Regulatory Requirements**

The IND associated with this study (113137), in which the NIDDK is the sponsor, is associated with lonafarnib. 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 by independent monitors and auditors to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIH 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.

#### **P. Recruitment strategy**

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

#### **Q. 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.

#### **R. Consent Process**

The investigational nature and research objectives of this trial will be carefully explained to the subjects during the preliminary visit evaluations and subjects will be offered multiple opportunities to ask protocol specific questions. These discussions will be documented in the patient's chart at each visit. Additionally, a copy of the consent will be provided to the patient for review during the pretreatment visits prior to obtaining a signed informed consent before dosing. Once a subject is determined to be eligible to participate in this study, they will be invited to complete the evaluations aforementioned in this protocol. Consent will be obtained by the NIDDK principal investigator or one of the NIDDK associate investigators identified on the cover page.

We do not plan or anticipate the enrollment of non-English speaking subjects; however they are not excluded from participation either. Should a non-English speaking subject be eligible for enrollment, IRB approval will be obtained for use of the short form consent process in the absence of a fully translated consent document as outlined in SOP 12.9.1, under the provisions of 45 CFR 46.117(b)(2). IRB approval will be obtained

according to IRB guidance prior to obtaining informed consent from the potential study participant/s.

**S. 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. Dr. Jeffrey S. Glenn at Stanford University School of Medicine will perform all HDV RNA quantitative testing for this protocol. All samples sent to outside collaborators and Dr. Glenn 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

and Ritonavir), 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.

#### **T. Risk/Benefit Assessment**

The research risk associated with this study is greater than minimal risk with potential prospect of direct benefit to the subject. We expect to acquire generalizable knowledge about lonfarnib and ritonavir as a therapy of chronic delta hepatitis infection.

#### **U. 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).

#### **Q. Remuneration/Compensation**

No compensation is offered to study participants.

## References

1. Glenn JS, Marsters JC, Jr., Greenberg HB. Use of a prenylation inhibitor as a novel antiviral agent. *J Virol* 1998;72:9303-6.
2. Rizzetto M, Ponzetto A, Forzani I. Hepatitis delta virus as a global health problem. *Vaccine* 1990;8 Suppl:S10-4; discussion S21-3.
3. Rosina F, Rizzetto M. Treatment of chronic type D (delta) hepatitis with alpha interferon. *Semin Liver Dis* 1989;9:264-6.
4. Rosina F, Pintus C, Meschievitz C, et al. A randomized controlled trial of a 12-month course of recombinant human interferon-alpha in chronic delta (type D) hepatitis: a multicenter Italian study. *Hepatology* 1991;13:1052-6.
5. Borghesio E, Rosina F, Smedile A, et al. Serum immunoglobulin M antibody to hepatitis D as a surrogate marker of hepatitis D in interferon-treated patients and in patients who underwent liver transplantation. *Hepatology* 1998;27:873-6.
6. Bordier BB, Ohkanda J, Liu P, et al. In vivo antiviral efficacy of prenylation inhibitors against hepatitis delta virus. *J Clin Invest* 2003;112:407-14.
7. Niro GA, Smedile A, Ippolito AM, et al. Outcome of chronic delta hepatitis in Italy: a long-term cohort study. *J Hepatol* 2010;53:834-40.
8. Caredda F, Rossi E, d'Arminio Monforte A, et al. Hepatitis B virus-associated coinfection and superinfection with delta agent: indistinguishable disease with different outcome. *J Infect Dis* 1985;151:925-8.
9. Verme G, Brunetto MR, Oliveri F, et al. Role of hepatitis delta virus infection in hepatocellular carcinoma. *Dig Dis Sci* 1991;36:1134-6.
10. Yurdaydin C, Bozkaya H, Onder FO, et al. Treatment of chronic delta hepatitis with lamivudine vs lamivudine + interferon vs interferon. *J Viral Hepat* 2008;15:314-21.
11. Kabacam G, Onder FO, Yakut M, et al. Entecavir treatment of chronic hepatitis D. *Clin Infect Dis* 2012;55:645-50.
12. Farci P, Mandas A, Coiana A, et al. Treatment of chronic hepatitis D with interferon alfa-2a. *N Engl J Med* 1994;330:88-94.
13. Battegay M, Simpson LH, Hoofnagle JH, et al. Elimination of hepatitis delta virus infection after loss of hepatitis B surface antigen in patients with chronic delta hepatitis. *J Med Virol* 1994;44:389-92.
14. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009;50:661-2.
15. Wedemeyer H, Yurdaydin C, Dalekos GN, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;364:322-31.
16. Wedemeyer H, Yurdaydin C, Ernst S, et al. 96 weeks of pegylated-Interferon-alpha-2 alpha plus tenofovir or placebo for the treatment of hepatitis delta: the HIDIT-2 study. *Hepatology* 2013;58:222A-223A.
17. Heller T, Rotman Y, Koh C, et al. Long-term therapy of chronic delta hepatitis with peginterferon alfa. *Aliment Pharmacol Ther* 2014;40:93-104.
18. Bogomolov P, Voronkova N, Allweiss L, et al. A proof-of-concept Phase 2a clinical trial with HBV/HDV entry inhibitor Myrcludex B. *Hepatology* 2014;60:1267A-1290A.

19. Einav S, Glenn JS. Prenylation inhibitors: a novel class of antiviral agents. *J Antimicrob Chemother* 2003;52:883-6.
20. Bordier BB, Marion PL, Ohashi K, et al. A prenylation inhibitor prevents production of infectious hepatitis delta virus particles. *J Virol* 2002;76:10465-72.
21. Tomaru A, Takeda-Morishita M, Banba H, et al. Analysis of the pharmacokinetic boosting effects of ritonavir on oral bioavailability of drugs in mice. *Drug Metab Pharmacokinet* 2013;28:144-52.
22. Zeldin RK, Petruschke RA. Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients. *J Antimicrob Chemother* 2004;53:4-9.
23. Lawitz E, Sullivan G, Rodriguez-Torres M, et al. Exploratory trial of ombitasvir and ABT-450/r with or without ribavirin for HCV genotype 1, 2, and 3 infection. *J Infect* 2014.
24. Kwo PY, Mantry PS, Coakley E, et al. An Interferon-free Antiviral Regimen for HCV after Liver Transplantation. *N Engl J Med* 2014.
25. Eron JJ, Lalezari J, Slim J, et al. Safety and efficacy of ombitasvir - 450/r and dasabuvir and ribavirin in HCV/HIV-1 co-infected patients receiving atazanavir or raltegravir ART regimens. *J Int AIDS Soc* 2014;17:19500.
26. Casado JL, Banon S, Moreno A, et al. Lamivudine plus darunavir boosted with ritonavir as simplification dual regimen in HIV-infected patients. *J Int AIDS Soc* 2014;17:19801.
27. Clotet B, Clumeck N, Katlama C, et al. Safety of etravirine in HIV-1/hepatitis B and/or C virus co-infected patients: pooled 96 week results from the Phase III DUET trials. *J Antimicrob Chemother* 2010;65:2450-4.
28. Gane EJ, Rouzier R, Stedman C, et al. Antiviral activity, safety, and pharmacokinetics of danoprevir/ritonavir plus PEG-IFN alpha-2a/RBV in hepatitis C patients. *J Hepatol* 2011;55:972-9.
29. Reddy MB, Chen Y, Haznedar JO, et al. Impact of low-dose ritonavir on danoprevir pharmacokinetics: results of computer-based simulations and a clinical drug-drug interaction study. *Clin Pharmacokinet* 2012;51:457-65.
30. Everson GT, Cooper C, Hezode C, et al. High SVR24 rates with ritonavir-boosted danoprevir plus Peg-IFN alpha-2a ( 40KD)/RBV in HCV genotype 1 or 4 patients in the DAUPHINE study. *Hepatology* 2012;56:552A-552A.
31. Goelzer P, Morcos PN, Tran J, et al. Coadministration of ritonavir with the HCV protease inhibitor danoprevir substantially reduces reactive metabolite formation both in vitro and in vivo. *Hepatology* 2012;56:580A-580A.
32. Ghosal A, Chowdhury SK, Tong W, et al. Identification of human liver cytochrome P450 enzymes responsible for the metabolism of lonafarnib (Sarasar). *Drug Metab Dispos* 2006;34:628-35.
33. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. *Gastroenterology* 2001;120:1828-53.
34. European Association For The Study Of The L. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012;57:167-85.
35. Lau DT, Khokhar MF, Doo E, et al. Long-term therapy of chronic hepatitis B with lamivudine. *Hepatology* 2000;32:828-34.

36. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-9.
37. Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. *Hepatology* 2000;31:241-6.
38. Wei X, Yan X, Zou D, et al. Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach. *BMC Gastroenterol* 2013;13:175.
39. Xu M, Wang B, Fu Y, et al. Changes of fecal Bifidobacterium species in adult patients with hepatitis B virus-induced chronic liver disease. *Microb Ecol* 2012;63:304-13.
40. Bajaj JS, Heuman DM, Hylemon PB, et al. The Cirrhosis Dysbiosis Ratio defines Changes in the Gut Microbiome Associated with Cirrhosis and its Complications. *J Hepatol* 2013.
41. Duffield JS, Forbes SJ, Constandinou CM, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest* 2005;115:56-65.
42. Seki E, De Minicis S, Osterreicher CH, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007;13:1324-32.
43. Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012;482:179-85.
44. Balagopal A, Philp FH, Astemborski J, et al. Human immunodeficiency virus-related microbial translocation and progression of hepatitis C. *Gastroenterology* 2008;135:226-33.
45. Parlesak A, Schafer C, Schutz T, et al. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000;32:742-7.
46. Sandler NG, Koh C, Roque A, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. *Gastroenterology* 2011;141:1220-30, 1230 e1-3.
47. Everson GT, Shiffman ML, Morgan TR, et al. The spectrum of hepatic functional impairment in compensated chronic hepatitis C: results from the Hepatitis C Antiviral Long-term Treatment against Cirrhosis Trial. *Aliment Pharmacol Ther* 2008;27:798-809.
48. Tonan T, Fujimoto K, Qayyum A, et al. Correlation of Kupffer cell function and hepatocyte function in chronic viral hepatitis evaluated with superparamagnetic iron oxide-enhanced magnetic resonance imaging and scintigraphy using technetium-99m-labelled galactosyl human serum albumin. *Exp Ther Med* 2011;2:607-613.
49. Ichikawa S, Motosugi U, Ichikawa T, et al. Magnetic resonance elastography for staging liver fibrosis in chronic hepatitis C. *Magn Reson Med Sci* 2012;11:291-7.
50. Eskens FA, Awada A, Cutler DL, et al. Phase I and pharmacokinetic study of the oral farnesyl transferase inhibitor SCH 66336 given twice daily to patients with advanced solid tumors. *J Clin Oncol* 2001;19:1167-75.
51. Harousseau JL. Farnesyltransferase inhibitors in hematologic malignancies. *Blood Rev* 2007;21:173-82.

52. Canini L, Koh C, Cotler SJ, et al. Understanding hepatitis virus dynamics and antiviral efficacy of the prenylation inhibitor lonafarnib. *Hepatology* 2014;60:32A-91A.
53. Koh C, Yurdaydin C, Cooper S, et al. Prenylation inhibition with lonafarnib decreases hepatitis D levels in humans. *Hepatology* 2014;60:92A-196A.
54. Awada A, Eskens FA, Piccart M, et al. Phase I and pharmacological study of the oral farnesyltransferase inhibitor SCH 66336 given once daily to patients with advanced solid tumours. *Eur J Cancer* 2002;38:2272-8.
55. Feldman EJ, Cortes J, DeAngelo DJ, et al. On the use of lonafarnib in myelodysplastic syndrome and chronic myelomonocytic leukemia. *Leukemia* 2008;22:1707-11.
56. Ravoet C, Mineur P, Robin V, et al. Farnesyl transferase inhibitor (lonafarnib) in patients with myelodysplastic syndrome or secondary acute myeloid leukaemia: a phase II study. *Ann Hematol* 2008;87:881-5.

***Lonafarnib + Ritonavir for Chronic Hepatitis Delta***

***Patient Name*** \_\_\_\_\_

***Enrollment Criteria***

<b><i>No</i></b>	<b><i>Criteria</i></b>	<b><i>Yes/No</i></b>	<b><i>Comment</i></b>
<b><i>1</i></b>	<b><i>Is patient 18 years or above?</i></b>		<b><i>Age = _____ years</i></b>
<b><i>2</i></b>	<b><i>Is mean ALT level <math>\geq 30</math> U/L in males or <math>\geq 20</math> U/L in females?  Or mean AST level <math>\geq 30</math> U/L in males or <math>\geq 20</math> U/L in females??</i></b>		<b><i>Mean ALT = (_____ + _____ + _____)=_____</i></b> <b><i>Mean AST = (_____ + _____ + _____)=_____</i></b>
<b><i>3</i></b>	<b><i>Are anti-HDV and HDV RNA positive?</i></b>		<b><i>Date of sample: ___/___/___</i></b>
<b><i>4</i></b>	<b><i>Is consent form signed?</i></b>		<b><i>Date of signature ___/___/___</i></b>
	<b><i>The answers to these questions should all be yes for the patient to be enrolled in this study.</i></b>		

***The answers to the above questions are all yes.***

\_\_\_\_\_  
***Signature and Date***

## **PRENYLATION INHIBITORS FOR CHRONIC HEPATITIS D**

*Patient Name* \_\_\_\_\_

### *Exclusion Criteria*

<i>No</i>	<i>Criteria</i>	<i>Yes/No</i>	<i>Comment</i>
<b>1</b>	<b><i>Is ALT &gt; 1000U/L?</i></b>		
<b>2</b>	<b><i>Is serum bilirubin &gt; 4 mg%?</i></b>		<b><i>Bilirubin =</i></b>
<b>3</b>	<b><i>Is serum albumin &lt; 3 gm%?</i></b>		<b><i>Albumin =</i></b>
<b>4</b>	<b><i>Is protime &gt; 2 seconds prolonged or INR &gt; 1.7?</i></b>		<b><i>Protime = INR</i></b>
<b>5</b>	<b><i>Is there a history of bleeding varices, ascites or encephalopathy?</i></b>		
<b>6</b>	<b><i>For females with childbearing potential: Is the patient pregnant, actively breast-feeding, or unable to practice birth control?</i></b>		<b><i>Not applicable if patient has had a tubal ligation ____</i></b>
<b>7</b>	<b><i>For males: Is the patient able to practice birth control?</i></b>		<b><i>Not applicable if patient has had a vasectomy _____</i></b>
<b>8</b>	<b><i>Are there significant other medical illnesses such as CHF, renal failure, transplant, psychiatric dx, and angina?</i></b>		
<b>9</b>	<b><i>Has the patient been on systemic immunosuppressive therapy within the previous 2 months?</i></b>		
<b>10</b>	<b><i>Does the patient have another form of liver disease in addition to coinfection of HBV and HDV?</i></b>		
<b>11</b>	<b><i>Has the patient undergone any experimental therapy or pegylated interferon within 6 months prior to starting the study?</i></b>		
<b>12</b>	<b><i>Has the patient had a diagnosis of malignancy within 5 years prior to enrollment with the exception of superficial dermatologic malignancies?</i></b>		
<b>13</b>	<b><i>Has patient had active substance abuse in the last one year?</i></b>		<b><i>Alcohol, inhaled or injection drugs</i></b>
<b>14</b>	<b><i>Does the patient have evidence of HIV co-infection?</i></b>		
<b>15</b>	<b><i>Is AFP &gt; 200 ng/ml and does the ultrasound/MRI/CT show evidence of a mass in the liver?</i></b>		<b><i>AFP = _____ US/MRI/CT date __/__/__</i></b>

<b>16</b>	<b><i>Is the patient currently on statin medications?</i></b>		
<b>17</b>	<b><i>Is the patient currently on medications that would alter metabolism of ritonavir?</i></b>		<b><i>Alpha-1-adrenoreceptor antagonist Antiarrhythmic medications Pimozide Sildenafil Sedative and hynotics Ergot St. John's Wort</i></b>

***The answer to all of the above questions is no:***

---

**Signature and Date**

Appendix 1

**SYMPTOM SCALE: Delta Hepatitis**

**Name:** \_\_\_\_\_ **Date:(M/D/Yr)** \_\_\_/\_\_\_/\_\_\_

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

<b>None</b>	<b>Worst ever</b>
<b>Fatigue</b>	
_____	
<b>Nausea</b>	
_____	
<b>Diarrhea</b>	
_____	
<b>Confusion</b>	
_____	
<b>Loss of Appetite</b>	
_____	
<b>Indigestion</b>	
_____	

*In general, how do you feel overall?*

<b>Very Good</b>	<b>Awful</b>
_____	

*Are you having side effects with Prenylation Inhibitor therapy?*

Yes \_\_\_ No \_\_\_

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

	<b>Not at all</b>	<b>A little</b>	<b>A lot</b>	<b>Extremely</b>
<b>Responsibilities at home</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Ability to work</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Enjoyment of life</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

## 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 [ ]