**CLINICAL RESEARCH PROTOCOL**

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

DATE:November 2, 2016 CLINICAL PROTOCOL NO.: 07-DK-0207

IND NO: 78,112
IND Name: Tenofovir, Emtricitabine
IND HOLDER: Marc G. Ghany

TITLE: **Tenofovir Disoproxil Fumarate Alone Versus its Combination with Emtricitabine for Treatment of Chronic Hepatitis B**

SHORT TITLE: **Tenofovir and Emtricitabine for Hepatitis B**

IDENTIFYING WORDS: Tenofovir disoproxil fumarate, Emtricitabine, FTC, Nucleoside/Nucleotide analogue, Chronic Hepatitis B, Hepatitis B mutants, Liver Biopsy.

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ESTIMATED DURATION OF STUDY: 12 Years

ANTICIPATED STUDY END DATE: 2019
NUMBER AND TYPE OF PATIENTS: 100 patients with chronic hepatitis B, ages above 18 years, both male and female

| SUBJECTS OF STUDY: | Number | Sex | Age Range |
|---|---|---|---|
| Patients | 100 | Male & Female | Above 18 years |
| Volunteers | None | | |

PROJECT USES IONIZING RADIATION: Yes, for medical indications only.
PROJECT USES "DURABLE POWER OF ATTORNEY": No
OFF-SITE PROJECT: No MULTI-INSTITUTIONAL PROJECT: No

## Précis

Chronic hepatitis B is a major cause of cirrhosis, end-stage liver disease and hepatocellular carcinoma and affects approximately 1.25 million Americans. Six medications have been licensed for use in chronic hepatitis B in the United States, but their relative benefit and long-term efficacy remain unclear. In previous studies, we have shown that maintained suppression of HBV DNA can be achieved with nucleoside analogues and that suppression is associated with marked improvements in disease. In this randomized study, we propose to evaluate long-term therapy with tenofovir alone or in combination with emtricitabine (FTC). Forty treatment-naive patients with chronic hepatitis B will be enrolled in the primary study. After medical evaluation and liver biopsy, patients will be stratified by hepatitis B e antigen (HBeAg) status and randomized to receive either tenofovir alone or in combination with FTC. Treatment will be continued long-term (at least four years) and patients will be carefully monitored for side effects, serum aminotransferase and HBV DNA levels. Patients will undergo repeat liver biopsy and assessment of antiviral resistance at 1 and 4 years. The primary endpoint of therapy will be the maintained suppression of HBV DNA to below $10^2$ IU/ml (lower limit of detection of current assays). The study will assess the relative efficacy and safety of combination versus mono-therapy. A separate group of 60 previously treated patients will also be enrolled and randomized to mono- or combination-therapy to assess the safety profile of these agents. The primary analysis will be conducted on the entire study cohort.

[Word count = 250]

## Background

### Chronic Hepatitis B and its Therapy:

Chronic hepatitis B is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma affecting close to 400 million persons worldwide. In the United States an estimated 1.25 million persons are chronically infected with the hepatitis B virus (HBV).[1,2]

Currently there are six therapies approved by the Food and Drug Administration (FDA) for chronic hepatitis B including: interferon alfa-2b, peginterferon alfa-2a, lamivudine, adefovir dipivoxil, entecavir and telbivudine.[3] All licensed therapies have been shown to be superior to placebo and both entecavir and telbivudine have been shown to be superior to lamivudine using several parameters to assess outcome, including: histological improvement (defined as improvement in necroinflammatory score by two or more points with no worsening of fibrosis), suppression of HBV DNA to undetectable levels by sensitive, polymerase chain reaction (PCR)-based assays, and normalization of serum alanine aminotransferase levels.[4-12] Additionally, in HBeAg-positive patients, higher rates of HBeAg loss and HBeAg seroconversion were observed in active treatment arms. Long-term follow-up studies have shown that a defined course of therapy with interferon and long-term treatment with lamivudine are associated with lower rates of cirrhosis, hepatocellular carcinoma and death.[13-15]

Despite these successes, current therapy remains less than optimal due to low rates of HBsAg loss, high relapse rates, the need for long-term therapy and subsequent development of antiviral resistance. The optimal regimen has not been defined: which agent/s to use, for how long and in which patients.[16-22] Therapy with any of the six agents for one year leads to sustained improvements in only a small proportion of patients. Among HBeAg-positive patients, between 17% and 33% of patients become HBeAg negative on therapy and therefore are candidates for withdrawal of therapy. Of these, relapse (reappearance of HBeAg in serum) may occur in up to 50% of patients necessitating reinstitution of therapy.[3,23] In patients who do not become HBeAg negative and in persons with HBeAg-negative chronic hepatitis B, the majority (~90%) relapse when therapy is stopped.

Long-term antiviral therapy of hepatitis B is possible with nucleoside analogues (lamivudine, adefovir, entecavir and telbivudine), but is impractical with interferon or peginterferon because of their frequent side effects. Several small studies have shown that extending therapy using the oral nucleoside analogues is associated with improved rates of HBeAg loss, maintained suppression of HBV DNA and improvements in both biochemical and histological features of disease.[24-28] However, these beneficial effects are often negated by a parallel increase in rates of viral resistance.[29] The rate of viral resistance varies among the various agents. With lamivudine monotherapy, rates of viral resistance increase from 14-24% at year 1 to approximately 70% by years 4 to 5. Similarly, recent data on long-term use of adefovir reports rates of resistance that increase from 2% at 2 years to 29% at 5 years. Resistance has been reported in <1% with entecavir

use in nucleoside analogue-naïve patients at three years but reaches 15% at three years in those with a history of lamivudine resistance.[30,31]

Continuation of therapy after the development of resistance is associated with loss of clinical response in the majority of patients.[25] Furthermore, development of resistance makes subsequent therapy difficult.[29,30] While both adefovir and entecavir have activity against lamivudine resistance HBV mutants, this activity is relatively reduced and the presence of lamivudine resistance appears to increase the rate of resistance to adefovir and entecavir. Treatment options for managing antiviral resistance are limited and the preferred strategies include adding a second agent or switching therapy. Decompensation and/or death have been associated with stopping therapy, especially in those with advanced liver disease and these patients need to be monitored closely.[32] A small minority of patients may continue to have clinical improvement compared to baseline values but the disease continues to worsen in the majority of patients.[25]

Post-hoc analyses of several treatment trials suggest that sustained suppression of HBV DNA to low levels (at least $<10^4$ and preferably to $< 10^2$) is associated with improved biochemical and histological outcomes and should be the primary surrogate goal of antiviral therapy. Maintained, long-term suppression of HBV DNA correlates well with observed clinical outcomes. Suppression of HBV DNA by oral nucleoside analogues (lamivudine) has also been shown to restore the HBV-specific CD4 and CD8 T cell responsiveness, which is typically weak in chronic hepatitis B.[33-35] Restoration of HBV-specific T cell responses was transient,[35,36] however, and recurring decreased T cell responsiveness during prolonged therapy was associated with increased prevalence of lamivudine-resistant HBV mutants and increased HBV titers.[35] Thus, treatment strategies are needed to improve long-term, sustained anti-viral efficacy as well as prevention of antiviral resistance. An attractive option borrowing from the HIV paradigm is to treat patients with drug combinations, using agents with different mechanisms or sites of action. This approach might be beneficial in both preventing resistance as well as heightening antiviral activity (through synergy or additive antiviral activity). However, results of trials of combination therapy in chronic hepatitis B have been disappointing.

**Combination Therapy of Chronic Hepatitis B**

There have been several prospective studies comparing the combination of interferon (or peginterferon) and nucleoside analogues (usually lamivudine) to either agent alone.[8,9,37] These studies have demonstrated that there is improved viral suppression (partial additive activity) and that lamivudine resistance is less common when it is combined with interferon, however sustained response rates have not been higher with combination therapy compared to interferon (or peginterferon) monotherapy. The most recent, large scale study evaluated one-year courses of therapy in HBeAg-positive patients and long-term response rates after peginterferon monotherapy as well as after peginterferon and lamivudine combination therapy and combined were in the range of 30 to 35%.[8] Thus, combination of interferon and nucleoside analogues appears to result in additive antiviral activity and some degree of delay in development of antiviral resistance, but when given in one year courses, combination therapy appears no more effective than monotherapy

with interferon alone. Long-term combination therapy has not been pursued because of the systemic side effects of interferon.

In contrast to interferon, the oral nucleoside analogues are very well tolerated and have few if any side effects in most patients, making long-term administration of these agents practical. There have, however, been few prospective trials comparing monotherapy with nucleoside analogues to combination therapy and none that have analyzed long-term results. Most combination studies have been conducted in patients with lamivudine resistance or conducted for short periods (4 to 12 months).[38-40] However, these studies have shown that combination of two nucleosides does not appear to increase antiviral activity; that the degree of viral suppression matches that of the most potent agent. Thus, when adefovir was used to treat patients with lamivudine resistance, the degree of HBV DNA suppression and loss of HBeAg was similar in patients switched to adefovir (monotherapy) to those in whom adefovir was added to lamivudine (combination therapy).[38] Furthermore, when patients were treated with telbivudine (LdT, a potent lamivudine-like nucleoside analogue) alone, lamivudine alone or the combination of telbivudine and lamivudine, the degree of HBV DNA suppression was the same with telbivudine alone as with the combination.[39,40]

The Liver Diseases Branch of NIDDK has been conducting prospective studies of long-term therapy of chronic hepatitis B with nucleoside analogues since 1995. The first study (95-DK-199) enrolled 42 patients with chronic hepatitis B (22 with HBeAg-positive, 20 with HBeAg-negative infection).[25] At one year, a majority of patients showed evidence of clinical improvement –in HBV DNA levels, ALT elevations and liver histology. However, the rate of antiviral resistance was high, reaching 25% by one year and ultimately 75% in HBeAg positive and 50% in HBeAg-negative patients. Patients enrolled in this study have continued to be followed and those still receiving lamivudine monotherapy have been enrolled in a long-term study (05-DK-0195). As of September 2006, only five of the initial 22 HBeAg-positive patients continue to have a maintained virological response (HBV DNA levels $< 10^4$ IU/ml) to lamivudine monotherapy. The remaining 17 HBeAg-positive patients required therapy with other agents (peginterferon or adefovir) because of failure of lamivudine and evidence of progressive disease. Importantly, the 5 patients with a maintained virological response all had marked improvement in liver histology, normalization of ALT elevations, and loss of HBeAg. In addition, 3 of the 5 have become HBsAg-negative and have been able to stop lamivudine therapy without evidence of relapse in follow up (for 0.5, 6 and 7 years). Among the 20 HBeAg-negative patients in that initial study, 16 continued on lamivudine monotherapy and 15 have a maintained response (HBV DNA $<10^4$), normal ALT levels and improved histology. In this cohort, 4 have become HBsAg-negative and have been able to stop lamivudine therapy without relapse. The 5 HBeAg-negative patients who failed to have a maintained response on lamivudine monotherapy included one who was lost to follow up (returned to Iran), one who underwent liver transplantation (and is alive and without HBsAg on long-term lamivudine provided by her transplant physicians), one who died of end-stage liver disease (with lamivudine resistance and intolerance to adefovir due to renal disease), one who is on lamivudine but has viral levels above $10^4$ (with normal serum aminotransferase levels) and one who is currently on adefovir (and has a

maintained response with no detectable HBV DNA and normal ALT levels). These results, while based on few patients, demonstrate that the rate of resistance to lamivudine monotherapy is unacceptably high (particularly in HBeAg-positive patients) but that maintained suppression of HBV DNA $<10^4$ IU/ml is associated with marked improvements in serum biochemical abnormalities and liver biopsy. Furthermore, long-term suppression of HBV DNA to $<10^2$ IU/ml can be followed by loss of HBsAg in a moderate proportion of patients. Loss of HBsAg is associated with resolution of the chronic hepatitis and permits discontinuation of therapy.

These findings led to a second study that started in 2001 and was a prospective, randomized trial of adefovir monotherapy versus the combination of adefovir and lamivudine (01-DK-246). This study did not include a lamivudine monotherapy arm because of the historical information available on lamivudine monotherapy and the unacceptable rate of antiviral resistance from that and other studies.[25,29] At the time that this study was initiated, adefovir had been shown to be effective against hepatitis B and no instances of adefovir resistance were reported. In the interim, it is clear that adefovir resistance does occur, but generally only after 1 to 2 years of therapy, reaching 2% at 2 years, 18% at 4 years and 29% at 5 years.[27,41] Thus, studies of adefovir for one-year only are inadequate to assess the rate or prevention of resistance and the overall rate increases gradually thereafter, although 10 year results have yet to be reported.

Results of our study of adefovir have yet to be published, but 39 patients have been enrolled including 26 HBeAg-positive patients who have been treated for at least one year. Twenty subjects were randomized to the combination of lamivudine and adefovir and nineteen to adefovir monotherapy. Thirty-one subjects have completed one year of therapy, 26 HBeAg-positive patients including 27 males, mean age 45 years, 10 of whom were previously treated and had lamivudine resistance. Four patients had cirrhosis at baseline. Undetectable HBV DNA by PCR was observed in 75% of patients receiving combination therapy versus 47% receiving monotherapy. Sixty-nine percent and 86% of patients receiving combination therapy had normalization of ALT and a histological response compared to 47% and 67% respectively receiving monotherapy. Primary non-response defined as $<3$ $\log_{10}$ HBV DNA reduction at 24 weeks was observed in 6 of 15 (40%) patients randomized to adefovir but in no patient randomized to combination therapy. Among the treatment naïve cohort who extended therapy, long-term suppression of HBV DNA to $<10^4$ IU/ml could be achieved in 100% of patients receiving the combination of adefovir and lamivudine (14 of 14 patients) but in only 44% (5 of 12) of patients receiving adefovir alone. Furthermore, among previously untreated patients with HBeAg-positive disease, combination therapy was associated with loss of HBeAg and no detectable HBV DNA ($<10^2$ IU/ml) in 9 of 10 patients at the time of their last clinic visit. Failure of adefovir therapy occurred early, during the first year of treatment, and was not associated with classical adefovir resistance, but rather with apparent suboptimal antiviral activity, levels of HBV DNA never falling below $10^6$ IU/ml. After a full year of treatment, patients who failed to respond to adefovir monotherapy were switched to combination therapy. Striking, all four patients who were switched to combination therapy and have had a full year of treatment have failed to have an adequate response, in that all four still have HBV DNA levels above $10^4$ IU/ml and elevations in serum ALT

levels. This latter result was unexpected and disturbing, as it appears to show that combination therapy is more effective than monotherapy only if started initially. Thus, a clinically attractive approach to therapy of treating all patients with monotherapy and switching those who fail treatment to combination therapy may not be as effective as starting with combination therapy. While based upon small numbers, the results of this trial have led us to plan to initiate a new study using tenofovir (an agent similar to adefovir but more potent) comparing tenofovir monotherapy to the combination of tenofovir and emtricitabine (FTC: an agent similar to lamivudine) in chronic hepatitis B.

**Why tenofovir? And why FTC rather than lamivudine?**

Tenofovir disoproxil fumarate is an acyclic nucleotide analogue that is structurally related to adefovir dipivoxil and shows overlap in mechanism of HBV DNA inhibition and in antiviral resistance patterns with adefovir.[29] *In vitro* tenofovir has a similar potency as adefovir, but in small human studies appears to be ~30 more potent than adefovir perhaps due to the higher dose administered.[42-44] The side effect profile and safety of tenofovir (given in doses of 300 mg once daily) is similar to that of adefovir (given in doses of 10 mg daily). Tenofovir is FDA-approved for therapy of HIV infection and is widely used in situations of HIV infection with multiple resistance patterns. Recently, a series of small studies has shown that tenofovir has potent activity against hepatitis B and has been used to rescue patients with end-stage liver disease in both lamivudine- and adefovir-resistant HBV and after liver transplantation.[45]

Adefovir and tenofovir were directly compared in a retrospective case series in 53 patients with lamivudine resistance and HBV DNA levels above $10^6$ IU/ml. The study was not randomized, but 35 patients received tenofovir and 18 received adefovir, therapy lasting for more than one year.[42] HBV DNA levels fell more rapidly in tenofovir- than adefovir-treated patients and after 28 weeks, all patients who received tenofovir had HBV DNA levels < $10^5$ IU/ml compared to only 44% of adefovir-treated patients. No patient developed tenofovir resistance, and only rare case reports of tenofovir resistance have been reported despite extensive use of this agent, particularly in HIV-HBV co-infected individuals. Side effects were also uncommon. Tenofovir shares a pattern of renal toxicity with adefovir, a side effect that is observed largely in patients with pre-existing renal disease or in those who are treated with higher doses of these agents. Additionally, the majority of HIV-HBV co-infected individuals who developed renal toxicity were also receiving ritonovir, the combination of lopinavir or ddI which may increase proximal renal tubular concentrations of tenofovir and increase the risk of renal toxicity.

Tenofovir has also been used to treat patients who fail to respond to adefovir. In a recent study, 20 patients with chronic hepatitis B (19 HBeAg-positive) and lamivudine resistance who failed to respond to a 4 to 28 month course of adefovir monotherapy were switched to tenofovir. All patients had a subsequent decrease in HBV DNA and 19 became HBV DNA negative by PCR (<$10^3$).[43]

We have experience with treating 7 patients with tenofovir who failed to respond adequately to lamivudine or the combination of lamivudine and adefovir. All seven

patients became HBV DNA negative (<$10^2$ IU/ml) with decrease in serum ALT levels to normal within 24 weeks. No toxicity or side effects were noted. Thus, tenofovir is a promising oral nucleotide analogue with potent activity against hepatitis B and a low rate of toxicity and resistance. The sponsor of tenofovir (Gilead Sciences) is currently conducting a multicenter randomized controlled trial in chronic hepatitis B with the intention of obtaining FDA approval for this indication.

Five year continuous therapy with tenofovir with viral suppression has been associated with histological improvement including reversal of fibrosis and cirrhosis.[46] Long-term histological data was reported on 348 patients with paired biopsy results from baseline and week 240 of therapy. This cohort represents about half of the cohort who were enrolled in the phase 3 trial. 304 (87%) of the 348 had histological improvement, and 176 (51%) had regression of fibrosis at week 240 ($p<0{\cdot}0001$).[46] Of the 96 (28%) patients with cirrhosis (Ishak score 5 or 6) at baseline, 71 (74%) no longer had cirrhosis (≥1 unit decrease in score), whereas three of 252 patients without cirrhosis at baseline progressed to cirrhosis at year 5 ($p<0{\cdot}0001$).[46]

Emtricitabine (FTC) is a cytosine analogue with potent in vitro and in vivo activity against hepatitis B.[47] FTC is similar to lamivudine (3TC) and has a similar profile of activity and viral resistance. Thus, FTC has little activity against lamivudine resistant strains of HBV and FTC-resistance is associated with similar mutations in the HBV polymerase gene as occur with lamivudine therapy (rtM204I/V and rtL180M). FTC was evaluated in a randomized controlled trial comparing a 48-week course of FTC (200 mg daily) to placebo in cohorts of HBeAg-positive and HBeAg-negative patients.[48] In this study, the average suppression of HBV DNA (4.5 $\log_{10}$ IU/ml) and rate of clearance of HBeAg (14%) was similar to that reported for lamivudine. Side effects were few and the major complication was withdrawal of FTC therapy after a year, which was associated with transient flares in disease activity in 23% of patients and one patient suffered a severe flare of disease and required liver transplantation. Similar instances of life-threatening flares of hepatitis after withdrawal of lamivudine have been reported.[8,37] At present, FTC is approved for use in HIV infection but has yet to be approved for the indication of chronic hepatitis B. Thus, FTC appears to be a lamivudine-like agent with a similar pattern of antiviral activity, resistance and side effects.

The combination of tenofovir and FTC has been evaluated in HIV infection and is approved for use under the trade name "Truvada".[49,50] Indeed, this combination is widely used as a convenient single, once-a-day treatment for HIV infection. The activity of Truvada against hepatitis B is well established and this combination is an attractive approach to the treatment of patients with HIV-HBV co-infection. The combination of tenofovir and FTC has yet to be formally assessed in patients with chronic hepatitis B without HIV infection. The drug sponsor (Gilead Sciences) has planned a limited study in patients with chronic hepatitis B and high levels of HBV DNA, but this trial has yet to be initiated.

In this protocol, we plan to evaluate the relative efficacy and safety of tenofovir monotherapy versus the combination of tenofovir and emtricitabine (Truvada) as long-

term maintenance therapy for patients with chronic hepatitis B (both HBeAg-positive and -negative). The results of this trial will be compared to historical controls from a NIH trial evaluating the combination of lamivudine and adefovir versus adefovir monotherapy and published multicenter randomized control trials of lamivudine and adefovir for HBeAg positive chronic hepatitis B. The primary endpoint of this trial will be a maintained suppression of HBV DNA below $10^2$ IU/ml and secondary endpoints will be normalization of serum ALT levels and improvement in histological features of chronic hepatitis. A separate group of patients will be treated with either tenofovir alone or tenofovir and emtricitabine as a "salvage" protocol for patients who have received nucleoside analogue therapy and have failed to have complete viral suppression despite at least 48 weeks of therapy or who have relapsed following withdrawal of therapy. This "salvage" arm will allow for further evaluation of safety and viral suppression and will be used in the primary analysis of response to tenofovir monotherapy vs. combination therapy of tenofovir and emtricitabine.

**Hypotheses and Issues:** This protocol will address the following questions.

Treatment naïve patients:

1. Will combination therapy using tenofovir and emtricitabine have superior antiviral effects against HBV compared to tenofovir alone? To address this issue, HBV DNA and ALT levels will be monitored frequently and both the absolute decrease and the slope (kinetics) of decrease will be compared. The investigators believe that the patterns of decrease of HBV DNA and ALT levels will be similar between tenofovir and combination arms, the pattern reflecting the more potent activity of tenofovir and the absence of a synergy or additive effect of these two nucleos(t)ide analogues.
2. Will combination therapy prevent or delay the appearance of antiviral resistance to tenofovir? To address this issue, the HBV polymerase gene will be sequenced from all serum samples at yearly intervals that are HBV DNA positive. Rates of development of mutations shown to be associated with tenofovir resistance (rtA194T)[20] will be compared between the combination and monotherapy arms. The investigators believe that the rate of antiviral resistance will be low and that at 4 years, 20-25% of patients in the monotherapy arm but no patients in the combination arm will exhibit genotypic antiviral resistance.
3. Will combination therapy yield a higher rate of beneficial response than monotherapy? To address this issue, several definitions for response will be made and compared between the two arms. The primary response definition will be absence of detectable HBV DNA by PCR-based methods ($<10^2$ IU/ml) at 48 weeks and 192 weeks. Secondary response endpoints will be HBV DNA levels < $10^4$ IU/ml at 48 and 192 weeks as well as both HBV DNA level definitions at 96, 144 and 240 weeks and <u>at the time of last assessment</u> (= maintained response). Other secondary response endpoints will be clearance of HBeAg (among HBeAg-

positive patients), loss of HBsAg, normalization of ALT, and improvement in liver biopsy histology scores using two definitions: (1) decrease of 3 points in histological activity index (HAI) compared to pre-treatment biopsy results with no worsening of fibrosis and (2) decrease of 3 points in HAI compared to pre-treatment biopsy results and to a total HAI score of 3 or less with no worsening of fibrosis. The investigators believe that long-term tenofovir and emtricitabine will provide HBV DNA suppression response rates of greater than 90%. Non-responders will likely be patients who are not compliant. Thus, this study will be a designed to show superiority of combination therapy to monotherapy, and the investigators believe that this study will set the standard of care for chronic hepatitis B.

4. Will long term maintenance of HBV DNA suppression result in loss of HBsAg and the ability to safely stop therapy? To address this issue, patients will be tested for HBsAg at six monthly intervals. Patients who become HBsAg negative will be withdrawn from therapy (beginning 6 months after the loss of HBsAg) and monitored carefully for evidence of return of HBV DNA activity or ALT elevations. Relapse will lead to reinstitution of therapy. The investigators believe that loss of HBsAg will occur in 5% to 10% of patients yearly, so that one-third of patients will be HBsAg negative by 5 years and two-thirds by 10 years. This study will thus indicate whether therapy of chronic hepatitis B should be based upon maintained suppression of HBV DNA to undetectable levels until loss of HBsAg, which may occur after years of therapy. This study may help define the factors that predict loss of HBsAg, or rather the duration of therapy necessary to achieve loss of HBsAg.

Patients with previous lamivudine ± adefovir resistance:

1) Will combination therapy yield a higher response rate and prevent the development of further resistance than monotherapy in patients with previous antiviral resistance (lamivudine, adefovir and entecavir)? Response will be defined as HBV DNA $<10^2$ IU/ml and normal ALT at 48 and 192 weeks. Samples of blood at yearly intervals that are HBV DNA positive will be tested for tenofovir (rtA194T) and emtricitabine (rtM204V/I and rtL180M) resistant mutations by direct sequencing. The investigators believe that the maintained virological and biochemical response will be higher in the combination arm compared to the monotherapy arm and the rate of further resistance will be lower at 48 and 192 weeks.

Patients who relapsed after withdrawal of lamivudine, adefovir or the combination of lamivudine plus adefovir:

1) We hypothesize that combination therapy will result in a greater proportion of subjects re-responding to re-institution of therapy. Response will be defined as an HBV DNA $<10^2$ IU/ml and normal ALT at 48 and 192 weeks. For individuals who experienced HBeAg sero-reversion (HBeAg negative to positive) a response will also include loss of HBeAg or HBeAg seroconversion again. We further hypothesize that

combination therapy will prevent or delay the appearance of antiviral resistance compared to tenofovir monotherapy in persons who relapsed? To address this issue, the HBV polymerase gene will be sequenced from all serum samples at yearly intervals that are HBV DNA positive.

**Protocol**

Up to 100 patients with chronic hepatitis B will be enrolled including at least 40 patients who have not been previously treated (naïve patients: primary study) and up to 40 patients who have received or are receiving nucleoside analogue therapy and up to 20 patients who have relapsed following withdrawal of therapy (experienced patients: salvage study). After medical evaluation and liver biopsy, patients will be randomized and receive either monotherapy with tenofovir (300 mg) daily or combination therapy with tenofovir (300 mg) and emtricitabine (FTC: 200 mg) daily. Gilead Sciences will provide the combination of tenofovir and emtricitabine as a single tablet and tenofovir 300 mg as a single tablet. Neither the investigators nor the patients will be blinded to the investigational agents. Patients will be followed carefully on therapy at 2 to12 week intervals and undergo repeat medical evaluation and liver biopsy at 48 and 192 weeks. Therapy will be continued indefinitely, although the randomized phase of this trial will last for 192 weeks only. The definition of a successful endpoint of therapy will be the maintained suppression of HBV DNA below $10^2$ IU/ml by current PCR-based assays, Roche Amplicor assay). Secondary endpoints will be (2) normalization of ALT levels and (3) histological improvements which are expected to occur in all patients with full suppression of HBV DNA and in a proportion of those with partial suppression. Other endpoints will be (3) loss of HBeAg and (4) loss of HBsAg. Patients who have lost HBsAg will undergo a monitored withdrawal of therapy with reinstitution of treatment if relapse occurs.

**A1. Inclusion criteria: Primary Study (nucleoside analogue-naïve subjects)**

1) Age >18 years and older, male or female
2) Known serum HBsAg positivity for 24 weeks
3) Detectable **HBV DNA $>10^4$** IU/ml. For patients with cirrhosis **HBV DNA $>10^3$ IU/ml**
4) Serum ALT or AST levels 1.5 X ULN (for ALT: ≥62 U/L and for AST: ≥ 46 U/L) based on at least two determinations taken at least one month apart during the 24 weeks before study entry; no ALT requirement for patients with cirrhosis
5) Liver biopsy within 2 years of entry that is consistent with chronic hepatitis and with a histology activity index (HAI) score of 4 or more (scores range from 0-18) and an Ishak fibrosis score of at least 1 (scores range from 0-6). For patients who have had a liver biopsy at another institution, slides must be obtained for reading and scoring at the NIH.
6) Written informed consent

**A2. Inclusion criteria: Salvage Study (nucleoside analogue experienced subjects)**

1) Age >18 years and older, male or female
2) Known serum HBsAg positivity for 6 months

3) Detectable **HBV DNA $>10^2$** IU/ml.
4) Liver biopsy within 5 years of entry that is consistent with chronic hepatitis
5) Written informed consent

A.3 **Inclusion criteria: Salvage Study (relapsers)**

1) Age >18 years and older, male or female
2) Known serum HBsAg positivity for 6 months
3) Detectable **HBV DNA $>10^3$** IU/ml.
4) Liver biopsy within 5 years of entry that is consistent with chronic hepatitis
5) Written informed consent
6) Serum ALT or AST levels 1.5 X ULN (for ALT: $\geq$62 U/L and for AST: $\geq$ 46 U/L) based on at least two determinations taken at least 2 weeks apart

**B. Exclusion criteria**

1) Previous or current treatment with tenofovir or emtricitabine.
2) Co-infection with HDV as defined by the presence of anti-HDV in serum and/or HDV antigen in the liver.
3) Co-infection with HCV as defined by the presence of HCV RNA in serum.
4) Co-infection with HIV as defined by the presence of anti-HIV in serum.
5) Decompensated liver disease as defined by serum bilirubin >2.5 mg/dL (with direct bilirubin > 0.5 mg/dL), prothrombin time of greater than 2 seconds prolonged, a serum albumin of less than 3 g/dL, or a history of ascites, variceal bleeding or hepatic encephalopathy.
6) Presence of other causes of liver disease, (i.e. hemochromatosis, Wilson disease, alcoholic liver disease, nonalcoholic steatohepatitis, alpha-1-anti-trypsin deficiency).
7) A history of organ transplantation or in the absence of organ transplantation, any immunosuppressive therapy requiring the use of more than 5 mg of prednisone (or its equivalent) daily.
8) Significant systemic illness other than liver diseases including congestive heart failure, renal failure, chronic pancreatitis, diabetes mellitus with poor control that in the opinion of the investigator may interfere with therapy.
9) Pregnancy or inability to practice contraception in patients capable of bearing or fathering children and lactating women.
10) Hepatocellular carcinoma (HCC), or the presence of a mass on imaging studies of the liver that is suggest of HCC, or an alpha-fetoprotein level of greater than 500 ng/mL.
11) History of clinically apparent pancreatitis or evidence of subclinical pancreatitis as shown by serum amylase values twice the upper limits of the normal range and abnormalities of the pancreas on CT or other imaging studies of the abdomen.
12) Sensory or motor neuropathy apparent from medical history and physical examination.
13) Creatinine clearance < 50 ml/min, serum creatinine > 1.3 mg/dl or urine protein >1 gram/24-hours; creatinine clearance will be determined on the average of two 24 hour urine specimens. Accuracy of collection will be ensured by documenting

appropriate total creatinine excretion in the 24-hour urine specimen (15 mg/kg) and correcting for the patient's age, gender and body surface area.

14) Concurrent use of nephrotoxic agents (e.g., aminoglycosides, amphotericin B, vancomycin, foscarnet, cis-platinum, pentamidine, nonsteroidal anti-inflammatory agents) or competitors of renal tubular excretion (e.g., probenecid) within 2 months prior to study screening or the expectation that the subject will receive these during the course of the study.
15) History of hypersensitivity to nucleoside analogues.
16) Active ethanol/drug abuse/psychiatric problems such as major depression, schizophrenia, bipolar illness, obsessive-compulsive disorder, severe anxiety, personality disorder that, in the investigator's opinion, might interfere with participation in the study.
17) History of renal tubular acidosis.
18) History of malignancy or treatment for a malignancy within the past 5 years.
19) Presence of conditions that, in the opinion of the investigators, would not allow the patient to be followed in the current study for at least 5 years.

**C. Initial evaluation**

Patients will be seen in the outpatient clinic of the NIH Clinical Center and undergo the following evaluation before starting therapy. Patients requiring a liver biopsy will be admitted to the Clinical Center for a percutaneous liver biopsy under ultrasound guidance.

1. History and physical examination.
2. Blood tests – complete blood count (CBC with differential and platelet count), prothrombin time, partial thromboplastin time, sedimentation rate, Chem-20 (which includes alanine aminotransferase [ALT], aspartate aminotransferase [AST], direct and total serum bilirubin, albumin, total protein, lactic dehydrogenase [LDH] creatine phosphokinase [CK], sodium, chloride, bicarbonate, potassium, blood urea nitrogen, creatinine, uric acid, calcium, and phosphorus), alpha-fetoprotein, amylase, lipase, immunoglobulin G, A and M, thyroid stimulating hormone (TSH), antinuclear antibody (ANA), rheumatoid factor, lipid panel (triglycerides, total cholesterol, high density lipoprotein associated cholesterol, low density lipoprotein associated cholesterol), hepatitis B viral markers (HBsAg, anti-HBs, HBeAg, and anti-HBe), anti-HCV, anti-HDV, and anti-HIV. An additional 10 ml. of serum will be collected and stored at –20 degrees Celsius for future research testing.
3. Baseline HBV DNA level by quantitative PCR (Amplicor™: Roche, lower limit of detection 20 IU/ml) and HBV genotyping (HBV Genotyping Line Probe Assay, Innogenetics, Belgium).
4. Routine urinalysis, random total protein/creatinine ratio, and two 24-hour urines for creatinine clearance and protein excretion.
5. Pregnancy test for women of childbearing potential.
6. Patients who have not had them within the previous year will undergo chest X-ray, electrocardiogram (EKG), and abdominal ultrasound.

7. Patients will undergo percutaneous liver biopsy unless they have had one within the past two years that is available for analysis or have specific contraindications to liver biopsy. Patients with known or suspected portal hypertension (previous liver biopsy showing Ishak stage 5 or 6 or platelet count less than 100,000) will undergo a transjugular liver biopsy with measurement of the hepatic venous pressure gradient (HVPG). The liver biopsy will be scored by an expert hepatopathologist blinded to the clinical information using the Ishak modification of the histology activity index (HAI) for inflammation and necrosis (scores ranging from 0 to 18). A fibrosis score using the Ishak staging of fibrosis (0-6) will also be assigned to each liver biopsy specimen. Slides will also be stained for HBsAg, HBcAg, and HDV antigen by peroxidase-antiperoxidase techniques and scored as 0, 1, 2 or 3+ based upon the percent of cells reactive for each viral antigen. Liver-infiltrating T cells will be isolated and assessed for frequency, function and HBV-specificity by Elispot and/or flow cytometry. m-RNA levels of T cell markers and chemokines/cytokines will be quantitated by real-time PCR.
8. Patients identified with portal hypertension (HVPG > 5 mm Hg) will undergo upper endoscopy for evaluation of endoscopic varices. Management of varices will be as per the standard guidelines of the American Association for the Study of Liver Diseases.
9. Fifty milliliters of whole blood will be drawn for baseline immunological studies: CD4+ and CD8+ T cell HBV specific responses.
10. Patients will undergo an ultrasound based fibroscan that measures elasticity of the liver and is an indirect measure of degree of liver fibrosis.
11. At baseline, all patients will undergo a three-phase computerized tomography of the abdomen to fully exclude the presence of hepatocellular carcinoma and provide a baseline assessment of all patients.
12. Patients will undergo a bone mineral density scan as a baseline to monitor for bone loss. Patients found to have osteoporosis or osteomalacia at baseline will be offered therapy with bisphosphonates and calcium.

**D. Treatment**

Patients will be stratified for randomization on the basis of HBeAg status and on the basis of previous therapy. The primary study will be in patients (Groups A and B) who have not been previously treated (treatment naïve) and who have active liver disease (based on ALT levels and liver biopsy) and high levels of HBV DNA ($>10^4$ IU/ml). The salvage study will be in patients (Groups C and D) who have received other nucleoside analogues and who continue to have HBV DNA detectable in serum ($>10^2/10^3$ IU/ml, respectively). A total of 100 patients will be enrolled including at least 60 patients total in Groups A and B and up to 40 patients in Groups C and D (previously treated).

***Group A:*** Up to 20 patients with detectable HBeAg in serum and no previous therapy with nucleoside analogues.

***Group B:*** Up to 20 patients without detectable HBeAg in serum and no previous therapy with nucleoside analogues.

***Group C:*** Up to 20 patients who have previously received lamivudine, adefovir or entecavir therapy but who have persistence of HBV DNA in serum as detected by PCR-based assay (>$10^2$ IU/ml) and HBeAg in serum for at least six months.

***Group D:*** Up to 20 patients who have previously received lamivudine, adefovir or entecavir therapy but who have persistence of HBV DNA in serum as detected by PCR-based assay (>$10^2$ IU/ml) and absence of HBeAg in serum for at least six months.

***Group E:*** Up to 20 patients who have previously received lamivudine, adefovir or the combination of lamivudine and adefovir and who relapsed after therapy was stopped

After medical evaluation, liver biopsy and informed written consent, patients will be randomized in double-blind fashion to receive either combination therapy with tenofovir and emtricitabine or mono-therapy with tenofovir. Patients with anti-viral resistance to lamivudine adefovir, entecavir or telbivudine or multi-drug resistance will continue on pre-study medication until the day of randomization. Randomization will be done by random numbers kept by the Pharmaceutical Developmental Service of the Clinical Center (A1-20, B1-20, C1-20, D1-20, E1-20). Both tenofovir (300 mg tablets) and Tenofovir (300 mg) / emtricitabine (200 mg) will be provided by Gilead Sciences. A 12 week supply of medications will be provided. Compliance will be monitored by medication diaries and counting of the residual tablets returned at outpatient visits.

Patients will be seen in the outpatient clinic at weeks 0, 2, 4, 8, 12, 24, 36, and 48 weeks during the first year of therapy and at 12 week intervals thereafter.

At 48 weeks, all patients will be readmitted to the NIH Clinical Center and undergo a repeat thorough medical evaluation and liver biopsy. Patients in the salvage arm will be excluded from undergoing the 48 week liver biopsy. Continuation of therapy thereafter will depend upon both tolerance and benefit. All patients will undergo a 192 week follow-up biopsy to assess long-term efficacy and durability of response. Benefit is described in Section F "Assessment of Response" and tolerance in Section G "Assessment of Toxicity."

The side effects will be monitored regularly. Tenofovir and emtricitabine will be stopped if moderate side effects attributable to either or both medications develop and persist for at least one week or on repeat testing. The medication(s) can be restarted if the adverse event resolves and does not appear to be related to either or both medications.

In the absence of toxicity attributable to either agent, combination therapy with tenofovir and emtricitabine or monotherapy with tenofovir will be continued for 192 weeks. Study medication/s will be discontinued if there is serological evidence of resolution of chronic hepatitis B, as shown by loss of HBsAg, documented on two serum samples taken at least six months apart.

Patients on therapy who develop evidence of drug resistance with elevations of serum aminotransferase levels twice above normal and HBV-DNA levels greater than $10^6$ IU/ml will be switched to alternative therapies if mutually agreed between the patient and the investigators.

**E. Monitoring**

Patients will be seen, interviewed, and have blood and urine tests taken at regular intervals according to the following schedule (Appendix A): on at least two occasions during the six months before treatment (Screen 1 and 2) including at the time of liver biopsy and initial evaluation, on day 0 (before first administration of drug) and at weeks 2, 4, 8, 12, 24, 36, and 48 after starting therapy. Thereafter, the patients will be seen every 12 weeks while on therapy. At each visit, the patients will be asked about symptoms and possible side effects and fill out a symptom and side effect questionnaire. Blood samples will be drawn for CBC and biochemical tests including chemistry-20 panel (ALT, AST, alkaline phosphatase, creatine kinase, lactic dehydrogenase, direct and total bilirubin, sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, albumin, total protein, calcium, and phosphate), and 10 ml of research blood. HBV DNA will be measured at every clinical visit during therapy and more often if needed, such as to investigate viral breakthrough. In addition, HBsAg, HBeAg and anti-HBe will be tested every 12 weeks during therapy and anti-HBs if appropriate. An additional sample will be collected for routine storage to allow for repeat testing. Urine will be collected for urinalysis and random total protein/creatinine ratio at every visit. Twenty-four hour urine for creatinine clearance and total protein will be done at 48 weeks and 192 weeks. Women of child bearing potential will have a pregnancy test at each study visit. Women with a positive pregnancy test will be immediately withdrawn from study drug/s. Patients may be offered alternative approved therapy if clinically indicated. Immunological testing for CD4+ and CD8+ T cell responses to HBV antigens will be done before treatment for all previously untreated patients and repeated at 2, 4, 8, 12, 36 and 48 weeks for HLA-A2 positive patients. Alpha-fetoprotein levels will be measured every 24 weeks. Ultrasound or computerized tomography of the liver will be done every 24 weeks months in patients with advanced fibrosis or cirrhosis; in other patients these tests will be done at the time of 48 and 192 week liver biopsies. Fibroscan of the liver will be performed yearly. Bone mineral density will be re-assessed at 48 and 192 weeks.

At the end of the initial 48 week period of treatment, patients will be readmitted to the NIH Clinical Center and undergo the same evaluation as outlined under initial evaluation above. This evaluation will include a repeat liver biopsy. Patients in the salvage arm will be excluded from undergoing the 48 week liver biopsy. Patients who underwent hepatic venous pressure gradient measurements at the start will undergo repeat pressure measurement with concurrent transjugular liver biopsy. Patients judged to have a beneficial response to therapy will be continued on tenofovir or combination therapy and followed at 12-week intervals. If therapy is stopped (either for toxicity, resolution of hepatitis B or at the end of the trial), patients will be seen in the outpatient clinic every 4 weeks for 6 months. Thereafter, patients will be followed at 3 to 12 month intervals as is customary for the Liver Diseases Branch patients in protocol 91-DK-214 (“Evaluation of

Patients with Liver Disease"). Patients who have not had a beneficial response or a sustained response will be eligible for antiviral therapy in other protocols.

**F. Assessment of response:**

In this study, the combination of tenofovir and emtricitabine and tenofovir monotherapy will be evaluated for long-term effects on chronic hepatitis B in both HBeAg-positive and -negative cohorts and in treatment naïve and those with resistance to lamivudine, adefovir, entecavir or all agents.

Definitions:

a) ***Full virological response***: absence of detectable HBV DNA by PCR ($<10^2$ IU/ml)

b) ***Partial virological response:*** suppression of HBV DNA to $<10^4$ IU/ml but $>10^2$ IU/ml

c) ***Serological response (HBeAg-positive patients only)***: loss of detectable HBeAg.

d) ***Full biochemical response***: fall of serum ALT into the normal range (<41 IU/L)

e) ***Partial histological response:*** improvement in liver histology as defined by a decrease in the inflammatory component of the histological activity index (HAI) score by 3 points or more and no worsening of the Ishak fibrosis score. Histological responses will be assessed after 48 and 192 weeks of therapy.

f) ***Complete histological response:*** improvement in liver histology as defined by a decrease in the inflammatory component of the histological activity index (HAI) score by 3 points or more and no worsening of the Ishak fibrosis score and a decrease of the HAI score to 3 or less. Histological responses will be assessed after 48 an 192 weeks of therapy.

g) ***Combined response***: the combination of a virological, serological, biochemical and histological response.

h) ***Complete Response***: Loss of HBsAg. A complete response is the major focus and most hoped for outcome of long-term antiviral treatment of hepatitis B. A complete response is reason for stopping all antiviral treatment.

Beneficial responses are also characterized as initial, maintained or sustained:

a) ***Initial response:*** full virological or biochemical response occurring within the first 24 weeks of therapy.

b) ***Maintained response:*** virological, serological, biochemical, histological, or combined response present at the time of last observation in a patient still receiving therapy.

c) ***Sustained response:*** virological, serological, biochemical, histological or combined response present at the time of last observation and at least six months after stopping therapy.

The primary study endpoints will be a maintained full virological response after 48 weeks and 192 weeks of therapy (the two points of formal statistical analysis). The major secondary endpoint will be absence of a failure of therapy and absence of resistance mutations. Other endpoints to therapy will be loss of HBeAg, loss of HBsAg, normalization of serum ALT, improvements in histology activity index, improvements in hepatic fibrosis, improvement in Fibroscan scores and decrease in HVPG. Other endpoints will be improvement in symptom scores at 48 and 192 weeks.

Failure of therapy may be due to primary non-response, lack of virological response, breakthrough, or relapse, as defined below.

A ***primary non-response*** is defined as lack of decrease in HBV DNA levels by at least 1 $\log_{10}$ after at least 24 weeks of therapy in the absence of non-compliance.

A ***failure in virological response*** is defined as failure to suppress HBV DNA below $10^4$ IU/ml after 48 weeks of therapy in the absence of non-compliance.

A ***breakthrough*** is defined as a $\geq$1 $\log_{10}$ IU/L increase in HBV DNA level from nadir in two consecutive samples at least one month apart in a patient with an initial full virological response in the absence of non-compliance.

A ***relapse*** is defined as a rise in HBV DNA levels to above $10^4$ IU/ml or of ALT to above the normal range after therapy is stopped in a patient who previously had a maintained full virological response.

These definitions are all interlocking but help to define response and non-response rates and whether therapy has a sustained effect.

**Stopping therapy:** Therapy will be continued indefinitely or until one of the following endpoints are reached:

1. ***Failure of therapy***: If HBV DNA levels remain consistently $>10^4$ IU/ml after one year of continuous therapy, patients will be offered alternative therapy or the option to stop therapy. Alternative therapies may include switching to combination therapy if they were receiving monotherapy or switching to entecavir if receiving combination therapy. Patients who fail to respond may also elect to receive a course of peginterferon. These options are available at the NIH Clinical Center on an open-label basis.

ii) ***Toxicity.*** Either intolerance to tenofovir or the combination of tenofovir and emtricitabine or development of worsening renal function would be a reason to stop therapy (see below **The risks and hazards of tenofovir and emtricitabine combination therapy** for management of tenofovir toxicity.)

In either situation, entecavir or peginterferon would be options for alternative therapy. Discontinuation of therapy due to toxicity will be considered failure of therapy and, if treated, not as a part of this protocol.

iii) ***Clearance of HBsAg***. Patients who become and remain HBsAg and HBV DNA negative for at least six months will have therapy discontinued. These patients will be monitored at 4 week intervals for the next 6 months and then every 12 weeks. If HBsAg returns or if HBV DNA levels rise above $10^4$ IU/ml, therapy will be reinstituted. Patients who clear HBsAg who are anti-HBs negative will be given a course of HBV vaccine (0, 1 and 4-6 months).

## G. Assessment of Toxicity and modification of dose of tenofovir and emtricitabine

### The risks and hazards of tenofovir therapy

There is limited experience with the use of tenofovir in patients with chronic hepatitis B. More than 12,000 HIV positive patients have been treated with tenofovir alone or in combination with other antiretroviral drugs for periods of 28 days to 215 weeks in Phase I-III clinical trials and expanded access studies. A total of 1,544 patients have received tenofovir 300 mg once daily in Phase I-III clinical trials; over 11,000 patients have received tenofovir in expanded access programs. In study 903, a double-blind, active controlled multicenter study comparing tenofovir 300 mg daily administered in combination with lamivudine and efavirenz versus stavudine (d4T), lamivudine and efavirenz in 600 antiretroviral naïve patients, the most common adverse reactions seen were mild to moderate gastrointestinal events and dizziness. Mild adverse events (Grade 1) were common with a similar incidence in both arms and included dizziness, diarrhea and nausea. Selected treatment emergent moderate to severe adverse events (Grades 2-4) reported in ≥5% of the tenofovir versus non-tenofovir arms respectively, included headache 14% vs. 17%, pain 13% vs. 12%, fever 8% vs. 7%, abdominal pain 7% vs. 12%, back pain 9% vs. 8%, asthenia 6% vs. 7%, diarrhea 11% vs. 13%, nausea 8% vs. 9%, dyspepsia 4% vs. 5%, vomiting 5% vs. 9%, lipodystrophy 1% vs. 8%, arthralgias 5% vs. 7%, myalgias 3% vs. 5%, depression 11% vs. 10%, insomnia 5% vs. 8%, dizziness 3% vs. 6%, peripheral neuropathy 1% vs. 5%, anxiety 6% vs. 6%, pneumonia 5% vs. 5%, rash 18% vs. 12%. With the exception of fasting cholesterol and fasting triglyceride elevations that were more common in the stavudine group (40% and 9%) compared with tenofovir (19% and 1%) respectively, laboratory abnormalities observed in study 903 occurred with similar frequency in the tenofovir and non-tenofovir treatment arms. Grade 3/4 laboratory abnormalities reported in ≥1% of treated patients include creatinine kinase 12% vs. 12%, serum amylase 9% vs. 8%, serum AST 5% vs. 7%, serum ALT 4% vs. 5%, hematuria (>100 RBC's/HPF) 7% vs. 7% and neutropenia (<750/$mm^3$) 3% vs. 1%. In

study 934, 511 antiretroviral naive patients received either tenofovir and emtricitabine administered in combination with efavirenz or zidovudine/lamivudine administered in combination with efavirenz. Adverse events and laboratory abnormalities observed in this study were generally consistent with those seen in previous studies in treatment-experienced or treatment naïve patients. In addition to adverse events reported from clinical trials, the following events have been identified during post-approval use of tenofovir. Because they are reported from a population of unknown size, estimates of frequency cannot be made. These events include allergic reactions, hypophosphatemia, lactic acidosis, dyspnoea, abdominal pain, increased amylase, pancreatitis, increased liver enzymes, hepatitis, renal insufficiency, renal failure, acute renal failure, Fanconi syndrome, proximal tubulopathy, proteinuria, increased creatinine, acute tubular necrosis, nephrogenic diabetes insipidus, polyuria and nephritis.

Tenofovir has a black box warning indicating that lactic acidosis and severe hepatomegaly with steatosis including death have been reported with the use of nucleoside analogues alone or in combination with other antiretrovirals. Additionally severe acute exacerbations of hepatitis B have been reported in patients who are coinfected with HBV and HIV and have discontinued tenofovir. Tenofovir is principally eliminated by the kidney. The dosing interval needs to be adjusted in all patients with creatinine clearance <50 ml/min.

The majority of safety data on tenofovir has come from HIV populations on multidrug regimens. The few series of patients with chronic hepatitis B treated with tenofovir have not reported any serious side effects. The major safety concern with tenofovir is renal impairment. Cases of acute renal failure and Fanconi syndrome (renal tubular injury with severe hypophosatemia) have been reported in association with the use of tenofovir. The majority of these cases occurred in patients with underlying systemic or renal disease, or in patients taking nephrotoxic agents, however, some cases occurred in patients without identified risk factors.

One other potential problem identified with the use of tenofovir in the HIV population is a decline in bone mineral density. In a 144-week study of HIV positive treatment naïve patients, decreases in bone mineral density (BMD) were seen at the lumbar spine and hip in both arms of the study (study 903 see above). At Week 144, there was a significantly greater mean percentage decrease from baseline in BMD at the lumbar spine in patients receiving tenofovir plus lamivudine plus efavirenz compared with patients receiving stavudine plus lamivudine plus efavirenz. Changes in BMD at the hip were similar between the two treatment groups. In both groups, the majority of the reduction in BMD occurred in the first 24–48 weeks of the study and this reduction was sustained through 144 weeks. Twenty-eight percent of tenofovir-treated patients vs. 21% of the comparator patients lost at least 5% of BMD at the spine or 7% of BMD at the hip. Clinically relevant fractures (excluding fingers and toes) were reported in 4 patients in the tenofovir group and 6 patients in the comparator group. Tenofovir disoproxil fumarate was associated with significant increases in biochemical markers of bone metabolism (serum bone-specific alkaline phosphatase, serum osteocalcin, serum C-telopeptide, and urinary N-telopeptide), suggesting increased bone turnover. Serum parathyroid hormone levels

and 1,25 Vitamin D levels were also higher in patients receiving tenofovir. The effects of tenofovir-associated changes in BMD and biochemical markers on long-term bone health and future fracture risk are unknown.

The risk of carcinogenesis, mutagenesis and impairment in fertility has been studied. Long-term oral carcinogenicity studies of tenofovir in mice and rats were carried out at exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV infection. At the high dose in female mice, liver adenomas were increased at exposures 16 times that in humans. In rats, the study was negative for carcinogenic findings at exposures up to 5 times that observed in humans at the therapeutic dose.

Tenofovir was mutagenic in the in vitro mouse lymphoma assay and negative in an in vitro bacterial mutagenicity test (Ames test). In an in vivo mouse micronucleus assay tenofovir was negative when administered to male mice.

There were no effects on fertility, mating performance or early embryogenic development when tenofovir was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 15 days prior to mating through day seven of gestation. There was however an alteration of the estrous cycle in female rats.

Reproduction studies have been performed in rats and rabbits at doses up to 14 and 19 times the human dose based on the body surface area comparisons and revealed no evidence of impaired fertility or harm to the fetus due to tenofovir. There are however, no adequate and well-controlled studies in pregnant women.

Women of child bearing potential will have a pregnancy test at each study visit. They will be counseled to use at least two forms of adequate birth control while receiving study medications. Any female patient found to be pregnant will stop antiviral therapy and be followed in the Liver Diseases Clinic.

**The risks and hazards of emtricitabine therapy**

More than 2000 adult patients with HIV infection have been treated with emtricitabine either alone or in combination with other antiretroviral agents for periods of 10 days to 200 weeks in Phase I-III clinical trials. Assessment of adverse reactions is based on data from studies 301A and 303 in which 571 treatment naïve (301A) and 440 treatment experienced (303) patients received emtricitabine 200mg (n-580) or comparator drug (n=431) for 48 weeks. The most common adverse events that occurred in patients receiving emtricitabine with other antiretroviral agents in clinical trials were headache, diarrhea, nausea and rash that were generally of mild to moderate severity. Approximately 1% of patients discontinued participation in the clinical trials due to these events. All adverse events were reported with similar frequency in emtricitabine and control treatment groups with the exception of skin discoloration which was reported with higher frequency in the emtricitabine treated group. Skin discoloration manifested

by hyperpigmentation on the palms and /or soles were generally mild and asymptomatic. The mechanism and clinical significance are unknown.

Selected treatment emergent adverse events (all grades regardless of causality) reported in ≥3% of emtricitabine-treated patients in either study 301A or 303 include abdominal pain 8-14% vs. 11-17%, asthenia 12-16% vs. 10-17%, headache 13-22% vs. 6-25%, diarrhoea 23% vs. 18-32%, dyspepsia 4-8% vs. 5-12%, nausea 13-18% vs. 12-23%, vomiting 9% vs. 7-12%, arthralgia 3-5% vs. 4-6%, myalgia 4-6% vs. 3-4%, abnormal dreams 2-11% vs. <1%-19%, depressive disorders 6-9% vs. 10-13%, dizziness 4-25% vs. 5-26%, insomnia 7-16% vs. 3-21%, nuropathy/peripheral neuritis 4% vs. 3-13%, paresthesias 5-6% vs. 3-13%, increased cough 14% vs. 8-11%, rhinitis 12-18% vs. 10-12% and skin rash 17-30% vs. 14-33% compared to comparator arm respectively. Treatment emergent grade ¾ laboratory abnormalities reported in >1% of emtricitabine treated patients in either study 301A or 303 included ALT >5X ULN 2% vs. 1%, AST >5X ULN 3% vs. <1%, bilirubin >2.5 X ULN 1% vs. 2%, creatine kinase >4 ULN 11% vs. 14%, neutropenia <750 $mm^3$ 5% vs. 3%, pancreatic enzyme elevation either amylase or lipase 1-2% vs. <1-2%, serum glucose 3% vs. 3% and triglycerides 10% vs. 8% compared to comparator arm.

Emtricitabine carries a black box warning advising of lactic acidosis and severe hepatomegaly with steatosis including death associated with its use.

Emticitabine was evaluated in a randomized placebo controlled trial in 167 patients with chronic hepatitis B. The incidence of clinical adverse events during treatment was similar in the 2 groups. Severe (grade 3 or 4) adverse events were reported in 14 (8%) of 167 emtricitabine-treated patients and 7 of 81 patients given placebo (9%), and serious adverse events occurred in 8% (n = 13) and 9% (n = 7), respectively. Related serious adverse events were rare, 4 (2%) of 167 emtricitabine and 3 (4%) of 81 placebo patients. During treatment, the incidence of grade 3 or 4 laboratory abnormalities was significantly reduced in the emtricitabine group, 31 (19%) of 167 patients vs. 33 (41%) of 81 in the placebo group (P<.001), which was due to the lower incidence of grade 3 or 4 transaminase, ALT (7% emtricitabine [n = 11], 26% placebo [n = 21]) and aspartate aminotransferase (2% emtricitabine [n = 3], 12% placebo [n = 10]) (P≤.001).

Post treatment follow-up safety data were available from 145 patients in the emtricitabine group (median follow-up, 110 days) and 63 patients in the placebo group. Post treatment exacerbation of CHB developed in 33 patients (23%) who had received emtricitabine and 3 placebo patients (5%) (P = .001), with a median time to onset of 10 weeks following the end of treatment (interquartile range, 8-16 weeks). One patient randomized to emtricitabine with marked bridging fibrosis at entry developed severe icteric post treatment exacerbation of CHB and required liver transplantation. All other patients recovered without clinical complications (overall, 14 with and 19 without antiviral therapy).

Long-term carcinogenicity studies of emtricitabine in rats and mice are in progress. Emtricitabine was not toxic in the reverse bacterial test (Ames test), mouse lymphoma or mouse micronucleus assays.

Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60-fold higher exposures than in humans given the recommended 200 mg dose. Fertility was normal in the offspring of mice exposed daily from before birth (in utero) through sexual maturity at daily exposures of approximately 60-fold higher exposures than in humans given the recommended 200 mg dose.

The incidence of fetal variations and malformations was not increased in embroyonal toxicity studies performed with emtricitabine in mice at exposures approximately 60 fold higher and in rabbits at approximately 120 fold higher than in humans exposures given the recommended 200 mg dose. There are however, no adequate and well-controlled studies in pregnant women.

**The risks and hazards of tenofovir and emtricitabine combination therapy**

Four hundred and forty-seven HIV-1 infected patients have received combination therapy with Emtriva and Viread with either a non-nucleoside reverse transcriptase inhibitor or protease inhibitor for 48 weeks in clinical studies. In study 934, a randomized, open-label, active controlled multicenter study comparing Emtriva and Viread administered in combination with efavirenz versus zidovudine/lamivudine fixed-dose combination administered in combination with efavirenz in 511 antiretroviral-naïve patients, adverse events observed were generally consistent with those seen in other studies in treatment experienced or treatment-naïve patients receiving Viread and/or Emtriva. Selected treatment emergent adverse events (Grades 2-4) reported in ≥3% in the Emtirva and Viread arm compared to the non- Emtirva and Viread arm include diarrhea 7% vs. 4%, nausea 8% vs. 6%, vomiting 1% vs. 4%, fatigue 7% vs. 6%, sinusitis 4% vs. 2%, upper respiratory tract infections 3% vs. 3%, nasopharyngitis 3% vs. 1%, somnolence 3% vs. 2%, headache 5% vs. 4%, dizziness 8% vs. 7%, depression 4% vs. 7%, insomnia 4% vs. 5%, abnormal dream 4% vs. 3%, skin rash 5% vs. 4%. Laboratory abnormalities observed in this study were generally consistent with those seen in other studies of tenofovir and/or emtricitabine. Significant laboratory abnormalities reported in ≥1% of patients in any treatment arm (tenofovir/emtricitabine arm 25% vs. non-tenofovir/emtricitabine arm 22%) included elevated fasting cholesterol 15% vs. 17%, elevated creatine kinase 7% vs. 6%, elevated amylase 7% vs. 3%, elevated AST 3% vs., 2%, elevated ALT 2% vs. 2%, hemoglobin <8 mg/dl 0% vs., 3%, hyperglycemia 1% vs. 1%, hematuria 2% vs. 2%, neutropenia 3% vs. 4% and elevated fasting triglycerides 4% vs. 2%.

In addition to the events described above for Study 934, other adverse events that occurred in >5% of patients receiving emtricitabine with other antiretroviral agents in clinical trials include abdominal pain, asthenia, arthralgia, increase cough, depressive disorder, dyspepsia, myalgia, paresthesia, rhinitis and rash event (including rash, pruritus, maculopapular rash, urticaria, vesiculobullous rash, pustular rash and allergic reaction).

Skin discoloration has been reported with higher frequency among emtricitabine treated patients. Skin discoloration, manifested by hyperpigmentation on the palms and/or soles was generally mild and asymptomatic. The mechanism and clinical significance are unknown. No additional adverse events were reported in post marketing experience with emtricitabine.

In addition to the laboratory abnormalities described above for study 934, grade 3/4 elevations of bilirubin (>2.5 x ULN), pancreatic amylase (>2.0 x ULN), serum glucose (<40 or >250 mg/dL) and serum lipase (>2.0 x ULN) occurred in <1–3% of patients treated with emtriciatbine.

In addition to the events described above for study 934, other adverse events that occurred in ≥5% of patients receiving tenofovir with other antiretroviral agents in clinical trials include arthralgia, anxiety, fever, pain, abdominal pain, back pain, peripheral neuritis, peripheral neuropathy, and pneumonia.

In addition to the laboratory abnormalities described above for study 934, grade 3/4 elevations of urine glucose (≥3+) occurred in 3% of patients receiving tenofovir with other antiretroviral agents in clinical trials the following events have been identified during post-approval use of tenofovir. Because they are reported from a population of unknown size, estimates of frequency cannot be made. These events include allergic reactions, hypophosphatemia, lactic acidosis, dyspnoea, abdominal pain, increased amylase, pancreatitis, increased liver enzymes, hepatitis, renal insufficiency, renal failure, acute renal failure, Fanconi syndrome, proximal tubulopathy, proteinuria, increased creatinine, acute tubular necrosis, nephrogenic diabetes insipidus, polyuria and nephritis.

The combination of tenofovir and emtricitabine carries a black box warning as for tenofovir and other nucleos(t)ide analogues highlighting that lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleos(t)ide analogs alone or in combination with other antiretrovirals. A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors. Particular caution should be exercised when administering nucleoside analogs to any patient with known risk factors for liver disease; however, cases have also been reported in patients with no known risk factors.

A major safety concern with tenofovir is renal impairment. Cases of acute renal failure and Fanconi syndrome (renal tubular injury with severe hypophosatemia) have been reported in association with the use of tenofovir. The majority of these cases occurred in patients with underlying systemic or renal disease, or in patients taking nephrotoxic agents, however, some cases occurred in patients without identified risk factors. Patients will be monitored by a series of tests for renal toxicity including calculated GFR using the Modification of Diet in Renal Disease (MDRD) formula, urine analysis and serum phosphate. Dose adjustments or cessation will be based upon any of the following criteria:
1) Absolute decline in GFR by 25 mls/min/1.73 $m^2$ confirmed on two occasions 2 weeks apart. GFR will be calculated using the Modification of Diet in Renal Disease (MDRD)

formula: (MDRD GFR (ml/min/1.73m$^2$=170 x[PCr]$^{-.999}$ X [Age]$^{-0.176}$ X[.0762 if patient is female] X [1.180 if patient is black] X [SUN]$^{-0.170}$ X[Alb]$^{+.318}$ where PCr=serum creatinine concentration (mg/dl) (alkaline picrate method); SUN=serum urea nitrogen concentration (mg/dl) (urease method); Alb=serum albumin concentration (g/dl) (bromocresol green method)).
2) Abnormality in any one of the following 4 parameters calculated GFR, urine glucose, serum phosphate and a routine urine analysis will trigger a formal renal evaluation. This will include including assessment of GFR by 24 urine hour creatinine clearance, urine glucose, fractional excretion of phosphate, 24 hour urine for amino acids and serum uric acid, If the evaluation is consistent with Fanconi's syndrome, patients will be discontinued from therapy indefinitely.
3) For Grade 1 toxicity serum creatinine ≥ .5 above baseline, the patient will be monitored weekly until the creatinine returns to baseline or ≤ 0.3 mg/dL of the baseline value. For confirmed Grade 2 and ≥ 0.5 increases from baseline, therapy will be permanently discontinued.

In a 144-week study of HIV positive treatment naïve patients, decreases in bone mineral density (BMD) were seen at the lumbar spine and hip in both arms of the study (study 903 see above). At Week 144, there was a significantly greater mean percentage decrease from baseline in BMD at the lumbar spine in patients receiving tenofovir plus lamivudine plus efavirenz compared with patients receiving stavudine plus lamivudine plus efavirenz. Changes in BMD at the hip were similar between the two treatment groups. In both groups, the majority of the reduction in BMD occurred in the first 24–48 weeks of the study and this reduction was sustained through 144 weeks. Twenty-eight percent of tenofovir-treated patients vs. 21% of the comparator patients lost at least 5% of BMD at the spine or 7% of BMD at the hip. Clinically relevant fractures (excluding fingers and toes) were reported in 4 patients in the tenofovir group and 6 patients in the comparator group. Tenofovir disoproxil fumarate was associated with significant increases in biochemical markers of bone metabolism (serum bone-specific alkaline phosphatase, serum osteocalcin, serum C-telopeptide, and urinary N-telopeptide), suggesting increased bone turnover. Serum parathyroid hormone levels and 1,25 Vitamin D levels were also higher in patients receiving tenofovir. The effects of tenofovir-associated changes in BMD and biochemical markers on long-term bone health and future fracture risk are unknown.

The risk of carcinogenesis, mutagenesis and impairment in fertility has been studied. Long-term oral carcinogenicity studies of tenofovir in mice and rats were carried out at exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV infection. At the high dose in female mice, liver adenomas were increased at exposures 16 times that in humans. In rats, the study was negative for carcinogenic findings at exposures up to 5 times that observed in humans at the therapeutic dose.

Tenofovir was mutagenic in the in vitro mouse lymphoma assay and negative in an in vitro bacterial mutagenicity test (Ames test). In an in vivo mouse micronucleus assay tenofovir was negative when administered to male mice.

There were no effects on fertility, mating performance or early embryogenic development when tenofovir was administered to male rats at a dose equivalent to 10 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 15 days prior to mating through day seven of gestation. There was however an alteration of the estrous cycle in female rats.

The incidence of fetal variations and malformations was not increased in embryofetal toxicity studies performed with emtricitabine in mice at exposures (AUC) approximately 60-fold higher than human exposures at the recommended daily dose.

Reproduction studies have been performed in rats and rabbits at doses up to 14 and 19 times the human dose based on the body surface area comparisons and revealed no evidence of impaired fertility or harm to the fetus due to tenofovir. There are however, no adequate and well-controlled studies in pregnant women.

**H. Viral resistance**

Tenofovir resistance is defined phenotypically as: (1) rise in serum HBV-DNA levels to levels above $10^4$ IU per ml (by quantitative PCR) after they had fallen below this level during therapy in patients who have been compliant with their regimen and genotypically as (2) documented mutation/s in the reverse transcriptase region of the HBV polymerase gene (thus far the only documented mutation is rtA194T), and biochemically as (3) rise in serum aminotransferases to twice the upper limit of normal range. Patients who develop resistance will be continued on the therapy if they agree to stay on therapy and if serum aminotransferases and HBV-DNA are consistently lower than pretreatment levels or changed to other available agents if clinically indicated.

Emtricitabine resistance in this study can only be defined phenotypically as a rise in serum aminotransferases to twice the upper limit of normal range. Genotypic resistance is defined by the typical mutations in the conserved catalytic region of the HBV polymerase gene (YMDD region).

Monitoring for mutations in the reverse transcriptase domain of the HBV-DNA polymerase on combination therapy will be performed on serum samples obtained at baseline, and yearly on all serum samples that have detectable HBV DNA from patients receiving tenofovir plus emtricitabine and tenofovir monotherapy. The monitoring will be done by sequence analysis of the reverse transcriptase region of the HBV DNA polymerase gene and will be performed by LDB, NIDDK. Novel mutations will be correlated with the clinical response. Antiviral susceptibility and cross resistance testing using in vitro assays will be conducted by Gilead Sciences using stored serum samples. Resistance data will be submitted to the FDA according to the FDA guidance document entitled, “Guidance for submitting HBV Resistance Data.”

I. **Statistical analysis**

Superiority of combination therapy compared to monotherapy with tenofovir will be tested. The primary analysis will be conducted at 1 and 4 years on the entire study cohort (Groups A-D). A separate analysis will be conducted on previously treated subjects with resistance combining all HBeAg-positive and -negative cases. We predict the rate of full virological response to combination therapy will be 94% and to tenofovir monotherapy to be 70% at 4 years. A Fisher's exact test with a .05 two-sided significance level will have 86% power to detect the difference between a combination therapy proportion of $\pi_1$ of .94 and a monotherapy proportion of $\pi_2$ .70 if the sample size in each group is 50. Secondary analyses will be done within the HBeAg positive and negative stratum and the nucleos(t)ide naïve and nucleos(t)ide treated stratum. Variables to be analyzed in association with a virological response will include: baseline ALT and HBV DNA level, liver histology, HBV genotype and previous resistance. Patients with missing data or who drop out of the study will be considered as treatment failures on an intention to treat basis. For comparison of means, Student's t-test will be used. All reported p-values will be 2-sided.

## J. Hazards and Discomforts

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 combination of tenofovir and FTC, frequent blood sampling will be required. Patients will have between 11 and 12 venipunctures during the first 48 weeks of the study and 4 to 6 each year thereafter. Each venipuncture will be for approximately 20 to 75 cc of blood. The total amount of blood drawn during an 8 week period will not exceed 10.5 ml/Kg or 550 mls, whichever is smaller.

2. **The risks and discomforts of HIV testing.** Patients will have blood tested for anti-HIV at entry. Mention of anti-HIV is made in the consent form which includes the exact language used in the standard consent form used for anti-HIV testing at the Clinical Center. It is important to test for HIV infection as both tenofovir and emtricitabine have activity against HIV and a treatment regimen using only two drugs against HIV would not be considered standard of care.

3. **The risks and discomfort of percutaneous liver biopsy.** Patients will undergo up to three liver biopsies in this protocol namely: one before therapy, a second at 48 weeks, and a third at 192 weeks. Patients will not have to undergo the initial liver biopsy if they had a biopsy within the previous 2 years that is adequate and available for analysis. In addition, patients with inadequate platelet counts ($<70{,}000/mm^3$) or coagulation parameters (prothrombin time > 15 seconds, INR>1.7) will not undergo liver biopsy as a part of this protocol. Patients requiring liver biopsy will be admitted to the Clinical Center for two days for this procedure and other blood testing. The major side effects of liver biopsy are pain, bacteremia, puncture of another organ and bleeding. Local pain and discomfort at the liver biopsy site occurs in about 20% of persons undergoing percutaneous liver biopsy. This is transient (lasting one to twelve hours) and is usually mild, rarely requiring analgesics. Bacteremia occurs in 1-2% of persons undergoing liver biopsy. In the absence of bile duct obstruction, this is

almost always self-limited and is rarely symptomatic. Significant bleeding after liver biopsy is the most serious side effect of this procedure. In the absence of a blood coagulation defect or hepatic malignancy, significant bleeding is rare, occurring in less than one in a thousand cases of liver biopsy. Death due to bleeding after liver biopsy has been reported in less than 1/10,000 cases. At the NIH Clinical Center, the Liver Diseases Branch has performed approximately 150 liver biopsies each year for the last 20 years. During this time, only two patients died as a complication of biopsy. One of these patients had cirrhosis and advanced hepatocellular carcinoma and the second had severe coagulation disorders. Both bled from the liver biopsy site and died after surgical attempts to stop the bleeding were unsuccessful.

4. **The risks and hazards of tenofovir therapy.** See section G.

5. **The risks and hazards of tenofovir and emtricitabine combination therapy.** See section G.

## L. Data and Safety Monitoring

The adequacy of data acquisition and storage and the monitoring of safety in this trial will be done by the principal investigator in collaboration with the associate investigators. Data and safety are reviewed weekly in clinical research rounds by the Liver Diseases Branch, NIDDK. These rounds are separate from regular clinical rounds and consist of review of all study patients including flow sheets of major safety and efficacy measurements without knowledge of which therapy is used. Monitoring of this study will also be conducted by the statistical department of NIDDK who will have knowledge of the patient allocation. Reports of serious adverse events are made to the Clinical Director NIDDK, the IRB, study monitor and the FDA.

## M. Adverse Event Reporting

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), 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 at least possibly related to the patient's participation in the research protocol, 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 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 and CD as soon as possible but not more than 14 days after the PI first learns of the event.

Non-serious protocol deviations will only be reported to the IRB (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.

All serious adverse events that are assessed as possibly, probably or definitely related to tenofovir or tenofovir plus emtricitabine will be reported to the FDA and Gilead within 15 days by submission of a serious adverse event form and a written report for events occurring within this protocol. Adverse events that are expected as a part of treatment or procedures outlined in the protocol will not be reported unless they occur at a rate or severity greater than known to occur in patients undergoing the treatment or procedures. If AEs associated with the treatment and procedures in the study occur at a rate or severity greater than known to occur, they will be reclassified as a UP and reported as such. AEs with known relation to the natural history of chronic hepatitis B or to other pre-existing conditions will not be reported unless they occur at a rate or severity greater than known to occur in patients with hepatitis B or the subject's other pre-existing conditions. AEs that are unrelated to the research will not be reported. The PI is responsible for summarizing all reportable serious adverse events and adverse events at the time of Continuing Review. Deaths and life-threatening events will be reported to the Clinical Director and IRB within 7 days after the PI first learns of the event. Any pregnancies that occur during the study and of the outcome of those pregnancies with relevant information will be reported to Gilead on a timely basis.

Any information in the literature, or that has evolved from similar research, and that might affect the IRB's analysis of risk/benefit for the protocol will be reported to the IRB at the time of Continuing Review. If such information is obtained before the time of continuing review, it should be reported to the IRB at the time that it becomes known, and summarized at the time of continuing review.

Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. 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.

## N. Enrollment of Children, Women and Minority Individuals

The current protocol excludes children (age limit > 18 years) as tenofovir has not been used in children for the treatment of chronic hepatitis B. Currently lamivudine is approved for use in children. Chronic hepatitis B is rare in children in the U.S. and the implications of long-term therapy are great.

Chronic hepatitis B is much more common among men than women. We expect to include women in the proportions that occur in this disease. Thus, we expect 10-20% of patients to be women. The exclusion of women interested in getting pregnant or who are not able to practice adequate birth control would be the only possible bias introduced in attaining adequate representation of women in this study.

The distribution of minority individuals in recent studies of nucleoside therapy of hepatitis B at the NIH Clinical Center has been reported yearly to the NIDDK IRB. In our study of adefovir and lamivudine (01-DK-0246), 62% of 39 enrolled patients were minority individuals, including 46% Asian-Americans, 13% African-Americans and 3% Hispanic Whites. Chronic hepatitis B is far more common among Asian Americans (2 to 10% of adults) than African Americans (1-2%) and Caucasians (~0.5%) and we expect that at least one-third of patients enrolled in this study will be Asian-Americans. There is no reason to suspect that response rates are different in different racial or ethnic groups.

**O. Research Use, Storage and Disposition of Human Samples and Data:**

Patients will have serum stored from selected time points during this study. These specimens will be used for repeat virological testing and special tests as needed (such as for viral levels, HBV genotype or serotype, HBV DNA sequence or testing for serum levels of cytokines or interferon induced genes). Samples may be used to assess factors associated with response or non-response to antiviral therapy. Liver biopsy tissue may be stored if a biopsy is done before therapy and if residual tissue is available after routine samples are taken for routine histological staining and evaluation. These samples will be tested in the Liver Diseases Branch or the routine clinical and surgical pathology services of the Clinical Center. Stored serum will be used to perform in vitro antiviral susceptibility and cross resistance testing. Gilead Sciences will conduct these assays and serum samples will not contain any patient identifiers. Samples will be sent as soon as virologic breakthrough is identified and Gilead will have one year after receipt of the sample in which to provide LDB, NIDDK with the results. Research records and data as well as liver biopsy slides, biopsy reports, liver tissue and sera with the patient's name and a unique identifier will be stored indefinitely in our locked offices and freezers, the medical record department and the pathology department. These materials will be protected and tracked by standard operating procedures in the medical record and pathology departments as well as a compulsive filing system in our locked offices and freezers. There will be redundant storage of clinical information in the medical record department and our offices. Likewise, there will be redundant storage of biopsy information and materials in the pathology department and our offices. This should minimize the risk of loss or destruction of information and specimens. If that were to occur we would report it to the IRB. We do not plan to destroy this personal medical information or the liver biopsy specimens or research subject sera after completion of the

study because it may be critically important for physicians (here or elsewhere) to have access them when caring for these patients in the future.

**P. Consent process**

Written informed consent will be obtained from the participant prior to any screening visits, study procedures or treatments. The Principal Investigator or other designated qualified protocol investigators (listed on the protocol's face page) will explain the study in language understandable to the subject. Potential study subjects will be informed of study rationale, design, participation burden, risks, benefits and side effects of therapy during routine LDB clinic visits and will have an opportunity to ask questions about the proposed study. They will be provided a copy of the study consent form to take home and review in more detail. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. The participant and the investigator will sign the current IRB-approved informed consent document. A witness will also sign the consent document to attest only to the validity of the signature of the subject, not the validity or quality of the consent. A copy of the consent will be given to the subject for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the medical record.

If a subject indicates a desire to participate in the research study, he/she will have all screening laboratories, radiologic imaging and if necessary, liver biopsy, performed through our natural history protocol 91-DK-0214. Hepatitis B can be a very dynamic disease and liver pathology can change rapidly as opposed to the scenario with hepatitis C. In contrast to other chronic liver diseases a liver biopsy is very helpful and in some cases essential to manage patients with chronic hepatitis B. How often one should perform a follow-up biopsy is open to debate and is influenced by many factors. For clinical investigation the ideal duration to perform a baseline liver biopsy would be within one year prior to enrollment, however a period of 2 years seems a reasonable compromise without being arbitrary. In our experience 2 years provides enough time for clinically significant changes to be observed without subjecting patients to unnecessary risk. We believe that it is medically indicated for every patient with chronic hepatitis B to undergo a liver biopsy before embarking on therapy that may last more than a decade. Potential study subjects who meet study inclusion and exclusion criteria will sign the consent form on day 0 of therapy, prior to randomization.

P.2 Short Form Consent

We do not plan to enroll non-English speaking subjects; however they are not excluded from participation either. Should a non-English speaking subject be enrolled, IRB approval will be obtained to use the short form process in the absence of a fully translated consent document. Requests for IRB approval will be obtained prior to implementing the short form consent process. The short form consent process will be incompliance with SOP 12.9.1 of 45 CFR 46.117(b)(2).

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**APPENDIX A.** Flow sheet for follow-up clinic visits, medication schedule, and diagnostic tests for patients on combination therapy with tenofovir and emtricitabine.

| | Week of therapy | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Pre-1* | Pre-2* | 0 | 2 | 4 | 8 | 12 | 24 | 36 | 48 | q 12 | 192 |
| Clinic visit | X | X | X | X | X | X | X | X | X | X | X | X |
| Admission | | X | | | | | | | | X | | X |
| Routine bloods† | X | X | X | X | X | X | X | X | X | X | X | X |
| Urine† | | | X | X | X | X | X | X | X | X | X | X |
| Extended tests¶ | | X | X | | | | X | X | X | X | X | X |
| Creatinine Clearance | | X | | | | | | | | X | | X |
| Ultrasound‡ | | X | | | | | | | | X | | X |
| Liver biopsy | | X | | | | | | | | X | | X |
| Alpha fetoprotein‡ | | X | | | | | | | | X | | X |
| Immunologic tests | | X | | X | X | | X | | | X | | |
| Fibroscan‡ | | X | | | | | | | | X | | X |
| Bone mineral density | | X | | | | | | | | X | | X |
| FTCvsPlacebo/Tenofovir | | X | X | X | X | X | X | X | X | X | X | X |

* Pre is the initial evaluation which will be an outpatient clinic visit for those who do not require a pretreatment biopsy and a clinical center admission for those who require a pretreatment liver biopsy.

† Routine bloods include CBC, chemistry-20 panel, serum lactic acid and HBV DNA by quantitative PCR.

† Urine tests include standard urine analysis and spot urine protein/creatinine ratio and a pregnancy test for women of child bearing potential.

¶ Extended tests include: INR, alphafetoprotein, HBsAg, anti-HBs, HBeAg and anti-HBe and are measured every 12 weeks.

‡ Ultrasound and fibroscan will be measured every 48 weeks.

**Appendix B.**
Table 1 Common Toxicity Criteria

| Grade | | | | | |
|---|---|---|---|---|---|
| Adverse event | 0 | 1 | 2 | 3 | 4 |
| Nausea | None | Able to eat | Oral intake significantly decreased | No significant intake, requiring IV fluids | - |
| Diarrhea patients without colostomy: | none | increase of <4 stools/day over pre-treatment | increase of 4-6 stools/day, or nocturnal stools | increase of 7 stools/day or incontinence; or need for parenteral support for dehydration | Physiologic consequences requiring intensive care; or hemodynamic collapse |
| Fatigue (lethargy, malaise, asthenia) | None | increased fatigue over baseline, but not altering normal activities | moderate (e.g., decrease in performance status by 1 ECOG level or 20% Karnofsky or Lansky) or causing difficulty performing some activities | severe (e.g., decrease in performance status by 2 ECOG levels or 40% Karnofsky or Lansky) or loss of ability to perform some activities | bedridden or disabling |
| Myalgia (muscle pain) | none | mild pain not interfering with function | moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living | severe pain: pain or analgesics severely interfering with activities of daily living | disabling |
| Confusion | normal | Confusion or | Confusion or | Confusion or delerium | Harmful to others or |

| | | | | | |
|---|---|---|---|---|---|
| | | disorientation or attention deficit of brief duration; resolves spontaneously with no sequelae | disorientation or attention deficit interfering with function but not interfering with activities of daily living | interfering with activities of daily living | self; requiring hospitalization |
| Mood alteration-anxiety, agitation | normal | mild mood alteration not interfering with function | moderate mood alteration interfering with function, but not interfering with activities of daily living | severe mood alteration interfering with activities of daily living | suicidal ideation or danger to self |
| Mood alteration- | normal | depression mild mood alteration not interfering with function | moderate mood alteration interfering with function, but not interfering with activities of daily living | severe mood alteration interfering with activities of daily living | suicidal ideation or danger to self |
| Mood alteration-euphoria | normal | mild mood alteration not interfering with function | moderate mood alteration interfering with function, but not interfering with activities of daily living | severe mood alteration interfering with activities of daily living | danger to self |
| Vision- | normal | - | symptomatic | symptomatic | - |

| | | | | | |
|---|---|---|---|---|---|
| blurred vision | | | and interfering with function, but not interfering with activities of daily living | and interfering with activities of daily living | |
| Vision-double vision (diplopia) | normal | - | symptomatic and interfering with function, but not interfering with activities of daily living | symptomatic and interfering with activities of daily living | - |
| Inner ear/hearing | normal | hearing loss on audiometry only | tinnitus or hearing loss, not requiring hearing aid or treatment | tinnitus or hearing loss, correctable with hearing aid or treatment | severe unilateral or bilateral hearing loss (deafness), not correctable |
| Fever (in the absence of neutropenia, where neutropenia is defined as AGC <1.0 x 10 9 /L) | none | 38.0 - 39.0°C (100.4-102.2°F) | 39.1 - 40.0°C (102.3 - 104.0°F) | >40.0°C (>104.0°F ) for<24hrs | >40.0°C (>104.0°F ) for>24hrs |
| Leukocytes (total WBC) | WNL | <LLN - 3.0 x $10^9$ /L<LLN 3000/$mm^3$ | 2.0 - <3.0 x $10^9$ /L 2000 - <3000/$mm^3$ | 1.0 - <2.0 x $10^9$ /L 1000 - <2000/$mm^3$ | <1.0 x $10^9$ /L<1000/$mm^3$ |
| Neutrophils/ granulocytes (ANC/AGC) | WNL | 1.5 - <2.0 x $10^9$ /L 1500 - <2000/$mm^3$ | 1.0 - <1.5 x $10^9$ /L 1000 - <1500/$mm^3$ | 0.5 - <1.0 x $10^9$ /L 500 - <1000/$mm^3$ | <0.5 x $10^9$ /L<500/$mm^3$ |
| Hemoglobin | (Hgb) WNL | <LLN - 10.0 g/dL | 8.0 - <10.0 g/dL | 6.5 - <8.0 g/dL | <6.5 g/dL |
| Platelets | WNL | <LLN - 75.0 | 50.0 - | 10.0 - | <10.0 x $10^9$ |

| | | | | | |
|---|---|---|---|---|---|
| | | x $10^9$ /L<LLN - 75,000/$mm^3$ | <75.0 x $10^9$ /L 50,000 - <75,000/$mm^3$ | <50.0 x $10^9$ /L 10,000 - <50,000/$mm^3$ | /L<10,000/ $mm^3$ |
| Alkaline phosphatase | WNL | >ULN – 2.5X ULN | >2.5 – 5.0 X ULN | >5.0 – 20.0 x ULN | >20.0 X ULN |
| SGPT (ALT) (serum glutamic pyruvic transaminase) | WNL | >ULN – 2.5X ULN | >2.5 – 5.0 X ULN | >5.0 – 20.0 x ULN | >20.0 X ULN |
| SGOT (AST) (serum glutamic oxaloacetic transaminase) | WNL | >ULN – 2.5X ULN | >2.5 – 5.0 X ULN | >5.0 – 20.0 x ULN | >20.0 X ULN |
| Bilirubin | WNL | >ULN – 1.5 X ULN | >1.5 – 3.0 x ULN | >3.0 – 10.0 x ULN | >10 x ULN |
| Hypoalbuminemia | WNL | <LLN – 3 g/dL | ≥2 - <3 g/dL | <2 g/dL | - |
| Prothrombin time (PT) | WNL | >ULN - 1.5 x ULN | >1.5 - 2 x ULN | >2 x ULN | |
| Liver dysfunction/ failure (clinical) | normal | - | - | asterixis | Encepalopathy or coma |
| Hypokalemia | WNL | <LLN - 3.0 mmol/L | - | 2.5 - <3.0 mmol/L | <2.5 mmol/L |
| Hypophosphatemia | WNL | <LLN -2.5 mg/dL <LLN - 0.8 mmol/L | 2.0 - <2.5 mg/dL 0.6 - <0.8 mmol/L | 1.0 - <2.0 mg/dL 0.3 - <0.6 mmol/L | <1.0 mg/dL <0.3 mmol/L |
| Creatinine | WNL | >.5 above baseline | >1.5 – 3.0 x ULN | >3.0 – 6.0 x ULN | >6.0 x ULN |
| Bicarbonate | WNL | <LLN – 16 mEq/dL | 11 – 15 mEq/dL | 8 – 10 mEq/dL | <8 mEq/dL |
| CPK (creatine phosphokinase) | WNL | >ULN - 2.5 x ULN | >2.5 - 5 x ULN | >5 - 10 x ULN | >10 x ULN |
| Amylase | WNL | >ULN - 1.5 | >1.5 - 2.0 x | >2.0 - 5.0 x | >5.0 x ULN |

| | | | | | |
|---|---|---|---|---|---|
| | | x ULN | ULN | ULN | |
| Lipase | WNL | >ULN - 1.5 x ULN | >1.5 - 2.0 x ULN | >2.0 - 5.0 x ULN | >5.0 x ULN |
| Sinus bradycardia | None | asymptomatic, not requiring treatment | symptomatic, but not requiring treatment | symptomatic and requiring treatment | life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock) |
| Sinus tachycardia | None | asymptomatic, not requiring treatment | symptomatic, but not requiring treatment | symptomatic and requiring treatment of underlying cause | - |
| Conduction abnormality Atrio-ventricular heart block | None | Asymptomatic, not requiring treatment (e.g. Mobitz type 1 second-degree AV block, Wenkebach) | Symptomatic, but not requiring treatment | Symptomatic and requiring treatment (e.g. Mobitz type II second degree AV block, third degree AV block | Life-threatening (e.g. arrhythmia associated with CHF, hypotension, syncope, shock |
| Supraventricular arrhythmias( SVT/atrial fibrillation /flutter) | None | asymptomatic, not requiring treatment | symptomatic, but not requiring treatment | symptomatic and requiring treatment | life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock) |
| Cardiac-ischemia/ infarction | None | non-specific T–wave flattening or changes | asymptomatic, ST– and T-wave changes suggesting ischemia | angina without evidence of infarction | acute myocardial infarction |