Observational Study of Hepatitis B Virus (HBV) in Patients Coinfected with Human Immunodeficiency Virus (HIV)

Abstract

Since the introduction of highly active antiretroviral therapy (ART) in 1996, there has been a dramatic reduction in morbidity and mortality among those living with HIV. However, chronic liver disease due to coinfection with hepatitis B (HBV) or C (HCV) virus has emerged as the second leading cause of mortality among HIV-infected persons. The natural history of HBV infection is altered in those with HIV. Current guidelines recommend that most coinfected patients be treated for both HIV and HBV infection using combinations of ART that include tenofovir (TDF). Despite widespread adoption in the US, the effect of this regimen on long-term outcomes of HBV disease such as histologic severity, progression, and risk of emergence of resistant HBV variants, and the long term risks of TDF therapy remains unanswered. Further investigation is required to address the following important questions: **(1)** what is the proportion of HIV-coinfected patients who have incomplete viral suppression on TDF?; **(2)** is incomplete suppression of HBV acceptable in HIV coinfected persons and if so, what threshold HBV DNA level constitutes an adequate clinical goal?; **(3)** in view of the lack of acceptance of liver biopsy among HIV practitioners, can noninvasive markers accurately assess HBV disease activity and the impact of ART on disease progression?; **(4)** What are the long term risks of TDF-based therapy for HBV in HIV coinfection? In short, what are the risks and benefits of TDF-based therapy for CHB in patients with HIV coinfection?

The NIH Hepatitis B Research Network (HBRN) is the first major effort to elucidate the natural history and treatment outcomes of persons with chronic HBV in the US. The current proposal, an approved ancillary study of the HBRN, offers a unique opportunity to fill major gaps in HBV-HIV knowledge and to compare HBV-HIV infected persons to those with HBV monoinfection participating in the HBRN.

Aims

1. To clinically, histologically, serologically, and virologically characterize a well-defined cohort of HBV-HIV patients in North America in a cross-sectional manner;

2. To longitudinally determine the impact of complete vs. incomplete viral suppression on clinical and serologic outcomes, and histologic progression by paired biopsy and
    - Define a threshold HBV DNA level associated with disease progression;
    - Establish the utility of noninvasive assessment of hepatic fibrosis compared with biopsy; and
    - Define the frequency of genotypic and phenotypic TDF resistance with long term therapy.

3. To define the risk of long term therapy. We will assess the long term renal and bone effects of TDF-based therapy in the HBV-HIV cohort.

Collectively, this study will fulfill many of the key priorities outlined in the NIH Action Plan for Liver Disease for HBV-HIV coinfection.

Type of study
Observational

HBV-HIV Cohort Study
Patients with HIV/HBV will be recruited in this prospective cohort study at 8 sites in the US and Canada. Patients will have baseline and follow-up evaluations every 24 weeks for up to 4 years. Histologic, virologic, serologic, and biochemical assessments will be made at study entry and every 24 weeks for up to four (4) years.

We will enroll HBV-HIV patients into a longitudinal cohort study. Liver histology at entry or within 36 months before entry will be assessed by the central pathologist of the HBRN for activity, fibrosis, and steatosis in a manner blinded to patient identity and HIV coinfection status and stained for HBV core and surface antigen. It is recognized that incomplete HBV suppression occurs frequently in HIV coinfected persons (up to 50% in our preliminary studies). What is not known are the clinical, histologic, and virologic outcomes of complete vs. incomplete suppression. To answer this aim, patients will be seen every 24 weeks for repeat assessments and monitored for outcomes. Those patients with compensated liver disease, who have had at least three years of observation, will have a follow-up liver biopsy to assess disease progression.

Patients with HBV-HIV infection will be identified by sites participating in the NIH-funded Hepatitis B Research Network. Consenting patients who meet entry criteria will undergo a baseline evaluation. They will then be followed longitudinally to observe clinical outcomes and changes in their virologic and immunologic status.

Inclusion Criteria
- Male and female subjects ≥ 18 years of age
- Serologic evidence of HIV infection by HIV antibody positivity or history of positive HIV-RNA prior to screening
- Serologic evidence of chronic hepatitis B infection by HBsAg positivity
- Currently receiving any type of anti-retroviral therapy for HBV or HIV
- Willingness to provide informed consent.

Exclusion criteria.
- Estimated life expectancy of less than one year based on clinical judgment of the investigator
- History of hepatic decompensation based on clinical or laboratory criteria
- Hepatocellular carcinoma (HCC)
- HCV RNA positive within 6 months prior to the baseline biopsy
- History of solid organ or bone marrow transplantation
- Pregnant women
- Medical or social condition which, in the opinion of the study physician, would make the patient unsuitable for the study or interfere with or prevent follow-up per protocol
- Unable or unwilling to return for follow-up visits
- Contraindications to liver biopsy.

We have kept the inclusion criteria broad to allow our results to be generalizable to the majority of HBV-HIV patients. We have excluded those who have limited life expectancy, have already reached an outcome of hepatic decompensation or HCC, or who are unwilling to return for follow up visits.

Briefly, the standard visit schedule for participants will be at baseline and then at six (6) month intervals for up to four years (up to 192 weeks). Variable follow up visits may be required if certain outcome events are encountered. The following **outcome** events will be noted:

- ALT Flare.
- HBsAg or HBeAg loss
- Cirrhosis
- Hepatic decompensation
- Hepatocellular carcinoma (HCC)
- Death

1. Background and rationale

1.1. Historical background and goals

Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) infect 400 million and 42 million persons worldwide, respectively[1]. Due to shared routes of transmission, coinfection with HBV and HIV is common. Cohorts of HIV-infected individuals assembled in Europe and the USA have noted HBV coinfection in 5-15% [2-7] and antibody to HIV is associated with a 25 fold increase in HBV infection [8]. Initially, the morbidity and mortality associated with HIV infection dwarfed the complications of HBV infection. However, with major advances in antiretroviral therapy (ART), liver-related mortality has supplanted AIDS-related mortality as a major complication in HIV-infected persons[9]. Despite the widespread use of antiviral agents active against both HBV and HIV, liver-related mortality remains the second leading cause of death among HIV infected persons. The Multicenter AIDS Cohort Study (MACs) reported in 2002 that of the 2559 HIV infected individuals, 8.3% were HBV-coinfected. The liver-related mortality rate in this cohort was 1.1/1000 person years, and was higher in men with HBV-HIV coinfection (14.2/1000) than in those with only HIV (1.7/1000, $p<0.001$) or only HBV infection (0.8/1000, $p<0.001$) [10]. However, patients in this cohort were likely treated with lamivudine (3TC), a nucleoside reverse transcription inhibitor (NRTI) that was subsequently determined to have a high rate of treatment failure [11-13]. Current HIV treatment guidelines from the Department of Health and Human Services recommend that all persons with HBV-HIV coinfection should be treated with ART containing tenofovir (TDF) plus 3TC or emtricitabine (FTC) [2-4, 14,15].

1.2. Hepatitis B Research Network

The Hepatitis B Research Network (HBRN) is a cooperative network of Principal Investigators and co-investigators from thirteen sites comprising 21 clinical centers, one Data Coordinating Center (DCC) and one Immunology Center. Clinical centers are responsible for proposing protocols, participating in their overall development, recruiting participants, conducting the research, and disseminating research findings. The individual clinical centers participate in a cooperative and interactive manner with one another and with the DCC, the Immunology Center and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in all aspects of the HBRN. The DCC supports protocol development; provides sample size calculations, statistical expertise, forms, and data analysis; supports manuscript preparation; and provides overall study coordination and quality assurance, including coordinating the activities of the Steering Committee and other standing committees. The DCC also collaborates with the NIDDK Biosample (plasma, serum, and liver tissue) and Genetics (DNA) Repositories. A Steering Committee composed of the principal investigators of each clinical center in the Network, the principal investigator of the DCC, the principal investigator of the Immunology Center, and the NIDDK Project Scientist is the main governing body of the HBRN. The Steering Committee has primary responsibility for the general organization of the HBRN, finalizing common clinical protocols and facilitating the development of a standardized nomenclature, diagnostic criteria,

histological definitions, and the necessary components to the common database on participants. The Steering Committee is responsible for the conduct and monitoring of studies and reporting of study results.

1.3. Scientific background

1.3.1. HBV-HIV introduction

Despite the well established guidelines on what drugs to use in the management of individuals with HBV-HIV coinfection [2-4, 14, 15], there are limited prospectively collected data on the extent of HBV control. For example, a recent study from France suggests that HBV viremia was undetectable in the majority (71% of 205) [16]. A more recent study from the Netherlands showed 92% of 67 patients with HBeAg and 100% of 15 patients without HBeAg had undetectable HBV DNA after 5 years of TDF [17]. Our preliminary data in North American patients (see **table 1**) suggests that up to 50% of HBV-HIV patients have detectable HBV DNA in the setting of ART that includes TDF. In support of our data, a recent abstract in 142 HBV-HIV coinfected subjects initiating ART identified 56% had incomplete viral suppression including 41% in those with undetectable HIV RNA suggesting mechanisms other than adherence (CROI 2012). Whether this is due to suboptimal drug potency, or treatment failure due to drug resistance or other factors such as immune dysfunction remains unknown.

Table 1: Characteristics of the patients at participating sites

	UW/HMC	VCU	UTSW	UCSF	UCLA‡	Wash Univ	JHU
Total number	115	66	51	172	21	67	216
Age	46(25-71)	44(27-73)	46(27-67)	46(32-66)	44-67	44(20-61)	47(43–53)
Gender [n(%) male]	107(93)	61(92)	48(94)	157(91)	21(100)	5(88)	167 (77)
Race [n(%)]							
White	73(64)	27(41)	28(55)	104(61)	16(75)	14(21)	71(29)
Black or African American	33(29)	39(59)	22(43)	52(30)	5(25)	53(79)	145(71)
Asian/Pacific Islander	5(4)	0	1(2)	12(7)	0	0	0
American Indian/Alaska Native/other	4(3)	0	0	12(7)	0	0	0
On ART [n(%)]	100(87)	58(87)	49(96)	153(89)	21(100)	63(94)	196(91)
Current HBV treatment [n(%)]							
TDF and emtricitabine	54(47)	30(54)	27(53)	86(50)†	16(75)	49(73)	NA
TDF and lamivudine	10(9)	5(9)	14(27)		5(25)§	11(16)	NA
TDF	24(21)	17(31)	7(14)	67(39)		0	NA
Adefovir	0	0	0	0		2(3)	NA
Entecavir	8(7)	0	7(14)	0		1(1.5)	NA
Interferon (ever)	3(3)	0	0	0		1(1.5)	NA
ALT (U/L)	30(11-380)	39(9-336)	65(6-388)	39 (9-347)	18-58	30(7-373)	
HBeAg [n(%)] positive*	33(38)	9(13)	39(76)	52(58)	16(76)	20(30)	NA
HBV DNA (log10)	4.8 (1.7-8.5)	4.4(2.3-8.8)	6.3(2.6-7.3)	3.9(1.7-9)	NA-8.0	4.3(1.5-8.7)	NA
HBV DNA [n(%) undetectable]*	77(68)	34(52)	26(51)	73/127(57)	9(43)	28(42)	NA
HDV [n(%)] positive*	7(6)	6(9)	0	0	0	0	NA
HCV [n(%)] positive	3(3)	8(12)	9(18)	61(35)	0	2(3)	NA
Absolute CD4	459(21-1386)	387(24-1536)	522(17-1463)	391(6-1071)	230-514	337(20-1092)	413(233 – 603)
CD4%	27(2-54)	22(3-57)	26(4-47)	289(11-1069)	NA	19(1-47)	NA
HIV RNA [n(%) undetectable]*	86(75)	41(61)	26(51)	82(60)	10(48)	45(67)	NA

Because liver biopsy is rarely performed in HBV-HIV patients, the spectrum of disease severity in the era of TDF-containing ART is unknown. Prior to effective ART, Colin and colleagues compared HBV DNA and liver histology in 67 anti-HIV negative HBV+ patients with 65 HBV-HIV coinfected patients and found that coinfected patients had lower ALT (103 vs. 188 U/L; p=.0001), higher HBV DNA (p=.01), and were more likely to have cirrhosis (28% vs. 13%; p=.04) despite similar inflammation scores [18]. More recently, Lacombe and colleagues reported histologic findings on 104 HBV-HIV patients between 2002 and 2003 and found F2-4 (out of 4) fibrosis in 67% [19]. In a multivariate analysis, HBV genotype G (OR 12.6), use of efavirenz (OR 3.55), and HIV duration > 9.5 years (OR 3.86) were independent predictors of significant fibrosis. They did not find any association with use of "anti-HBV drugs" (3TC, TDF, or interferon) and fibrosis. No data on associations between HBV DNA or HBV e antigen/antibody and fibrosis were given. A later report from the same French group of 134 HBV-HIV patients found HCV and HDV coinfections but not use of 3TC or TDF to be associated with increased fibrosis [20]. Finally, another study from France in 59 HBV-HIV patients (33 of whom were on LAM or FTC combined with TDF), found that 40% had inflammation (A1-A3), 61% had fibrosis (34% with advanced fibrosis), and 10% had >10% steatosis [21]. Therefore, there are limited data on the histologic spectrum of liver disease in HBV-HIV patients treated with long-term TDF and no data from North America.

1.3.2. Predictors of advanced fibrosis

Table 2 compares the characteristics from our preliminary data cohort (Table 1) of those with advanced fibrosis (stage 3-4) to those with less fibrosis (stage 0-2). Patients with HCV were excluded. Those with advanced fibrosis tended to have higher AST, lower HBV DNA levels and CD4 counts. Importantly, there was no difference between groups with respect to HBeAg status or presence of HDV co-infection suggesting that these factors are not useful in identifying those with significant disease. In a subset of this group with available retrospective data (n=23), HBV DNA <1000 IU/mL was not associated with disease severity, illustrating the importance of studying a larger, well characterized group of patients to further assess the impact of incomplete HBV suppression on disease severity. Although the APRI, a non-invasive model of fibrosis used in HCV, was higher in those with advanced fibrosis, the AUROC was only moderate for differentiating advanced fibrosis or cirrhosis from more mild disease [0.68 (95% CI 0.48-0.89) and 0.68 (0.61-0.75), respectively]. A second model, FIB-4, had slightly better performance for differentiating advanced fibrosis from lesser disease [0.78 (0.71-0.85)]. There were insufficient data to assess the performance of FIB-4 in predicting cirrhosis.

Table 2 . Characteristics of HBV-HIV co-infected patients who underwent liver biopsy

	All patients	Stage 0-2	Stage 3-4	P value
Number	53	22	31	
Age	42(10)	43(9)	41(10)	.63
Gender [n(%) male]	51(96)	22(100)	28(90)	.26
Race [n(%)]				
White	34(64)	14(64)	19(61)	.03
Black or African American	16(30)	4(18)	12(39)	
Other	3(6)	4(18)	0	
ART [n(%)]	35(66)	10(45)	25(81)	.02
HBV treatment [n(%)]				
TDF and emtricitabine	17(32)	7(32)	10(32)	.11
TDF	5(9)	0(0)	5(16)	
Lamivudine	15(28)	5(23)	10(32)	
ALT	57(73)	54(48)	65(106)	.35
AST	52(68)	47(30)	65(131)	.08
Platelet count	176(70)	210(91)	167(54)	.07
APRI	.8(1.2)	.5(.9)	1.2(1.5)	.02
FIB-4	1.8(1.5)	1.7(1.0)	1.9(2.8)	.16
eAg [n(%)]				
Positive	33(62)	13(59)	20(65)	.76
Negative	15(28)	7(32)	8(26)	
Unknown	5(9)	2(9)	3(9)	
HBV DNA (log10)*†	5.5(2.9)	7.3(2.5)	4.7(3.4)	.03
HBV DNA [n(%) <1000 IU/mL]†	16(35)	5(23)	11(39)	.53
HBV DNA [n(%) undetectable]†	14(30)	5(28)	9(32)	.51
HDV [n(%)]				
Positive	3(6)	2(9)	1(3)	1.0
Negative	27(51)	13(59)	14(45)	
Unknown	23(43)	7(32)	16(52)	
HIV CDC class (% A/B/C)**	57/9/34	79/0/21	43/14/43	.14
Absolute CD4	420(288)	496(368)	389(284)	.08
CD4%	25(13)	28(12)	25(14)	.14
CD4 nadir‡	164(356)	309(357)	110(144)	.04
HIV RNA (log10)*§	4.4(1.5)	4.3(1.5)	4.5(.4)	.47
HIV RNA [n(%) undetectable] §	20(63)	6(46)	14(74)	.15

Data listed as median (interquartile range) unless specified otherwise; stage assigned by Knodell in 24, Batts-Ludwig in 7, Metavir in 19. *In those with detectable virus; †Available in 46 patients; **Available in 35 patients; ‡Available in 23 patients; §Available in 32 patients.

1.3.3. Natural History and progression of HBV-HIV coinfection

It is known that HIV infection adversely influences the natural history of chronic HBV infection. HIV coinfection is associated with increased level of HBV DNA and higher risk of liver-related mortality [18]. Importantly, the frequency of mutation in the HBV genome differs between mono-infected and co-infected patients, even after adjustment for HBV DNA levels [22]. In a recent study from China in 846 HBV monoinfected patients with genotypes B and C, the authors identified several core promoter region mutations in genotype C associated with advanced disease and HCC [23,24]. However, the association between the mutational patterns and liver disease progression and liver-related clinical outcomes in HBV-HIV co-infected is not known. Furthermore, correlation between genotypic and phenotypic mutational patterns of HBV

genomes in those on TDF is poorly understood. Similarly, because not all patients completely suppress HBV DNA on TDF-based ART, a threshold HBV DNA level associated with liver disease progression is unknown. This lack of knowledge of the impact of HBV resistance patterns in incomplete HBV suppressors has limited our understanding of the drivers of HBV evolution under the selection pressure of drugs in HBV mono-infection and HIV-coinfections in patients on long-term TDF and is a major gap in knowledge that will be addressed in this study.

1.3.4. Liver fibrosis assessment

Liver biopsy is the gold standard for assessment of hepatic fibrosis or cirrhosis but it is an invasive procedure with a risk of significant bleeding of 1 in 2,500 to 1 in 10,000 and a risk of death of < 1 in 10,000. Liver biopsy is also subject to sampling error[25]; this is most likely why there are no prospective data, utilizing paired biopsy analysis or non-invasive markers, on liver disease progression in HBV-HIV subjects.

In view of the general lack of acceptance of liver biopsy among HIV practitioners, it is unknown whether there are noninvasive markers to accurately assess HBV disease activity and the impact of ART. During the last 15 years, there has been extensive research into non-invasive tests for hepatic fibrosis or cirrhosis. These tests include indices or algorithms based on routine laboratory tests, panels of serum fibrosis markers, liver stiffness measurement, and radiologic imaging [26]. Most of these studies were performed in patients with hepatitis C and showed that non-invasive tests are more accurate in detecting cirrhosis than in differentiating the earlier stages of fibrosis and that liver stiffness is more accurate than blood tests. Data on the performance of these non-invasive tests in patients with hepatitis B are limited [27-30]. Several European studies using liver stiffness measurements by elastography (Fibroscan) as a surrogate for histologic stage have been reported. Maida et al studied 37 HBV-HIV Italian patients on ART (26 on TDF); 57% had F0-F1 while 24% had bridging fibrosis (13%) or cirrhosis (11%). There was poor correlation between AST and liver stiffness and no association of advanced fibrosis with age, gender, HBV e antigen status, CD4 level, or HBV replication [31]. In a multicenter French study, Fibroscan was performed in over 1000 patients with viral hepatitis, of which 110 had HIV and coinfected with HCV or HBV [32]. The overall accuracy of Fibroscan was high (AUROC 0.77-0.86) with somewhat better results in those with coinfection compared to those with HCV or HBV alone. In a recent French study, Fibroscan in 59 HBV-HIV patients showed an AUROC of 0.85 for ≥ F2. 0.92 for ≥ 3, and 0.96 for ≥ 4 fibrosis [21]. Subjects with an elevated ALT (32% of the cohort) tended to have higher liver stiffness compared to those with normal ALT, especially in those with more fibrosis. The elevated ALT, a surrogate of liver inflammation, is known to increase liver stiffness and can result in misclassification of fibrosis stage [33]. Therefore, there are limited data on non-invasive assessment of disease progression in patients receiving long-term antiviral therapy in patients with HBV infection alone or with HIV coinfection.

1.3.5. New markers of HBV in monitoring disease activity with long-term treatment

There are few if any data on correlates of quantitative HBsAg (qHBsAg), HBV DNA and histology in those with HBV-HIV coinfection. A recent study in 149 HBV monoinfected patients showed good correlation of qHBsAg with serum HBV DNA (r=0.69), hepatic cccDNA (r=0.71), and total hepatic HBV DNA (r=0.76) in those who were HBeAg positive. However, in those who were HBeAg negative, the relationship of HBsAg to serum HBV DNA was poor (r=0.28) as HBsAg did not correlate with hepatic cccDNA or total circulating HBV DNA [34]. This study, however, did not examine the relationship of qHBsAg, hepatic HBV DNA and liver histology. Furthermore, it did not include HIV patients and no data on prior HBV therapy was given. Because many coinfected patients with viral suppression may continue to experience active

liver injury, novel markers of disease activity, such as qHBsAg may have an important role in this population.

1.3.6. Treatment of HBV in HIV

Despite its widespread use in HIV, the long-term renal and bone effects of TDF in HBV-HIV are unclear, specifically:

Renal Toxicity of TDF. In clinical trials and cohort studies, exposure to TDF for the treatment of HIV has been associated with a modest decrease in renal function as well as proximal renal tubular dysfunction which may be rarely (< 0.1%) associated with Fanconi syndrome (urinary loss of amino acids, glucose, uric acid, phosphorus, bicarbonate, and low molecular weight proteins)[35]. In a meta-analysis of 17 studies, Cooper et al found there was a significantly greater loss of kidney function among the TDF recipients, compared with control subjects (mean difference in calculated creatinine clearance, 3.92 mL/min; 95% confidence interval [CI], 2.13-5.70 mL/min [36]. In one small study, the effect of TDF on renal function was greater in HIV-HBV coinfected patients with advanced fibrosis [37]. The mechanism of renal impairment may be related to the effect of TDF on the proximal tubule which may be subclinical in nature. TDF use in HIV-infected patients has been associated with markers of tubular dysfunction including increased risk of proteinuria, phosphaturia and glucosuria [38]. The mechanism of renal tubular dysfunction is unclear but may due to specific mitochondrial DNA toxicity or polymorphisms and/or drug interactions with HIV protease inhibitors related to the transport of TDF by multidrug resistance-associated protein 4 (MRP4) [39].

Bone Toxicity of TDF. TDF has been consistently associated with decreased bone mineral density in multiple randomized, clinical trials in HIV-infected persons, both with initiation of ART [40-43] or switching ART regimens [44]. Most recently, TDF exposure was found to decrease BMD compared to placebo in high risk HIV-uninfected persons receiving TDF for pre-exposure prophylaxis [45]. The clinical impact of this effect is not clear, but may contribute to the high prevalence of osteoporosis and fracture observed among HIV-infected patients [46, 47]. Also unclear from existing data are the mechanisms underlying the effect of TDF on BMD. One of the leading hypotheses is that TDF induces subclinical phosphate wasting leading to impaired bone mineralization and lower BMD. TDF is associated with impaired bone mineralization in non-human primates [48]. In clinical studies, subclinical urinary phosphate wasting was seen in 41% of TDF-treated individuals and is correlated with increased alkaline phosphatase [49, 50]. TDF may also exert a more direct effect on bone metabolism. In *in vitro* models, TDF has been shown to alter gene expression in both osteoclasts and osteoblasts [51]. Although TDF is used commonly in HBV-infected patients both with and without concomitant HIV, there is very little data regarding the renal and skeletal effects in this population.

2. Overall Study Design

2.1. Participant enrollment procedures

Clinical center investigators and coordinators must be certified by the DCC to begin screening participants. Prior to implementing this protocol at an individual site, there must be Institutional Review Board (IRB) or Research Ethics Board (REB) for Human Research approval. Once a potential participant has been identified, details of the study, including possible risks and benefits, will be carefully discussed and he or she will be asked to sign a consent form. Treatment for HBV or HIV will not be offered as part of this HBV-HIV Cohort Study.

2.2. Screening

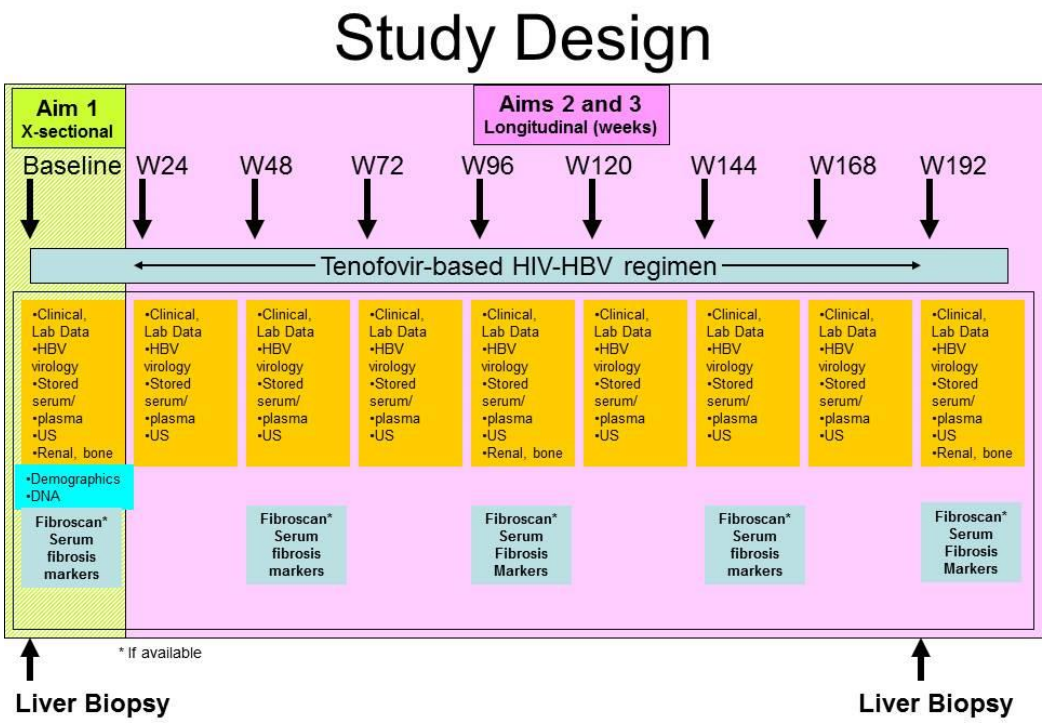
While many of the potential participants likely will be current patients of the HBRN investigators, patients also may be referred from physicians outside the HBRN and some patients may refer themselves. Existing patients who are familiar to the site Hepatitis B investigator may be

consented and screened at a visit that is part of the ongoing clinical care of the patient. Patients who are new to the HBRN center will undergo initial clinical evaluation to verify HBsAg and HIV status and other inclusion/exclusion criteria. The site investigators have extensive experience in enrolling patients with chronic viral disease in epidemiologic and natural history studies and clinical trials. A screening log will be maintained to capture the reasons for ineligibility of study subjects. Monthly reports that compare recruitment goals with actual enrollment will be compiled and consortium sites failing to meet their recruitment targets will undergo detailed review by the executive committee to identify additional methods to enhance enrollment.

2.2.1. Anticipated Barriers to Recruitment

Although we anticipate that some HIV care providers and HIV-infected patients with hepatitis B viral suppression may be reluctant to participate in liver biopsy, we will work to overcome this barrier through education about study goals and how the study will be used to advance knowledge of hepatitis B. Most patients with HBV-HIV have had HBV for a period of time when they were either not on any HIV or HBV therapy or on ineffective HBV therapy as part of ART (such as 3TC). During this period of ineffective HBV suppression, patients could have developed significant liver damage that is not reflected by current laboratory studies. Our preliminary data support this concept (see below).

2.3. Study Design. We will enroll HBV-HIV patients into a longitudinal cohort study. At entry, the data will be collected (**Table 3 and detailed in Appendix D**). Liver histology at entry or within 36 months before entry will be assessed by the



HBRN central pathologists for activity, fibrosis, and steatosis in a manner blinded to patient identity and HIV coinfection status and stained for HBV core and surface antigen. Patients will undergo ultrasound (US) for HCC screening every 24 weeks and Fibroscan every 48 weeks (if available). All participants will contribute to aim 1. Those with adequate follow-up will contribute

to aims 2 and 3. The preexisting HBRN infrastructure will be a significant asset for the accomplishment of these aims.

Table 3: Data Collection

Data type	Specific Details
Demographics	Age, race, gender, ethnicity, country of origin, education, family history, HIV risk factors, health behaviors, medical history, history of AIDS defining illness(es)
Clinical exam	Physical exam, height, weight, waist circumference, BMI, assessment for lipodystrophy
HBV treatment history	Specific agents and their duration
HIV treatment history	Current and past ART
Alcohol history Quality of Life (QOL) Symptom assessments	AUDIT and health behaviors SF-36 version 2 Brief symptom and fatigue questionnaires
Routine local labs	CBC, ALT, AST, ALP, Bilirubin (total, direct, indirect), albumin, creatinine, calcium, phosphate, anti-HCV, abdominal imaging
HIV-related	HIV-1 RNA quant, entry and nadir (by chart review) CD4, CD4%, CD8, CD8%
HBV-related	HBsAg, HBeAg, Anti-HBe, and HBV DNA quant, and anti-HDV, HBV genotype and subtype and HBV precore/BCP, HBV genotypic and phenotypic resistance (central virology lab and CDC)
Renal	Urinalysis for protein, creatinine, and glucose, fasting urine and serum phosphate and creatinine
Bone-related	C-terminal telopeptide of type I collagen (CTx), procollagen type I N-terminal propeptide (P1NP), 25 hydroxyvitamin D, intact Parathyroid Hormone (PTH)
Metabolic	fasting glucose, insulin, lipids, Triglycerides,
Liver histology	At entry or within 36 months before entry
Non-invasive assessment	Fibroscan (if available), fasting serum for noninvasive markers of disease severity
Stored blood	Fasting serum/plasma and DNA banking

It is recognized that incomplete HBV suppression occurs frequently in HIV coinfected persons (up to 50% in our preliminary studies (**Table 1**). What is not known are the clinical, histologic, and virologic outcomes of complete vs. incomplete suppression. To answer this aim, patients will be seen every 24 weeks for repeat assessments (see figure above and **Table 4 and detailed in Appendix D**) and for outcomes defined below.

Patients with compensated liver disease, who have had at least three years of observation, will have a follow-up liver biopsy to assess disease progression. This biopsy will be covered by the study.

Table 4. Schedule of tests and visits

Tests/Measures	Baseline	Frequency
Medical history, medication history, physical exam, anthropometrics, outcomes	X	Every 24 weeks
Questionnaires: QOL, health behaviors, AUDIT, symptoms, fatigue (baseline, weeks 96 and 192)	X	Every 48 weeks
Routine laboratory and virologic tests, CTP, MELD, AFP	X	Every 24 weeks
Liver biopsy*	X	Week 192 ± 52 weeks
Liver ultrasound	X	Every 24 weeks
Fibroscan (if avialable), serum for non-invasive models	X	Every 48 weeks

Urinalysis, fasting urine and serum creatinine, protein and phosphate	X	Every 48 weeks
Stored serum/plasma	X	Every 24 weeks

*A follow-up liver biopsy will not be performed for participants who are in the study for less than three years.

2.3.1. Evaluation of the clinical relevance of quantitative HBsAg (qHBsAg), quantitiatve HBeAg (qHBeAg) and novel noninvasive markers to assess disease severity and progression of liver disease

For both the cross sectional and longitudinal Aims, serum qHBsAg, qHBeAg and other non-invasive markers will be assessed at baseline and every 24 weeks and concurrent with the second biopsy. Non-invasive models will include the APRI, FibroSURE®, FIB-4, Fibroscan and non-invasive studies that synergize testing for "Liver fibrosis severity" in parallel with the HBRN main study. The longitudinal assessment of noninvasive markers will permit the accomplishment of two important goals: (1) to assess the accuracy of noninvasive markers in a paired biopsy study, and (2), to assess the performance of these markers in defining the natural history of HBV related liver disease in persons with HIV coinfection on TDF. Because our follow-up period is only up to 4 years, we will assess the association or prediction of non-invasive tests on all clinical events. Because direct comparison to the HBV monoinfected cohort would be biased by the frequent receipt of antiretroviral therapy with dual anti-HBV effects in the HIV+ subjects, the HIV-negative comparison group will consist of those subjects entering and being assigned to receive TDF in one of the arms of the randomized clinical trial being performed by the HBRN.

3. Main Outcomes to be assessed

3.1. Histologic: Liver disease progression. Once scored, inflammation, fibrosis, steatosis, and core and surface antigen will be assessed for changes between initial and follow-up histology.

3.1.1. Serologic: HBeAg and HBsAg loss are considered to be significant events in the natural history of HBV infection and are usually associated with biochemical and histologic improvement. However, the impact of changes in qHBsAg and qHBeAg that do not result in HBeAg or HBsAg loss on clinical and histologic outcomes are unknown. These tests will be done at the core virology laboratory of the HBRN.

3.1.2. Virologic: HBV DNA level, HBV genotype and subtype and HBV precore/BCP mutations will be assessed by the core virology lab of the HBRN at the CDC (see letter of support). Using standard techniques, genotypic substitutions and phenotypic TDF resistance patterns (change in TDF IC50 of isolate harboring the genotypic substitution) will be assessed in those with detectable HBV DNA.

3.2. Liver-related clinical events

3.2.1. Hepatitis exacerbation marked by ALT Flare. A flare is defined as serum ALT greater than or equal to 10 times the upper limit of normal (30 IU/L for male and 20 U/L for female) which corresponds to 300 IU/L in males or 200 IU/L in females. Detailed history for precipitating etiologies will be performed to determine if the flare is due to non-compliance (concurrent increase in HIV RNA), immune reconstitution (marked increase in CD4), inadvertent omission of TDF (pharmacy records), drug induced liver injury- DILI (patient history and pharmacy records), alcohol use, HBsAg or eAg seroconversion, or HAV, HCV, or HDV superinfection.

3.2.2. Antigen loss: e and s

HBeAg or HBsAg loss are considered to be significant events in the natural history of HBV infection and are usually associated with biochemical and histologic improvement. Loss of these viral markers may also be associated with appearance of corresponding antibodies in

serum (anti-HBe or anti-HBs). HBsAg loss appears to represent a “cure” of HBV infection and is associated with reduction, but not necessarily elimination, of the risk of future complications, such as HCC which may still occur, particularly in those who lose HBsAg at an older age (e.g. after 50 years) or after the development of cirrhosis. When HBeAg or HBsAg loss occurs, participants will be followed more closely initially and then return to the regular follow-up schedule.

3.2.3. Cirrhosis
The diagnosis of cirrhosis will be made by (1) liver histology, when available or (2) clinical criteria. Once cirrhosis is diagnosed, follow-up will include HCC surveillance. HCC surveillance will also be performed in non-cirrhotic participants who meet AASLD (95) guidelines criteria.

3.2.4. Hepatic decompensation.
Development of hepatic decompensation will be defined by any of the following events:

- Ascites or hepatic hydrothorax
- Variceal or portal hypertensive bleeding
- Hepatic encephalopathy
- Child-Turcotte-Pugh (CTP) score of 7 or above

3.2.5. Hepatocellular carcinoma (HCC). HCC may be detected by routine surveillance or may become clinically apparent. The diagnosis of HCC will be made using the AASLD criteria.

3.2.6. Death. Death may occur related to liver disease (typically hepatic decompensation or HCC) or may occur unrelated to hepatitis B or liver disease. Date and cause of death (liver or non-liver) will be recorded.

3.3. Renal complications. Creatinine clearance (CrCl) will be calculated by CKD-EPI [52-54] to accurately assess changes in CrCl above a GFR of 60 ml/min/1.73m^2. In those with a CrCl less than 60, urine protein/creatinine ratio (24-hour urine) and glucose will be measured to assess proximal tubular function. Excretion of phosphate (described below) will also be measured, as this relates to bone disease. TDF dose modification due to decreased eGFR will be correlated with HBV suppression and disease outcomes.

4. Additional laboratory studies
4.1. Measures of Phosphate Homeostasis, Tubular phosphate reabsorption will be estimated by determination of the ratio of maximal reabsorption capacity (TmPO4)/glomerular filtration rate (GFR) which is calculated using fasting serum and urine creatinine (sCr, uCr) and phosphate (sPh, uPh) in the following formula: sPh - ((uPh*sCr) /uCr)))[55]. A $TmPO_4$/GFR ratio lower than 2.5 indicates significant urinary phosphate wasting. This measurement will be done at baseline and 48 week intervals.

4.2. Bone markers: We will measure C-terminal telopeptide of type I collagen (CTx) as an established marker of bone resorption and osteocalcin and procollagen type I N-terminal propeptide (P1NP) as markers of bone formation[56]. These markers will be measured at the VCU Laboratory. These measurements will be used to assess the relationship of baseline and changes in BMD to these markers.

4.3. 25 hydroxyvitamin D: 25(OH)D is the major circulating form of vitamin D and precursor to the active form. It is most commonly used for the determination of vitamin D deficiency because the serum half-life is long (3 weeks); therefore, this form serves as an adequate measure of vitamin D stores obtained from both UV light and dietary intake over time. In addition, recent

data suggests that the effect of TDF on calcitropic hormones may differ by vitamin D status [57]. Liquid chromatography-tandem mass spectrometry will be used for determination of 25(OH)D levels which is thought to be the gold standard [58].

4.4. Parathyroid Hormone (PTH): PTH is one of the major regulators of calcium and phosphate metabolism. Cross-sectional studies and clinical trials have demonstrated that PTH is increased with TDF-exposure compared to other regimens [59,60]. We will measure intact PTH at baseline and year 4 using a commercially available assay (ALPCO).

5. Selection and enrollment of participants

5.1. Sources of participants

Participants will be recruited from the HBRN centers participating in this ancillary study. Patients who have been seen and are being followed in existing GI and liver clinics as well as those who are newly referred for consultation and seen for the first time at a HBRN center will be recruited.

Some HBRN investigators may engage in outreach activities from which HBV-HIV patients seen in primary care settings outside the HBRN centers may be accessed.

5.2. Inclusion criteria

- Male and female subjects ≥ 18 years of age
- Serologic evidence of HIV infection by HIV antibody positivity or positive HIV-RNA prior to screening
- Serologic evidence of chronic hepatitis B infection by HBsAg positivity
- Currently receiving any type of anti-retroviral therapy for HBV or HIV
- Willingness to provide informed consent.

5.3. Exclusion criteria

- Estimated life expectancy of less than one year based on clinical judgment of the investigator
- History of hepatic decompensation based on clinical or laboratory criteria
- Hepatocellular carcinoma (HCC)
- HCV RNA positive within 6 months prior to the baseline biopsy
- History of solid organ or bone marrow transplantation
- Pregnant women
- Medical or social condition which, in the opinion of the study physician, would make the patient unsuitable for the study or interfere with or prevent follow-up per protocol
- Unable or unwilling to return for follow-up visits
- Contraindications to liver biopsy.

5.4. Participant enrollment procedures

Prior to implementing this protocol at an individual site, there must be Institutional Review Board (IRB) or Research Ethics Board (REB) for Human Research approval. Once a potential participant has been identified, details of the study, including possible risks and benefits, will be carefully discussed and he or she will be asked to sign a consent form. Treatment for HBV will not be offered as part of this Cohort Study.

6. Baseline evaluation

After informed consent is obtained, the Baseline Evaluation will be done and may, in some instances, be completed over more than one visit; however, all baseline procedures must be

completed within 12 weeks. The participant will be instructed to come fasting for the baseline visit. The Baseline Evaluation will consist of:

- Questionnaires to be completed by the participant, including Alcohol Health behavior questionnaire (AUDIT)[61], Brief symptom questionnaire, QOL, Fatigue questionnaire
- Questionnaire to be completed by the coordinator
- Questionnaire to be completed by a Physician Investigator
- Medical history
- Medication history
- Brief physical examination
- Measurement of vital signs
- Demographics
- Family history, risk factors
- Blood will be drawn for laboratory tests related to HIV, hepatitis B and liver disease
- Blood will be drawn to prepare and obtain serum or plasma for storage
- Blood will be drawn (with consent) for DNA extraction and genetic testing

6.1. Participant-completed forms

The questionnaire to be completed by the participants collects data and information on basic demographics and health behaviors (e.g. smoking and consumption of coffee, tea, and alcohol). In addition, participants will complete a brief survey of symptoms related to liver disease, a fatigue questionnaire, and a QOL form. In non-English speaking participants, a version translated into the participant's native language may be used. If a participant requires assistance completing these forms, this will be recorded.

6.2. Coordinator interview and forms

A coordinator will collect information such as family history, past medical history, antiviral therapy for HBV and HIV and medication history. Given practical considerations, medication history will be obtained for various classes of medications taken by participants.
In non-English speaking participants, the interview will be collected through an interpreter. While a trained translator is preferred, a family member may be acceptable for this role.

6.3. Physician Investigator completed form

A physician investigator will record his or her opinion of items such as the most likely source of the HIV and HBV infection, the most likely time of onset of the HIV and HBV infection, whether the participant is symptomatic of hepatitis B, or HIV, and the HBV phenotype that best describes the participant.

6.4. Physical examination

Vital signs will be done and recorded by the coordinator. A physical examination will be done by a physician or other qualified practitioner focusing on physical signs associated with HIV and liver disease.

6.5. Laboratory data (see Appendix D)

6.5.1 Blood tests related to the standard of care of HIV, viral hepatitis and liver disease

When the following laboratory tests are done as a part of routine care, the results will be obtained from the participant's records. Data available 12 weeks or less prior to the date of the initial baseline visit may be used.

- CBC
- Hepatic panel (ALT, AST, ALP, bilirubin, albumin)

- Basic metabolic (fasting)
- Serum calcium and phosphate
- INR
- AFP
- Urinalysis
- HIV-RNA quantitation
- CD4, CD4%, CD8, CD8%

6.5.2. **Historical lab data**
The following serologic/virologic data may be obtained from participant records.

- HBV DNA level
- HBsAg
- HBeAg
- anti-HBe
- anti-HDV
- anti-HCV
- anti-HBs
- anti-HIV or positive HIV-RNA at or prior to Baseline
- Autoimmune markers (if available).

The most recent serologic/virologic results will be recorded along with the month and year when the sample was drawn. If recent HBV and HIV results are not available from the participant's records within the prior 6 months, it is recommended that blood be drawn for these tests. For other serologies (anti-HCV, anti-HIV) and for HBV genotype, there will be no time limits.

6.5.3. Lab tests
Samples for the following study-related lab tests will be collected and batch shipped to a central laboratory:

Fasting insulin
Fasting total cholesterol, LDL, HDL, triglyceride
Quant HBsAg, HBeAg
Fibrosure
25 OH-Vitamin D
Serum for HBV tests (HBsAg/anti-HBs/HBeAg/anti-HBe/HBVDNA, HBV Genotype)

The following study-related lab tests will be done locally:

Fasting urine calcium, creatinine and phosphate

6.5.4.Serum/plasma for banking
Blood will be drawn at the Baseline Evaluation and plasma and serum samples will be banked. Standardized methods for plasma and serum processing will be followed. Plasma and serum samples will be stored locally and ultimately shipped to a central repository for storage to be used for centralized tests and future studies to determine phase of hepatitis B and possibly to predict disease outcomes and response to antiviral therapy.

6.5.5. Liver histology
Information about liver biopsies obtained as part of standard of care will be collected.
For participants who have had a liver biopsy within 36 months of enrollment in the HBV-HIV Cohort Study, unstained slides of the biopsy specimen will be requested from the institution where it was done, and shipped to the central pathology site for staining and ultimately

central review. For participants in whom no liver biopsy has been done within 36 months of enrollment, a liver biopsy will be scheduled during the Baseline visit window. Biopsy slides will be sent for central pathology reading. In situations in which adequate tissue is obtained (greater than 2.5 cm), the residual tissue will be banked for future studies.

6.5.6. DNA for banking

For participants who consent, blood samples will be drawn and DNA will be banked. Standardized methods for DNA processing will be followed. If not at baseline, this can be done at any follow up visit.

7. Follow-up evaluation

Routine follow-up visits Participants will be followed at predetermined intervals planned to coincide with accepted standard of care visits for managing patients with HBV-HIV
Routine follow-up will be every 24 weeks for up to 192 weeks.

The Baseline visit may be completed in more than one visit; however, all of the baseline procedures outlined in Appendix D of the protocol must be completed within 12 weeks
Additional follow-up visits may be performed under special circumstances or when clinically indicated

7.1. Routine scheduled follow-up data items to be completed every 24 or 48 weeks, or as noted in Appendix D

Participant assessments including: Health behaviors, AUDIT, QOL, Fatigue, symptoms
Medication history
Height, weight, waist circumference (yearly)
Vital signs
Brief physical exam
Blood tests (see Appendix D)

7.2. Unscheduled follow-ups

Potential reasons for unscheduled visits may include abnormal liver tests including ALT flares, hepatic decompensation, suspicion of hepatocellular carcinoma, change in HBeAg or HBsAg status, development of significant non-hepatic condition that affects the management or prognosis of their hepatitis B, and pregnancy. Data items captured at the unscheduled visits will be a subset of those items captured at the routine scheduled follow-up visits, unless specified otherwise in the sections below. Questionnaires will be administered to pregnant patients (who become pregnant after Baseline) during pregnancy and following delivery. Blood specimens for central testing and storage may be collected at the time of unscheduled visits (e.g. liver biopsy, ALT flare). These specimens will be processed according to the same procedure as routine follow-up samples.

7.2.1. Participants experiencing an ALT flare or HBeAg or HBsAg loss

When the routine follow-up schedule is interrupted by an ALT flare or HBeAg/HBsAg loss, a data collection mechanism will be activated as indicated below. Once the flare is resolved, the participant will return to the routine protocol follow-up schedule and return for the protocol evaluation that is closest to, but after, the flare resolution date. If the flare resolution date occurs within 4 weeks of the next scheduled protocol visit, the protocol evaluation visit may be performed in conjunction with the unscheduled visit.

7.2.1.1. Evaluating participants experiencing an ALT flare

Flares are defined in section A.4. and Appendix B. Examples of data to be collected include:

- Medications
- Other risk factors for acute viral hepatitis or other liver disease
- Precipitating event information to determine whether the flare is HBV-related or due to another event
- Clinical decisions (e.g., start on antiviral treatment, list for transplantation) made in response to the flare will be recorded at the end of the flare

7.2.1.2. Follow-up for participants experiencing an ALT flare

- Once an ALT flare is diagnosed, the follow-up intervals will be every 4 weeks unless ALT is greater than 1000 IU/L or total bilirubin is greater than 2.5 mg/dl in the absence of Gilbert's syndrome, then follow-up intervals will be every 2 weeks.
- An ALT flare is considered to be resolved when the participant no longer meets the ALT definition of flare, ALT must drop to below 300 IU/L in males or below 200 IU/L in females. When the ALT flare is considered to be resolved, the follow-up will revert to the original follow-up schedule.

7.2.1.3. Follow-up in participants achieving HBeAg or HBsAg loss

The participant will be seen 12 weeks after the visit during which loss occurred, and again 24 weeks after the visit during which loss occurred, and then revert to their original follow-up schedule. The unscheduled follow-up data collection adequately captures information of interest.

7.2.2. Participants who become pregnant

Participants who become pregnant after enrollment will be followed more closely for the duration of their pregnancy and for 72 weeks after delivery. For these participants, additional questionnaires will be completed. At the visit 72 weeks post-delivery, a questionnaire on immunization and HBV testing of the infant will be completed.

7.2.2.1. Follow-up in participants who are pregnant

Participants who are pregnant will return for visits that may coincide with a scheduled protocol visit or require extra visits. A visit will occur the first or second trimester and on or after 28 weeks gestation. After delivery, data will be collected 12, 24, and 72 weeks later. Should a woman become pregnant more than once during the study, special visits and data collection will be performed for each pregnancy as described above.

7.3. Follow-up in participants newly diagnosed with cirrhosis

There are no specific data to be collected in participants who are newly diagnosed with cirrhosis. The regular follow-up data collection adequately captures information of interest. The diagnosis of cirrhosis does not change the follow-up interval. However, all participants with cirrhosis will undergo HCC surveillance consisting of serum AFP measurement and liver ultrasound every 24 weeks.

7.4. Follow-up in participants experiencing clinical events

Hepatic decompensation and HCC are expected to be rare. Systematic follow-up more than once every 24 weeks is not mandated for participants experiencing any of these events. As discussed below, detailed information at the time of the diagnosis of these complications will be obtained. There will also be data collection when liver transplantations or deaths become known.

7.4.1. Participants experiencing hepatic decompensation
Systematic follow-up more than once every 24 weeks is not mandated for participants experiencing hepatic decompensation. Information about the event(s) that defined hepatic decompensation including hepatic encephalopathy, ascites, hepatic hydrothorax, and variceal or portal hypertensive bleeding will be obtained.

7.4.2. Participants newly diagnosed with HCC
Examples of information to be collected:

- Data to verify that the diagnosis of HCC could be established based on the AASLD criteria [72]. This includes imaging modality, characteristics of the lesions, serum levels of AFP and histology, when available.
- Information about the extent of the tumor and clinical staging.

7.5. Additional information collected at liver biopsy
Information will be collected regarding the technique and possible complications of the procedure.

7.6. Follow-up in participants undergoing liver transplantation
Date of transplantation, indication for transplantation and whether an incidental HCC was found. If an HCC was found, the HCC form will be completed. Follow-up ends with liver transplantation.

7.7. Participants who die
Date of death and cause of death will be collected.

8. Informed consent
In addition to a consent form for participation in the HBV-HIV Cohort Study, subjects may be asked to sign a separate consent for genetic testing.

9. Statistical and design considerations
9.1. Statistical analyses
9.1.1. Analysis Plan for Aim 1
Determine the clinical, virological and histological characteristics of a well-defined cross-sectional cohort of HBV-HIV patients. Baseline data will provide detailed characteristics of patients with HBV-HIV co-infection at the participating centers. Participants will be characterized by the distribution of demographic characteristics such as age, gender, race, ethnicity; medical history and sources of infection, CD4, CD4%, HIV RNA levels, presence or incidence of AIDS defining illnesses, antiretroviral therapy choice and duration, as well as baseline variables including liver histology, HBV DNA level and genotype, and HBV antiviral resistance pattern. Distribution of participants across different subgroups (e.g. race, ethnicity, HBV genotype, co-morbidities, high/low HBV DNA, high/low HIV RNA) will be reported using group-specific proportions and their 95% exact or approximate confidence intervals as appropriate. Continuous characteristics such as age, HBV DNA levels, HIV RNA levels, CD4 count and ALT will be summarized using mean and standard deviations or median and inter-quartile range depending on the distribution of the data. Distribution of ALT levels will also be investigated using the new lower limit of normal cut-offs of ≤ 30 IU/L for males and ≤ 20 IU/L for females. Comparison of such variables across subgroups (e.g. race, genotype) will be done by Wilcoxon (Kruskal-Wallis) or t-test (F-test), as appropriate. For assessing the association between categorical variables such as race and genotype, contingency tables will be used and Chi square or its exact equivalent for formal testing.

Liver Histology. Liver biopsy will be performed in the standard fashion at entry or within 36

months before entry and scored by the HBRN central pathologist for activity, fibrosis, and steatosis[62,63] (**Appendix E** for details of histologic assessment). Each of these three scores will be used separately as outcomes and their relationship to HBV DNA levels, presence or absence of TDF genotypic and phenotypic resistance, qHBsAg and qHBeAg levels, estimated duration of disease, HDV coinfection status, and alcohol history (by AUDIT) [61] duration of antiretroviral therapy, CD4, CD4%, HIV RNA levels and other demographic and clinical characteristics will be assessed. The Spearman correlation coefficient will be used to describe the correlation between each individual outcome and the covariates HBV DNA levels and quantitative serologies performed in the virology lab of the HBRN. We will investigate the association between the outcomes and discrete covariates such as gender, race, ethnicity, presence or absence of AIDS defining illnesses using Wilcoxon's rank sum test or Kruskal-Wallis test. We will use multiple regression analysis to adjust for important histologic confounders. We will use Ishak fibrosis score above 2 to define significant fibrosis and a score above 3 to define advanced fibrosis. The association between advanced fibrosis and patient characteristics and laboratory studies will be investigated using logistic regression analysis. Similar analysis will be conducted for inflammatory activity and the steatosis outcomes.

9.1.2. Analysis Plan for Aim 2

Longitudinally assess the clinical, histological, and virologic outcomes of patient with complete vs. incomplete viral suppression. For this analysis, the frequency and correlates of complete (<1000 IU/mL) vs. incomplete (≥1000 IU/mL) viral suppression will be assessed to coincide with the HBRN protocol. This will allow direct comparison to HBV monoinfection subjects who will receive 4 years of TDF as part of an HBRN clinical trial. We have chosen 1000 IU/mL as a definition for complete suppression because most inactive HBV carriers harbor DNA levels below this threshold [64]. We will estimate the percentage of patients with complete suppression of HBV in this cohort with a 95% confidence interval at each time point. The trend in HBV suppression over time will be investigated using generalized linear model with logit link to provide predicted odds of viral suppression over time. To correlate the viral suppression with the outcomes, a multivariable generalized linear logistic regression analysis that accounts for correlation among repeated observations will be performed. Potential predictors would include CD4, CD4%, HIV RNA levels, presence or incidence of AIDS defining illnesses, antiretroviral therapy choice and duration, as well as baseline variables including liver histology, HBV DNA level and genotype, HBV antiviral genotypic and phenotypic resistance pattern, and compliance with HIV medications. However, because the threshold associated with active hepatic inflammation and fibrosis is unknown, we will also compare those with active histology to those without to define the HBV DNA threshold associated with liver disease progression (see below).

Clinical outcomes, defined above, will be assessed at each visit and compared between complete and incomplete HBV suppression. We will use generalized linear mixed model with the incidence of event (e.g. HCC) as outcome and longitudinal data on complete viral suppression (binary) of HBV DNA as covariates adjusting for baseline characteristics to assess the association between incomplete suppression and the incidence of such events. The results will be summarized using adjusted odds ratios (OR) and 95% CI.

Liver disease progression. For analysis of liver specimens, biopsy material will be obtained from the entry biopsy of HIV coinfected subjects enrolling in the present protocol. If possible, we will compare their liver histology to HIV negative subjects in the parent HBRN cohort, matched for duration of TDF therapy. We anticipate that entry biopsies from the two groups will have a comparable duration of TDF use, consistent with the generally long term use of this agent.
The primary outcome for disease progression/regression will be a ≥ 2 point change in fibrosis between the baseline and follow-up liver biopsy to minimize the impact of sampling error.

Because some patients will have undergone biopsy up to 36 months prior to entry, we will also calculate a fibrosis progression rate as change in fibrosis/months x 12 (to be reported as change in fibrosis/yr) between biopsies. Change will be correlated with clinical and virologic data assessed both at baseline and during follow-up to compare complete vs. incomplete HBV suppression. Specifically, we will quantify the proportion of evaluation times for which there was a complete suppression and use it as a covariate in the regression model to assess its association with the histological progression.

Histologic Staining for HBcAg and HBsAg. Tissue staining for HBsAg and HBcAg on baseline and follow up biopsies will be compared between suppressors and incomplete suppressors.

Define threshold HBV DNA level associated with liver disease progression. To determine a threshold of HBV DNA beyond which improvement in histology is unlikely to be achieved, we will consider three measurement: (a) peak of HBV DNA (with an evaluation period of at least two years), (b) nadir of HBV DNA (with an evaluation period of at least two years), and (c) HBV DNA years, defined as the area under the HBV DNA curve (time expressed in years) divided by the number of evaluation years. We will conduct ROC analysis to obtain cut-offs for each of these three measurements above which no improvement in histological outcome (improvement defined as 2 point reduction in fibrosis) is likely. We will validate these cut-offs using cross-validation. A similar analysis will be conducted to identify cut-offs beyond which a reduction in the fibrosis score is unlikely. This will be conducted using a discriminant analysis.

Clinical relevance of qHBsAg, qHBeAg and novel noninvasive markers to assess disease severity and progression. The clinical relevance of qHBsAg and qHBeAg will be assessed by correlation with liver histology, HBeAg status, HBV DNA, and liver enzymes. We will construct non-invasive models for predicting hepatic fibrosis based on patient characteristics described in Aim 1 (excluding biopsy related variables) using multivariable step-wise logistic regression. All serum tests collected as part of the study will be assessed for normality and log transformed if necessary. We will assess all models using two binary endpoints: Ishak 0-1 vs. 2-6 and Ishak 4-6 vs. Ishak 0-3. We will compare the accuracy of this model with those of non-invasive tests of the HBRN. Published serum models will be assessed including APRI [65], FIB-4 [66], Forns Index [67], and Fibrosure® [68] based on published cut-offs. Because these models have not been validated in HBV-HIV patients, we will use area under ROC (AUROC) to determine optimal cut-points for each model for maximal sensitivity and specificity. Fibroscan, if available will be performed every 48 weeks and compared to baseline histology. We will also compare changes in liver fibrosis by biopsy to Fibroscan and both baseline and change in fibrosis of HBV-HIV subjects to the HBV monoinfected patients. Novel models that include Fibroscan alone and in combinations with demographics (age, gender) and serum tests (liver enzymes, qHBsAg, qHBeAg, platelets, CD4) will be developed.

Adherence vs. incomplete suppression. Adherence to medication will be assessed by detailed patient history and pharmacy records at each visit [69,70]. Persons with undetectable HIV RNA < 50 copies/mL will be defined as adherent to ART and those with undetectable HIV RNA and detectable HBV DNA on TDF-based ART will be defined as incomplete HBV suppressors. If both HIV RNA and HBV DNA are increased despite patient reported compliance, then HIV and HBV resistance testing will be performed to define adherence.

Sample size and Power Analyses. The sample size and power calculations are based on the primary aim of liver fibrosis progression. Based on our preliminary data, of over 500 HBV-HIV subjects at the 8 participating clinical sites, we anticipate approximately 250 subjects will

undergo liver biopsy for the longitudinal cohort study. We expect 5% of the cohort to drop out each year, so by year 4, at least 160 patients will be evaluable. Of these, we expect at least 100 to undergo a follow-up biopsy. Based on a range of percent of patients with complete HBV DNA suppression between worst case (50%) and best case (80%) scenarios, the shaded areas in Table 5 show several sample sizes (N) according to power (70-90%) and proportion of subjects (15-30%) showing a clinically significant (≥ 2 point change) in Ishak fibrosis score, assuming that those with complete suppression have ≤ 5% (essentially no change) chance of fibrosis progression over 4 years.

Table 5: Sample size (N), shaded cells	50% HBV DNA Suppression			80% HBV DNA Suppression		
	Power (%)			Power (%)		
% that will show a difference in ≥ 2 point change in Ishak fibrosis	70	80	90	70	80	90
15%	148	185	244	209	268	366
20%	98	122	161	136	175	238
25%	72	89	117	99	126	171
30%	56	69	90	77	97	131

Darker shaded cells represent N we expect to be able to enroll

9.1.3. Analysis Plan for Aim 3

Baseline Analyses. Our baseline analyses regarding bone metabolism and TDF will address the following questions: Is renal phosphate wasting (TmP/GFR) and higher markers of bone turnover in HIV/HBV patients treated with TDF? Are vitamin D deficiency (25OHD < 20 ng/mL) and secondary hyperparathyroidism associated with markers of bone turnover? Linear regression models will be used to assess the relationship between bone turnover (CTx, P1NP, OC)) and independent variables TmP/GFR, 25OHD, PTH, demographic variables (age, race, sex, smoking, alcohol use), HIV variables (CD4 cell count, duration of known HIV infection), body composition variables (BMI, lean mass, trunk fat, extremity fat, trunk fat/lower extremity fat ratio), liver disease severity by biopsy, and presence of concomitant medications that may affect bone metabolism, such as corticosteroids, proton pump inhibitors, warfarin. Results will be presented as regression coefficients and their 95% confidence intervals (CI). With respect to renal disease we will address the following: What is the prevalence of reduced eGFR (< 60 ml/min/1.73m^2) and dose modification of TDF due to renal disease? What is the prevalence of subclinical and clinical proximal tubule dysfunction? The association between reduced eGFR (dependent variable) and liver disease stage and duration of TDF exposure will be investigated using logistic regression models. In all analyses, stepwise methods will be used for independent variable selection.

Longitudinal Analyses. Our longitudinal analyses will address the following questions: We will use linear regression analysis to identify factors associated with TmP/GFR, vitamin D deficiency, bone turnover markers, demographic and HIV-related variables, and liver disease severity. What is the natural history of renal function in HIV-HBV coinfected patients treated with TDF? Changes in renal complications over time and its association with independent baseline variables such as age, race, sex, smoking, alcohol use, BMI, baseline TmP/GFR, vitamin D deficiency, bone turnover markers, and HIV-related variables will be investigated by fitting repeated measures linear mixed models with GFR as the dependent variable. Similarly, a generalized linear mixed model with appropriate link function (log or logit) will be used to analyze absolute $TmPO_4$/GFR ratio and significant urinary phosphate wasting ($TmPO_4$/GFR ratio < 2.5) over time adjusting for baseline demographic, clinical, virologic and treatment-related variables such as length of TDF use.

Expected results. We expect to describe the clinical, laboratory, and histologic characteristics of a heterogeneous cohort of HBV-HIV subjects in North America. We expect the majority to be on anti-retroviral therapy that includes TDF and that at least half of this cohort will have adequate HBV suppression (<2000 IU/mL). We expect that liver disease severity and progression will be worse in those with incomplete viral suppression and related to HBV mutations. Finally, we predict that routine tests do not adequately predict liver histology and that non-invasive models will be clinically useful. It is expected that these data will provide information critical to the ongoing management of HBV-HIV coinfected patients.

Potential Pitfalls and Alternative Strategies. Because this is an observational study, we will not be able to advance the treatment paradigm of HBV in the setting of HIV. However, we will be able to understand the histologic spectrum of disease and the factors that affect it. We will provide important information on histologic liver disease in patients with complete vs. incomplete viral suppression. However, we recognize that there may have been improvement in histology including reversal of fibrosis in patients with suppressed HBV DNA [71]. Because not all HBV-HIV patients seen at each site may be willing to undergo biopsy, especially those with normal ALT and suppressed HBV DNA, our population may be biased. To minimize this, we have included experienced hepatitis investigators who have a track record for recruitment into studies that have included liver biopsy. We recognize that Fibroscan as a non-invasive marker of disease severity is not available at all centers However, because it was recently FDA approved our data will have an impact . Furthermore, by incorporating HBV tests (qHBsAg, qHBeAg, HBeAg/Ab status, and HBV DNA) new models will be developed. We acknowledge that a small proportion of subject may undergo biopsy within the 3 years of entry and not have contemporaneous Fibroscan and serum. Lastly, our ability to compare our patients to those with HBV alone depends on the HBRN. It is possible that there will be a very small number of patients in the HBRN monoinfection trial who undergo a year 4 biopsy. Nonetheless, we believe that with a projected 90% suppression rate in HBV monoinfected subjects, there will still be a finite number of non-suppressed subjects among the 150 enrolling in the TDF monotherapy arm of the HBRN trial. In the event that the numbers do lag, we will also enroll patients from the second arm of the HBRN Trial, those who will receive PEGIFN x 24 weeks, together with 4 years of TDF.

10. Multiple project director leadership plan

10.1. Overall Study PI: Dr. Sterling will serve as the overall PI of this Ancillary Study of the HBRN. He will be responsible for all aspects of the study and chair the Executive Committee. He will be responsible for communicating with the NIDDK and serve as the contact PI.

10.2. Executive Committee: This will be chaired by Dr. Sterling (VCU), and include Drs. Chung (Harvard), Sulkowski (Johns Hopkins), and King (DCC). The purpose of the committee is to assure the progress of the project, adjudicate complex issues that arise during the study, and interact with the Steering Committee of the HBRN. They will teleconference monthly and report back to the study team.

10.3. Data Coordinating Center: The Epidemiology Data Center of the University of Pittsburgh School of Public Health (Steve Belle, PI), which serves as the DCC for the HBRN, will also serve as the DCC for this Ancillary Study of the HBRN. They will work with Dr. Sterling (VCU) to develop the data collection forms (modified from those of the HBRN to assure consistency) and expand the HBRN data system to accommodate entry of these data into the HBRN database. They will also be responsible for data analysis.

10.4. Site Investigators: Each site investigator will be responsible for the conduct of the study at his/her site. These responsibilities will include patient recruitment and enrollment, oversight of data collection and integrity, transmission of data to the DCC, compliance with regulatory guidelines, and patient safety. If any investigator leaves his/her site during the course of the study, another site PI will be nominated by the site, upon approval of the overall PI, the HBV-HIV Executive Committee, and the NIDDK. In the event that a site is unable to participate, a non-HBRN participating site that has sufficient expertise and patient population will be considered by the HBV-HIV Executive Committee and the NIDDK. This step would be taken only if our recruitment numbers were insufficient to maintain power of the study.

10.5. Communications: The Executive Committee will hold teleconferences approximately monthly and report back to the Ancillary Study Committee of the HBRN on the progress of the study and any issues that have arisen since the last report. There will be conference calls every 3 months of all site PIs and a face to face meeting at least twice a year for all investigators and coordinators to coincide with the planned face to face meetings of the parent HBRN. In the event that the HBRN does not meet face to face in the course of a year, we will schedule an ad hoc face to face meeting of site investigators. Study wide issues will be discussed and progress presented to the HBRN Steering Committee.

10.6. Data Sharing Plan: All data from this proposal will be made available to the HBRN and NIDDK. Once the study is completed, stored specimens will be retained by NIDDK and made available for future studies.

10.7. Alternative Leadership Plan: In the event that Dr. Sterling is unable to fulfill the role of overall PI, responsibility will be transferred to Dr. Chung and his NIH effort will be adjusted accordingly. A site PI will then be nominated from VCU upon approval of NIDDK. Because the grant will be awarded to VCU (the submitting institution), the grant could be re-directed to Harvard (Dr. Chung) if the site PI for VCU is unable to maintain the grant. Dr. Chung will adjust his other effort to be able to take on the full responsibility for successful completion of this project. If Dr. Chung is unable serve as overall PI, then it will be transferred to Dr. Sulkowski and Johns Hopkins in a similar fashion. Both Drs. Chung and Sulkowski are established investigators on the Executive Committee of the HBV-HIV Study and will be well qualified to assume these duties of overall PI.

10.8. Relationship to the HBRN: The current proposal is an Ancillary Study to the HBRN and uses common resources as outlined in the proposal. However, because HIV patients are excluded from the HBRN, a separate R01 proposal was requested by NIDDK. Because not all site PIs and co-PIs in this proposal are coinvestigators currently supported by the HBRN, effort support for investigator for this proposal is being requested. This also allows for additional site coinvestigators (such as Infectious Disease Investigators) to participate and brings added expertise to the study. Independent funding is also required for this study for each site, the central laboratories and DCC to ensure the successful completion of this study in the event that the HBRN funding ends.

11. Data management

Data will be submitted to the Data Coordinating Center (DCC) via a distributed web-based data entry system. Clinical center coordinators and other project personnel will be trained and certified to collect and enter data using established systems. Clinical centers will have the option to use any front end device (ex. TabletPC, laptop, desktop) that provides access to the Internet.

DCC personnel will closely monitor clinical center adherence to study protocol and data collection practices for complete and accurate research data. Monitoring will be performed via established data management procedures with on-site monitoring visits conducted at designated intervals, or as needed, to facilitate the smooth conduct of the study. At the time of the on-site visit, DCC personnel will have access to all study and participant documents and to clinical center personnel. All participant and study documents will be kept confidential. Identifiers such as participant name and address will not be included on any data sent to the DCC. DCC personnel meet weekly to discuss study status, recruitment, compliance, review data issues, clinical center participation, and other issues that arise during the course of the study.

12. Study Organization – Sites

This study will be conducted at approximately 8 clinical centers within the United States and Canada. A Data Coordinating Center (DCC) will coordinate operations, develop and implement data and other systems, maintain the database and perform data analyses. This study will use a biospecimen and a genetic repository. A central virology lab, pathology lab, and one or more central testing labs will be utilized to perform tests and to store specimens identified in the protocol.

13. HUMAN SUBJECTS AND DATA MONITORING/INTEGRITY

13.1. Protection of Human Subjects

13.2. Human subjects involvement and characteristics. In this study, we will recruit approximately 250 men and women adult volunteers that are infected with HIV and hepatitis B. The study protocol, study consent, and any recruitment materials will be submitted to the Institutional Review Board (IRB)/Research Ethics Board (REB) at the following participating sites: VCU, JHU, Mass General, UTSW, UCSF, WashU, NIH, and University of Toronto. No potential study subject will be contacted until formal approval is received from the site IRB/REB as well as the NIH. Within the context of the proposed studies, we anticipate recruiting a significant proportion of males and females. The sex and race/ethnicity distribution in our study population is expected to reflect the diversity evident in the general population of the different participating States in the USA as well as that of the province of Ontario, Canada and that females will be well represented among the participants (See Inclusion of Women; Inclusion of Minorities, and Targeted Enrollment Table).

13.3. Sources of materials. Blood for serum, plasma, and DNA, and liver tissue will be collected from the participants during their clinical visits. Existing clinical and laboratory data will be collected from individual medical records.

13.4. Handling of research samples. Serum, plasma, whole blood, and liver tissue will be collected at study visits, and the laboratories at the eight participating institutions will complete specimen processing and provide temporary storage for specimens until shipping to the designated HBRN central repository or laboratory. Standardized methods for sample processing will be included in the Manual of Operations and uniformly applied to all participating institutions. The liver biopsy specimens will be transferred to the NIDDK Repository and then the central staining lab in the form of unstained slides for histologic evaluation. Each subject will be assigned a study identification number for the purpose of labeling of the specimens. The patient's name and record will be kept confidential.

13.5. Data management and quality assurance. Data collection forms to be used by the study personnel for screening and study visit will be developed. The Manual of Operations will explicitly detail how, as well as what, data are collected. The original data collection forms and original laboratory results will be stored at the participating study sites that will be kept in a locked file cabinet. The study patient files will also include details of any contact with the patient (by phone or mail) occurring outside the scheduled study visit. The research assistants designated for this study will perform data entry at each institution. Data will be entered to the DCC data system in real time. Data clean-up, including summary statistics, will be done on a routine basis.

13.6. Minimizing risk/Insuring confidentiality. Study physicians and study personnel who are on site during the study visits will be prepared to provide medical care if any adverse events should arise. All forms used for the study data will be only identified by coded identifiers to

maintain subject confidentiality. All records will be kept in locked file cabinets at the clinical centers with access limited to HBRN study staff. All study staff will identify participants by the participant identifier number generated at the study sites. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB or Data and Safety Monitoring Board (DSMB). Clinical information may be reviewed during site visits by the HBRN DCC and the NIH. Participants grant permission to share research data with these entities in the consent document. Federal regulations govern the protection of participant's rights relative to data confidentiality and use of research data.

13.6.1. Risks of frequent phlebotomy. The risks of routine blood drawing are rare but can include pain, bleeding, bruising at the site, anemia, dizziness, and very rarely an infection at the site.

13.6.2. Risks of liver biopsy. A liver biopsy is done at the beginning of the study IF NOT ALREADY PERFORMED WITHIN THE PAST 36 MONTHS to assess severity of liver disease as well as liver disease progression when evaluating the natural history of HBV-induced liver disease. About 20% of persons having a liver biopsy have some degree of pain at the biopsy site or in the right shoulder over the liver that may last a few minutes to several hours. This may require pain medication and usually resolves within a day or two. A rare complication of liver biopsy is severe bleeding such that a blood transfusion is required or a procedure or an operation is needed to stop the bleeding. These complications occur in less than one in 1,000 times. Very rarely (in less than one in 10,000 reported cases), death has occurred from bleeding after a liver biopsy. Other rare complications of liver biopsy include infection, and puncture of the gallbladder, lung or kidney. These complications occur in less than one in 1,000 times.

13.6.3. Adequacy of protection against risks

13.6.3.1 Recruitment and informed consent: The study protocol, study consent, and any recruitment materials will be submitted to the IRB/REB of each participating site. No subjects will be contacted regarding the study until formal approval is received from the IRB/REB as well as the NIH. Volunteers will be recruited from each participating site and other local community clinics and through community advertisement. A full explanation of the study's goals and the details of study procedures with risks and benefits will be included in the consent. Once the subject has read the consent form, the principal investigators will review the study with the subjects. A copy of the signed informed consent and the Bill of Rights will be given to the subjects and the original will be kept in the subject's record in a LOCKED CABINET. All study personnel will or have completed training in the Protection of Human Subjects per NIH guidelines.

13.6.4. Potential benefits of the proposed research to the subjects and others. There may be no benefit to patients by participating in this study. However, patients who will be followed in our longitudinal study may potentially benefit from close monitoring of their liver disease.

13.6.5. Importance of the knowledge to be gained. The information gained from this study will substantially increase our understanding of the natural history and management/treatment of patients with chronic hepatitis B within the context of HIV coinfection.

13.6.6. Data and Safety Monitoring Plan: A DSMB will be established from the HBRN of non-participating sites. They will review collected data every 6 months. Since this is largely an observational cohort study, we do not expect any adverse events related to the study, with the exception of possible liver biopsy complications arising in biopsies performed for research

indications only.

13.7. Inclusion of women. Of the estimated 250 patients participating at our sites, we estimate that 15% will be females representing the distribution of women among the HBV-HIV coinfected population of the study sites. A similar proportion of HBV mono-infected females will be identified from the HBRN. Thus, we anticipate that females will be represented in our study population (see Targeted Enrollment Table).

13.8. Inclusion of minorities. The various clinical study sites participating in this study represent ethnic and racial diversity of the general population within North America (see minority and gender mix table). It is anticipated that the racial/ethnicity distribution of the States in the USA and the province of Ontario, Canada will be represented in the study population (see Targeted Enrollment Table).

13.9. Inclusion of children: The HBRN is conducting a trial in children with chronic HBV. Because of the rarity of HIV in children with chronic HBV, children under the age of 18 years will not be included in this protocol. Furthermore, because most children with HBV are immune tolerant and have minimal liver inflammation and fibrosis, they are not appropriate for inclusion in this study.

Minority and gender mix table

	American Indian or Alaska Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female			4.0%	0.5%	10.0%	0.5%	15%
Male			26.0%	4.0%	50.0%	4.5%	85%
Unknown							
Total			30.0%	5.0%	60.0%	5.0%	100%

14. Standard of care

All participants will receive standard of care for HBV-HIV infection and identified associated medical problems outside of the study protocol. This will include providing health care, laboratory testing, HCC surveillance, counseling and educational materials at enrollment and on an ongoing basis during follow-up.

15. Subject confidentiality

Clinical sites are responsible for the confidentiality of the data associated with participants in the HBRN in the same manner they are responsible for the confidentiality of any patient information within their sphere of responsibility. All forms used for the study data will be only identified by coded identifiers to maintain subject confidentiality. All records will be kept in locked file cabinets at the clinical centers with access limited to HBRN study staff. All study staff will identify participants by the participant identifier number generated at the clinical center. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB or Data and Safety Monitoring Board (DSMB). Clinical information may be reviewed during site visits by the DCC and the NIDDK Project Officer. Participants grant permission to share research data with these entities in the consent

document. Federal regulations govern the protection of participant's rights relative to data confidentiality and use of research data.

Consent procedures and forms, and the communication, transmission and storage of participant data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA). The DCC will require that clinical centers provide documentation from the site IRBs with the appropriate authorization or consent form.

16. Participant withdrawal

If a participant chooses to withdraw, all data collected up to the point of withdrawal will remain in the study database, but no further data may be collected. The participant must submit a written request to withdraw to the clinical center personnel. This is consistent with HIPAA guidelines and regulations. A participant may also withdraw consent for use of data or stored specimens – in this case, any specimens collected from this subject will be destroyed and data deleted.

17. Data and safety monitoring

Data and safety will be monitored by the NIDDK in conjunction with an NIDDK appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the study. The description of DSMB activities is included in the DSMB Charter. The DSMB charter will be developed by the NIDDK. It will monitor all aspects of the study (e.g., recruitment, protocol deviations, breeches of confidentiality, data quality, attrition, descriptive characteristics), and recommend protocol modifications, including early study termination. Reports will be prepared by the DCC. Tables showing study progress will be presented by clinical center and overall. These will include recruitment, protocol deviations, attrition, breeches of confidentiality, and data quality. The DCC will maintain a cumulative summary of breeches of confidentiality to be forwarded to the DSMB for their meetings via conference call or in person. Based on the data presented, the DSMB will recommend continuation or termination of the study. A summary of the DSMB findings will be forwarded to all investigators for submission to their respective IRBs.

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Appendix A: Definitions

A.1. Acute Hepatitis B:

Presence of HBsAg and IgM anti-HBc with serum ALT values greater than 300 IU/L and absence of known history of HBsAg positivity. Probable acute hepatitis B is when all above criteria are met except serum ALT is less than or equal to 300 IU/L or if there is any suspicion of chronic disease.

A.2. Phases or phenotypes of chronic hepatitis B

Immune tolerant: Presence of HBsAg and HBeAg and normal ALT levels on two occasions or more at least 6 months apart. HBV DNA levels of greater than 1,000,000 IU/mL.

HBeAg-positive chronic hepatitis: Definite: presence of HBsAg and HBeAg and abnormal serum ALT levels (at least twice the ULN) on two occasions or more at least 6 months apart. HBV DNA levels of greater than 10,000 IU/mL. **Probable:** Presence of HBsAg and HBeAg and HBV DNA greater than 10,000 IU/mL, but ALT levels between 1-2 times the ULN.

HBeAg-negative chronic hepatitis: Definite: presence of HBsAg without HBeAg but with abnormal serum ALT levels (at least twice the ULN) on two occasions or more at least 6 months apart. HBV DNA levels of greater than or equal to 1,000 IU/mL.

Probable: Presence of HBsAg without HBeAg and HBV DNA greater than or equal to 1,000 IU/mL, but ALT levels between 1-2 times the ULN.

Inactive carrier: Definite: presence of HBsAg without HBeAg and normal ALT levels and HBV DNA levels of less than 1,000 IU/mL on two occasions or more at least 6 months apart. **Probable:** Presence of HBsAg without HBeAg and HBV DNA between 1,000-10,000 IU/mL, but ALT levels normal.

Indeterminate: Does not fit into any of the above categories.

Criteria for phases or phenotypes of chronic hepatitis B (CHB)

	HBeAg	**ALT**	**HBV DNA (IU/mL)**
Immune tolerant	Positive	Normal	>1,000,000
HBeAg+CHB	Positive	Elevated*	>10,000
HBeAg- CHB	Negative	Elevated*	≥1,000
Inactive carrier	Negative	Normal	<1,000**

HBeAg ALT HBV DNA (IU/mL)

Immune tolerant Positive Normal >1,000,000

HBeAg+ CHB Positive Elevated* >10,000

HBeAg- CHB Negative Elevated* ≥1,000

Inactive carrier Negative Normal <1,000**

* > 2 times ULN = definite, 1-2 times ULN = probable (ULN=30 IU/L for males, 20 IU/L for females)

** HBV DNA 1,000-10,000 IU/mL with normal ALT and no HBeAg-probable

Notes:

• The phase will be assigned based on HBeAg, ALT and serum HBV DNA level, regardless of the presence or absence of anti-HBe in serum

• Probable: 1) if all other criteria for chronic hepatitis B are met and serum ALT is elevated 1-2x ULN, participant will be assigned to “probable” chronic hepatitis B (HBeAg positive or negative) or 2) if all other criteria for immune tolerant are met and serum HBV DNA is between 100,000 and 1,000,000 IU/mL, participant will

be assigned to “probable” immune tolerant or 3) if all other criteria for inactive carrier are met and serum HBV DNA is between 1,000 and 10,000 IU/mL, participant will be assigned to “probable” inactive carrier

• Indeterminate: The phase or phenotype will be “indeterminate” if the participant does not clearly fall into any one of the above definite or probably categories or two or more sets of

results at baseline are contradictory.

A.3. Normal ALT

≤ 30 IU/L for males and ≤ 20 IU/L for females regardless of the laboratory at which the test is done.

A.4. ALT flare

Serum ALT greater than or equal to 10 times the upper limit of normal corresponding to 300 IU/L or greater in males or 200 IU/L or greater in females.

A.5. Loss/Seroconversion/Seroreversion

A.5.1. HBeAg

In a participant who is previously HBeAg-positive and anti-HBe-negative, disappearance of HBeAg from the serum is HBeAg *loss*. If this is accompanied by emergence of anti-HBe, it is HBeAg *seroconversion*. Among HBeAg-negative participants, reappearance of HBeAg is considered HbeAg *seroreversion*.

A.5.2. HBsAg

By definition, a participant with HBV infection is HBsAg-positive. Anyone who is HBsAg-positive regardless of the presence or absence of anti-HBs is considered to be HBV-infected. A subsequent disappearance of HBsAg from the serum is HBsAg *loss*. If this is accompanied by emergence of anti-HBs, it is referred to as HbsAg *seroconversion*.

A.6. Clinical events

Clinical events will be adjudicated by an Outcomes Subcommittee consisting of at least three clinical investigators and representatives from the DCC and the NIDDK. The subcommittee will develop criteria for judging outcomes and adjudicate them as definite, probable or unlikely. Cases to be adjudicated include etiology of ALT flares, cirrhosis, hepatic decompensation, HCC, and cause of death.

A.6.1. Cirrhosis

In the absence of histological diagnosis, cirrhosis is defined as:

• Any one of the following
o Presence of ascites or hepatic hydrothorax
o Variceal or portal hypertensive bleeding
o Hepatic encephalopathy
o Child-Turcotte-Pugh (CTP) score of 7 or above
or in the absence of hepatic decompensation
• Any two of the following (in the absence of another explanation)
o Splenomegaly
o Nodular liver
o Platelet count below 120,000/mm3

A.6.2. Hepatic decompensation

Development of hepatic decompensation will be defined by any of the following events:

• Ascites or hepatic hydrothorax
• Variceal or portal hypertensive bleeding
• Hepatic encephalopathy
• Child-Turcotte-Pugh (CTP) score of 7 or above

A.6.3. Hepatocellular carcinoma (HCC)

The diagnosis of HCC will follow the AASLD guidelines[72].

A.7. Hepatitis exacerbation marked by ALT Flare

Serum ALT greater than or equal to 10 times the upper limit of normal corresponding to 300 IU/L or greater in males or 200 IU/L or greater in females.

A.8. HBeAg or HBsAg loss

In a participant who is previously HBeAg-positive and anti-HBe-negative, disappearance of HBeAg from the serum is HBeAg *loss*. If this is accompanied by emergence of anti-HBe, it is HBeAg *seroconversion*.

Among HBeAg-negative participants, reappearance of HBeAg is considered HBeAg *seroreversion*. By definition, a participant with HBV infection is HBsAg-positive. Anyone who is HBsAg-positive regardless of the presence or absence of anti-HBs is considered to be HBV-infected. A subsequent disappearance of HBsAg from the serum is HBsAg *loss*. If this is accompanied by emergence of anti-HBs, it is referred to as HBsAg *seroconversion*.

Follow-up in participants achieving HBeAg or HBsAg loss

The participant will be seen 12 weeks after the visit during which loss occurred, and again 24 weeks after the visit during which loss occurred, and then revert to their original follow-up schedule. The unscheduled follow-up data collection adequately captures information of interest.

A.9. Death

Death may occur related to liver disease (typically hepatic decompensation or HCC) or may occur unrelated to hepatitis B or liver disease. Date and cause of death will be recorded.

Appendix B: Evaluation and Follow-up for participants experiencing an ALT flare
Evaluation and Follow-up of participants experiencing an ALT flare

- Once an ALT flare is diagnosed, the follow-up intervals will be every 4 weeks unless ALT is greater than 1000 IU/L or total bilirubin is greater than 2.5 mg/dl in the absence of Gilbert‟s syndrome, then follow-up intervals will be every 2 weeks.
- An ALT flare is considered to be resolved when the participant no longer meets the ALT definition of flare, ALT must drop to below 300 IU/L in males or below 200 IU/L in females. When the ALT flare is considered to be resolved, the follow-up will revert to the original follow-up schedule.
- ALT ≥ 300 IU/L for males
- ALT ≥ 200 IU/L for females
- Evaluation:
- History, physical
- Blood draw
- Follow up:
- ALT ≤ 1000 IU/L: Every 4 weeks
- ALT > 1000 IU/L or total bilirubin > 2.5 mg/dl: Every 2 weeks

Grades of Hepatotoxicity used for Hepatic Flares

Patients with Normal Baseline AST/ALT				
Grade of Hepatoxicity				
0	1	2	3	4
< 1.25 x ULN	1.25 - 2.5 x ULN	2.6 - 5.0 x ULN	5.1 - 10 x ULN	>10 x ULN
Patients with Elevated Baseline AST/ALT				
< 1.25 x BL	1.25 - 2.5 x BL	2.6 - 3.5 x BL	3.6 - 5 x BL	>5 x BL

ULN = upper limit of normal; BL = baseline

Appendix C: Participating centers

Baltimore, MD: Johns Hopkins University
Bethesda, MD: National Institutes of Health (NIH) Clinical Center
Boston, MA: Massachusetts General Hospital
Pittsburgh, PA: University of Pittsburgh Graduate School of Public Health (DCC)
San Francisco, CA: UCSF at SFGH
Texas: University of Texas Southwestern
Toronto, Ontario, Canada: University of Toronto
Virginia: Virginia Commonwealth University
Saint Louis, Mo. Washington University

Appendix D: Schedule of events

	Baseline	Week							
		24	48	72	96	120	144	168	192
Informed consent	X								
Demographics (year of birth, sex, race)	X								
Country of origin, education	X								
Family Hx, risk factors	X								
Health behaviors (AUDIT) ; QOL	X		X		X		X		X
Symptoms	X	X	X	X	X	X	X	X	X
Fatigue questionnaire	X				X				X
Medical history	X	X	X	X	X	X	X	X	X
Medication history (HBV Tx and antiretroviral therapy)	X	X	X	X	X	X	X	X	X
Medication history	X	X	X	X	X	X	X	X	X
Height, Weight, Waist circumference	X		X		X		X		X
Vital signs	X		X		X		X		X
Brief physical exam	X		X		X		X		X
CBC	X		X		X		X		X
Hepatic panel (ALT, AST, ALP, bilirubin, albumin)	X	X	X	X	X	X	X	X	X
Serum Creatinine	X	X	X	X	X	X	X	X	X
Serum calcium and phosphate	X		X		X		X		X
Fasting insulin	X		X		X		X		X
Fasting total cholesterol, LDL, HDL, triglyceride	X				X				X
INR	X		X		X		X		X
Urinalysis	X		X		X		X		X
Fasting urine calcium, creatinine and phosphate	X		X		X		X		X
HBsAg	Xa		X		X		X		X
HBeAg	X	Xc	Xc	Xc	Xc	Xc	Xc	Xc	Xc
Anti-HBe	X	Xc	Xc	Xc	Xc	Xc	Xc	Xc	Xc
Quant HBV DNA	X	X	X	X	X	X	X	X	X
HBV genotype and subtype	Xf								
HBV precore/BCP	Xf								
Anti-HCV	Xa								
Anti-HDV	Xa								
Anti-HIV	Xa								
Autoimmune markers	Xe								
AFP	X	X	X	X	X	X	X	X	X
Abdominal imaging	X	Xd	Xd	Xd	Xd	Xd	Xd	Xd	Xd

Liver biopsy	X								X
Non-fasting or fasting serum banking		X		X		X		X	
Fasting serum/plasma banking	X		X		X		X		X
DNA banking	Xb								
HIV-1 RNA quantitation	X	X	X	X	X	X	X	X	X
Quant HBsAg, HBeAg	Xf	X	X	X	X	X	X	X	X
CD4	X	X	X	X	X	X	X	X	X
CD4%	X	X	X	X	X	X	X	X	X
CD8	X	X	X	X	X	X	X	X	X
CD8%	X	X	X	X	X	X	X	X	X
History of AIDS defining illnesses	X	X	X	X	X	X	X	X	X
APRI (calculated)	X		X		X		X		X
FibroSURE (determined from stored serum)	Xf		X		X		X		X
Fib-4 (calculated)	X		X		X		X		X
Fibroscan (if available)	X		X		X		X		X
25OH-Vitamin D (done centrally at VCU)	X				X				X
Serum for serologies done at central HBRN lab ; fasting serum required at baseline & annual visits	Xf	Xf	Xf	Xf	Xf	Xf	Xf	Xf	Xf
Fasting Serum for bone studies done at VCU	X				X				X

X: Record available data

a: Results at any time prior to baseline are acceptable

b: Can be obtained at any visit if not at baseline

c: May not be repeated in HBeAg- participants unless a flare

d: For HCC surveillance in at risk individuals, imaging can range from 6-12 mo

e: Only if clinically indicated

f. Done by central labs of HBRN: Serologies and HBV DNA, HBV genotype and mutations

Additional samples may be collected at the time of a special visit (e.g. liver biopsy, ALT flare)`

Appendix E

***Liver Biopsy and Histology*.** Liver biopsy will be performed in the standard fashion at entry or within 36 months before entry. Ten unstained slides will be sent to the central pathology lab. Five slides will be stained (H&E, Masson trichrome, Iron, HBsAg, HBcAg). Five unstained slides will be retained for future study as part of the HBV CRN biospecimen collection. Original slides are reviewed if necessary and then returned to contributing hospital. Additional stains for opportunistic infections (Ziehl-Nelsen, Gomori Methenamine Silver), will be performed locally as indicated. Liver histology will be interpreted blindly with respect to clinical data, HIV status, or sequence (initial vs. follow up) by the HBRN central pathologists using the exact same scoring methodology. Histology will be systematically assessed for inflammation, fibrosis, steatosis, steatohepatitis, dysplasia and findings associated with HBV infection. Other findings (e.g. granulomas, viral inclusions) will be noted in comments. Necroinflammatory activity will be assessed by the Ishak Histologic Activity Index (HAI) and fibrosis by Ishak score [62].

Steatosis/Steatohepatitis: Nonalcoholic fatty liver disease will be graded by methods similar to those used in the HALT-C trial [63] Specifically, grade of steatosis (None, < 5%, 5-33%, 34-66%, and > 66%), hepatocyte ballooning (absent or present), presence of Mallory's hyaline, and for the presence of perisinusoidal fibrosis away from septa will be assessed. A diagnosis of steatohepatitis will be based on the recognition of the distinctive pattern of injury.

Immunostaining for HBcAg and HBsAg: The fraction of hepatocytes positive will be scored semi quantitatively as 0: negative; 1: <10%; 2: 10-50%; 3: 50-90%; 4: >90%; and the patterns of HBcAg staining (nuclear, cytoplasmic or mixed) and HBsAg staining (inclusion-like, granular cytoplasmic, membranous) recorded.

Appendix F

Missing data: In general, missing outcome data will be handled as follows in statistical analyses. First, we will explore the pattern of missing data by comparing characteristics of participants with available data to those with missing data. For data missing completely at random (does not depend on observed outcome; MCAR) standard analytical techniques described earlier would produce unbiased results when the analysis is performed on the complete cases. For the time-to-event data analyses, analytical methods such as Kaplan-Meier estimation and Cox proportional hazards model account for MCAR dropouts. However, in other cases where the dropout depends on observed outcome or covariates (missing at random, MAR) or unobserved outcome and/or covariates (missing not at random, MNAR), we will use selection models such as MNAR Dale model and Diggle-Kenward model. These models often require strong assumptions on the dropout mechanism which are primarily unverifiable based on the observed data. We will conduct sensitivity analyses to investigate the sensitivity of our conclusions to possible violation of such assumptions by fitting models under MCAR, MAR and MNAR assumptions and compare the model fits using log-likelihoods. We will consider other approaches to analyzing longitudinal data with informative censoring including modeling the dropout process jointly with the fitted model of interest and weighted estimating equations using inverse probability of missingness to account for the potential bias that arises due to the missing outcomes.