Official Study Title: Combination Therapy of Peginterferon Alfa-2a and Tenofovir versus Tenofovir Monotherapy in HBeAg-positive and HBeAg-negative Chronic Hepatitis B

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Combination Therapy of Peginterferon Alfa-2a and Tenofovir versus Tenofovir Monotherapy in HBeAg-positive and HBeAg-negative Chronic Hepatitis B

Hepatitis B Research Network

Synopsis

Study Aims
To define the role of limited duration peginterferon-alfa 2a in the presence of tenofovir in treating patients with chronic hepatitis B by:

- Comparing the long-term efficacy of initial treatment with combination therapy consisting of peginterferon alfa-2a plus tenofovir DF versus tenofovir DF monotherapy
- Evaluating "off treatment" sustained responses following combination therapy with peginterferon alfa-2a plus tenofovir DF versus tenofovir DF monotherapy

Type of Study
- Randomized treatment trial

Clinical Trial
This is a randomized (1:1) parallel group design trial comparing (i) tenofovir DF 300 mg daily for 192 weeks (4 years) and (ii) peginterferon alfa-2a 180 µg weekly for 24 weeks plus tenofovir DF 300 mg daily for 192 weeks (4 years). Enrolled participants will be stratified by HBeAg status (positive/negative), known genotype (A vs. all others) and cirrhosis (present vs. absent). After 192 weeks of treatment, participants meeting criteria for treatment discontinuation will stop treatment and be followed for up to 48 weeks (total duration of treatment and follow up is up to 240 weeks). Emtricitabine/tenofovir coformulated as Truvada, approved for treating HIV but not for treating HBV infection, will be offered to participants with primary nonresponse, partial virological response or confirmed virologic breakthrough.

1. Background
Chronic infection with the hepatitis B virus (HBV) is prevalent world-wide (estimated to affect 360 million individuals) and in the United States (estimated to affect 2 million individuals) (1). Chronic hepatitis B may result in progressive liver disease that leads to cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). Chronic hepatitis B can also be benign and non-progressive, evolving into an inactive carrier state that rarely leads to significant liver injury or HCC. Over the last few years, several highly effective antiviral agents have been developed and approved for use in the treatment of chronic hepatitis B (2). Nevertheless, uncertainty remains about which patients warrant therapy with which agent or agents, for how long, and with what ultimate endpoint as a measure of clinical benefit.

Indications and approaches to therapy of hepatitis B are based upon knowledge of the features of its natural history. The natural history of chronic hepatitis B, particularly if acquired in early childhood, runs through several stages or phases that have different clinical manifestations and implications for long-term hepatic injury (3, 4). Typically, there is an early inactive "immune tolerant" phase marked by the presence of high levels of HBsAg, HBeAg and HBV DNA, but minimal or no elevations in serum alanine and

aspartate aminotransferase levels (ALT, AST) and minimal activity or injury on liver biopsy (5). The immune tolerant phase is variable in duration, but is typically followed in adolescence or early adulthood by an active "immune clearance" phase, marked by rises in serum ALT and AST, inflammation and hepatocellular necrosis on liver biopsy and somewhat lower levels of serum HBV DNA with persistence of HBsAg and HBeAg. This phase is what is commonly referred to as "typical" HBeAg-positive chronic hepatitis B, and is also variable in duration and severity. If severe and prolonged, HBeAg positive chronic hepatitis B can lead to progressive hepatic fibrosis and cirrhosis. If milder in course or shorter in duration, this phase can eventually resolve, leading to an inactive HBsAg carrier phase with minimal liver fibrosis or injury. This transition from immune active to inactive chronic hepatitis B is usually marked by a sudden fall in HBV DNA levels and loss of HBeAg despite persistence of HBsAg (often referred to as HBeAg seroconversion because of development of anti-HBe). The resulting inactive HBsAg carrier state is marked by the presence of normal ALT and AST levels with minimal activity (inflammation and necrosis) on liver biopsy, absence of HBeAg and presence of HBsAg. Once HBeAg has been cleared and HBV DNA falls to low levels, the disease becomes inactive or mild and does not progress further (6). However, in some patients, the loss of HBeAg is not associated with complete resolution of disease and HBV DNA levels remain moderately elevated. These patients have developed HBeAg-negative chronic hepatitis B, which can be progressive and is marked by fluctuating and moderate levels of HBV DNA in serum. Changes in the hepatitis B virus sequence as well as changes in immune reactivity appear to govern the course and outcome of chronic hepatitis B and whether the disease goes into long-term remission and an inactive carrier state or evolves into an HBeAg-negative chronic hepatitis B or continues in the HBeAg-positive chronic hepatitis B. Furthermore, some patients with chronic HBV infection who become HBeAg negative, later also lose HBsAg and appear to be cured of the chronic viral infection. In actuality, these patients usually harbor low levels of HBV DNA in an inactive state in the liver, but they usually are spared further hepatic injury or long-term consequences of the disease.

These features of the natural history of chronic hepatitis B underlie the criteria for therapy of this disease and the endpoints of treatment (2, 7, 8). Thus, therapy of typical HBeAg-positive chronic hepatitis B is generally aimed at clearance of HBeAg and attainment of the subsequent inactive carrier state with normal serum ALT levels and inactive liver histology. Treatment of HBeAg-negative chronic hepatitis B is generally aimed at long-term suppression of HBV DNA levels and improvements in serum ALT levels and liver histology. Of course, clearance of HBsAg is a much more desired endpoint of therapy. Unfortunately, loss of HBsAg is rarely achieved, at least with therapy for a limited period of time and with current treatment options. Nevertheless, endpoints short of loss of HBsAg have to be considered surrogate endpoints of therapy and of uncertain long-term benefit (9). Thus, while many patients who clear HBeAg during antiviral therapy of hepatitis B seem to attain a long-term remission in disease, a proportion do not improve or later relapse either re-developing HBeAg or evolving into HBeAg-negative chronic hepatitis B with fluctuating moderate levels of HBV DNA, elevations in serum aminotransferase levels, and inflammation, necrosis and potentially progressive fibrosis on liver biopsy.

Thus, the aim of antiviral therapy of chronic hepatitis B is to achieve control of HBV replication and improve disease activity. Suppression of HBV DNA to a low level is usually followed by improvements in serum ALT and AST and amelioration of the

underlying hepatic inflammation and hepatocellular injury. This often follows loss of HBeAg induced by antiviral therapy, but most reliably occurs with loss of HBsAg.

There are two forms of licensed treatment for chronic hepatitis B: parenterally administered peginterferon and orally administered nucleos(t)ide analogues (lamivudine adefovir dipvoxil, telbivudine, entecavir and tenofovir disoproxil fumarate [tenofovir DF]). The mechanisms of action of these two forms of therapy are quite different, as are the route of administration and frequency and severity of side effects (10, 11). Mainly because of their ease of administration and lack of side effects, the nucleos(t)ide analogues have become the preferred means of treatment. Nevertheless, several prospective trials have shown that the rate of response, defined by HBeAg loss, to a 48 week course of peginterferon is significantly higher than that to a 48 week course of nucleos(t)ide analogues; with rates of HBeAg loss at one year ranging from 12-24% for nucleos(t)ide analogues compared to rates of 30-35% with peginterferon (12-16). However, the nucleos(t)ide analogues are typically administered long-term rather than for one year only; and rates of clearance of HBeAg during nucleos(t)ide therapy typically increase with more prolonged therapy. In contrast, the expense and side effects of peginterferon makes long-term therapy impractical. Thus, it appears that rates of clearance of HBeAg achieved with peginterferon can be achieved with the better tolerated nucleos(t)ide analogues with longer courses of treatment. Nevertheless, the rates of clearance of HBeAg after stopping peginterferon have not been assessed adequately and long-term follow up of 48 weeks of therapy with peginterferon may be associated with a gradual rise in rates of loss of HBeAg. These factors make it difficult to discern whether peginterferon has any benefit above and beyond what can be achieved with the oral nucleos(t)ide analogues. Furthermore, long-term therapy with nucleos(t)ide analogues is often associated with a high rate of improvement in liver histology, HBV DNA suppression and normalization of ALT even without change in HBeAg status. The long-term improvement in these features is particularly evident in therapy of patients with HBeAg-negative chronic hepatitis B (17, 18), in which peginterferon therapy is associated with only a modest long-term effect on HBV DNA and ALT levels (19).

A feature of peginterferon treatment that is distinctly greater than with nucleos(t)ide analogue therapy is the loss of HBsAg. Thus, in studies of HBeAg-positive chronic hepatitis B with a one-year course of peginterferon, rates of loss of HBsAg have ranged from 3% to 7%, whereas loss of HBsAg with a 48 week course of nucleos(t)ide analogues is <1% to 3% (12-16). Furthermore, long-term follow up of patients who have become HBeAg-negative after interferon therapy has shown that the majority of these patients eventually become HBsAg negative as well (20). This gradual loss of HBsAg is greater than occurs spontaneously and appears to be a special characteristic of responses to peginterferon therapy. Emerging data suggests that ultimately prolonged suppression of viral replication with a nucleos(t)ide analogue leads to HBsAg loss. In one study loss of HBsAg after 1 year of tenofovir DF was observed in 3% of those treated for HBeAg-positive CHB and this figure increased to 6% after 2 years of therapy (only in genotypes A and D). In another study with adefovir for 4-5 years for HBeAg-negative CHB, HBsAg clearance occurred in a third of the patients followed for up to 5 years after stopping therapy. It is unknown whether even longer duration of therapy with nucleoside(t)ide analogues will result in ever increasing HBsAg loss or whether the rate of loss will level off.

Thus, the advantages of nucleos(t)ide analogue therapy are the ease of administration, the lack of significant side effects and the consistent suppression of HBV DNA that can be achieved, particularly with the newer agents such as tenofovir DF and entecavir. While emergence of drug resistance can limit the efficacy of oral agents, a low rate of antiviral resistance (1% or less even with prolonged therapy for 4 to 5 years), is seen with entecavir and tenofovir DF, reflective of their greater potency and higher genetic barrier to resistance. As a consequence 80-90% of patients treated long-term with these agents achieve rates of marked suppression of HBV DNA levels and normal ALT levels. The advantage of peginterferon therapy is that it is administered for a limited period (6 to 12 months) and results in loss of HBsAg in a significant proportion of patients. Previous studies have shown that a one-year course of combination therapy of peginterferon and nucleos(t)ide analogue does not yield a higher response rate than a one-year course of either agent alone (21). However, these studies have been limited by the use of a nucleos(t)ide analogue with high risk of resistance (e.g. lamivudine) and a relatively short course of treatment. Thus, an obvious question is whether the combination of a limited course of peginterferon (6 or 12 months) with long-term therapy with a potent nucleos(t)ide analogue (tenofovir DF or entecavir for at least 4 years) will provide a higher rate of loss of HBsAg and ability to stop therapy without relapse. This clinical trial compares the efficacy of peginterferon plus a potent nucleotide analogue, tenofovir DF, for 24 weeks followed by monotherapy with tenofovir DF for a further 3.5 years to the efficacy of that same potent nucleotide analogue, tenofovir DF, given for a full 4 years. The primary measure of outcome will be HBsAg loss in serum 240 weeks after starting antiviral therapy**.**

2. Study objectives

To define the role of limited duration peginterferon alfa-2a in the presence of tenofovir for treating people with chronic hepatitis B by:

- Evaluating “off treatment” sustained responses following combination therapy consisting of peginterferon alfa-2a plus tenofovir DF versus tenofovir DF monotherapy.
- Comparing the on-treatment efficacy of initial treatment with combination therapy consisting of peginterferon alfa-2a plus tenofovir DF versus tenofovir DF monotherapy.

2.1. Primary Endpoint

HBsAg loss at week 240.

2.2. Secondary endpoints

2.2.1. <u>At week 192</u> :

a. Cumulative HBsAg loss.
b. HBeAg loss.
c. HBsAg seroconversion.
d. HBeAg seroconversion.
e. ALT normalization (males ≤30 U/L, females ≤20 U/L).
f. ALT ≤38 U/L (males), ≤25 U/L (females) corresponding to approximately 1.25 xULN.
g. HBV DNA <1000 IU/mL.
h. HBV DNA <20 IU/mL (Lower limit of quantification [LLOQ]) of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test).
i. Absence of detectable antiviral drug-resistance HBV mutations.
j. Sustained HBV DNA <1000 IU/mL

2.2.2. Sustained <u>end of follow-up responses</u> at week 240

a. Cumulative HBsAg loss.
b. HBeAg loss.
c. HBsAg seroconversion.
d. HBeAg seroconversion.
e. ALT normalization (males ≤30 U/L, females ≤20 U/L).
f. HBV DNA <1000 IU/mL.
g. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test).

2.2.3. <u>Sustained off treatment (36 and 48 weeks after end of treatment) responses</u>

a. ALT ≤38 U/L (males), ≤25 U/L (females) corresponding to approximately 1.25 xULN.
b. HBV DNA <1000 IU/mL.
c. HBV DNA <1000 IU/mL AND ALT ≤38 U/L (males), ≤25 U/L (females) corresponding to approximately 1.25 xULN.
d. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test).

2.2.4. Sustained off treatment (36 and 48 weeks after end of treatment) responses in participants with low end of treatment HBsAg levels (<2000 IU/mL) versus participants with high end of treatment HBsAg levels (≥2000 IU/mL)

a. Cumulative HBsAg loss.
b. HBeAg loss.
c. HBsAg seroconversion.
d. HBeAg seroconversion.
e. ALT normalization (males ≤30 U/L, females ≤20 U/L).
f. HBV DNA <1000 IU/mL.
g. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test).

2.2.5. Sustained off-treatment (36 and 48 weeks after end of treatment) responses in participants with a substantial drop of HBsAg levels (≥1 log from baseline to end of treatment) versus participants who did not have substantial drop of HBsAg levels (<1 log from baseline to end of treatment)

a. Cumulative HBsAg loss.
b. HBeAg loss.
c. HBsAg seroconversion.
d. HBeAg seroconversion.
e. ALT normalization (males ≤30 U/L, females ≤20 U/L).
f. HBV DNA <1000 IU/mL.
g. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test).

2.2.6. Sustained off-treatment (36 and 48 weeks after end-off treatment) responses in participants with end of treatment HBsAg level <2000 IU/mL *and* HBV DNA below 1000 IU/mL versus participants with end of treatment HBsAg levels ≥2000 IU/mL and HBV DNA ≥1000 IU/mL

a. Cumulative HBsAg loss.
b. HBeAg loss.

c. HBsAg seroconversion.
d. HBeAg seroconversion.
e. ALT normalization (males ≤30 U/L, females ≤20 U/L).
f. HBV DNA <1000 IU/mL.
g. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test).

2.2.7. Treatment failure- Cumulative rates of primary nonresponse, partial virologic response and confirmed virologic breakthrough

2.2.8. Rate of discontinuation of treatment due to adverse events.

2.2.9. Rates of adverse events and serious adverse events.

3. Study design

This is a randomized (1:1) parallel group design trial comparing outcomes of treatment with (i) monotherapy, i.e., tenofovir DF 300 mg daily for 192 weeks (4 years) and (ii) combination therapy, i.e., peginterferon alfa-2a 180 µg weekly for 24 weeks plus tenofovir DF 300 mg daily for 192 weeks (4 years). Enrolled participants will be stratified by HBeAg status (positive/negative), known genotype (A vs. all others) and cirrhosis (present vs. absent). After 192 weeks of treatment, participants meeting criteria for treatment discontinuation will stop treatment and be followed for up to 48 weeks (total duration of treatment and follow up is up to 240 weeks). Emtricitabine/tenofovir coformulated as Truvada, approved for treating HIV but not for treating HBV infection, will be offered to participants with primary nonresponse, partial virological response or confirmed virologic breakthrough.

4. Study population

4.1 Inclusion criteria

1. Enrolled in the Hepatitis B Research Network (HBRN) Cohort Study or completed the necessary components of the Cohort baseline evaluation by the end of the baseline visit for this study.
2. At least 18 years of age at the time of randomization (day 0).
3. Chronic HBV infection as evidenced by **at least one** of the following:
    a. HBsAg positive result within 8 weeks prior to randomization and another time at least 24 weeks prior to randomization with no HBsAg negative result in between.
    b. HBsAg positive plus absence of detectable anti-HBc IgM in serum within 8 weeks prior to randomization.
    c. HBsAg positive within 8 weeks prior to randomization and HBV DNA ≥1,000 IU/mL on 2 occasions at least 24 weeks apart (can include result from screening visit within 8 weeks of randomization).
    d. HBsAg positive within 8 weeks prior to randomization plus evidence of chronic hepatitis B infection as indicated by a liver biopsy within 144 weeks of randomization.
4. HBeAg positive or negative.
5. Serum HBV DNA ≥1000 IU/mL on 2 occasions at least 4 weeks apart within the 32 weeks prior to randomization (can include result from screening visit within 8 weeks of randomization).

6. At least two elevated serum ALT levels (>45 U/L for males and >30 U/L for females) at least 4 weeks, and no more than 32 weeks apart with the second being within 8 weeks of randomization.
7. Compensated liver disease, with total bilirubin ≤2 mg/dL (except if Gilbert's syndrome), direct bilirubin ≤0.5 mg/dL, INR ≤1.5, and serum albumin ≥3.5 g/dL
8. No evidence of HCC based upon alpha-fetoprotein (AFP) ≤20ng/mL within 8 weeks prior to randomization:
    a. Participants who meet AASLD criteria for HCC surveillance must have negative liver imaging by ultrasound (US), computerized tomography (CT) or magnetic resonance imaging (MR) within 28 weeks of randomization as part of standard of care.
    b. Participants with AFP >20 ng/mL must be evaluated clinically with additional imaging and shown not to have HCC on CT or MRI.
9. Liver biopsy that shows findings consistent with chronic hepatitis B with the Modified Ishak histology activity index (HAI) ≥3 (necroinflammatory component only) or Ishak fibrosis score ≥1 or both, as assessed by the local consortium pathologist on review of a liver biopsy done within 144 weeks of randomization. Slides must be available for review by the local consortium pathologist and meet adequacy requirements. If the participant had received previous treatment for hepatitis B, the biopsy must have been done after discontinuation of treatment.
10. Females of child bearing potential must agree to use an adequate method of contraception throughout the study and must have a negative pregnancy test immediately prior to the start of treatment.
11. Provide informed consent and agree to adhere to the requirements of the study.

4.2 Exclusion criteria

1. Serum ALT >450 U/L for males and >300 U/L for females (participants are eligible for re-screening if ALT levels fall to the range of eligibility).
2. Treatment with interferon or nucleos(t)ide analogues for hepatitis B within 48 weeks of randomization.
3. More than 48 weeks of therapy with nucleos(t)ide analogues for hepatitis B at any time in the past.
4. History of hepatic decompensation including, but not limited to, ascites, variceal bleeding, or hepatic encephalopathy.
5. Known allergy or intolerance to any of the study medications.
6. Females who are pregnant or breastfeeding.
7. Previous organ transplantation including engrafted bone marrow transplant.
8. Any other concomitant liver disease, including hemochromatosis or hepatitis C or D. Non-alcoholic fatty liver disease (NAFLD) with steatosis and/or mild to moderate steatohepatitis is acceptable but NALFD with severe steatohepatitis is exclusionary.
9. Positive anti-HIV (test to be completed within 8 weeks prior to randomization).
10. Renal insufficiency with calculated (by MDRD method) creatinine clearance <60 mL/min within 8 weeks prior to randomization.
11. Platelet count <90,000 /mm^3, hemoglobin <13 g/dL (males) or <12 g/dL (females), absolute neutrophil count <1500 /mm^3 (<1000/mm^3 for African-Americans) within 8 weeks prior to randomization.
12. History of alcohol or drug abuse within 48 weeks of randomization.
13. Pre-existing psychiatric condition(s), including, but not limited to:

   a. Current moderate or severe depression as determined by the study physician.
   b. History of depression requiring hospitalization within past 10 years.
   c. History of suicidal or homicidal attempt within the past 10 years.
   d. History of severe psychiatric disorders including, but not limited to, schizophrenia, psychosis, bipolar disorder as determined by a study physician.
14. History of immune-mediated disease, or cerebrovascular, chronic pulmonary or cardiac disease associated with functional limitation, retinopathy, uncontrolled thyroid disease, poorly controlled diabetes or uncontrolled seizure disorder, as determined by a study physician.
15. Any medical condition that would, in the opinion of a study physician be predicted to be exacerbated by therapy or that would limit study participation.
16. Any medical condition requiring, or likely to require, chronic systemic administration of corticosteroids or other immunosuppressive medications during the course of this study.
17. Evidence of active or suspected malignancy, or a history of malignancy within the last 144 weeks prior to randomization (except adequately treated carcinoma in situ or basal cell carcinoma of the skin).
18. Expected need for ongoing use of any antivirals with activity against HBV during the course of the study.
19. Participation in any other clinical trial involving investigational drugs within 30 days of randomization or intention to participate in another clinical trial involving investigational drugs during participation in this study.
20. Any other condition that in the opinion of a study physician would make the participant unsuitable for enrollment or could interfere with the participant participating in and completing the study.

5. Study drugs and drug management

5.1. Dosage and administration

Participants will be randomly assigned to receive one of the following treatments during the treatment period of the study:

- Treatment A: Tenofovir DF 300 mg by mouth once daily for 192 weeks.
- Treatment B: Peginterferon alfa-2a 180 µg by subcutaneous injection once weekly in combination with tenofovir DF 300 mg by mouth once daily for 24 weeks followed by tenofovir DF 300 mg once daily for 168 weeks (192 weeks total therapy).

All participants will be instructed to take one tablet of tenofovir DF once daily without regards to meals. Participants in treatment arm B will also be instructed to take 180 µg of peginterferon alfa-2a subcutaneously once weekly. Participants in treatment arm B will be instructed to inject the peginterferon alfa-2a on the same day each week.

5.2. Tenofovir disoproxil fumarate

5.2.1 General information

Tenofovir disoproxil fumarate (tenofovir DF) is a prodrug of tenofovir. It is an acyclic nucleotide phosphonate analogue of adenosine 5 monophosphate. The medication is stored at room temperature.

5.2.2 Pharmacokinetic considerations

Tenofovir DF is well absorbed from the gastrointestinal tract and after being taken up intracellularly is hydrolyzed rapidly to tenofovir and phosphorylated by cellular nucleotide kinases to the active form, tenofovir diphosphate. Tenofovir diphosphate competes with the natural intracellular deoxyadenosine triphosphate for uptake into the nascent viral DNA molecule and once incorporated causes DNA chain termination. Tenofovir diphosphate may also interfere with the HBV DNA polymerase by binding to the active catalytic site. The intracellular half-life of tenofovir is prolonged and is in excess of 60 hrs. The half-life of tenofovir in serum is about 17 hours following a single dose. Multiple dose pharmacokinetics suggests that the median steady state tenofovir C_{max} values are 355 mg/L [0.33 µg/mL] after 48 weeks. Food does not affect absorption or pharmacokinetics. No demographic variables appear to affect tenofovir pharmaco-kinetics across either healthy participants or participants infected with HIV or HBV. Tenofovir is excreted unchanged in the urine and dose adjustments are needed in participants with renal insufficiency.

Dose adjustments are required for creatinine clearance (CrCl) of <50 mL/min.

	Creatinine Clearance (mL/min)			Hemodialysis
	>50	30-50	10-29	
Recommended 300 mg dosing interval	Every 24 hours	Every 48 hours	Every 72 to 96 hours	Every 7 days or after a total of approximately 12 hours of dialysis

Only when there is moderate CrCl (30-50 mL/min) or severe reduction in CrCl (<30mL/min) is there the chance for drug toxicity after a single 300 mg dose.

In vitro, tenofovir DF at expected concentrations with once a day dosing does not inhibit the metabolizing ability of major human cytochrome P-450 isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) responsible for phase I metabolism of most drugs.

Drug interactions potentially could occur with other drugs which are eliminated by proximal tubular secretion e.g. cidofovir, acyclovir and other members of this group of agents. Such a drug interaction may result in increased blood levels of tenofovir DF or the competing agent. Similarly there may be some interactions with drugs used in the treatment of HIV e.g. didanosine, atazanavir and lopenavir/ritonavir.

There is no substantial effect of TDF on methadone, estrogens or birth control pill pharmacokinetics.

5.2.3 Safety

Tenofovir DF is freely excreted unchanged by the kidneys by glomerular filtration and tubular secretion. High levels of tenofovir DF in renal tubules may interfere with renal function in a dose-dependent manner. Reports of hypophosphatemia, renal tubular toxicity and renal failure exist. This tubular toxicity manifests as renal tubular acidosis, a Fanconi-like syndrome or nephrogenic diabetes insipidus (22). Probably as a consequence of the nephrotoxic effects of tenofovir DF, osteomalacia has been reported in young macaques given 25 times the recommended dose. The osteomalacia observed may have been the result of chronic hypophosphatemia caused by tubular phosphate leakage. For this reason, use of tenofovir DF currently is not recommended in children.

To date most studies examining bone density in participants on prolonged therapy with tenofovir DF have been in participants with HIV infection. Osteopenia and osteoporosis have been reported but with no significant difference in the frequency of these complications in participants with HIV who are and who are not taking tenofovir.

Reproductive Toxicity: Tenofovir DF is a Pregnancy Category B drug. No adverse effects on embryo-fetal development in rats were observed with long-term, high-dose therapy; nor were there adverse effects on growth, development, and behavior. In this study, females of childbearing potential will be enrolled only if they agree to use effective contraception during therapy and pregnancy testing will occur at every visit during the peginterferon treatment phase.

5.3 Peginterferon-alfa-2a

5.3.1 General description

Peginterferon alfa-2a is a covalent conjugate of recombinant alfa-2a interferon with a single branched molecule of polyethylene glycol with a molecular weight of approximately 40,000 daltons. Peginterferon alfa-2a is produced using recombinant DNA technology and contains 180 µg/1.0 mL in vial form and the same amount per 0.5 mL in a prefilled syringe. The drug must be kept refrigerated at +4C until use.

Interferon inhibits viral replication by inducing an antiviral state in cells. Interferon does not enter the hepatocyte, but rather binds to specific receptors on the cell surface, which initiates intracellular signaling that leads to rapid activation of multiple “interferon-stimulated genes (ISGs); the encoded proteins of these genes inhibit viral replication in infected hepatocytes by multiple mechanisms (including inhibition of viral protein synthesis and breakdown of viral RNA). Peginterferon alfa-2a also has immunomodulatory activity that is thought to be important in obtaining a virological response. Steady-state serum concentrations of peginterferon alfa-2a are reached within 5 to 8 weeks using once weekly dosing. The mean terminal half-life after subcutaneous dosing is 160 hours compared to 5 hours for standard alpha interferon.

5.3.2 Pharmacokinetic considerations

In participants with end-stage-renal-disease undergoing hemodialysis, there is a 25% to 45% reduction in clearance of peginterferon alfa-2a. The effect of milder renal impairment has not been studied but it is advised that in participants with impaired renal function, signs and symptoms of interferon toxicity should be closely monitored. It is also recommended that peginterferon alfa-2a be used with caution in participants with creatinine clearance < 50 mL/min.

There is no known effect of peginterferon alfa-2a on the pharmacokinetics of drugs metabolized by the cytochrome P-450 system. There are no known clinically significant interactions with nucleos(t)ide analogue therapy, but a phase IV registration trial of peginterferon alfa-2a and telbivudine was prematurely discontinued due to a higher than anticipated incidence of peripheral neuropathy.

5.3.3. Safety

As with standard alpha interferon, treatment with peginterferon alfa-2a is associated with many troublesome and occasionally serious, or even life-threatening, side effects. Dose discontinuation has been reported in 6% to 9% and dose modification in 31% to 47% of participants treated in the peginterferon alfa-2a registration trials for hepatitis B. The most frequent causes of dose adjustment were laboratory abnormalities such as

thrombocytopenia or leukopenia. These side effects are particularly common in patients with advanced liver disease and hypersplenism. Peginterferon alfa-2a is contraindicated in participants with decompensated cirrhosis due to the possibility of serious infections, flares of disease, and worsening decompensation. In this study, patients with any clinical or laboratory features suggestive of hepatic decompensation will not be enrolled.

The majority of clinical experience in the use of peginterferon alfa-2a has been in chronic hepatitis C in which peginterferon is combined with ribavirin. In the few studies of peginterferon alfa-2a in chronic hepatitis B it has appeared that side effects are less frequent than in chronic hepatitis C. The overall incidence of serious adverse events was less in studies in hepatitis B (4%-5%) than in those with hepatitis C (7% to 16%), and fewer drug withdrawals were reported (6% to 8% versus 17% to 33%, respectively) despite similar doses and durations of therapy. Depression was also less frequently reported in studies done in hepatitis B (4%) than hepatitis C (22%, $p < 0.001$).

Peginterferon should be avoided in participants with other serious co-morbid illnesses, including, but not limited to, coronary artery disease, cerebrovascular disease, serious autoimmune conditions and severe depression.

Pregnancy:
Peginterferon alfa-2a is a Pregnancy Category C drug that has not been adequately evaluated in humans for its teratogenic effect. Standard interferon has been shown to increase the rate of abortion in Rhesus monkeys when given approximately 20 to 500 times the human weekly dose. Interferon, however, is highly species specific in its effects and animal studies may not reliably reflect the potential of side effects in humans. There have been no adequate and well-controlled studies of peginterferon alfa-2a in pregnant females. Therefore, in this study, females of childbearing potential will be enrolled only if they agree to use effective contraception during therapy and pregnancy testing will occur at every visit during the peginterferon treatment phase.

5.4. Administration and drug accountability
Study drugs (peginterferon alfa-2a, tenofovir DF, and emtricitabine/tenofovir) will be shipped to study sites from the drug distribution center. Staff at each site will be responsible for drug accountability. This will include documenting drugs received, study drug dispensed to participants, used and unused study drug returned, and accounting of any drug destroyed by the site. An accurate and up-to-date accountability log will be maintained by each site.

5.5. Concomitant medications
All concomitant medications (prescription and non-prescription) being taken by each participant will be queried and recorded by the study coordinator at each visit.

5.6. Prohibited medications
Use of the following medications is prohibited while participants are on study drugs:

1. Antivirals with activity against HBV, including famciclovir or valacyclovir. The need for ongoing use of these antivirals would be exclusionary at study entry, but allowed if medically indicated and prescribed during the course of the study.
2. Nephrotoxic agents including aminoglycoside antibiotics, amphotericin, cidofovir, and foscarnet.
3. Competitors of renal excretion such as probenecid and sulfinpyrazone.
4. Systemic chemotherapeutic agents (i.e., cancer treatment medications).

5. Systemic corticosteroids. The need for ongoing use of systemic corticosteroids would be exclusionary at study entry, but would be recorded as a concomitant medication if medically indicated and prescribed during the course of the study.
6. Any investigational agents.
7. Use of growth factors such as GCSF, erythropoietin, and eltrombopag.

6. Study procedures

6.1. Study enrollment

Participants thought to be eligible for participation will be asked to give their consent to participate by discussions with study physicians and coordinators and by signing a current consent form approved by the IRB/REB at that institution. Participants will be required to sign the consent form before any study procedures are performed.
They will then undergo the screening (8 weeks prior to randomization) procedures, including medical history, physical examination and blood tests as summarized in the Time and Event chart (Appendix 3). Once eligibility is confirmed, they will complete pre-randomization tests and then be randomly assigned (at baseline visit, day 0) to one of the two treatment groups using a system established by the Data Coordinating Center (DCC). Patients who are not currently enrolled in the HBRN Cohort study may be enrolled concurrently with the screening visit for this trial, or if they choose not to enroll into the Cohort Study, the necessary components on the Cohort baseline evaluation will be completed prior to enrolling in this study at the clinical trial screening or baseline visit.

6.2. Pre-randomization (screening) assessment

All participants are required to have a liver biopsy that is adequate for assessment within 144 weeks of randomization. The pre-treatment biopsies will be read by the local consortium pathologist, judged with respect to adequacy for assessing stage and grade, and whether it meets histologic criteria for entry. All slides will be re-reviewed centrally for subsequent data analysis.

The following assessments must be obtained within 8 weeks (56 days) prior to randomization.

1. Written informed consent.
2. Detailed medical history.
3. Physical examination, including pulse, blood pressure, temperature.
4. Limited clinical evaluation including anthropometric measures.
5. Depression assessment.
6. If baseline evaluation for Cohort Study is combined with trial screening visit, complete the Cohort Screening Log and Baseline Evaluation forms (coordinator, participant, and investigator).
7. Blood tests including CBC with differential, routine biochemical tests (including ALT, AST, total bilirubin, direct bilirubin, albumin, total protein, alkaline phosphatase, creatinine [including calculated CrCl], serum uric acid, BUN, calcium, phosphate, vitamin D, triglycerides, glucose, TSH, PT/INR, alpha-fetoprotein, anti-HCV and anti-HDV (if not available within the prior 144 weeks), anti-HIV, and HBV serologies
8. Urinalysis.
9. Pregnancy test for females of childbearing age.
10. Blood samples for qualitative HBsAg and HBeAg, anti-HBs, anti-HBe, HBV DNA, HBV precore/BCP, and antiviral resistance. Plasma and serum samples will be stored at each visit.

11. Blood samples for HBV genotype and/or subtype will be obtained if not available from samples drawn for the HBRN Cohort study.
12. Dual energy X-ray absorptiometry (DEXA) scan for bone density if clinically indicated (as standard of care).
13. A CT or MRI is required for patients with alpha fetoprotein greater than 20 ng/mL as clinically indicated to rule out HCC.
14. In participants with diabetes or hypertension, a retinal examination will be performed as standard of care unless done in the previous 48 weeks.

There are two ALT levels and HBV DNA levels required to qualify for randomization, with the most recent for ALT being within 8 weeks prior to randomization and the most recent for HBV DNA being within 32 weeks of randomization.

6.3. Baseline visit (day 0)

The baseline visit (day 0) will coincide with randomization and the start of therapy. The baseline visit will consist of:

1. Limited clinical evaluation.
2. Review of adverse events and concomitant medications.
3. Drug dispensing; instructions on their use, including education on self-injection with peginterferon alfa-2a (if appropriate).
4. Questionnaires (symptoms, quality of life, health behaviors, and fatigue).
5. Depression assessment.
6. Blood tests including CBC with differential, hepatic and renal panel, albumin, total protein, alkaline phosphatase, and PT/INR.
7. Urinalysis, urine creatinine, and urine phosphate.
8. Pregnancy test for females of childbearing potential.
9. A blood sample for storing serum and plasma for future research.
10. Blood samples for qualitative HBeAg, anti-HBe, quantitative HBsAg, quantitative HBeAg, and HBV DNA testing.
11. Blood sample (with consent) for DNA extraction and genetic testing will be drawn if not obtained as part of the Cohort Study. This may be collected at a future visit if not obtained at baseline.

6.4. On-Treatment assessments

After randomization, participants will be seen in follow up at 4-week intervals for the first 12 weeks and then at 12 week intervals until therapy is completed, as shown in the Time and Event Chart (Appendix 3). For participants having side effects related to treatment or adherence issues, more frequent visits can be undertaken at the discretion of the study physician. These visits will be conducted as an “Unscheduled Visit” and tests/procedures performed will be at the discretion of the study physician (with the exception of adverse events to be managed per guidelines specified in the protocol or manual of operations, i.e. hepatitis flare or hypophosphatemia).

1. Each outpatient visit will include assessment of compliance, adverse events reporting, vital signs, weight, symptoms evaluation, record of concomitant medications, drug dispensing, and blood testing as detailed in the Time and Event Chart (Appendix 3).
2. Symptom questionnaire at each visit.
3. Depression assessments at weeks 12, 24, 36, 48, and 96.
4. Fatigue questionnaire at weeks 24 and 96.
5. Health Behavior and quality of life questionnaires at weeks 48, 96, and 144.

6. Blood will be drawn for CBC with differential at each visit until week 36 and then CBC without differential at week 48, 96, and 144.
7. Hepatic panel (AST, ALT, total bilirubin) at each visit.
8. A renal panel (creatinine, calculated creatinine clearance, phosphate, blood urea nitrogen, and calcium) at every visit from week 12 on.
9. TSH at weeks 12 and 24.
10. Glucose and triglycerides at week 24.
11. There will be a PT/INR, serum uric acid, and other liver tests (albumin, total protein, alkaline phosphatase, direct bilirubin) at weeks 48, 96, and 144.
12. There will be a urinalysis done at weeks 48, 96, and 144.
13. A fasting urine creatinine and phosphate will be done at week 96.
14. For females of childbearing age, adherence to contraception will be reviewed and a pregnancy test repeated at each visit during the peginterferon treatment phase.
15. Qualitative HBeAg (in patients who were HBeAg-positive at baseline visit) and HBsAg at weeks 48 and 96. If there is HBeAg or HBsAg loss then anti-HBe or anti-HBs will be tested.
16. Qualitative HBeAg and anti-HBe (regardless of HBeAg status at the baseline visit) at weeks 144 and 180.
17. Qualitative HBsAg at week 180.
18. Quantitative HBsAg and HBeAg will be tested at every visit from week 12 on.
19. HBV DNA will be drawn at every visit.
20. A blood sample for storing serum and plasma for future research will also be drawn.
21. Antiviral resistance testing at weeks 48, 96, and 144.
22. Ultrasound exam (If clinically indicated, per AASLD guidelines, done as standard of care) at weeks 48, 96, and 144.
23. Fibroscan (if available) no earlier than week 180 visit and no later than week 192 visit

6.4.1 Review criteria for discontinuing treatment and resumption of treatment and post treatment follow up schedule

At the week 180 visit, the scheduled visit prior to the visit at which eligible participants are scheduled to discontinue treatment, a study physician will review the rationale for antiviral withdrawal, criteria for treatment discontinuation, potential benefits and risks, criteria for resuming treatment, and the post treatment follow up schedule with the participants. The study PI will document this discussion in the participant's record. The participant will be provided with a fact sheet that will be reviewed by a study physician and a signed copy maintained in the study record. Participants should be reminded to bring all remaining study medication to the next visit (week 192). Blood will be drawn for qualitative HBeAg, anti-HBe, and HBsAg testing so results are available at week 192. Eligibility to discontinue treatment will be reconfirmed by the study PI on week 192.

6.5 End of Treatment (Week 192)

At week 192, all participants will complete the following:

1. Physical exam.
2. Assessment of adverse events, concomitant medications and adherence
3. Questionnaires (symptoms, quality of life, health behaviors, and fatigue)
4. Depression assessment.
5. Blood will be drawn for CBC, hepatic panel (including albumin, total protein, alkaline phosphatase), renal panel, serum uric acid, PT/INR, anti-HIV, qualitative HBeAg, anti-HBe, anti-HBs, HBsAg, and HBV DNA. A sample of blood will be

stored for eventual assessment of HBeAg and HBsAg concentration, precore/BCP analysis, and antiviral resistance testing.
6. A blood sample for storing serum and plasma for future research will also be drawn
7. Urinalysis and fasting urine creatinine and phosphate.
8. Ultrasound exam (if clinically indicated, per AASLD guidelines, done as standard of care).
9. Fibroscan if available and not performed at, or since, the week 180 visit

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At week 192 (year 4), participants who meet study criteria for discontinuing therapy (section 6.5.1) will have treatment stopped.

6.5.1 Eligibility criteria for discontinuing treatment at week 192 (participants must meet all criteria)

Participants will be discontinued from treatment if they meet all of the following criteria as determined based on laboratory results at the week 180 visit and any additional test results and clinical assessment up until the week 192 visit:

1. HBV DNA <1000 IU/mL for the previous 24 weeks (i.e. starting at or before week 156 and continuing through week 180).
2. No cirrhosis (Ishak score 5 or 6) on baseline biopsy
3. Normal indices of liver function (CTP =5), albumin ≥3.8 g/dL, INR ≤1.3, direct bilirubin ≤0.5 mg/dL
4. No clinical evidence of decompensation
5. No clinical or radiologic evidence of portal hypertension
6. Platelet count ≥120,000/mm^3
7. HBeAg-negative at baseline visit and confirmed at week 180, or HBeAg-positive at baseline visit with HBeAg loss at or before week 144 and confirmed at week 180. In either situation, there can be no HBeAg positive result at or after week 144.
8. HBsAg-negative (regardless of anti-HBe status), or if HBsAg-positive, HBeAg-negative and anti-HBe-positive at week 180

Participants who do not meet criteria for discontinuing treatment will continue treatment with tenofovir DF until Week 240 or end of the study. Tenofovir DF for the period Week 192 to Week 240 will be provided by the study. Follow up visits and testing will continue every 12 weeks (204, 216, 228) to week 240 or end of the study. If tenofovir DF will be continued beyond Week 240 or end of the study, medication must be obtained per standard of care.

Participants who meet criteria for discontinuing treatment but continue treatment will have follow-up visits and testing per the schedule for participants who discontinue treatment. No study medications (Tenofovir DF) will be provided beyond Week 192 and any antiviral therapy must be obtained per standard of care.

6.6 Post Treatment assessments (Appendix 4)

1. Outpatient visits will occur at weeks 196, 200, 204, 208, 212, 216, 228, and 240 for participants in both treatment groups.
2. Laboratory testing (liver panel) plus telephone assessment can replace an outpatient visit at weeks 208 and 212 if ALT at the preceding assessment (week 204 and week 208, respectively) is <300 U/L for men or <200 U/L for women and bilirubin is normal (total bilirubin <1.5 mg/dL or direct bilirubin <0.5 mg/dL)

3. Medical history and physical exam at week 240.
4. Clinical evaluation at weeks 196, 200, 204, 208, 212, 216, and 228.
5. Assessment of adverse events and concomitant medications at each visit.
6. Questionnaires (quality of life, depression assessment, fatigue, and health behaviors) at week 240.
7. Symptom questionnaire at each visit.
8. CBC including platelets, alkaline phosphatase, albumin, total protein, and creatinine at week 240.
9. Renal panel (creatinine, calculated creatinine clearance, phosphate, blood urea nitrogen, and calcium) at each study visit for participants who are on Tenofovir
10. Hepatic panel (ALT, AST, albumin, total protein, alkaline phosphatase, total and direct bilirubin) at each visit.
11. Urinalysis at week 240.
12. PT/INR at weeks 196, 200, 204, 216, and 240.
13. Blood samples for serum/plasma at each outpatient visit.
14. Qualitative HBsAg, HBeAg, and anti-HBe at weeks 216 and 240.
15. Anti-HBs and antiviral drug resistance testing at week 240.
16. HBsAg quantification at weeks 204, 216, 228, and 240.
17. HBeAg quantification at weeks 204, 216, 228, and 240.
18. HBV DNA at each outpatient visit
19. Ultrasound exam (If clinically indicated, per AASLD guidelines, done as standard of care) at week 240.
20. Fibroscan (if available and obtained at, or since, the week 180 visit but not later than the week 192 visit) at week 240.

6.7 Assessment of adherence
At each study visit, study personnel will review the study drug dosing schedule with participants and counseling on adherence will be provided. At each study visit while on therapy, participants will be asked to return all bottles of tenofovir and used vials of peginterferon alfa-2a to document how much study medications were used. Serum drug levels may be measured on stored serum samples.

6.8. Management of non-response
Definition: Primary non-response is defined as a failure to achieve a >1-$\log_{10}$ IU/mL decline in HBV DNA after the first 24 weeks of treatment. HBV DNA will be measured by the quantitative HBV DNA assay which will be reconfirmed with re-testing 4 weeks later (week 28). Compliance with study medications will be assessed and documented. Primary non-response is considered to be treatment failure.

Management of non-response
6.9.1. Reinforce compliance when non-response is initially suspected.
6.9.2. Testing of Tenofovir DF levels may be performed (samples will be archived for analysis and not available in real-time).
6.9.3. If serum HBV DNA report confirms that participant meets criteria for virological non-response, change to combination emtricitabine/tenofovir within 2 weeks of obtaining the week 28 result.
6.9.4. Participants will be assessed at weeks 4, 8 and 12 and then every 12 weeks after initiation of rescue therapy to document the response. Thereafter, participants will be followed per protocol.

6.9.5. If serum HBV DNA is ≥1000 IU/mL by HBV DNA quantitation assay after 24 weeks of rescue therapy, subsequent treatment will be altered at the discretion of the study physician.

6.10 Management of a partial virological response
Definition: A partial virological response is defined as a decrease of serum HBV DNA >1 $\log_{10}$ IU/mL by week 24 but a level ≥100,000 IU/mL confirmed on two consecutive visits (weeks 36 and 48). A partial virological response is considered to be treatment failure.
6.10.1. Reinforce compliance when partial response is initially suspected.
6.10.2. Testing of Tenofovir DF levels may be performed (samples will be archived for analysis).
6.10.3. If serum HBV DNA report confirms participant meets criteria for partial virologic response, change to combination emtricitabine/tenofovir within 2 weeks of obtaining the week 48 result
6.10.4. Participants will be assessed at weeks 4, 8 and 12 weeks and then every 12 weeks after initiation of rescue therapy to document the response. Thereafter, participants will be followed per protocol.
6.10.5. If serum HBV DNA is ≥1000 IU/mL by HBV DNA quantitation assay by week 24 of rescue therapy, subsequent treatment will be altered at the discretion of the study physician.

6.11. Management of virological breakthrough
Definition: Virological breakthrough is defined as a >1-$\log_{10}$ IU/mL increase in HBV DNA level from nadir or redetection of serum HBV DNA after disappearance that is confirmed on re-testing 4 weeks later in a participant verified to be medication compliant. If the re-test confirms virological breakthrough, a blood sample will be tested for genotypic resistance.

6.11.1. Reinforce compliance when virological breakthrough is initially suspected. The importance of compliance with study medication will be reinforced with study participants at each visit.
6.11.2. Testing of Tenofovir DF levels may be performed (samples will be archived for analysis).
6.11.3. Resistance mutations will be evaluated by sequencing stored samples.
6.11.4. Selection of rescue therapy will be based upon the cross-resistance profile of the mutation(s) present when available. If unavailable, emtricitabine/tenofovir will be used. Rescue therapy should be started within 4 weeks of confirming the virological breakthrough. The time to start of rescue therapy may be adjusted depending upon the time required for resistance testing to be completed.
6.11.5. Participants will be assessed at weeks 4, 8 and 12 weeks and then every 12 weeks after initiation of rescue therapy to document the response. Thereafter, participants will be followed per protocol.
6.11.6. If serum HBV DNA is ≥1000 IU/mL by week 24 of rescue therapy, treatment will be changed at the discretion of a study physician.

6.12 Definition and management of on-treatment hepatitis flares
On-treatment hepatitis flare is defined as increase in ALT to ≥300 U/L for men and ≥200 U/L for women AND >3 times baseline (day 0) value. Participants who meet criteria of a flare will be seen every 4 weeks until ALT decreases to <300 U/L for men and <200 U/L for women. Severe on-treatment hepatitis flare is defined as ALT ≥1000 U/L regardless

of gender. Participants who meet criteria of a severe flare will be seen every week until ALT decreases to <1000 U/L, at which point they will be evaluated every 4 weeks until ALT <300 U/L for men and <200 U/L for women and thereafter at scheduled visits per study protocol. Participants who experience hepatitis flares may continue treatment if total bilirubin is <2.0 mg/dL and direct bilirubin <1.0 mg/dL. Participants who experience severe hepatitis flares may continue treatment at the discretion of the investigator if total bilirubin is <2.0 mg/dL and direct bilirubin <1.0 mg/dL.

6.13. End-of-treatment (prior to week 192) assessment

6.13.1. Eligibility criteria for discontinuation of treatment prior to week 192

Participants who meet either of the criteria below will be discontinued from treatment:

- Grade IV toxicity (laboratory or clinical)
- Hepatic decompensation while on peginterferon defined by:
    - Total bilirubin ≥2.0 mg/dL and direct bilirubin ≥1.0 mg/dL
    - Any clinical decompensation
- Participant refuses to continue treatment/participation in study

6.13.2. End of treatment assessment

Participants who stop treatment early will complete assessments specified for the week 192 visit (see section 6.5 and Appendix 3).

Participants who discontinue treatment due to Grade IV toxicity or hepatic decompensation will continue to be followed until these events are resolved or stabilized, according to the post-treatment follow-up schedule.

6.13.3. Follow-up visit schedule after week 192

All participants who are eligible to stop treatment at week 192 will be seen at weeks 196, 200, 204, 208, 212, 216, 228, and 240 (corresponding to weeks 4, 8, 12, 16, 20, 24, 36, and 48 post-treatment for those whose treatment is discontinued). This is the minimal follow up required. Laboratory testing (liver panel) plus telephone assessment can replace an outpatient visit at weeks 208 and 212 if ALT at the preceding assessment (week 204 and week 208, respectively) is <300 U/L for men or <200 U/L for women and bilirubin is normal (total bilirubin <1.5 mg/dL or direct bilirubin <0.5 mg/dL). Additional visits for safety monitoring should be included as needed. Participants who are not eligible to stop treatment at week 192 and who remain on treatment will be seen at weeks 204, 216, 228 and 240 (i.e., 12, 24, 36 and 48 weeks after week 192). Laboratory testing will be performed as outlined in Appendix 4. Blood samples for serum/plasma will be collected at each outpatient visit.

6.14. Assessment and management of relapse after stopping therapy

6.14.1. Definition of relapse

Virological relapse: Persistent HBV DNA ≥1000 IU/mL during the follow-up period (on at least two samples at least 12 weeks apart, with one of them being more than 24 weeks after stopping therapy) in HBeAg-positive or HBeAg-negative participants.

Biochemical relapse will be defined as an elevated ALT (>30 U/L for females and >45 U/L for males if normal at end of treatment or an increase of 1.5 X end of treatment level if abnormal at end of treatment) on at least two samples at least 12 weeks apart, with one of them being at least 24 weeks after stopping therapy.

An ALT flare will be defined as an ALT level ≥300 U/L for males or ≥ 200 U/L for females.

These definitions will be used for data analyses, see section 6.14.2 for criteria to reinitiate treatment.

Monitoring of participants

- Participants with an increase in total bilirubin to ≥3 mg/dL or direct bilirubin to ≥1 mg/dL or INR ≥1.3 (regardless of ALT and HBV DNA levels) must be reassessed as soon as possible and within 1 week for liver panel, PT/INR, CBC, and creatinine. The laboratory testing should continue every week until INR<1.3 and either total bilirubin <3 mg/dL or direct bilirubin <1 mg/dL.
- Participants with an ALT >1000 U/L for both males and females (regardless of HBV DNA levels) must be reassessed as soon as possible and within 1 week. Thereafter, weekly monitoring of liver panel, bilirubin (total or direct), PT/INR, HBV DNA should continue until ALT $\leq$1000 U/L.
- Participants with an ALT ≥300 U/L for males or ≥200 U/L for females (regardless of HBV DNA levels) must be reassessed within 1 week and thereafter, every 2 weeks for liver panel, HBV DNA, bilirubin (total or direct), and PT/INR. The laboratory testing should continue until ALT <300 U/L for males or <200 U/L for females.
- Participants with an ALT ≥150 U/L for males or ≥100 U/L for females (regardless of HBV DNA levels) must be reassessed within 4 weeks and thereafter every 4 (±1) weeks for liver panel, HBV DNA, bilirubin (total or direct), and PT/INR. The laboratory testing should continue until ALT <150 U/L for males or <100 U/L for females.

6.14.2. Criteria for treatment reinitiation in the Post-Treatment Follow up Period

Tenofovir DF will be restarted for any one of the following:

- Any one of the three criteria: INR ≥1.3, total bilirubin ≥3.0 mg/dL or direct bilirubin ≥1.0 mg/dL, regardless of HBV DNA or ALT level.
- Any clinical decompensation, regardless of HBV DNA or ALT level.
- HBV DNA ≥10,000 IU/mL and ALT >1000 U/L (male or female) (i.e. only one ALT value >1000 U/L is needed to qualify).
- HBV DNA ≥10,000 IU/mL and ALT ≥300 U/L for males, ≥200 U/L for females. A total of one HBV DNA ≥10,000 IU/mL and any three ALT values ≥300 U/L (male) or ≥200 U/L (female) over the 4-week (or longer) time frame are needed to qualify. Treatment will be resumed if the third ALT remains ≥300 U/L (male) or ≥200 U/L (female).
- HBV DNA ≥10,000 IU/mL and ALT ≥150 U/L for males or ≥100 U/L for females. A total of one HBV DNA ≥10,000 IU/mL and any three ALT values ≥150 U/L (male) or ≥100 U/L (female) over the 12 week (or longer) time period are needed to qualify. Treatment will be resumed if the third ALT remains ≥150 U/L (male) or ≥100 U/L (female).
- HBsAg-positive and HBeAg-positive at week 192.
- HBsAg-positive, HBeAg-negative, and anti-HBe-negative at week 192

6.14.3. Management after reinitiation of treatment

Participants who meet criteria for treatment reinitiation specified in section 6.14.2 will be restarted on Tenofovir DF 300mg daily. Tenofovir DF will be provided by the study until week 240. Monitoring of AST, ALT and HBV DNA levels will be done at 4, 8 and 12 weeks after Tenofovir DF is restarted and then every 12 weeks. If bilirubin or INR is abnormal at the time of treatment reinitiation, it (they) should be monitored at 4, 8, and 12 weeks after Tenofovir DF is restarted and then every 12 weeks until it (they) return(s) to normal. If, after treatment reinitiation, the participant meets criteria for non-response (section 6.9) or partial response (section 6.10), the drug therapy will be changed as outlined in those sections.

7. Adverse events and toxicity management

7.1. Criteria for permanent and premature discontinuation of treatment

- Death
- Grade IV toxicity (laboratory or clinical)
- Hepatic decompensation defined by:
    - Total bilirubin ≥2.0 mg/dL and direct bilirubin ≥1.0 mg/dL
    - Any clinical decompensation

7.2.a. Adverse effects of peginterferon alfa-2a

Common side effects of peginterferon alfa-2a include influenza-like symptoms, particularly with the first few injections, fatigue, neuropsychiatric symptoms such as depression and reduction in white blood cell counts and platelet counts. Hyper- or hypothyroidism has been noted to occur in up to 5% of participants receiving peginterferon alfa-2a.

Some rare but potentially serious side effects include:

- neuropsychiatric complications, which may include suicide, suicidal ideation, acute psychosis and severe depression
- serious and severe bacterial infections; fatal infections have been reported during treatment with peginterferon alfa-2a
- pancreatitis
- interstitial pneumonitis, fatal cases have been reported with peginterferon alfa 2a use

Other possible but uncommon adverse events associated with peginterferon alfa-2a include:

- development or exacerbation of existing autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenic purpura, hemolytic anemia, type 1 diabetes, psoriasis, etc.
- pulmonary disorders including dyspnea, pulmonary infiltrates, and sarcoidosis
- exacerbations of inflammatory bowel disease
- eye disorders, including decrease or loss of vision, retinal hemorrhages
- hearing disorders, including loss of hearing and tinnitus
- hypersensitivity reactions including severe skin reactions, in the spectrum of Stevens Johnson syndrome

7.2.b. Peginterferon alfa-2a dose reductions

Factors that will lead to a reduction in the dose of peginterferon alfa-2a include:

1. Disabling symptoms, which, in the opinion of the study physician, are related to peginterferon alfa-2a treatment and prevent the participant from performing his/her occupation or daily tasks.
2. A rash consistent with allergic reaction or vasculitis.
3. Reductions in the neutrophil count according to the guidelines in Appendix 2.
4. A reduction in platelet count according to the guidelines in Appendix 2.
5. Any adverse reaction, which, in the opinion of the study physician, places the participant at increased risk of a serious adverse effect.

The dose of study medications may be decreased due to adverse events or laboratory abnormalities. The weekly dose of peginterferon alfa-2a will be reduced in graduated steps as follows:

- Level 1 decrease from 180 μg to 135 μg
- Level 2 decrease from 135 μg to 90 μg
- Level 3 decrease from 90 μg to 45μg

For participants who require a downward dose adjustment for neutropenia or thrombocytopenia, laboratory values should be repeated, if practical, prior to the next dose of peginterferon alfa-2a to confirm the result and dictate the course of action. Participants who remain with a neutrophil count below 500/mm^3 (but above 250/mm^3) or a platelet count below 50,000/mm^3 (but above 25,000/mm^3) should receive stepwise dose reductions until levels of neutrophils and platelets are ≥ 500/mm^3 or 50,000/mm^3, respectively, or until a dose of 45 μg of peginterferon alfa-2a is reached. Participants who have a neutrophil count below 250/mm^3 or a platelet count below 25,000/mm^3 should have their peginterferon alfa-2a dose held. Peginterferon alfa-2a may be resumed at the previous reduced dose when the neutrophil count has been above 500/mm^3 or platelet count above 50,000/mm^3 on 2 occasions at least 1 week apart.

7.2.c. Guidelines for subsequent peginterferon alfa-2a dose adjustments

Once a participant's dose has been decreased for laboratory abnormalities or adverse events, the study physician may attempt to increase the dose back to or toward the previous stable level only if the following conditions are satisfied:

1. The event or circumstance responsible for the dosage adjustment has resolved or improved;
2. The participant has been at the lower dose for ≤ 4 consecutive doses;
3. The participant had ≤ 6 total doses administered at the lower level during the entirety of the treatment period.

If four or more consecutive doses of peginterferon alfa-2a are held or otherwise not administered (i.e., the participant has not received test medication for more than 28 days), the participant will be considered intolerant of the test medication or non-compliant, whichever is more appropriate to the clinical situation. In such cases, the study physician will discontinue peginterferon alfa-2a but tenofovir DF treatment will continue.

Every attempt will be made to keep those participants randomized to treatment with peginterferon alfa-2a on therapy by dose reduction. The use of hematological growth factors during the study will not be allowed.

7.2.d. Screening and management for depression

The CES-D will be used, along with the study physician's judgment, to screen participants for depression at the screening visit (8 weeks prior to randomization) and to monitor for onset or worsening of symptoms during the treatment.

Screening Visit (8 weeks prior to randomization): If the participant has a CES-D score >15 at the screening visit (8 weeks prior to randomization), clearance from a mental health provider may be considered at the discretion of the study physician. In addition to clarifying possible psychiatric disorders, the mental health provider may advise on the need for adjuvant medical or counseling therapy and suitability for enrollment.

During treatment: Potential mood disturbance/medication intolerance will be assessed at each visit by study personnel. The CES-D will be administered every 12 weeks for 48 weeks. If the participant has a score >15, the study physician will determine whether referral to a mental health provider is indicated. If the participant has a score of at least 27, the study physician will refer to a mental health provider for evaluation and management. Any participant who develops recurrent suicidal ideation, a suicide plan and/ or makes a suicide attempt should have peginterferon alfa-2a immediately discontinued and be referred to a mental health provider for further management.

Suggested guidelines for peginterferon alfa-2a dose reduction:

- Unpleasant and/or disabling side effects might prompt dose reduction, but this is not required.
- A CES-D score of 16-26 might prompt further consideration of dose reduction, but is not a requirement for dose reduction.
- A score of at least 27 should prompt attention from the PI and dose reduction or discontinuation be considered.

If the management of the depression is not successful after eight weeks or if the participant develops suicidal or homicidal ideation, the peginterferon alfa-2a will be stopped but tenofovir DF continued until the end of the treatment period. The participant will be referred to a mental health provider.

7.3.a. Adverse effects of tenofovir DF

All nucleoside and nucleotide analogues carry a warning about the risk of lactic acidosis and severe hepatomegaly and steatosis because of the possibility of mitochondrial injury.

Tenofovir DF has been associated with instances of lactic acidosis, but only in participants receiving other medications that have been associated with this complication, such as didanosine, stavudine and zidovudine. It is not clear whether tenofovir DF when used by itself or in combination with peginterferon alfa-2a, is associated with mitochondrial toxicity either to the liver (lactic acidosis, acute fatty liver and hepatic failure), muscle (myositis), nerves (neuropathy) or pancreas (acute pancreatitis).

In addition, all agents with activity against HBV replication carry a warning label about the risk of exacerbation of hepatitis after discontinuation of treatment. This issue is addressed by the protocol design and close follow-up of participants after stopping therapy (see section 6.6, 6.14.1, and Appendix 4).

7.3.b. Tenofovir DF and renal function

Tenofovir DF is principally eliminated by the kidney. Renal impairment, including cases of acute renal failure and a Fanconi-like syndrome (proximal renal tubular dysfunction with severe hypophosphatemia), has been reported.

Adverse Event Monitoring and Management Related to Elevated Serum Creatinine (Cr)
Nephrotoxicity will be based upon serum creatinine results:

- If Cr ≥0.5 mg/dL above baseline, repeat testing within 72 hrs will be performed and drug continued unless the study physician considers drug discontinuation necessary.
- If Cr increases to ≥1.2 mg/dL and by ≥0.3 mg/dL from baseline, additional testing at the next scheduled visit should be considered (see Manual of Operations). If Cr ≥1.2 mg/dL and >0.5 mg/dl above baseline on two consecutive occasions, the study drugs will be discontinued and not restarted unless another, reversible cause of creatinine elevations is identified. If the study physician identifies an alternative cause for the increase in Cr, study drugs may be resumed at discretion of study physician if calculated Cr clearance is >30 ml/min with tenofovir DF dose adjustment.
- Any study participant discontinued from the study will be recommended to commence anti-HBV therapy with an alternative HBV drug immediately (this drug will not be provided by the study).

Tenofovir DF Dosing is Based Upon Creatinine Clearance (CrCL)
Creatinine clearance will be calculated in all participants prior to initiating therapy and at each study visit to determine tenofovir DF dose.

- Participants whose calculated creatinine clearance decreases to <50 ml/min should have this value confirmed within 72 hrs. If confirmed to be 30-50 ml/min, the dosing frequency for tenofovir DF should be adjusted from every 24 hours to every 48 hours until the calculated creatinine clearance returns to >50 ml/min.
- Participants whose calculated creatinine clearance decreases to <30 ml/min, will be permanently discontinued from treatment and enter the follow-up phase.

Adverse Event Monitoring and Management of Hypophosphatemia:
If serum phosphate falls below 2.5 mg/dL and by at least 0.5 mg/dL from baseline, consider phosphate supplementation. Additional testing at the next scheduled visit should be considered (see Manual of Operations).

- If the serum phosphate is <1.4 mg/dL, repeat testing within one week will be performed and supplementation provided as per manual of operations. If serum phosphate is <1.4 mg/dL on repeat testing, urine and serum phosphate and creatinine will be determined to assess maximal phosphate tubular reabsorption and blood test should be performed to check potassium, calcium, bicarbonate, BUN and Cr.
- Tenofovir DF dosing may be interrupted and aggressive supplementation with intravenous (if symptomatic) or oral (if no symptoms) phosphorus should be used. Repeat phosphate testing weekly until corrected to lower limit of normal range. If this intervention does not correct phosphate level in 2 weeks, Tenofovir DF should be permanently discontinued.
- If serum phosphate is <1.0 mg/dL, participant will be asked to return immediately for further evaluation and treatment and Tenofovir DF dosing will be discontinued.

Any study participant discontinued from the study will be recommended to commence anti-HBV therapy with an alternative HBV drug immediately (this drug will not be provided by the study).

7.3.c. Tenofovir DF and bone mineral density
Decreases in bone mineral density have been observed in participants taking tenofovir DF for prolonged periods in the context of treatment for HIV infection. These changes have been associated with significant increases in biochemical markers of bone metabolism, suggesting increased bone turnover. Although the pathogenesis of these changes are not certain, they may be related to proximal renal tubulopathy, phosphate loss and a form of secondary hyperparathyroidism.

Serum calcium, phosphate and creatinine levels will be monitored. Serum vitamin D levels will be measured at baseline and supplements prescribed for the participants as clinically indicated. Participants will have bone densitometry done prior to initiating therapy if clinically indicated (as standard of care). If either osteopenia or osteoporosis is present on the bone densitometry done prior to initiation of treatment, the participant can be enrolled as long as he/she is concurrently treated for osteopenia or osteoporosis. Repeat bone densitometry may be performed if clinically indicated (as standard of care).

7.3.d. Discontinuation of tenofovir DF for selected biochemical abnormalities
Tenofovir DF will be permanently discontinued and the participant considered a treatment failure for Grade 3 hypophosphatemia (1.0-1.4 mg/dL) that does not correct within 2 weeks with phosphate supplements or Grade 4 hypophosphatemia (<1.0 mg/dL) or otherwise unexplained serum creatinine elevations by ≥1.2 mg/dL and ≥0.5 mg/dl above baseline on two consecutive occasions or calculated creatinine clearance <30 ml/min that develop on therapy. For lesser degrees of renal insufficiency not requiring discontinuation, the dose of tenofovir DF will be adjusted according to the manufacturer's recommendation.

7.4. Adverse events of emtricitabine and emtricitabine/tenofovir
Emtricitabine alone or in combination with tenofovir coformulated as Truvada (emtricitabine 200 mg/tenofovir DF 300 mg) has not been approved for the treatment of chronic hepatitis B. Emtricitabine belongs to a class of anti-HIV drugs (nucleoside reverse transcriptase inhibitors). The most common side effects seen in ≥10% patients treated with emtricitabine in combination with other anti-HIV drugs are: headache, diarrhea, nausea, and elevation in creatine kinase. These side effects were generally mild to moderate in severity.

Lactic acidosis (increase in lactic acid) is a very serious reaction, which is characterized by symptoms of nausea, vomiting, abdominal pain, general discomfort and tiredness that do not get better and also by severe hepatomegaly (enlargement of the liver) with steatosis (fatty deposits). Lactic acidosis, including fatal cases, has been reported with the use of nucleoside and nucleotide reverse transcriptase inhibitors.

For people who are infected with HIV as well as hepatitis B, other possible risks of treatment with Truvada include making their hepatitis B worse. The potential risk relates to HBV flares induced by the discontinuation of Truvada and careful monitoring is required when the drug is stopped. There is no evidence of drug-drug interactions when emtricitabine and tenofovir are used in combination.

Truvada dosing is based upon CrCL
Creatinine clearance will be calculated by the MDRD method in all participants prior to initiating therapy and at each study visit to determine Truvada dose.

- Participants whose calculated creatinine clearance decreases to <50 ml/min should have this value confirmed within 72 hrs. If confirmed to be 30-50 ml/min, the dosing frequency for Truvada should be adjusted from every 24 hours to every 48 hours until the calculated creatinine clearance returns to >50 ml/min.
- Participants whose calculated creatinine clearance decreases to <30 ml/min, will discontinue Truvada. Subsequent management will be at the discretion of the investigator, alternative antiviral therapy should be considered and the participant monitored until creatinine clearance returns to baseline or stabilizes.

7.5. Pregnancy

Female participants will be asked to practice effective birth control while they are taking antiviral agents in this study. However, given the very long duration of therapy and the fact that many of the participants are expected to be females of child bearing potential, the following precautions will be taken:

All female participants of child bearing potential will undergo a urine or serum pregnancy test prior to starting therapy and every visit during the peginterferon treatment phase. If a participant is found to be pregnant, therapy will be adjusted as follows:

- Peginterferon alfa-2a will be permanently discontinued
- Tenofovir DF can be continued throughout the pregnancy at the discretion of the study physician.
- Emtricitabine/tenofovir (Truvada) can be continued throughout the pregnancy at the discretion of the study physician.

The study will collect data on the outcomes of any pregnancies that occur in females who conceived while taking study medication.

7.6. Adverse events – definition and management

7.6.1. Definitions

An adverse event (AE) is any adverse change from the participant's baseline (pre-treatment) condition, including intercurrent illness which occurs during the course of the trial, after the consent form has been signed, whether the event is considered related to treatment or not.

The Common Terminology Criteria from the National Cancer Institute (NCI) of the National Institutes of Health will be used for grading severity of AEs. For AEs not outlined in the Common Terminology Criteria, study specific definitions will be identified in the Manual of Operation (MOP).

<u>Serious Adverse Event:</u>
A serious adverse event is an untoward medical occurrence that results in any of the following:

1. Death
2. Is life threatening (risk of death at the time of the event)
3. Requires in-patient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability/incapacity
5. Congenital abnormality or birth defect

Important medical events that do not result in one of the events listed above may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

A serious adverse event which is unexpected and is related will require expedited reporting to the DCC and the NIDDK and appropriate oversight committees or entities.

Disease related outcomes, such as the following events, will not be considered to be serious adverse events:

1. Development of HCC
2. Hepatic decompensation

Although pregnancy, overdose, cancer (excluding HCC), and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs.

Unexpected:
An adverse event that is not listed in the Investigator's Brochure (or package insert) or is not listed at the severity that has been observed.

Suspected Adverse Reaction:
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event (i.e., there is evidence to suggest a causal relationship between the drug and the adverse event).

SAE Reporting:
This trial will be conducted under U.S. IND. Therefore, the safety data required to meet IND regulatory requirements will be collected through adverse event reporting by the clinic investigators and will be provided by the Data Coordinating Center to the NIDDK for transmission to the FDA.

A serious adverse event which is unexpected and is drug related (even remotely) will require expedited reporting to the drug manufacturer, the DCC and to local IRB/REBs (per local IRB/REB policy).

Only serious and unexpected suspected adverse reactions with evidence of a causal relationship to the study and/or study drug will be reported to the FDA as an IND safety report according to 21 CFR 312.32.

Unexpected fatal or life-threatening suspected adverse reactions must be reported to FDA no later than 7 calendar days after the sponsor's initial receipt of the information.

All other unexpected serious suspected adverse reactions must be reported to FDA no later than 15 calendar days after the sponsor's initial receipt of the information.

A summary of adverse events will be reported to the FDA as part of the IND annual report.

7.6.2. Data collection procedures for adverse events

Participants will be interviewed regarding medical conditions, medication changes, and

symptoms that have occurred at each study visit. An Adverse Event form will be completed if any adverse event is reported. If the Study Coordinator or Principal Investigator learns of any hospitalizations or other adverse events between study visits, an Adverse Event form will be completed. All adverse events and Serious Adverse Events from time of study entry (consent) up to the end of follow-up (week 240 or end of the study) will be reported to the DCC.

A Serious Adverse Event form will be completed for all adverse events rated as serious.

Participants will be followed for all ongoing unresolved adverse events until they are either resolved, or in the opinion of the Principal Investigator, the participant is medically stable.

The investigator will assess the relationship of each adverse event to the use of study drug, based on available information, using guidelines outlined in the MOP.

7.6.3. Reporting procedures

All serious adverse events that are unexpected and related to study drug(s) will be reported to the DCC within 24 hours of knowledge of the event, via the Serious Adverse Event form. This reporting includes serious adverse events that occur from the time the participant has signed the clinical trial consent.

The DCC will distribute the expedited report to the NIDDK, the appropriate oversight committees or entities, and clinical centers. Status reports on serious adverse events will be generated by the DCC and will include the relationship of the adverse event to trial medications, the severity of the event and if the event is resolved or ongoing.

All deaths will be reported to the DCC within 24 hours of knowledge of the death via the Serious Adverse Event form. This reporting begins at the time the participant has signed the informed consent up to the last scheduled participant visit. The report will include the relationship of the death to trial medications. A Clinical Outcome form will also be completed and sent to DCC for distribution. Deaths will be reported immediately to the NIDDK, the appropriate oversight committees or entities, and the clinical centers. A death will be reported as an expedited report only if it is unexpected and drug related. A death must also be reported in accordance with local law and regulations.

The HBRN will comply with Genentech/Roche SAE reporting requirements (see Manual of Operations).

8. Statistical considerations

8.1. Statistical analysis

Summary statistics will be generated to describe the study sample at baseline. Descriptive statistics, including measures of central tendency (e.g. means, medians) and estimates of spread (e.g., standard deviations, quartiles) will be used for continuous variables such as age and baseline HBV DNA and HBsAg levels. Frequency distributions will be used for categorical variables such as HBV genotype and HBeAg status. Ninety-five percent confidence intervals will be calculated for all point estimates of continuous data. Graphical displays (e.g. histograms, box plots) will also be used to describe the data. The baseline demographic information and viral characteristics will be compared between the two treatment arms using the chi-square test for association or exact tests for differences in proportions for categorical variables or using a test of trend

in ordinal data. Continuous variables may be transformed, where appropriate, to meet the assumptions of parametric tests. The Student's t-test or a non-parametric alternative, the Wilcoxon rank-sum test, will be used to determine differences in the distribution of continuous data between the two treatment arms.

Primary analyses will be performed using all enrolled participants (intention-to-treat analysis). Participants will be considered enrolled in the study upon being randomized to one of the two treatment arms.
For all analyses, a p-value of less than 0.05 will be used to indicate statistical significance.

8.1.1. Analysis of primary endpoint

The primary endpoint is loss of HBsAg at week 240. The cumulative proportion of HBsAg loss at the designated time-point will be estimated using the product-limit (Kaplan-Meier) estimate. HBsAg loss between two treatment groups will be compared using a Wald-type two-sided two-sample proportion test (chi-square test).

8.1.2. Analysis of secondary endpoints

Analysis of secondary endpoint listed in 2.2.1a: *To compare the cumulative proportion HBsAg loss over time during and after treatment*
Cumulative proportion of HBsAg loss over time will be calculated using the product limit (Kaplan-Meier) method. HBsAg loss between two treatment groups will be compared using a two-sided two-sample stratified log-rank test. We will also describe the treatment effects in terms of hazard rates of HBsAg-loss and corresponding 95% confidence intervals. To take into account the stratification at randomization, we will use stratified Cox proportional hazard models. Comparison of hazard rates of HBsAg loss across two treatment groups adjusting for other baseline demographic, virological and clinical characteristics such as age, race, genotype, HBeAg status, initial HBV DNA level, and baseline histology will be performed using Cox proportional hazard regression. The results will be presented as incidence rate ratios of HBsAg loss and 95% confidence intervals.

8.1.2.1. Analyses of other secondary endpoints

Secondary endpoints for this clinical trial are listed in sections 2.2.1 – 2.2.9. These endpoints could be grouped into several different classes. The first group of endpoints we will deal with are binary endpoints characterized by the occurrence of some event by a fixed time point. These events include HBeAg loss, HBeAg seroconversion, HBsAg seroconversion, normalization of ALT, ALT ≤1.25 X ULN, HBV DNA <1000 IU/mL, HBV DNA <20 IU/mL, absence of detectable antiviral drug-resistance HBV mutations, treatment discontinuation, and treatment failure. Analysis will be conducted separately for these events at the designated time points listed in sections 2.2.1-2.2.9

Proportion of such an event (e.g. proportion of HBeAg loss) between two treatment groups will be summarized using 95% confidence intervals and will be presented graphically by using bar charts. The rate of occurrence of an event between two treatment groups will be compared using a two-sided two-sample proportion test (chi-square test or Fisher's exact test as appropriate). We will also describe the treatment effects using relative risk of the event and corresponding 95% confidence interval. In order to take into account the stratification at randomization, we will use Mantel-Haenszel chi-square test. Comparison of rates of an event across two treatment groups adjusting for other baseline demographic, virological and clinical characteristics such as

age, race, genotype, HBeAg status, initial HBV DNA level, and baseline histology will be performed using multivariable log-linear (Poisson) regression, with HBsAg loss (Yes/No) as the outcome. The results will be presented as relative risks of the event and their 95% confidence intervals.

Time to such events may also be of interest (e.g. cumulative HBeAg loss, time to treatment failure). For time-to event analysis, Kaplan-Meier curves will be used to plot the cumulative proportion of events over time by treatment groups. The cumulative proportions will then be compared using two-sided stratified log-rank test. The treatment effect will be described using hazard rate of the event and corresponding 95% confidence interval. In order to take into account the stratification at randomization, we will use stratified Cox proportional hazard models. Comparison of hazard rates of an event across two treatment groups adjusting for other baseline demographic, virological and clinical characteristics such as age, race, genotype, HBeAg status, initial HBV DNA level, and baseline histology will be performed using Cox proportional hazard regression. The results will be presented using hazard rates and 95% confidence intervals.

The second group of endpoints is continuous variables such as changes in ALT, HBV DNA levels from baseline at designated treatment or follow-up time points. Descriptive statistics, including measures of central tendency (e.g. means, medians) and estimates of spread (e.g., standard deviations, quartiles) will be used to characterize such changes. Student's t-test or a non-parametric alternative, the Wilcoxon rank-sum test, will be used to determine differences in the distribution of these changes between the two treatment arms. Multiple regression analyses will be conducted to assess the association between baseline characteristics and these changes. Spearman or Pearson correlation coefficients will be used to measure the strength and direction of the association between such changes and other participant characteristics. Scatter plots will be used to display the association between continuous variables.

The third group of variables consists of variables that are measured over time (longitudinal data) such as quantitative HBV DNA levels. For graphical representation over time, we will use spaghetti plots of original data (e.g. HBV DNA levels) and summarize the pattern of data across subgroups (e.g. genotype, treatment groups) over time by using means (for continuous outcome) or proportions (for binary outcomes). To determine the significance of change in continuous outcome over time across treatment group adjusting for other baseline and longitudinal participant characteristics, we will use mixed models if the data are normally distributed or generalized estimating equations (GEE) if deviation from normality is evidenced. These approaches simultaneously model the regression of the response variable on the independent variables and account for the correlated nature of the responses. Analyses using conventional regression methods could provide erroneous variance estimates and loss of statistical efficiency because of the conventional assumption that each response is statistically independent. As a result, invalid inferences could be made if the conventional approach were applied to these longitudinal data. A similar approach of analysis will be adopted (generalized linear mixed model or GEE) for longitudinal binary or count outcomes over time.

The results will be summarized using rate of change/odds ratio/relative risk over time and the corresponding confidence intervals. Interaction between treatment and participant characteristics will be investigated to assess the differential effect of treatments across subgroups of participants (e.g. genotypes).

Secondary endpoints that are defined within a specific subgroup will be analyzed only for participants in that subgroup. For example, sustained off treatment (36 and 48 weeks after end of treatment) responses will be assessed only among those whose treatment was discontinued at week 192.

8.2. Missing data

Primary analyses will be performed using all enrolled participants (intention-to-treat analysis). For the primary analysis, the proportion of patients with HBsAg loss at the end of follow-up (week 240) will be estimated using the product limit (Kaplan-Meier) method which includes drop outs for as long as they are followed. Since our sample size estimation accounts for participants lost to follow up for various reasons, and due to randomization, the rate of missing is expected to be similar in the two treatment groups, we will also conduct the analysis of primary endpoint on the available cases. For this analysis, we will describe the treatment effects in terms of relative risks and 95% confidence intervals. In order to take into account the stratification at the randomization, we will use Mantel-Haenszel chi-square test. Comparison of rates of HBsAg loss across two treatment groups adjusting for other baseline demographic, virological and clinical characteristics such as age, race, genotype, HBeAg status, initial HBV DNA level, and baseline histology will be performed using multivariable log-linear (Poisson) regression, with HBsAg loss (Yes/No) as the outcome. The results will be presented as relative risks of HBsAg loss and their 95% confidence intervals.

In general, the procedure of 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 data. For the time-to-event data analyses, the analytical methods such as Kaplan-Meier method, log-rank test, and Cox proportional hazard model automatically accounts for MCAR drop-outs. However, in other cases, we will assume that the dropout depends on observed outcome and/or covariates (missing at random, MAR). For such scenarios, likelihood-based methods such as mixed models, generalized linear mixed models and logistic regression will provide unbiased inference. For GEE, we will draw inference using inverse-probability-weighting approach, with models fitted using the reciprocal of the estimated probability of missing as weights. The probability of missing will be estimated using logistic regression.

It is unlikely that the probability of missing will depend on unobserved outcome and/or covariates (missing not at random, MNAR). However selection models such as MNAR Dale model and Diggle-Kenward model (23) can be used in such cases. 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 conclusion to possible violation of such assumptions by fitting models under MCAR, MAR and MNAR assumptions and compare the model fits using log-likelihoods.

8.3. Safety analysis

8.3.1. Adverse events

Summaries of adverse events (number adverse events, number and percentage of participants with adverse events, rates per person-years) will be provided by treatment

group. Events will be summarized based on the date of onset for the event. A treatment emergent adverse event will be defined as an adverse event that begins on or after the date of first dose of study drug. Events that occur prior to the first dose of study medication or after the last dose of study medication will be summarized separately. Summaries of the following are planned:

- all adverse events recorded between screening and first dose of study medication,
- all treatment emergent adverse events,
- all emergent and related adverse events,
- all treatment emergent renal adverse events,
- all treatment emergent and related renal adverse events
- combined Grade 2, 3 and 4 treatment emergent adverse events,
- combined Grade 2, 3 and 4 related treatment and emergent adverse events,
- combined Grade 3 and 4 treatment emergent adverse events,
- combined Grade 3 and 4 related treatment and emergent adverse events,
- all adverse events that caused permanent discontinuation of study drug,
- all adverse events that caused temporary interruption of study drug,
- all serious adverse events, and
- all serious and related adverse events.

In addition quarterly safety analysis will be reported to DSMB.

8.3.2. Laboratory abnormalities

Laboratory results will be presented as both actual value as well as a function of normal range. Selected laboratory data (using conventional units) will be summarized by the change from baseline in laboratory test. If baseline data are missing, then any graded abnormality is considered treatment emergent.

8.3. Sample size and power

The sample size for this study was determined based on the primary endpoint of HBsAg-loss at 48 weeks after treatment has been stopped. There are no data available on HBsAg loss for long-term treatment with tenofovir DF or tenofovir DF and peginterferon combination therapy. We anticipate that after 192 weeks of treatment and 48 weeks of follow-up post-treatment 5% of the participants in the tenofovir DF alone group will achieve HBsAg loss compared to 15% participants in the tenofovir DF and peginterferon combination therapy group. The clinical trial would require 141 participants per arm (282 participants in total) to detect this expected difference with 80% power at 5% level of significance using a two-sided test of equality between two proportions (chi-square test). However, for this long-term treatment trial, it is expected that approximately 25% of the participants will be lost to follow-up and hence the study will recruit a total of 376 participants (randomized 1:1 to two treatment arms, 188 per arm) to account for the missing participants.

9. Data and safety monitoring board

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.

9.1 Data and safety monitoring plan

This phase III randomized trial aims to compare the long-term efficacy of initial treatment with combination therapy with peginterferon plus tenofovir DF versus tenofovir DF monotherapy in the treatment of chronic hepatitis B. The individual drugs (peginterferon and tenofovir DF) are FDA-approved and have been well-studied. The risks to participants are outlined in sections 5.2.3 and 5.3.3 in the accompanied protocol. Section 7 of the protocol defines the toxicities associated with these drugs and outlines dose adjustment and discontinuation guidelines due to toxicity.

Emtricitabine/tenofovir DF coformulated as a single tablet Truvada is approved for HIV but not for HBV treatment. Emtricitabine/tenofovir DF will be offered to patients who have primary nonresponse, partial virologic response or confirmed virologic breakthrough. Adverse events and dose adjustment guidelines are described in section 7.4.

The data and safety monitoring plan (DSMP) for this study focuses on close monitoring by the principal investigators (PI) and prompt reporting of excessive adverse events and all serious adverse events to the DCC and the appropriate oversight committees or entities such as the NIDDK, the Data and Safety Monitoring Board (DSMB) and to the participating centers' IRB/REBs.

The Data Coordinating Center (DCC) will monitor clinical center performance (e.g., recruitment, retention, data completeness, timeliness of data collection and submission) and protocol compliance. These reports, with summaries of adverse event data, will be provided to the DSMB for their quarterly reports and biannual calls or meetings, and to the Steering Committee at its annual meeting. DSMB reports will include both open and closed session reports, with only the latter including information by treatment arm.

The DCC will work with the Safety Officer and the Steering Committee to maintain a cumulative summary of adverse events (overall and stratified by serious/non-serious status) that will be forwarded to the DSMB every three months. Safety reports will also be sent to the Principal Investigators and the NIDDK Project Officers. The Project Coordinator will be responsible for distributing these reports and assuring that all parties obtain copies of these reports.

The frequency of data review for this study differs according to the type of data and can be summarized in the following table:

Data type	Frequency of review
Recruitment Retention Protocol adherence(e.g., meeting inclusion/exclusion criteria, treatment compliance) Pregnancy and outcomes in female participants	Quarterly reports for NIDDK and the appropriate oversight committees
Adverse events and Serious Adverse Events (SAE) that do not meet expedited reporting criteria	Quarterly reports for NIDDK and the appropriate oversight committees
Serious Adverse Events that meet expedited reporting criteria*	As they occur for NIDDK, DSMB, appropriate industry partner(s), and

	cumulative reports quarterly
Laboratory data	Yearly for the DSMB

The definition of adverse and serious adverse events is provided in section 7.6.1 of this protocol along with the reporting procedures in the MOP.

Stopping rules during the treatment phase (up to Week 192)
No interim analysis for efficacy is planned for this clinical trial, since the primary endpoint is expected to occur very rarely during the treatment period. As mentioned above, adverse event reporting will be quarterly throughout the study. Additional analyses will be performed at the recommendation of the DSMB if there are any concerns raised by adverse events or serious adverse events reported. The following study-wide stopping rules have been agreed upon by the clinical investigators for this interim analysis. The study will be stopped if at least one of the following occurs:

1. If the incidence of renal toxicity (defined as a persistent increase in serum creatinine >1.0 mg/dL above baseline on two successive visits 4 weeks apart or persistent grade 3 or grade 4 hypophosphatemia (requiring TDF discontinuation) exceeds 10% in either of the two study arms.
2. If the incidence of peripheral neuropathy (documented by EMG) exceeds 10%.
3. If the incidence of grade IV toxicity exceeds 10% in either of the two study arms.

Stopping rules during treatment discontinuation phase (Weeks 192 to 240)
The following has been agreed upon by the clinical investigators

1. If more than 1 participant develops clinical decompensation, no further participants will undergo treatment withdrawal until the formal review by investigators and the DSMB
2. Resumption of treatment withdrawal may occur if the DSMB and investigators determine the safety measures are adequate AND that the benefits of continued study of treatment withdrawal outweighs the risks

9.2 Participant Confidentiality
The central database of the study is on a server at the Epidemiology Data Center (EDC) at the University of Pittsburgh secured behind locked doors and an alarm with password access provided only to authorized personnel. Backups are performed daily to guard against data loss due to an equipment or power failure. Scheduled backups and archives at the EDC protect central and local information from hard disk failures. Tape backup volumes and CD-ROM copies of critical project files are located in a secured off-site storage area to prevent data loss due to catastrophic events. Routine virus detection is also enforced for all EDC computers involved in the study. All critical information regarding database transactions is logged and stored in journal files. In the event of accidental corruption of the project database, a previous database state may be restored from backup volumes or journal files. All servers used for this project are connected to uninterrupted power supplies to protect equipment against electrical surges and outages. A secured, raised-floor computer room in an area with a burglar alarm houses all project server equipment.

Subject confidentiality is preserved by encoding subject names into alphanumeric IDs at the clinical centers. Data sent to the DCC are identified by alphanumeric ID only. No reports of this study will use names or other identifying information such as social security numbers or addresses. Data, with alphanumeric ID only, will be stored at the DCC indefinitely. In addition, following the completion of the study, data and information

with alphanumeric ID only, will be submitted to the NIDDK repository by a suitable date agreed upon by NIDDK and the steering committee.

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Appendix 1: Dosing of peginterferon if missed doses

Dose delayed 1 or 2 days: administer on usual dosing day of the week (e.g., if Monday is the usual dosing day and the dose is delayed until Wednesday, the next dose may be administered as usual on Monday).
Dose delayed 3-5 days: administer subsequent doses every 5th or 6th day until the participant is back to his or her original schedule (e.g., if Monday is the usual dosing day and the dose is delayed until Saturday, the next dose should be administered on Thursday, the following dose on Tuesday, then the dose after that as usual on Monday).
Dose delayed 6 days: hold the dose for that week then continue on the usual schedule the following week (e.g., if Monday is the usual dosing day but the participant is not ready to be dosed until the following Sunday, the dose is considered to have been held and the next injection should be for the following weeks; dose on Monday).
Dose delayed 7 or more days: the investigator may reintroduce study drug at any time and, if necessary, dose the participant every 5th or 6th day until the participant resumes weekly dosing on their usual scheduled day.

Appendix 2: Peginterferon alfa-2a dose adjustment guidelines

Specific dose adjustment guidelines for peginterferon alfa-2a are provided in the tables below for neutropenia, and thrombocytopenia. For other adverse effects considered to be possibly related to peginterferon, including laboratory abnormalities, adverse events, and vital signs changes, study physicians should utilize the table below labeled "General Dose Reduction Guidelines." When practicable, abnormal laboratory results should be confirmed as soon as possible following notification of the study physician. If appropriate, downward adjustments in one level reductions should be considered. The lowest dose of peginterferon alfa 2a that should be administered is 45 µg weekly. It should be kept in mind that whereas these guidelines should be generally followed to promote consistency across centers, other responses by a study physicians may be more appropriate in some circumstances.

a. General dose reduction guidelines

For Peginterferon alfa-2a, 1 dose reduction = 135 µg; 2 dose = 90 µg; 3 dose = 45 µg

Number of Dose Reduction Levels					
Mild	Moderate Limited	Moderate Persistent	Severe Limited	Severe Persistent	Life-Threatening
0	0	0-1	0-1	1-3	Stop drug

b. Dose adjustments for low absolute neutrophil and platelet counts for participants who enter the trial with ANC > 1500 and platelet count > 90,000.

Parameter	Downward Dose Adjustment or Delay in Dosing
ANC (cells/mm^3)	
≥500	No change
250-499	1 step initially; potentially up to 3 steps
<250	Stop drug
Platelet Count (cells/mm^3)	
>50,000	No change
25,000-50,000	1 step initially; potentially up to 3 steps
<25,000	Stop drug

Appendix 3: Time and event chart for screening, baseline, and up to week 192 while on treatment

Event	Screen (weeks)	Weeks on Treatment																		
Weeks of Treatment	-8	0[1]	4	8	12	24	36	**48**	60	72	84	**96**	108	120	132	**144**	156	168	180	**192**
Informed consent	X																			
Medical history	X																			
Physical exam	X																			X
Limited clinical evaluation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Assessment of AE, concomitant meds, adherence		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Symptom questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaires (QOL)		X						X				X				X				X
Depression assessment	X	X			X	X	X	X				X								X
Health behavior		X						X				X				X				X
Fatigue questionnaire		X				X						X								X
CBC with platelet (differential up to week 36)	X	X	X	X	X	X	X	X				X				X			X[a]	X
Hepatic panel (AST, ALT, bilirubin)[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Other liver tests (albumin, total protein, ALKP)	X	X						X				X				X			X[a]	X
Renal Panel (Creatinine, calculated CrCl, BUN, Ca, PO_4)	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vitamin D	X																			
Other Chemistries (triglycerides, glucose)	X					X														
TSH[3]	X				X	X														
Urinalysis	X	X						X				X				X				X
Urine creatinine, urine PO_4 [4]		X										X								X
Pregnancy test[5]	X	X	X	X	X	X														
PT/INR	X	X						X				X				X			X[a]	X
AFP	X																			
Anti-HCV[6]	X																			
Anti-HDV[6]	X																			
Anti-HIV[7]	X																			X
Serum/plasma banking[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBeAg	X	X						X				X				X			X	X
anti-HBe[9]	X	X						X				X				X			X	X
anti-HBs[9]	X	X						X				X				X				X
HBsAg	X							X				X				X			X	X
HBsAg quant		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBeAg quant		X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Quant HBV DNA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBV genotype and subtype (if not done in cohort study)[10]	X																			
Serum uric acid	X							X				X				X				X
HBV precore/BCP	X																			X

Antiviral resistance testing[11]	**X**							**X**				**X**				**X**				**X**
DNA banking[12]		**X**																		
DEXA scan (if participant has risk factors)[13]	**X**																			
Ultrasound exam/Imaging[14]	**X**							**X**				**X**				**X**				**X**
Liver Biopsy	**X**[15]																			
Eye examination (if participant has risk factors)[16]	**X**																			
Anti-HBc IgM	**X**[17]																			
Fibroscan (if available)																			**X**[18]	**X**[18]

[1] Day 0 is the Baseline visit, date of randomization, and start of study drug.
[a] Platelets, albumin, and INR required
[2] If total bilirubin elevated, obtain direct bilirubin at next blood draw.
[3] If abnormal, continue to follow until normalizes.
[4] Fasting urine tests at weeks 96 and 192.
[5] In females of childbearing potential during the peginterferon treatment phase.
[6] Anti-HCV and anti-HDV if results within 144 weeks of screening visit are available need not be repeated, these results may have been entered into cohort study database.
[7] Anti-HIV must be tested at screening (within 8 weeks prior to randomization) and again at week 196.
[8] 24mL of serum and 8mL of plasma at screening (within 8 weeks prior to randomization), week 0, 48, 96 144 and 192, 8mL tube of serum and 8mL of plasma at other time points. For patients not already enrolled in cohort, an additional 8 mL serum and 8 mL plasma should be collected at baseline visit (Day 0) for future research.
[9] Anti-HBe/Anti-HBs to be done at next visit if HBeAg/HBsAg becomes negative.
[10] The genotype result from the CDC should be used to randomize a participant; however, a result from a local lab may be used if the result from the CDC is not yet available. If a blood sample for CDC genotype testing was not collected as part of the Cohort Study, a sample should be obtained at the screening visit for the trial.
[11] Baseline and every 48 weeks in patients with detectable HBV DNA also performed if criteria for non-response, partial response or virologic breakthrough met.
[12] DNA – 5 mL whole blood at any time during the trial if not collected previously.
[13] If clinically indicated.
[14] Participants with AFP>20 ng/mL must be further evaluated by imaging (CT or MRI) as clinically indicated to rule out HCC before entry into the study. Participants meeting AASLD criteria for HCC surveillance will have follow-up imaging as standard of care.
[15] Slides of biopsy performed within 144 weeks of treatment start date (randomization) must meet criteria for adequacy by local consortium pathologist to confirm Modified Ishak HAI ≥3 or Ishak fibrosis ≥1 or both and the slides must be available for subsequent central read.
[16] In participants with diabetes or hypertension and no eye examination within past 48 weeks (standard of care).
[17] In participants who were not documented to be HBsAg positive at least 24 weeks prior to the positive result during screening.
[18] Fibroscan (if available) no earlier than week 180 visit and no later than week 192 visit

Appendix 4: Time and event chart after week 192 or treatment discontinuation *

Weeks Post Treatment Discontinuation	4	8	12	16[5]	20[5]	24	36	48
Study Week	196	200	204	208	212	216	228	240
Medical history								X
Physical exam								X
Clinical evaluation (limited)	X	X	X	X	X	X	X	
Assessment of AE, concomitant meds	X	X	X	X	X	X	X	X
Questionnaires (QOL, Depression assessment, Fatigue questionnaire, and Health behavior)								X
Symptom questionnaire	X	X	X	X	X	X	X	X
CBC including platelets								X
Hepatic panel (AST, ALT, total bilirubin, direct bilirubin)	X	X	X	X	X	X	X	X
Other liver tests (ALKP, total protein, and albumin)	X	X	X	X	X	X	X	X
Renal Panel (Creatinine, calculated CrCl, BUN, Ca, PO_4)**	X	X	X	X	X	X	X	X
Urinalysis								X
PT/INR	X	X	X			X		X
Serum/plasma banking[1]	X	X	X			X	X	X
HBeAg						X		X
Anti-HBe[2]						X		X
Anti-HBs[2]								X
HBsAg						X		X
HBsAg quant	X	X	X			X	X	X
HBeAg quant[3]			X			X	X	X
Quant HBV DNA	X	X	X			X	X	X
Antiviral drug resistance testing								X
Ultrasound exam[4]								X
Fibroscan (if available)								X[6]

*Schedule is used for participants:

(i) Those who meet criteria for stopping treatment at week 192 regardless of whether they stop treatment

(ii) Those who stopped treatment early due to meeting criteria for treatment failure (non-response, partial response, virologic breakthrough);

(iii) Those stopping treatment early due to an adverse event

(iv) For those not eligible to stop treatment, visits only occur at study weeks 204, 216, 228, and 240

** Renal panel should be obtained at each study visit for participants who are on treatment. All participants should have creatinine tested at week 240

[1]16mL of serum and 8mL of plasma at week 12, 24 and 48, 8mL of serum and 8mL of plasma at other time points

[2]Anti-HBe/Anti-HBs to be done at next visit if HBeAg/HBsAg becomes negative.

[3]In participants who are HBeAg positive in qualitative assay

[4]If clinically indicated, per AASLD guidelines, done as standard of care

[5]A hepatic panel and telephone follow-up (minimal assessment) may replace an outpatient visit if ALT at the preceding visit is <300 U/L for males or <200 U/L females and bilirubin is normal (total bilirubin <1.5 mg/dL or direct bilirubin <0.5 mg/dL)

[6]Fibroscan (if available) to be performed at week 240 if obtained at week 180 or 192 visits.

Appendix 5: Participating centers

Bethesda, MD: National Institutes of Health (NIH) Clinical Center

Boston, MA: Beth Israel Deaconess Medical Center, Massachusetts General Hospital

Los Angeles, CA: UCLA, Cedars Sinai Medical Center

Michigan Consortium: University of Michigan, Queen's Medical Center, Honolulu, Hawaii

Minnesota: Mayo Clinic Rochester, University of Minnesota

North Carolina: University of North Carolina, Duke University Medical Center

Pittsburgh, PA: University of Pittsburgh Graduate School of Public Health (DCC)

San Francisco, CA: UCSF, San Francisco General Hospital

St. Louis, MO: Saint Louis University, Washington University School of Medicine

Texas: University of Texas Southwestern, Baylor University Medical Center

Toronto, Ontario, Canada: University of Toronto

Virginia: Virginia Commonwealth University

Washington: University of Washington Medical Center, Virginia Mason Medical Center