

Official Study Title: Combination Entecavir and Peginterferon alfa-2a Therapy in HBeAg-Positive Immune Tolerant Adults with Chronic Hepatitis B

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Combination Entecavir and Peginterferon alfa-2a Therapy in HBeAg-Positive Immune Tolerant Adults with Chronic Hepatitis B

Synopsis

Study aims:

- To determine the safety and efficacy of treatment with 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a in the treatment of chronic hepatitis B in HBeAg positive adults who are in the immune tolerant phase.
- To evaluate “off treatment” sustained responses after treatment with entecavir and peginterferon alfa-2a in the treatment of chronic hepatitis B in HBeAg positive adults who are in the immune tolerant phase.

Type of study

- Single arm treatment study

Clinical trial

A single arm pilot study of 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a in adults with HBeAg-positive chronic hepatitis B with normal or near normal ALT levels and high serum levels of HBV DNA (“immune tolerant” HBeAg-positive chronic hepatitis B). All participants will be followed until week 96 (48 weeks after discontinuation of therapy) at which time the primary outcome will be measured.

1. Background

1.1. “Immune tolerant” HBV in adults

Chronic hepatitis B virus infection (CHB) affects 360 million people worldwide, including approximately 2.0 million in North America. Chronic hepatitis B may result in progressive liver disease that leads to cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). 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 (1, 2). The initial “immune tolerant” phase is variable in duration, but typically evolves during childhood or early adulthood to the “immune clearance” phase, marked by rises in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), increased inflammation and hepatocellular necrosis on liver biopsy, and an increase in cytoplasmic hepatitis B core antigen (HBcAg) expression. As the term ‘immune tolerant’ (IT) suggests this is a state where the host appears to exert no overt immune response to a chronic infection with Hepatitis B. The state is characterized by the presence of hepatitis B e antigen (HBeAg) and high levels of hepatitis B virus (HBV) DNA (typically HBV DNA $\geq 10^8$ IU/mL), but minimal or no elevations in serum ALT and AST levels and minimal activity or injury on liver biopsy. Most but not all individuals with ‘Immune Tolerant’ CHB are children or young adults, with a mean age of transition to the immune clearance phase of ~30 years. Thus, many adults with CHB who acquired their infection at birth or in early childhood may be in the immune tolerant phase of the infection up to the age of 30 or 40 years, or longer.

Adults in the immune tolerant CHB phase have variable histologic findings, largely dependent upon the definition of ALT used to define the population. For studies using the more stringent ALT criteria of ≤ 30 U/L for males and ≤ 19 U/L for females, histologic disease is minimal (majority fibrosis $< 2/4$ and necroinflammation 0-1/4) (3-5). Natural history studies of adults in the immune tolerant phase have shown that the ALT levels were significantly higher in patients with

subsequent loss of tolerance. For inclusion criterion, we propose to use ALT upper limits of 30 U/L for females and 45 U/L for males, and avoid the need for liver biopsy.

It has been hypothesized that the mother-to-child HBV transmission leads to transplacental transfer of maternal HBeAg that may induce a specific unresponsiveness of helper T cells to HBeAg in neonates. Because HBeAg and HBcAg are highly cross-reactive at the T-cell level, deletion of the helper T-cell response to HBeAg results in an ineffective cytotoxic T-lymphocyte response to HBcAg (6). During the immune tolerant phase of chronic infection, the continued secretion of HBeAg (the tolerogen) is necessary to maintain the tolerant state. Little is known about the mechanisms that regulate the loss of immune tolerance in chronic HBV infection. Recent studies have emphasized the importance of low expression of transporter associated with antigen processing (TAP1) and low molecular weight protein 2 (LMP2) compared with CHB patients in the immune clearance phase, suggesting a deficiency in antigen processing and transport to major histocompatibility complex class I molecules in IT adults (7). Point mutations and deletions in HBV epitopes that occur prior to or concurrent with the onset of the immunoactive phase during chronic HBV infection may contribute to a significant decrease in both viral replicative competence and HBV-specific CD8(+) T cell responses, and in turn, lead to maintenance of viral persistence (8). The proposed study will provide an opportunity to study the immunologic features and correlates of immune tolerant HBV infection in adults as well as the immune markers associated with breaking of tolerance in the context of antiviral therapy.

1.2 Treatment of CHB in adults

The known natural history of chronic hepatitis B underlies the current criteria for therapy and the endpoints of treatment. Treatment of adults with CHB targets those with active disease, characterized by elevated ALT levels and/or evidence of significant inflammatory activity or fibrosis on biopsy in conjunction with elevated HBV DNA levels. Treatment of adults in the immune tolerant phase of infection is not recommended (9). Reasons for not recommending treatment include the low frequency of histologic disease and complications and the lack of established effective therapies. Serum HBV DNA level has been found to be directly correlated with the development of HCC and cirrhosis independent of serum ALT level, HBV genotype, and HBeAg status (10,11). This has led to promotion of the use of serum HBV DNA concentration as an indicator for treatment regardless of serum ALT level, and even patients with normal serum ALT levels are to be treated with antiviral therapy with the primary aim of suppressing HBV DNA. In this context, the risks and benefits of treatment of immune tolerant adults is an important issue – not addressed by treatment studies to date.

Currently, there are two broad categories of HBV therapies available: pegylated interferon (peginterferon) and nucleos(t)ide analogues. The advantages of nucleos(t)ide analogue therapy is 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 and entecavir. While emergence of drug resistance can limit the efficacy of oral agents, entecavir and tenofovir are characterized by great potency and higher genetic barrier to resistance leading to a low rate of antiviral resistance (1% or less even with prolonged therapy for 4 to 5 years). 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. The latter feature may be particularly relevant in the treatment of immune tolerant adults, the target population for this study. 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. However, these studies have been limited by the use of a nucleos(t)ide analogue

with high risk of resistance (e.g. lamivudine) and may have been affected by the concurrent use of peginterferon and nucleos(t)ide analogue rather than sequential use.

1.3 Considerations in the treatment of immune-tolerant adults

There are no prior studies of treatment of immune tolerant adults. Data from the pediatric literature indicated that treatment with IFN alone during the immune tolerant phase of infection is rarely effective and loss of HBV DNA or anti-HBe seroconversion, with less than 10% of treated children achieving this outcome (12). However, the combination of a nucleoside analogue and IFN was examined in a pilot study of 23 children and appears encouraging (13). Lamivudine was given for 8 weeks and then combined IFN and lamivudine therapy for a further 44 weeks. HBV DNA by PCR was negative in 48% at end of treatment, and 5 (22%) children seroconverted to anti-HBe. Four (17%) children cleared HBsAg and seroconverted to anti-HBs. Six months after treatment, HBV DNA was again positive in all but the children who had seroconverted to anti-HBe and (presumably) anti-HBs.

Based upon these stimulating results, we propose to evaluate the safety and efficacy of a short lead-in course (8 weeks) of entecavir followed by combination of entecavir plus peginterferon alfa-2a for 40 weeks. We hypothesize that using a potent nucleos(t)ide analogue will provide a higher rate of HBeAg loss and suppression of HBV DNA. The primary measure of outcome will be HBeAg loss and suppression of HBV DNA to ≤ 1000 IU/mL in serum 48 weeks after stopping all antiviral therapy (sustained off-treatment response).

2. Study objectives

2.1 Primary objectives

- To determine the safety and efficacy of treatment with 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a in the treatment of chronic hepatitis B in HBeAg positive adults who are in the immune tolerant phase.
- To evaluate “off treatment” safety and sustained responses after treatment with entecavir and peginterferon alfa-2a in the treatment of chronic hepatitis B in HBeAg positive adults who are in the immune tolerant phase.

2.2. Primary endpoint

2.2.1 Safety: Adverse Events and Serious Adverse Events through the end of treatment and through the end of follow-up

2.2.2 Efficacy: HBeAg loss (lack of detectable HBeAg) AND HBV DNA $\leq 1,000$ IU/mL 48 weeks after stopping therapy.

2.3. Secondary endpoints

2.3.1. At the end of treatment:

- a. Cumulative HBsAg loss
- b. HBeAg loss
- c. HBeAg seroconversion
- d. HBsAg seroconversion
- e. ALT < 45 U/L for men, < 30 U/L for women (approximately 1.5 ULN)
- f. ALT normalization (men < 30 U/L, women < 20 U/L)
- g. HBV DNA $\leq 1,000$ IU/mL
- h. HBV DNA < 20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test)
- i. Absence of detectable antiviral drug-resistance HBV mutations

2.3.2. Sustained end of follow-up responses 48 weeks after end-of-treatment (week 96)

- a. Cumulative HBsAg loss
- b. HBeAg loss
- c. HBeAg seroconversion
- d. HBsAg seroconversion
- e. ALT <45 U/L for men, <30 U/L for women (approximately 1.5 ULN)
- f. ALT normalization (men <30 U/L, women <20 U/L)
- g. HBV DNA ≤1000 IU/mL
- h. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test)

3. Study design

A single arm pilot study of 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a in adults with HBeAg-positive chronic hepatitis B with normal or near normal ALT levels and high serum levels of HBV DNA ("immune tolerant" HBeAg-positive chronic hepatitis B). Participants will be followed until week 96 (48 weeks after discontinuation of therapy). Screening can include historical values up to 52 weeks prior to the baseline visit, but there must be a screening visit within 6 weeks of the baseline visit.

4. Study population

4.1. Inclusion criteria

2. 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 ≥18 years of age at the baseline visit (day 0). Patients >50 years of age at the baseline visit need to have a liver biopsy as standard of care with HAI ≤3 and Ishak fibrosis score ≤1 within 96 weeks prior to the baseline visit.
3. Documented chronic HBV infection as evidenced by detection of HBsAg in serum for ≥24 weeks prior to the baseline visit **OR** at least one positive HBsAg and negative anti-HBc IgM within 24 weeks prior to the baseline visit **OR** at least one positive HBsAg and two positive HBV DNA over a period of ≥24 weeks prior to the baseline visit.
4. Presence of HBeAg in serum at the last screening visit within 6 weeks of the baseline visit.
5. Serum HBV DNA level >10⁷ IU/mL on at least two occasions at least 12 weeks apart during the 52 weeks before the baseline visit. One of the two HBV DNA levels must be within 6 weeks of the baseline visit.
6. ALT levels persistently ≤45 U/L in males, ≤30 U/L in females (approximately 1.5 times the upper limit of normal [ULN] range) as documented by at least three
7. values: one taken 28-52 weeks before the baseline visit, one taken 6 to 28 weeks before the baseline visit, and the final value within 6 weeks prior to the baseline visit. No evidence of HCC based upon alpha-fetoprotein (AFP) ≤20 ng/mL at the screening visit (up to 6 weeks prior to the baseline visit):
 - a. Participants who meet AASLD criteria for HCC surveillance must have negative liver imaging as shown by ultrasound (US), computerized tomography (CT) or magnetic resonance imaging (MRI) within 28 weeks prior to the baseline visit 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 before they can be enrolled.
8. Provide informed consent and agree to adhere to the requirements of the study.

4.2. Exclusion criteria

1. History of hepatic decompensation, including but not limited to ascites, variceal bleeding, or hepatic encephalopathy.

2. Evidence of decompensated liver disease prior to or during screening, including direct bilirubin >0.5 mg/dL, INR >1.5, or serum albumin <3.5 g/dL.
3. Platelet count <120,000/mm³, hemoglobin <13 g/dL (males) or <12 g/dL (females), absolute neutrophil count < 1500 /mm³ (<1000/mm³ for African-Americans) at the last screening visit.
4. Previous treatment with medications that have established activity against HBV including, but not limited to, interferon and nucleos(t)ide analogs ≥24 weeks. Patients with <24 weeks of prior HBV treatment and a wash-out period >24 weeks are not excluded. Brief and episodic use of famciclovir or valacyclovir for herpes infection is not exclusionary.
5. Known allergy or intolerance to any of the study medications.
6. Females who are pregnant or breastfeeding.
7. Females of childbearing potential unable or unwilling to use a reliable method of contraception during the treatment period.
8. Renal insufficiency with calculated creatinine clearance <50 mL/min at the last screening visit.
9. History of alcohol or drug abuse within 48 weeks of the baseline visit.
10. Previous liver or other organ transplantation including engrafted bone marrow transplant.
11. Any other concomitant liver disease, including 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.
12. Presence of anti-HDV or anti-HCV (unless HCV RNA negative) in serum on any occasion in the 144 weeks prior to the baseline visit.
13. Presence of anti-HIV (test to be completed within 6 weeks prior to the baseline visit).
14. 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.
15. History of immune-mediated or cerebrovascular disease, 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.
16. 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.
17. Any medical condition requiring, or likely to require, chronic systemic administration of corticosteroids or other immunosuppressive medications during the course of this study.
18. Evidence of active or suspected malignancy, or a history of malignancy within the 144 weeks prior to the baseline visit (except adequately treated carcinoma in situ or basal cell carcinoma of the skin).
19. Expected need for ongoing use of any antivirals with activity against HBV during the course of the study.
20. Concomitant use of complementary or alternative medications purported to have antiviral activity.
21. Participation in any other clinical trial involving investigational drugs within 30 days of the baseline visit or intention to participate in another clinical trial involving investigational drugs during participation in this study.
22. Any other condition or situation 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

Treatment Entecavir 0.5 mg daily orally for 48 weeks plus peginterferon alfa-2a 180 µg subcutaneously (sq) weekly during weeks 9-48 of treatment. After treatment discontinuation, follow-up off treatment will be for 48 weeks.

Participants will be instructed to take one tablet of entecavir daily on an empty stomach at least 2 hours prior to or after meals. Participants will be instructed to take 180 µg of peginterferon alfa-2a subcutaneously once weekly. The participant will be instructed to inject the peginterferon alfa-2a on the same day each week.

5.2 Entecavir

5.2.1 Mechanism of action

Entecavir is a novel carbocyclic 2'-deoxyguanosine analogue with potent and selective anti HBV activity. Entecavir blocks three phases of HBV replication: (1) priming of the HBV polymerase, (2) reverse transcription of the negative strand DNA synthesis, and (3) DNA polymerase activity responsible for positive strand DNA synthesis. Entecavir functionally inhibits episomal viral replication rather than genomic transcriptional events mediated by cellular polymerases. Entecavir is converted to the triphosphate, which then acts as an inhibitor of hepadnaviral polymerase. Entecavir is not recognized by mitochondrial DNA polymerase and cannot be incorporated into (human) DNA or inhibit a polymerase assay system even when tested at very high doses (14). EC_{50} falls by 50% with wild type HBV DNA but there is a reduction 8-30 fold in entecavir phenotypic susceptibility with LAM resistance. In treatment naïve patients the mean log fall in HBV DNA in patients on entecavir is 5-6 logs.

Entecavir is not recommended in patients with HIV who are not on HAART as it has weak activity against HIV. All study participants will be tested for HIV within the 6 weeks prior to the baseline visit. Participants who self-report acquiring HIV will be removed from the study and referred to an HIV specialist.

5.2.2 Metabolism

The intracellular half-life is about 15 hrs. Oral entecavir given to subjects with CHB in doses ranging from 0.05 to 1mg daily over 28 days lead to a mean log fall in HBV DNA of 2.8. Absorption appears to be rapid – oral bioavailability estimated to be >70%. Entecavir is not affected by the co-administration of agents that are metabolized by inhibit or induce CYP450 system.

5.2.3 Safety of entecavir

In animal studies life time entecavir administration gave rise to an increased incidence of lung tumors when given at low multiples of human exposure and more with high exposure multiples. These tumors may be species specific and not relevant to humans. Clinical experience from 1755 patients treated with entecavir is available and does not indicate an increased risk of malignancies. There is no evidence in animal models for either genotoxicity or carcinogenicity.

Studies in rats and rabbits given in high dose during pregnancy indicated no embryotoxicity or maternal toxicity.

5.2.4 Entecavir resistant mutations

Entecavir has a very high genetic barrier when given in treatment naïve patients in a dose of 0.5mg/d. Resistance to entecavir after 5 yrs of therapy is reported to be 1.5%. When given to patients with lamivudine-resistance, a higher dose of 1mg/day is recommended and the cumulative probability of entecavir resistance is detailed below:

	1 yr	2 yr	3 yr	4 yr	5 yr
Naïve to entecavir	0.2%	0.5%	1.2%	1.2%	1.2%
Previous lamivudine resistance	6%	15%	36%	47%	52%

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, non-peginterferon alfa-2a.

5.3.2 Pharmacokinetic considerations

In patients 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 patients 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 patients 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 patients 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 leucopenia. These side effects are particularly common in patients with advanced liver disease and hypersplenism. Peginterferon alfa-2a is absolutely contraindicated in patients with decompensated cirrhosis due to the possibility of serious infections, flares of disease and worsening decompensation.

The majority of clinical experience in the use of peginterferon alfa-2a has been in chronic hepatitis C in which peginterferon alfa-2a is combined with ribavirin. In the few studies of peginterferon 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 alfa-2a should be avoided in patients 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 women. Therefore, in this study, women 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 treatment phase.

5.4. Administration and drug accountability

Study drugs (peginterferon alfa-2a and entecavir) will be shipped to study sites from a drug distribution center. Staff at each site will be responsible for drug accountability. This will include the documentation of drugs received from the drug distribution center, of study drug dispensed to study participants, of used and unused study drug returned, and an 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 valacyclovir. The need for ongoing use of these antivirals 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.
2. Systemic chemotherapeutic agents (i.e., cancer treatment medications).
3. 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.
4. Any investigational agents.
5. Growth factors such as GCSF or epoetin alfa.

6. Study procedures

6.1. Study enrollment

Participants thought to be eligible for participation in this trial will be asked to give their consent to participate after discussions with study investigators and by signing a consent form approved by the IRB/REB at that institution. Patients who are not 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 of the Cohort baseline evaluation will be completed prior to enrolling in this study at the screening or baseline visit. They will then undergo the screening procedures, including medical history, physical examination and blood tests as summarized in the Schedule of Events (Appendix I).

6.2. Screening assessment

The following assessments must be obtained within 6 weeks (42 days) prior to the baseline visit (start of treatment)

1. Written informed consent
2. Detailed medical history
3. Physical examination
4. Depression assessment
5. Blood tests: CBC with differential, routine biochemical tests (including ALT, AST, total bilirubin, albumin, alkaline phosphatase, glucose, total protein, creatinine, [including estimated creatinine clearance], TSH, PT/INR, alphafetoprotein (AFP), HBsAg, HBeAg, quantitative HBV DNA, and anti-HIV (see Appendix I).
6. Anti-HCV and anti-HDV are to be tested unless results within 144 weeks of the baseline visit are available.
7. Urinalysis
8. Pregnancy test in women with reproductive potential (urine or blood)
9. Abdominal imaging as necessary
10. Plasma and serum samples will be stored at each visit.
11. Anti-HBs/HBe will be done at the next testing time point if HBsAg/HBeAg becomes negative

6.3. Baseline visit Baseline assessments (day 0) must be obtained prior to the initiation of study drugs. Participants will have a limited clinical evaluation and have blood drawn for complete blood count, hepatic panel, renal panel, PT/INR and HBV DNA. Urine for urinalysis will be obtained. A sample of blood will be stored for eventual assessment of HBeAg and HBsAg concentration, anti-HBs, anti-HBe, full HBV DNA sequence and presence of pre-core and core mutations as well as polymerase gene antiviral resistance mutations, and DNA extraction (if sample not already obtained as part of Cohort Study, and sample may be obtained at future visit if not obtained at baseline) (See Schedule of Events, Appendix 1). QOL and health behaviors will be assessed. A pregnancy test will be performed in women with reproductive potential prior to start of treatment. At the end of the baseline visit, study drug (Entecavir) will be provided to the participant with instructions on its use.

6.4. On-treatment assessments

After starting treatment, visits will occur at weeks 8, 12, 16, 20, 24, 30, 36, 42, and 48. Each visit will include assessment of compliance, adverse events reporting, vital signs, weight, symptoms evaluation, record of concomitant medications, and blood testing as detailed in Schedule of Events (Appendix I). Participants will be dispensed study medications.

1. At the Week 8 visit, peginterferon alfa-2a will be provided to subjects with instructions on its use, including education on self-injection, if appropriate. Subjects will have a limited clinical evaluation as well as blood and urine tests.

2. Pregnancy test will be done in women with reproductive potential at each visit until the end of treatment.
3. Depression assessment will occur at weeks 8, 12, 24, 36 and 48.
4. QOL assessments will occur at day 0, weeks 8 and 48. Health behaviors will be included at day 0 and week 48.
5. Fatigue questionnaire will occur at day 0.
6. CBC (with differential), hepatic panel (ALT, AST, total bilirubin), renal panel (Cr, CrCl) and HBV DNA quantitation at each visit.
7. TSH at week 12, 24, 36 and 48.
8. Albumin, alkaline phosphatase, glucose and total protein will be obtained at week 48
9. HBeAg at weeks 8, 12, 24, 36 and 48.
10. HBsAg at week 48.
11. Blood samples for serum/plasma at each visit.
12. HBsAg and HBeAg quantification at each visit.
13. Anti-HBs/HBe will be done at day 0 and week 48. Also at the next visit if HBsAg/HBeAg becomes negative.

6.5 End of treatment assessment

At week 48, all participants will complete the treatment phase. Laboratory testing will be performed as outlined in Appendix 1 Schedule of Events. A sample of blood will be stored for eventual assessment of HBeAg and HBsAg concentration.

6.6. Post-treatment assessments

1. Visits will occur at weeks 52, 60, 72, and 96 .
2. Depression assessment will occur at weeks 60 and 96.
3. QOL and health behaviors assessments will occur at week 96.
4. Fatigue questionnaire will be obtained at week 96.
5. CBC (without differential), hepatic panel (ALT, AST, total bilirubin), renal panel (Cr, CrCl), and HBV DNA quantitation at each visit.
6. TSH at week 60.
7. Albumin, alkaline phosphatase, total protein, glucose, INR/PT, and urinalysis at week 96.
8. HBeAg at weeks 60, 72, and 96.
9. HBsAg at weeks 72 and 96.
10. HBsAg and HBeAg quantification every visit.
11. Blood samples for serum/plasma at each visit.
12. Anti-HBs/HBe will be done at week 96. Also at the next visit if HBsAg/HBeAg becomes negative.

6.7 Assessment of adherence

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

6.8 Stopping rules

Therapy will only be stopped for the following indications:

- Grade IV toxicity (except hematologic, see appendix III.)
- Death
- Pregnancy

- Non-response per section 6.9.
- Virologic breakthrough per section 6.10.
- Hepatic decompensation defined by:
 - Total bilirubin ≥ 2.0 mg/dL and direct bilirubin ≥ 1.0 mg/dL
 - Any clinical measure of decompensation
- Participant refusal to continue treatment

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. Managing these participants including more frequent assessments and alternative treatment will be at the discretion of the investigator.

Participants who become pregnant while receiving treatment will stop treatment and subsequent management will be at the discretion of the investigator. The outcome of the pregnancy will be recorded.

6.9. Management of non-response

Definition: Primary non-response is defined as a failure to achieve at least $>1\text{-log}_{10}$ IU/mL decline in HBV DNA after the first 24 weeks of treatment by the quantitative HBV DNA assay, which will be confirmed on re-testing 4 weeks later. Compliance with study medications will be assessed and documented. Primary non-response is considered treatment failure. In the event of non-response, the following will be implemented:

6.9.1. Reinforce compliance upon initial determination of non-response.

6.9.2. Treatment (both entecavir and peginterferon alfa-2a) will be discontinued if non-response is confirmed on re-testing 4 weeks later.

6.9.3. Participants will be assessed at week 4 after treatment discontinuation and then be followed at weeks 12, 24, and 48 post-discontinuation .

6.10. Management of virological breakthrough

Definition: Virological breakthrough is defined as a $> 1\text{-log}_{10}$ IU/mL increase in HBV DNA level from nadir in a participant with an initial virological response or redetection of HBV DNA after becoming undetectable, and will be confirmed on re-testing 4 weeks later in a participant with either an initial or partial virological response and who has been compliant with study medications.

- Virologic breakthrough is considered a treatment failure.

Management algorithm if virological breakthrough **6.10.1.** Reinforce compliance. The importance of compliance with study medication will be reinforced with study participants at each visit.

6.10.2. Resistance testing will be performed but will not be used to guide decisions (as results will not be available in real-time). Participants meeting the definition for virologic breakthrough on repeat testing will stop all treatment.

6.10.3. Participants will be assessed at weeks 4, 12, 24, and 48 post-discontinuation.

6.11 Management algorithm for participants with ALT flare during the treatment phase

6.11.1. If ALT values increase to ≥ 300 U/L for males and ≥ 200 U/L for females, retest the hepatic panel within 4 weeks. Continue testing of hepatic panel every 4 weeks as long as the ALT remains >300 U/L for males and >200 U/L for females.

6.11.2. If ALT values decline below these levels, the frequency of testing the hepatic panel can return to the usual schedule.

6.11.3. Participants will remain on treatment until week 48 unless:

- i. there are signs of clinical decompensation or
- ii. total bilirubin ≥ 2.0 mg/dL and direct bilirubin ≥ 1.0 mg/dL or
- iii. ALT >450 U/L for males or >300 U/L for females for more than 12 weeks

Participants meeting any of these criteria will continue to be followed in the study but with any subsequent management at the discretion of the study physician.

6.12. Management algorithm for participants with ALT elevation during the follow-up phase (weeks 48-96)

6.12.1. If ALT increases to >300 U/L for males and >200 U/L for females, retest the hepatic panel in 4 weeks. Continue testing of hepatic panel every 4 weeks as long as the ALT >300 U/L for males and >200 U/L for females.

6.12.2. If ALT decline below these levels, the frequency of testing the hepatic panel can return to the usual schedule.

6.12.3. For participants with ALT persistently >150 U/L in males and >100 U/L in females for ≥ 24 weeks and HBV DNA $\geq 10,000$ IU/mL, they are eligible for retreatment with entecavir (up to one year or by December 31, 2015 (whichever comes first), or peginterferon alfa-2a or both drugs for one year if desired by participants and provider.

6.13. Participants previously randomized to control group

Participants enrolled in this trial prior to amendment 7.0 of this protocol who were randomized to the control (no treatment) group will be offered treatment if they still meet the entry criteria for this trial (i.e. HBV DNA and ALT results from the most recent IT trial visit meet the inclusion and exclusion criteria listed in sections 4.1 and 4.2), and are willing to receive treatment. These participants will be reconsented prior to starting treatment and follow the visit schedule and assessment beginning with baseline (Day 0) visit.

Participants who no longer meet entry criteria for this trial or are not interested in receiving treatment will complete a Termination Visit at the next scheduled visit for the control (no treatment) group. The procedures to be followed for this Termination Visit will be those specified for the Week 48 visit.

7. Adverse events and toxicity management

7.1. Managing peginterferon alfa-2a side effects

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 patients receiving peginterferon alfa-2a.

Some rare but potentially serious side effects include:

- neuropsychiatric complications, which may include suicide, suicidal ideation and severe depression
- serious and severe bacterial infections, some fatal 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, psoriasis etc
- other pulmonary disorders including dyspnea, pulmonary infiltrates, and sarcoidosis
- exacerbations of inflammatory bowel disease
- eye disorders, including decrease or loss of vision, retinal hemorrhages
- hypersensitivity including sometimes severe skin reactions in the spectrum of Stevens Johnson syndrome

7.1.1. Managing depression

When possible, the CES-D will be used along with the investigators judgment, to screen participants for depression at the screening visit (within 6 weeks of the baseline visit) and to monitor for onset or worsening of symptoms during the treatment and follow-up phases visits in all participants (See Schedule of Events). If a participant has a CES-D score >15 at the screening visit (within 6 weeks of the baseline visit), evaluation by a mental health provider should be considered before enrollment (start of treatment). During the treatment phase, if a participant has a CES-D score >15, the investigator will determine whether referral to a mental health provider is indicated.

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 entecavir continued until the end of the treatment period (see section 7.5.4) and the participant will be referred to a mental health provider.

7.2. Peginterferon alfa-2a dose reduction guidelines

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

1. Disabling symptoms, which, in the opinion of the investigator, 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 platelet count according to the guidelines in Appendix III.
4. A reduction in neutrophil count according to the guidelines in Appendix III.
5. Any adverse reaction, which, in the opinion of the study physician, places the participant at increased risk.

The dose of study medications may be decreased due to adverse events or laboratory abnormalities. In most instances the 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/mm³ (but above 250/mm³) or a platelet count below 50,000/mm³ (but above 25,000/mm³) should receive stepwise dose reductions until levels of neutrophils and platelets are ≥500/mm³ or ≥50,000/mm³, respectively, or until a dose of 45 µg of peginterferon alfa-2a is reached.

7.2.1. Guidelines for subsequent peginterferon 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 according to the following guidelines:

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 participants 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 entecavir treatment will continue (see section 7.5.4).

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

7.2.2. Peginterferon alfa-2a dosing and creatinine clearance (CrCL)

Creatinine clearance will be calculated in all participants prior to initiating therapy and at each study visit. The peginterferon alfa-2a dose does not require adjustment unless the patient is on dialysis. However, in this study, peginterferon alfa-2a will be discontinued if CrCl is ≤ 30 ml/min to be consistent with criteria for entecavir discontinuation (see below).

7.3. Managing adverse effects of entecavir

7.3.1. Adverse effects of entecavir

- Headache, fatigue, dizziness, nausea, upper abdominal pain, upset stomach, diarrhea, vomiting, trouble sleeping or sleepiness, changes to liver and pancreas enzymes. These side effects were generally mild to moderate in severity.
- Lactic Acidosis: Patients taking drugs similar to entecavir have experienced lactic acidosis. Symptoms of lactic acidosis include weakness/tiredness, muscle pain, trouble breathing, stomach pain with nausea and vomiting, feeling cold (particularly in extremities), dizziness/lightheadedness, and increased heart rate.
- Hepatitis "flares": In patients with chronic hepatitis B, liver enzyme changes are common.
- Laboratory studies have shown that entecavir does not interact with the enzymes in the liver that are responsible for most drug interactions. However, because the body gets rid of entecavir in the urine, it is possible that drugs which affect kidney function could increase the levels of entecavir when a patient uses both drugs. These types of drugs are not allowed in this study.
- When mice and rats were given entecavir for their entire adult life, these animals developed more tumors, including cancerous tumors, than are normally expected. Male mice developed non-cancerous lung tumors when they were exposed to entecavir at levels greater than or equal to 4 times higher what participants will be exposed to in this study. Cancerous lung tumors were also observed in male and female mice when they were given high levels of entecavir. Further animal studies have been performed to better understand these findings. These additional studies suggest that the lung findings in mice are unique for mice. The lung findings in mice did not occur in rats, dogs or

monkeys. It is not known whether these tumor findings in animals are relevant to humans who are treated with Entecavir.

7.3.2. Entecavir and renal function

Entecavir dosing is based upon CrCL: Creatinine clearance will be calculated for all participants prior to initiating therapy and at each study visit to determine Entecavir dose.

- Participants whose calculated creatinine clearance decreases to <50 ml/min should have this value confirmed within 72 hrs.
- Entecavir may be continued if the CrCL is ≥ 30 -50 ml/min at the adjusted dose of 0.5 mg every 48 hours.
- Entecavir will be discontinued if the CrCL is <30 ml/min.

7.4. Pregnancy

Participants will be asked to practice effective birth control while they are taking antiviral agents in this study. Additionally, following precautions will be taken:

- All female participants with reproductive potential will undergo pregnancy test on enrollment and will have pregnancy tests performed at each visit during treatment.
- If a participant on entecavir or peginterferon alfa-2a becomes pregnant, the study physician should be notified immediately and treatment will be discontinued immediately. Subsequent management will be at the discretion of the study physician. The study will collect data on the outcomes of any pregnancies that occur in women who conceived while taking study medication.

7.5. Adverse events – definition and management

7.5.1. 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 modified World Health Organization (WHO) grading system will be used for grading severity of AEs. For AEs not outlined in the modified WHO grading system, study specific definitions will be identified in the Manual of Operation (MOP).

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, 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.

7.5.2. 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 a study physician 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 96) 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 study physician, the participant is medically stable.

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

7.5.3 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 and Bristol-Myers Squibb SAE reporting requirements (see Manual of Operations).

7.5.4. Management if either entecavir or peginterferon alfa-2a is discontinued due to adverse events

If entecavir is discontinued due to adverse event, then peginterferon alfa-2a will be continued until the end of the treatment period. If peginterferon alfa-2a is discontinued due to adverse

event, then entecavir will be continued until the end of the treatment period.

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.

8.1.1. Analysis of primary endpoints

The primary endpoints include safety, measured by the rate of adverse and Serious Adverse Events, and efficacy, defined as loss of HBeAg and HBV DNA $\leq 1,000$ IU/mL at the end of follow-up (week 96). That is, only participants achieving HBeAg loss and having HBV DNA ≤ 1000 IU/mL at the end of follow-up will be considered as achieving the primary efficacy endpoint.

Safety

Summaries of adverse events (number of adverse events, number and percentage of participants with at least one adverse event, and for each adverse event, rate per person-years) will be provided. 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.

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.

We will provide 95% confidence intervals for the rate of adverse and serious adverse events.

Efficacy

The percentage of participants achieving the primary efficacy endpoint will be summarized with the point estimate and 95% confidence interval.

8.1.2. Analysis of secondary endpoints

8.1.2.1. Analysis of secondary endpoint listed in 2.3.1.: *Estimate the cumulative proportion of 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 and presented with point-wise 95% confidence interval.

8.1.2.2. Analyses of other secondary endpoints

For all of the secondary endpoints listed in sections 2.3.1. – 2.3.4, we will provide frequencies and percentages with corresponding 95% confidence intervals. Time to such events may also be of interest (e.g. cumulative HBeAg loss). For time-to event analysis, Kaplan-Meier curves will be used to plot the cumulative proportion of events over time.

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. .

8.2. Missing data

Primary analyses will be performed using all enrolled participants (intention-to-treat analysis). Participants who do not have primary endpoint data due to drop-out or other reasons at the end of follow-up will be considered as treatment failures. Since this is a pilot study with a small sample size, formal procedures for handling missing data such as inverse-weighting and multiple imputation will not have enough power, nevertheless, we will apply the following methods to assess the sensitivity of our primary analysis results to different missing data assumptions:

1. Single LOCF Imputation: In this case the primary endpoint analysis will be repeated by replacing missing endpoint data at follow-up using data from prior visits.
2. Inverse-probability-weighting: This analysis will be carried out in two stages. In the first stage, for each subject we will estimate the probability of having complete data at week 96 based on the data collected at prior visits using logistic regression. In the second stage, the primary endpoint analyses will be repeated by weighting each subject with complete data by the inverse of the corresponding estimated probability from the first stage.
3. Multiple Imputations: The missing values at week 96 will be imputed by Markov-Chain Monte-Carlo algorithm, based on the data collected prior to week 96. Five such imputations will be used and the results of the primary endpoints analyses will be repeated on each of these imputed datasets and averaged.

8.3. Sample size

This pilot study will enroll 40 participants into treatment. This sample size is considered to be achievable. We have calculated precision estimates based on various rates of primary endpoint of efficacy [loss of HBeAg and HBV DNA $\leq 1,000$ IU/mL at the end of follow-up (week 96)]. For example, if the expected proportion achieving primary efficacy endpoint is 25%, then we would be able to estimate it with 95% Clopper-Pearson confidence to ensure that the expected width of the interval does not exceed 29% (or equivalently, the estimated 95% confidence limits will be within $\pm 15\%$ of the estimate). Below are some further precision estimates:

Table: Precision (Width) Estimates for 95% Confidence Intervals under Different Primary Endpoint Proportions

Expected proportion with primary efficacy endpoint	95% lower	95% upper	Width
0.15	0.057	0.298	0.24
0.20	0.091	0.356	0.27
0.25	0.127	0.412	0.29

0.30	0.166	0.465	0.30
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9. Data and safety monitoring board

Data and safety will be monitored by the National Institute of Diabetes and Digestive and Kidney Diseases (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

This trial aims to examine the safety and efficacy of treatment with 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a in the treatment of chronic hepatitis B in HBeAg positive adults who are in the immune tolerant phase. The individual drugs (peginterferon alfa-2a and entecavir) are FDA-approved and have been well-studied except in the immune tolerant participants. The risks to participants are outlined in sections 5.2.3 and 5.3.3 of this protocol. Section 7 of the protocol defines the toxicities associated with these drugs and outlines dose adjustment and discontinuation guidelines due to toxicity. 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 and to the participating centers' IRBs.

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.

The DCC will work with the HBRN 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	Quarterly reports for NIDDK and the appropriate oversight committees
Retention	
Protocol adherence(e.g., meeting inclusion/exclusion criteria, treatment compliance)	
Pregnancy in female subjects receiving treatment	
Adverse events and Serious Adverse Events (SAE) that do not meet expedited reporting	Quarterly reports for NIDDK and the appropriate oversight committees

criteria

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 DSMB

Definitions of adverse and serious adverse events and their management guidelines are provided in section 7.5 of this protocol along with the reporting procedures and in the MOP. No interim analysis for efficacy is planned.

9.2 Participant confidentiality

The central database of the study is on a server at the Epidemiology Data Center (EDC) in the Graduate School of Public Health 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, computer room in an area with a burglar alarm houses all project server equipment.

Participant 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 in the NIDDK repository by a suitable date agreed upon by NIDDK and the steering committee.

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Appendix I: Schedule of events for treated participants

Phase of Study	Screening			Treatment										Follow-up			
Weeks of Study	-28 to -52	-6 to -28	up to -6	0 1	8	1 2	1 6	2 0	2 4	3 0	3 6	4 2	4 8	5 2	6 0	7 2	9 6
Informed consent			X														
Medical history			X														
Physical exam			X	X	X	X	X	X	X	X	X	X	X				X
Drug dispensing				X	X	X	X	X	X	X	X	X					
Adherence assessment/ Clinical evaluation including AEs, concomitant meds				X	X	X	X	X	X	X	X	X	X	X	X	X	X
Anthropometric measures				X									X				X
Questionnaires (QOL)				X	X								X				X
Health behaviors				X									X				X
Depression assessment			X		X	X			X		X		X		X		X
Symptom questionnaire				X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fatigue questionnaire				X													X
CBC, including platelets and differential ²			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hepatic tests (AST, ALT, and bilirubin; albumin and ALKP day 0 and weeks 48 and 96 only) ³	X ⁴	X ⁴	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Other chemistries (glucose, total protein)			X										X				X
TSH			X			X			X		X		X		X		
Renal tests (Creatinine and CrCl calculated MDRD)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis			X	X	X								X				X
PT/INR			X	X									X				X
AFP			X														
Pregnancy test (urine or blood) ⁵			X	X	X	X	X	X	X	X	X	X	X				

Phase of Study	Screening			Treatment										Follow-up			
	-24 to -52	-6 to -24	up to -6	0 1	8	1 2	1 6	2 0	2 4	3 0	3 6	4 2	4 8	5 2	6 0	7 2	9 6
HBsAg			X										X			X	X
HBsAg quant				X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBeAg			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
Anti-HBe ⁶				X									X				X
Quant HBV DNA	X ⁴		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Anti-HBs ⁶				X									X				X
HBV genotype and subtype ⁷				X													
HBV precore/BCP				X													X
Antiviral drug resistance mutation testing ⁹⁸				X									X				
Anti-HCV ⁹			X														
Anti-HDV ⁹			X														
Anti-HIV			X										X				
Abdominal imaging ¹⁰			X						X				X			X	X
Plasma/serum banking ¹¹			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DNA Banking ¹²				X													

1. Day 0 is the Baseline visit, and start of study drug.
2. CBC to include differential count up to week 48.
3. If total bilirubin elevated, obtain direct bilirubin at next blood draw.
4. Historical data acceptable—ALT (2 values, one 28-52 weeks before screening, one 6-28 weeks before screening) and HBV DNA (1 value, within 12-52 weeks before screening).
5. .
5. All women with reproductive potential prior to baseline visit; women on treatment with reproductive potential after start of treatment .
6. Anti-HBe/Anti-HBs to be done at next visit if HBeAg/HBsAg becomes negative.
7. For those who did not have genotype performed for the cohort study
8. Also if meets criteria for virologic breakthrough (results not available in real-time).
9. Anti-HCV and anti-HDV, if results within 144 weeks of baseline visit are available, tests do not need to be repeated.
10. Patients with AFP >20 should be further evaluated by imaging (US/CT/MRI) as clinically needed to rule out HCC before entry into the study. Patients meeting AASLD criteria for HCC surveillance should have follow-up imaging per standard of care.
11. 24mL of whole blood (for serum) and 8mL of whole blood (for plasma) at screening visit, day 0, 8, 48 and 96, and 8mL of whole blood (for serum) and 8mL of whole blood (for plasma) at other time points. These volumes include blood that will be collected for central testing. Patients who will be enrolled in HBRN Cohort Study concurrent with screening visit for this trial will have an additional 8 mL serum and 8 mL plasma at the baseline visit for future research.
12. 5 mL of whole blood if DNA sample (with consent) not previously collected. May be collected at any study visit if not at baseline.

Appendix II: 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 III: Peg-Interferon Dose Reduction

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, investigators 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 investigator. 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 an investigator 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

Parameter	Downward Dose Adjustment or Delay in Dosing
ANC (cells/mm³)	
≥500	No change
250-499	1 step initially; potentially up to 3 steps
<250	Stop drug
Platelet Count (cells/mm³)	
>50,000	No change
25,000-50,000	1 step initially; potentially up to 3 steps
<25,000	Stop drug

Appendix IV: 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, HI

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, California Pacific Medical Center

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