Observational Study of Persons with Hepatitis B Virus Infection in North America (Cohort Study)

Abstract

Aims

- Primary Aim:
  - To describe participants with hepatitis B virus (HBV) infection in a prospective cohort in the United States (US) and Canada and identify predictors of disease activation and progression

- Secondary Aims:
  - To describe clinical, virological, and immunological characteristics of participants with HBV in the US and Canada
  - To evaluate changes in HBV infection status and quantitative HBsAg levels and factors associated with those changes
  - To verify whether a baseline HBsAg below 1,000 IU/mL and HBV DNA below 1,000 IU/mL is an accurate predictor of people who are, or who will become, inactive carriers, defined as people who are HBsAg positive, HBeAg negative, have normal ALT and HBV DNA under 1,000 IU/mL on at least two occasions at least 6 months apart
  - To develop a bank of biospecimens (e.g., serum, plasma, DNA, lymphocytes, liver tissue) obtained from participants with HBV infection
  - To identify participants with HBV infection who are potential candidates in one of the treatment studies to be conducted by the Hepatitis B Research Network (HBRN)
  - To describe the natural history of hepatitis B infection in pregnancy including the frequency of, and clinical and virological characteristics associated with, hepatic flares during pregnancy and post-partum.

Type of study

- Observational

Cohort study

Patients with HBV infection will be identified by sites participating in the NIH-funded Hepatitis B Research Network (HBRN). Consenting patients who meet entry criteria will undergo a baseline evaluation. Those who are eligible for one of the treatment studies being conducted by the network will be approached about participating. If they are not eligible, unwilling to participate or have completed their participation in a treatment study, they will be followed longitudinally to observe clinical outcomes and changes in their virologic and immunologic status.

It is estimated that approximately 2,500 participants with HBV infection will be enrolled over a period of approximately 24 months. They will be followed indefinitely until the conclusion of the study.

- **Inclusion criteria**
  - Written informed consent
  - At least 18 years of age
  - HBsAg-positive and either
    - Pregnant
    - Anti-HDV positive
    - Diagnosed with acute HBV infection or experiencing a hepatitis flare
    - Immune tolerant or immune active phenotype
    - Potentially eligible for the Immune Regulation and Costimulation in Natural History of Chronic Hepatitis B ancillary study.

    OR completed the HBRN Adult Immune Active trial (regardless of HBsAg status)

- **Exclusion criteria**
  - History of hepatic decompensation based on clinical or laboratory criteria
  - Hepatocellular carcinoma (HCC)
  - History of solid organ transplantation or bone marrow transplantation
  - Current hepatitis B antiviral treatment (except pregnant women, patients who are anti-HDV positive, and participants who completed the HBRN Adult Immune Active trial)
  - Chronic immunosuppression therapy
  - Known HIV co-infection (patients with HDV or HCV co-infection are not excluded)
  - Medical or social condition which, in the opinion of the investigator, would make the patient unsuitable for the study or interfere with or prevent follow up per protocol.
  - Unable or unwilling to return for follow-up visits

Briefly, the standard visit schedule for participants will be at baseline, weeks 12, 24 and 48 in their first year of enrollment and every 24 weeks in subsequent years.

Variable follow up visits may be required if certain events are encountered. For example, for participants experiencing an ALT flare follow up will occur every 2-4 weeks until resolved and for HBeAg or HBsAg loss, participants will be asked to return in 12 and 24 weeks, and then resume follow-up of every 24 weeks.

The following events will be noted:

- ALT flare
- HBsAg or HBeAg loss
- Cirrhosis
- Hepatic decompensation
- HCC
- Liver transplantation
- Death

For women who are, or become, pregnant while enrolled in the cohort study, one visit will occur early in pregnancy (1st or 2nd trimester) and one later in pregnancy (at or after 28 weeks gestation). Such visits may coincide with a regularly scheduled visit or may be an additional visit. Following delivery, extra data will be collected at 12, 24, and 72 weeks post delivery (see Appendix E), either at a regularly scheduled visit or at a special visit.

1. Background and rationale

1.1. Historical background and goals

Chronic hepatitis B virus (HBV) infection is a leading cause of morbidity and mortality from end stage liver disease and hepatocellular carcinoma (HCC) in the US and worldwide. Of the estimated 1.4 million Americans with HBV infection, up to 25% are expected to die of these liver-related causes. Although significant progress has been made recently in HBV therapy, including approval of six new therapeutic agents, the current knowledge in the management of HBV infection is limited because treatment trials have utilized one to two years of therapy at most, whereas most patients require treatment of much longer duration for optimal long term outcome.

In 2008, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), through the U01 mechanism, established a Research Network, the goal of which is to facilitate and perform clinical, scientific, epidemiological and therapeutic research in HBV infection and chronic liver disease

1.2. Hepatitis B Research Network

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

The Steering Committee is responsible for the conduct and monitoring of studies and reporting of study results.

1.3. Scientific background

1.3.1. HBV introduction

HBV is a DNA virus, a member of the family hepadnaviridae that replicates via an RNA intermediate. Its genome is 3.2 kb in length, occurring within viral particles as a double-stranded, partially complete circle (1). The virus itself circulates in the blood of infected persons as the 42 nm Dane particle, comprising an outer envelope of hepatitis B surface antigen (HBsAg) and an inner nucleocapsid of hepatitis B core antigen (HBcAg). The genome of HBV encodes four proteins – HBsAg, HBcAg, polymerase and X protein. In addition, the core gene can produce hepatitis B e antigen (HBeAg), a soluble protein. HBV is not directly cytopathic to hepatocytes. Liver injury associated with chronic HBV infection appears to be immune mediated (2;3). Thus, ongoing or episodic cell-mediated immune responses which target viral antigens expressed on the surface of infected hepatocytes result in hepatocytolysis, inflammation and fibrogenesis. The mechanisms by which HBV predisposes an individual to HCC are not clearly understood, but may be mediated via inflammation, fibrosis and cirrhosis or may be more directly tied to viral factors such as the X protein or viral integration within the host genome (4). HBsAg is the main marker of HBV infection. HBeAg may be detectable in serum of some individuals. HBcAg is detectable within the liver by immunostaining (5;6). Serum HBV DNA levels have come to be recognized as a key determinant of liver injury, HCC and an important marker of response to therapy (7;8;9). Antibodies to hepatitis B antigens may also be detected in serum - anti-HBs and anti-HBe in those who have recovered from HBV infection. Anti-HBc is present both during and after HBV infection and the IgM form of anti-HBc is a useful clinical marker of acute HBV infection (6).

HBV has been classified into 8 genotypes A-H and most genotypes have been further divided into subgenotypes. Genotype A is found mainly in North America, Northern Europe, South Asia, and Africa; genotypes B and C are prevalent in Asia; genotype D is more common in Southern Europe, South Asia and the Middle East; genotype E is predominantly found in Africa and genotypes F and H in South and central America (9-12). In a study of 694 patients from 17 US liver centers conducted in 2001-2, 7 genotypes (A-G) were present with A (35%) and C (31%) being most common followed by B (22%) and D (10%) (13). HBV genotypes B and C were present in 75% of the patients on the west coast and genotypes A and D in 74% of the patients in the south. Genotype A was mainly seen among whites and African Americans while genotypes B and C were predominantly found among Asian Americans. Although this study included a fairly large number of patients from different regions of the country, some pockets of the US with more unique ethnic mix were not included. A recent study of 1157 Alaska Native people found that genotype D (57%) was most common followed by genotypes F (20%) and A (13%), while genotypes B and C were present in 4% and 6% only.(14) A study in Hawaii reported that the predominant genotype was C (70%) follow by genotypes A (23%) and B (7%) (15). These findings highlight the diversity of chronic HBV infection in the US, and the need to include clinical centers located in different

parts of the country and to enroll patients of all racial/ethnic background to obtain a full spectrum of HBV-related liver disease in the US.

The precore stop codon G1896A variant and the basal core promoter A1762T/G1764A variant are the most common naturally occurring HBV variants. These mutations abolish or decrease HBeAg production but HBV replication continues. Precore and/or core promoter variants are found in most patients with HBeAg-negative chronic hepatitis. Precore variant is most commonly associated with genotypes B, D, G and some subgenotypes of C; while core promoter variant is more often associated with genotypes A, C and D than B.(16-19) During the past decade, many investigators have observed a shift from predominantly HBeAg-positive to HBeAg-negative chronic hepatitis (20;21). This shift is likely related to a decrease in incidence of new HBV infection and aging of previously infected persons. Because of the need for very long duration of therapy in HBeAg-negative patients, monitoring the proportion of HBeAg-positive vs. HBeAg-negative chronic hepatitis is critical in estimating the costs of treating patients with chronic hepatitis B. In the study of HBV genotypes in the US cited above, precore and core promoter variants were present in 27% and 44% of patients, respectively (16). These data indicate that HBeAg-negative chronic hepatitis is not rare in the US.

1.3.2. Epidemiology

Hepatitis B virus (HBV) remains an important cause of acute and chronic liver disease globally and in the United States. World-wide, approximately 350 million persons are chronically infected with the hepatitis B virus (HBV) (22;23). HBV is transmitted by percutaneous and mucous membrane exposures to infectious body fluids, such as serum, semen, and saliva (24). Perinatal transmission is thought to be a major route by which HBV infection is perpetuated in endemic populations. Children born to HBsAg-positive mothers who do not become ‘vertically’ infected during the perinatal period remain at risk of infection during early childhood (25). Although the exact mechanism by which this ‘horizontal’ transmission of HBV occurs in children is not completely understood, contacts involving contaminated environmental objects may play an important role.

Among adults, high-risk sexual activity is one of the most frequent routes of transmission for HBV (26). Historically, male homosexual contacts have been associated with a high risk for HBV infection. More recently, heterosexual transmission is the most common cause of acute HBV infection in adults (23). Transmission of HBV via transfusion of blood and plasma-derived products has been essentially eliminated through donor screening and viral inactivation procedures. However, transmission of HBV may continue to occur in other health care settings (27). For example, non-adherence to isolation guideline in a hemodialysis unit or direct person-to-person exposure (e.g., surgeon or dentist to patients) may transmit HBV.

The Center for Disease Control and Prevention has estimated that 1.25 million persons in the United States are chronically infected (28). An encouraging trend is that the

incidence of acute hepatitis B in the United States declined as much as 80% between 1987 and 2004, attributable to effective vaccination programs as well as universal precautions in needle use and in healthcare in general (23).However, these decreases in acute infections have not translated into diminished prevalence or burden of chronic HBV infection. A part of the reason for this persistence is that chronic HBV infection affects certain groups disproportionately - for example, chronic hepatitis B in the United States affects 10 to 15% of foreign born and first generation Asian Americans and is common among recent immigrants from the Middle East, Africa and Eastern Europe (29-31). Approximately 2-3% of children adopted from Asia and Eastern Europe have chronic hepatitis B (32).

Chronic hepatitis B is a significant cause of death due to liver disease in the U.S. and accounts for a substantial number of cases of hepatocellular carcinoma (HCC) and liver transplantation each year (33); hepatitis B is the underlying cause of 2,000 to 4,000 deaths each year in this country (34). Furthermore, the recent immigration trends from regions of high HBV prevalence portend a substantial increase in the number of Americans with chronic HBV infection (30). This trend is indeed reflected in the health and economic burden associated with HBV infection. The number of outpatient visits and hospitalizations for a HBV-related diagnosis increased several fold during the 1990s. Similarly, the total charges for hospitalizations have been estimated to have increased from $357 million in 1990 to $1.5 billion in 2003 (34).

1.3.3. Natural history and progression of HBV

The natural history of chronic HBV infection is complex and differs widely from infected patient to patient (35). Overall, the lifetime risk of serious complications is approximately 25% for HCC and 10-15% for decompensated cirrhosis. Four clinical phases of chronic HBV infection have been identified. The immune tolerance phase occurs primarily in patients who have been infected at birth from hepatitis B “e” antigen (HBeAg) positive mothers and is characterized by presence of HBeAg, normal ALT, very high HBV DNA levels usually > 200,000 IU/mL and no or minimal histological inflammation or fibrosis. With host immune recognition of HBV, ALT levels rise, HBV DNA levels begin to decline and liver inflammation and fibrosis occur. This is called the immune active phase (36). Eventually at least 90% of persons will experience HBeAg seroconversion and develop antibody to HBeAg (anti-HBe). Most of these persons will go into the inactive hepatitis B phase characterized by normal ALT and low levels (usually below 2,000 IU/mL) or undetectable HBV DNA (37). However, as many as 20% will revert back to the HBeAg positive immune active phase and another 10-20% will remain in the anti-HBe immune active phase (38). Reversions and seroconversions are often accompanied by flares of ALT elevations that are usually asymptomatic. In addition, 10-20% of persons who appear to be in the inactive phase can reactivate to the anti-HBe immune active phase. For those patients who remain in the inactive phase, approximately 0.5%/year will lose HBsAg. Although the risk of HCC and cirrhosis decrease significantly, HCC can still occur years later (37). Thus, the natural history of chronic HBV infection is dynamic as patients can develop liver inflammation and fibrosis slowly or rapidly and can have periods of inactive disease where liver inflammation and fibrosis can improve or can have reactivations of liver disease throughout their lifetimes.

Outcomes of chronic HBV infection include progression to cirrhosis, liver failure, and HCC. The annual incidence of cirrhosis has been estimated to be 2-4% for HBeAg-positive and 3-10% for HBeAg-negative patients (39). The annual incidence of HCC has been estimated to be <1% for non-cirrhotic carriers and 2-4% for patients with cirrhosis (7). Factors associated with an increased risk of cirrhosis and HCC include older age, male gender, habitual alcohol consumption, and concurrent infection with HCV, HDV or HIV(39;40). Additional factors which influence disease progression include HBV viral genotype (especially genotypes C and F), high levels of HBV DNA, and certain HBV mutations (41). Recent studies indicate that persistent high level HBV replication is one of the most important risk factors for cirrhosis and HCC. These studies found that delayed HBeAg seroconversion (after age of 40) and HBV reactivation with or without HBeAg seroconversion after HBeAg loss were associated with increased risk of adverse outcomes (38;42;43). Several large cohort studies in Asia demonstrated that high baseline HBV DNA was associated with increased risk of cirrhosis, HCC and liver-related mortality regardless of ALT (7;8;44-46). However, it is not clear if this finding applies to patients with adult acquired HBV infection or to patients with perinatally acquired HBV infection that are younger than 40 years. Likewise, other factors that have not been well studied include HBV viral mutations, the host immune response and co-presence of hepatic steatosis (40).

HBV genotype has been reported to be associated with clinical outcome. To date, most studies were conducted in Asia. These studies showed that compared to genotype B, genotype C is associated with more rapid progression to cirrhosis and a higher rate of HCC(45), (47-51). The more aggressive course may be related to the observations that patients with genotype C undergo spontaneous HBeAg seroconversion later, are more likely to have abortive ALT flares before HBeAg seroconversion, and are less likely to have sustained biochemical remission after HBeAg seroconversion (52-55) . Data correlating disease progression and other genotypes are scanty. A study in Spain found that deaths related to liver disease occurred more often in patients with genotype F compared to those with genotypes A or D (56). Another study in sub-Saharan Africans showed that genotype A was associated with an increased risk of HCC (57) while a study of Alaska natives reported that genotype F was associated with the highest incidence of HCC (14).

Core promoter variants have been reported to be associated with more severe liver damage and HCC, independent of HBV genotype (46), (58-60). Whether this is mediated through enhanced HBV replication or changes in the overlapping X gene is unclear. Recent studies, mostly from Asia, found that HBV subgenotypes notably C_2, deletions in the pre-S region and other core promoter mutations such as C1763T and T1753V are also associated with HCC (7), (61-64) . These data indicate that variations in HBV sequences may contribute to progression of HBV-related liver disease and HCC development.

Host and environmental factors may also contribute to progression of HBV-related liver disease. Studies in East Asia have consistently reported higher rates of HCC among

patients with chronic HBV infection than those in Europe or the US, whether this is related to a longer duration of infection, virus factors (HBV genotype C, core promoter variant), or to host (genetics) or environmental (e.g. aflatoxin) factors is unclear. The diversity of chronic HBV infection (multiple race/ethnicity of patients and HBV genotypes) in the US provides an opportunity to determine if the rates of adverse outcomes such as HCC across racial/ethnic groups and among the various HBV genotypes and subgenotypes are different and the reasons (virus vs. host genetics) for those differences.

1.3.4. Immune pathogenesis

Since HBV is largely non-cytopathic, the pathogenesis of chronic hepatitis B is determined by the interactions between the virus and the host immune response. Although loss of HBsAg is often referred to as viral clearance, the rare but well documented reemergence of viremia in individuals who develop compromised immunity such as after allogeneic bone marrow transplant, demonstrates that complete viral clearance had not yet, and may never occur (65). Quiescent or inactive disease indicates that the virus has been suppressed by the healthy host immune response. (66;67) In the setting of established chronic infection, the natural history of hepatitis B has been described clinically as different phases of immune control with or without immune-mediated damage ranging from immunotolerance with high viral loads and normal liver histology, to immune clearance with fluctuating viral loads and active hepatitis to final immune control with suppression of viremia and inactive liver disease (40).

The immunological processes that underlie this proposed natural history have yet to be fully defined (66;68); (69). While HBV may remain invisible to the innate IFNα/β system until the onset of adaptive immune response in experimentally infected chimpanzees (70), it is nevertheless suppressed by the activation of toll-like receptor (TLR) stimulation (71) and activated NK or NKT cells that produce various antiviral factors (IFNγ, TNFα) (72-74). In acute infection that resolves, several studies have described the importance of a strong polyclonal and multi-specific CD8+ T cell response (66;67); (68) In chronically infected individuals, the T cell responses are weak and poorly functional. However, suppression of HBV DNA by lamivudine in human subjects can result in enhanced HBV-specific T cell responses (75;76). This suggested that HBV-specific T cells are indeed maintained in a dysfunctional state (but not deleted) during chronic HBV infection. However, enhancement of HBV-specific T cell responsiveness was only transient despite ongoing treatment (76). Decreased T cell responsiveness with prolonged therapy was also associated with increased lamivudine-resistant mutants and viral titers (77). The lack of sustained immune enhancement may contribute to the rebound viremia typically observed when lamivudine therapy is stopped.

The mechanism of T cell dysfunction during chronic HBV infection likely involves a number of immune regulatory pathways. In other infections such as chronic murine lymphocytic choriomeningitis virus (LCMV) and simian immunodeficiency virus (SIV) infection, blockade of immune regulatory pathways such as PD-1:PD-L1 (programmed

death 1:programmed death ligand 1) (78;79) or IL-10:IL-10R (73;80) could suppress viremia and restore antiviral T cell function in vivo. These results suggested that immune regulatory pathways contribute to reversible T cell dysfunction and the outcome of viral infection. Increased PD-1 expression with reversible antiviral effector function in vitro was also reported in HBV-specific T cells isolated from patients with chronic HBV infection (81). Additional immune inhibitory mechanisms may exist in chronic viral infection, including CTLA-4, Tim-3 and Lag-3. Furthermore, there may be qualitative differences in the Th1/Th2 balance of peripheral HBV-specific CD4+ and CD8+ T cell responses among untreated individuals in each of the different clinical phases (82;83) in which immune control (low viremia without disease) of HBV infection was associated with strong CD4+ Th1 and Th2 responses (including IL-10) and detectable CD8+ T cell responses. Furthermore, FoxP3 regulatory T cells have been associated with HBV-specific T cell dysfunction and disease pathogenesis in chronic HBV infection (84;85) . Collectively, the host immune response in chronic HBV infection may be ineffective in viral clearance (yet mediate liver damage) due to active immune inhibitory signals induced during active viremia. Based on this understanding of disease pathogenesis, current treatment guidelines suggest treatment for those with uncontrolled HBV infection with ongoing immune-mediated damage (as evidenced by elevated ALT). The balance between the immune effector and regulatory forces may ultimately mediate the natural history and treatment outcome of HBV infection, including acute hepatitis B, 4 phases of chronic hepatitis B and during/after flares with successful vs. abortive HBeAg seroconversion. The primary goal of the immunology study is to examine the immune regulatory mechanisms such as PD-1:PD-L1 pathway, IL-10:IL-10R pathway and FoxP3+ Tregs) that control HBV-specific immune effector function.

1.3.5. Treatment

The major goal of treatment for chronic hepatitis B infection is to prevent the development of cirrhosis, liver failure and hepatocellular carcinoma by sustained suppression of HBV replication. Current treatment endpoints are based on laboratory tests including reduction in HBV DNA levels, normalization of serum aminotransferase activities, HBeAg seroconversion and HBsAg seroconversion. Long-term suppression of HBV replication results in improvement in necro-inflammatory activity on liver biopsy and decrease in clinical hepatic decompensation.

Treatment guidelines for chronic hepatitis B infection have been published by various professional societies. While there is general consensus that treatment is indicated for patients with the greatest risk for disease progression based on HBV DNA levels and ongoing necroinflammatory activity measured by elevated ALT levels and/or liver biopsy findings, there is less agreement as to the definition of abnormal level of ALT and the level of HBV DNA when therapy is indicated. While recent retrospective epidemiological studies suggest that high HBV DNA level is an independent predictor of risk of cirrhosis and HCC, some clinicians advocate that treatment should be based on HBV DNA level. However, for most patients treatment is necessarily long-term, bringing with it concerns for the development of drug resistance, long-term drug safety and cost.

Currently, there are seven therapeutic agents which are approved for use in the United States and Canada for the treatment of chronic hepatitis B. These agents include two immune modulators, interferon-alfa and peginterferon-alfa and five nucleos(t)ide analogues, lamivudine, adefovir, entecavir, telbivudine, and tenofovir. By convention, treatment duration for peginterferon for chronic hepatitis B is 1 year. Among HBeAg-positive chronic hepatitis B patients, the rate of HBeAg seroconversion is 27% among patients treated with peginterferon with durable HBV DNA suppression. In contrast, HBeAg seroconversion associated with oral nucleos(t)ide analogues ranges from 12-22% with a high rate of relapse if treatment is discontinued after one year. However, for patients treated with oral nucleos(t)ide analogues, HBeAg seroconversion rates increase with time. Seroconversion rates ranging from 40-50% with lamivudine and adefovir, respectively, are achieved at the end of the fifth year and 31% with entecavir at the end of the fourth year of treatment. Among patients with chronic hepatitis B treated with nucleos(t)ide analogues, there is a high rate of relapse if therapy is discontinued shortly after HBeAg seroconversion. Relapse rates are lower if an additional 6-12 months of (consolidation) therapy is administered after seroconversion.

Among HBeAg-negative patients, HBV DNA suppression is achieved in 70% of patients treated with interferon and in 60-90% treated with nucleos(t)ide analogues. However, more than 90% of patients relapse when therapy is discontinued after one year. Therefore, it is recommended that treatment with oral nucleos(t)ide analogues for HBeAg negative patients with chronic hepatitis B should be given indefinitely.

Long-term treatment with individual nucleos(t)ide analogues is associated with drug resistance as a result of selection of HBV mutants. Resistance to nucleos(t)ide analogues is a common cause of treatment failure and ranges from 70% at five years with lamivudine, 29% at four years with adefovir, 22% at year two with telbivudine, and approximately 1% at year five with entecavir. No resistance has emerged with the use of tenofovir to-date but long-term data on tenofovir monotherapy is limited. Cross resistance exists between nucleoside analogues as well as between some nucleoside and nucleotide analogues. The likelihood of resistance relates to pretreatment viral load, prior exposure to other nucleos(t)ide analogues and the rate of viral suppression during therapy which is affected by potency of the agent and patient compliance. Emergence of resistance is associated with laboratory changes such as virological and biochemical breakthrough, and a reduction in clinical benefit of decreasing disease progression.

The viral target of current nucleos(t)ide analogues for HBV is the reverse transcriptase domain of the HBV DNA polymerase. Lamivudine, an L-enantiomer, inhibits HBV DNA polymerase by incorporating a monophosphate form into the viral DNA resulting in DNA chain termination. Lamivudine resistance is associated with M204V and M204I mutations along with compensatory V173L and L180M mutations which restore replicative fitness of the HBV mutant. The M204V / M204I mutations also confer resistance to telbuvidine. N236T and A181T/A181V mutations confer resistance to adefovir. Entecavir resistance is associated with lamivudine-resistance mutations with additional mutations at M250V, S202G, and T184.

The challenge of drug resistance associated with monotherapy with nucleos(t)ide analogues for patients with chronic hepatitis B is similar to the experience with anti-retroviral therapy for HIV infection over 20 years ago. Resistance emerged rapidly with monotherapy. As patients developed resistance to one drug and was then switched to another drug, patients developed resistance to successive drugs. Indeed, this pattern of resistance has been seen in patients with chronic HBV infection treated sequentially with lamivudine followed by adefovir or entecavir. When HIV infected patients were started on dual therapy, emergence of resistance was much slower, and even more slowly when three drugs were initiated.

There is a need for treatment strategies that provide durable suppression of HBV replication by preventing resistance. The experience of combination therapy for chronic hepatitis B infection is limited. The combination of lamivudine and adefovir after two years resulted in a lower incidence of lamivudine resistance compared to patients treated with lamivudine alone (15% vs. 43%) although virological responses were similar. In a small study, viral suppression occurred at a higher frequency among patients who were treated with combination emtricitabine and adefovir (78.6%) for 96 weeks compared to adefovir monotherapy (37.5%) (p=0.03). In this study, no resistance to either emtricitabine or adefovir was seen at 96 weeks. Combination therapy using two similar nucleoside analogues confers no benefit with respect to potency or resistance. Similarly, the combination of nucleoside analogue and interferon did not show improvement in HBeAg seroconversion rate compared to interferon monotherapy.

1.3.6. Liver fibrosis assessment

Liver biopsy is the gold standard for assessment of hepatic fibrosis or cirrhosis but it is an invasive procedure with a risk of significant bleeding of 1 in 2,500 to 1 in 10,000 and a risk of death of $\leq$ 1 in 10,000. Liver biopsy is also subject to sampling error (86). During the last 15 years, there has been extensive research into non-invasive tests for hepatic fibrosis or cirrhosis. These tests include indices or algorithms based on routine laboratory tests, panels of serum fibrosis markers, liver stiffness measurement, and radiologic imaging (87;88). Most of these studies were performed in patients with hepatitis C. They showed that non-invasive tests are more accurate in detecting cirrhosis than in differentiating the different earlier stages of fibrosis and liver stiffness is more accurate than blood tests. Data on the performance of these non-invasive tests in patients with hepatitis B are limited.

1.3.7. Hepatitis B and pregnancy

Although women infected with hepatitis B generally tolerate pregnancy (89), there are some women who experience ALT flares either during pregnancy or after delivery. Tan et al (90) observed that more HBsAg positive pregnant women had elevated ALT levels than controls, regardless of their HBeAg status. Nguyen et al (91) reported 3 cases of significant liver dysfunction during pregnancy with one case requiring liver transplantation when the patient failed a course of salvage antiviral therapy. Another study observed a post-partum flare in 38% of those who were not treated with lamivudine during pregnancy and among those treated, 62% flared after withdrawal of lamivudine (92). Post-partum flares have been associated with HBeAg seroconversion

rates of 12.5 to 17%(93). However, detailed data describing the natural history of hepatitis B in pregnancy are lacking.

2. Aims

- Primary Aim:
  - To describe participants with hepatitis B virus (HBV) infection in a prospective cohort in the United States (US) and Canada and identify predictors of disease activation and progression

- Secondary Aims:
  - To describe clinical, virological, and immunological characteristics of participants with HBV in the US and Canada
  - To evaluate changes in HBV infection status and HBsAg levels and factors associated with those changes
  - To verify whether a baseline HBsAg below 1,000 IU/mL and HBV DNA below 1,000 IU/mL is an accurate predictor of people who are, or who will become, inactive carriers, defined as people who are HBsAg positive, HBeAg negative, have normal ALT and HBV DNA under 1,000 IU/mL on at least two occasions at least 6 months apart
  - To develop a bank of biospecimens (e.g., serum, plasma, DNA, lymphocytes, liver tissue) obtained from participants with HBV infection
  - To identify participants with HBV infection who are potential candidates for one of the treatment studies to be conducted by the HBRN
  - To describe the natural history of hepatitis B infection in pregnancy: including the frequency of, and clinical and virological characteristics associated with, hepatic flares during pregnancy and post-partum.

The HBRN will evaluate a cohort of participants with all stages of HBV infection, including those with acute infection, categorize them into various phases of HBeAg-positive and HBeAg-negative HBV infection and stages of HBV disease, describe the characteristics of these phases, determine the factors associated with transition from one phase to another, and determine the rate of various clinical outcomes.

Specifically, this study will:

1. Describe the occurrence of transitions among various phases of infection and stages of disease in persons with HBeAg-positive and HBeAg-negative HBV infection
2. Identify immunologic and virologic changes associated with those transitions
3. Identify predictors of those transitions

Given that the majority of participants are expected to have normal ALT values, the study will focus on transition into phases with active liver disease. To accomplish these goals, projects with the following objectives will be pursued.

2.1.1. Descriptive epidemiology

Data collected at baseline will provide a snapshot of HBV patients seen at the HBRN centers. Descriptive characteristics, such as race, ethnicity, country of origin, co-morbidity, and disease and treatment history will be examined.

2.1.2. Categorize patients into different phases of HBV infection

Patients in the immune tolerant phase are believed to have mild histology and HBeAg-negative inactive carriers tend to have no or minimal hepatic inflammation; whereas HBeAg-positive and HBeAg-negative hepatitis B have active inflammation and variable fibrosis. Hepatitis B infection is a dynamic process and it is unclear how patients evolve from “quiescence” to an active phase of the disease, and vice versa. Likewise, the steps involved in viral clearance following acute infection have not been elucidated.

2.1.3. Relationship of HBV genotype to clinical, biochemical and histological characteristics

The relationship between genotype and participant characteristics including age, HBV DNA, ALT, and stage of liver disease for each phase of HBV infection will be analyzed. In addition the relationship between genotype and clinical outcome, including cirrhosis, hepatic decompensation and HCC will be studied.

2.1.4. Hepatitis B and pregnancy

The relationship between participant characteristics including age, HBeAg status, HBV genotype, HBV DNA, ALT, stage of liver disease and the occurrence of flares during pregnancy and post-partum will be analyzed. In addition, the use of antiviral therapy during pregnancy and the outcome of the infant will be examined.

2.2. Support of other studies of the HBRN

In addition to conducting its own studies as described above, the cohort study will establish and maintain data, biospecimen bank(s) and other resources for ancillary studies of the pathogenesis, diagnosis, natural history and treatment of HBV infection.

3. Screening and enrollment

HBRN sites will keep a screening log of HBsAg-positive patients who are seen at a recruitment site. This screening log will keep only minimal information and will be used to identify patients for eligibility for the Cohort Study and to allow comparing enrolled participants to all potentially eligible participants with respect to a few important characteristics. The presence of hepatic decompensation, HCC, current antiviral treatment, a history of liver transplantation or known HIV infection will be recorded. Only de-identified elements of the screening log will be forwarded to the DCC for inclusion in the HBRN database.

Patients who appear to meet the eligibility criteria will be approached to participate. Patients with hepatitis B who appear to be in one of the special populations will be considered for enrollment except those who have already reached significant outcomes (decompensation, HCC, liver transplantation), are known to be HIV infected or are currently on antiviral therapy (except pregnant women, anti-HDV positive patients, and participants who completed the HBRN Adult Immune Active trial).

3.1. Phases of HBV Infection ("phenotype")

A patient with HBV infection transitions through a number of phases of HBV infection. One of the aims of the HBRN is to define these phases with objective criteria. Therefore, at the outset of the study it is helpful to have some conceptual definitions of these phases, or phenotypes, which include:

- Acute hepatitis
- Immune tolerant
- Immune active
    - HBeAg positive chronic hepatitis B
    - HBeAg negative chronic hepatitis B
- Inactive carrier
- Indeterminate, i.e., participants who do not fall into one of these categories

Based on historical data and information obtained at the initial study evaluation, each participant will be assigned into one of these categories (see Appendix A, sections A.1 and A.2).

3.2. Outcomes

Once the initial evaluation is completed and a determination made of the phenotype of hepatitis B, participants will be observed over time and the following events recorded:

- ALT flare
- HBsAg or HBeAg loss
- Cirrhosis
- Hepatic decompensation
- HCC
- Liver transplantation
- Death

An Outcomes Committee will review the pertinent data and assign a date of occurrence. With regard to ALT flares and deaths, the committee will focus on assigning probable cause(s).

3.2.1. Hepatitis exacerbation marked by ALT Flare

A flare is defined as serum ALT greater than or equal to 10 times the upper limit of normal which corresponds to 300 U/L in males or 200 U/L in females. This definition will also be applied to HBsAg positive pregnant women whose ALT levels increase during pregnancy or postpartum. Once a flare is detected, participants will be followed more closely until its resolution (see section 6.2.1.2).

3.2.2. Antigen loss: e and s

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

HBsAg loss appears to represent a “cure” of HBV infection and is associated with reduction, but not necessarily elimination, of the risk of future complications, such as HCC which may still occur, particularly in those who lose HBsAg at an older age (e.g. after 50 years) or after the development of cirrhosis.

When HBeAg or HBsAg loss occurs, participants will be followed more closely initially and then return to the regular follow-up schedule (see section 6.2.1.4).

3.2.3. Cirrhosis
The diagnosis of cirrhosis will be made by (1) liver histology, when available or (2) clinical criteria (See Appendix A for definition).

Once cirrhosis is diagnosed, follow-up will include HCC surveillance. HCC surveillance will also be performed in non-cirrhotic participants who meet AASLD (95) guidelines criteria.

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

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

It is anticipated that there will be a small number of participants who will develop hepatic decompensation during follow-up.

3.2.5. Hepatocellular carcinoma (HCC)
HCC may be detected by routine surveillance or may become clinically apparent. The diagnosis of HCC will be made using the AASLD criteria (95).

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

4. Selection and enrollment of participants
4.1. Sources of participants
Participants will be recruited from all of the HBRN centers. Patients who have been seen and are being followed in existing GI and liver clinics as well as those who are newly referred for consultation and seen for the first time at a HBRN center will be recruited.

Some HBRN investigators may engage in outreach activities from which HBV patients seen in primary care settings outside the HBRN centers may be accessed. While such activities are allowed and encouraged, those patients need to be seen at a HBRN center for enrollment in the HBRN.

For the study of the effects of pregnancy on hepatitis B infection two sources of participants are anticipated:

1. Women of childbearing age already enrolled who become pregnant.
2. Pregnant women infected with hepatitis B who are referred to a hepatologist at a HBRN recruitment site and consent to enroll in this study.

4.2. Inclusion criteria

- Written informed consent
- At least 18 years of age
- HBsAg-positive and either
  - Pregnant
  - Anti-HDV positive
  - Diagnosed with acute HBV infection or are experiencing a hepatitis flare
  - Phenotype of immune tolerant or immune active
  - Potentially eligible for the Immune Regulation and Costimulation in Natural History of Chronic Hepatitis B ancillary study.

  OR completed the HBRN Adult Immune Active trial (regardless of HBsAg status)

- **Exclusion criteria**
  - History of hepatic decompensation (see 3.2.4 above)
  - Hepatocellular carcinoma (HCC)
  - History of solid organ transplantation or bone marrow transplantation
  - Chronic immunosuppression therapy
  - Current hepatitis B antiviral treatment (except pregnant women, patients who are anti-HDV positive, and participants who completed the HBRN Adult Immune Active trial)
  - Known HIV co-infection (patients with HDV or HCV co-infection are not excluded).
  - Medical or social condition which, in the opinion of the investigator, would make the patient unsuitable for the study or interfere with or prevent follow-up per protocol
  - Unable or unwilling to return for follow-up visits

4.3. Participant enrollment procedures

Clinical center investigators and coordinators must be certified by the DCC to begin screening participants. Prior to implementing this protocol at an individual site, there must be Institutional Review Board (IRB) or Research Ethics Board (REB) for Human Research approval.

Once a potential participant has been identified, details of the study, including possible risks and benefits, will be carefully discussed and he or she will be asked to sign a consent form. Treatment for HBV will not be offered as part of this Cohort Study.

5. Screening and baseline evaluation

5.1. Screening, consent, and follow-up overview

While many of the potential participants likely will be current patients of the HBRN investigators, patients also may be referred from physicians outside the HBRN and some patients may refer themselves. Existing patients who are familiar to the site investigator may be consented and screened at a visit that is part of the ongoing clinical care of the patient.

Patients who are new to the HBRN center will undergo initial clinical evaluation to verify HBsAg status and other inclusion/exclusion criteria.

5.2. Initial screening (Screening Log)

HBRN sites will keep a screening log of HBsAg-positive patients seen at their site, who appear to fall into one of the special populations. The following elements will be captured for these patients:

1. Date (month and year) assessed
2. Year of birth
3. Gender
4. Currently pregnant
5. Race
6. Presence of exclusion criteria: hepatic decompensation, HCC, liver transplantation, known HIV positivity or therapy for HIV, current therapy for HBV infection (except pregnant women and patients who are anti-HDV positive).
7. Date of enrollment in the Cohort Study or, if not enrolled due to consent not obtained, the reason (refused, language barrier, unable or unwilling to comply with follow up or other reason).
8. Special population group

Patient identifier and contact information associated with the log entry will be kept securely at the HBRN site to prevent repeated screening of the same subject. De-identified data will be entered into the study database so that a summary of those screened may be generated to monitor enrollment and to determine if participants enrolled are representative of patients seen at the sites.

5.3. Baseline evaluation

After informed consent is obtained, the Baseline Evaluation will be done and may, in some instances, be completed over two visits, with the second baseline visit occurring before, or at the time of, the 12 week follow-up visit. The participant will be instructed to come fasting for the baseline visit.

The Baseline Evaluation will consist of:

- Health behavior questionnaire to be completed by the participant (with translator or assistance if needed)
- Brief symptom questionnaire

- Quality of life (QOL) form (SF-36 version 2)
- Questionnaire to be completed by the coordinator
- Questionnaire to be completed by a Physician Investigator*
- Brief physical examination
- Blood will be drawn for laboratory tests related to hepatitis B and liver disease
- Blood will be drawn (with consent) to prepare and obtain serum or plasma for storage*
- Blood will be drawn (with consent) for DNA extraction and genetic testing**

* These may be collected at the 12 week follow-up visit if not obtained at baseline.
** May be collected at any visit if not obtained at baseline

5.3.1. Participant-completed forms

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

5.3.2. Coordinator interview and forms

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

5.3.3. Physician Investigator completed form

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

5.3.4. Physical examination

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

5.3.5. Laboratory data

Blood tests related to viral hepatitis and liver disease
When the following laboratory tests are done as a part of routine care, the results will be obtained from the participant's records. Data available 3 months or less prior to the date of consent may be used.

- Hemoglobin, hematocrit, WBC and platelet count

- Liver panel (AST, ALT, alkaline phosphatase, bilirubin [total, direct, indirect], total protein, albumin)
- Creatinine
- Alpha fetoprotein
- INR
- Lipid panel (total cholesterol, HDL, LDL, triglyceride)
- Fasting glucose and fasting insulin (must be performed at baseline or at follow-up week 12)

The following serologic/virologic data may be obtained from participant records.

- HBV DNA level
- HBsAg
- HBeAg
- anti-HBe
- anti-HDV
- anti-HCV
- anti-HBs
- anti-HIV
- IgM anti-HBc (if acute hepatitis B is suspected)
- Autoimmune markers

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

5.3.6. Serum/plasma for banking

For participants who consent, blood will be drawn at the Baseline Evaluation and plasma and serum samples will be banked. Standardized methods for plasma and serum processing will be followed. Plasma and serum samples will be stored locally and ultimately shipped to a central repository for storage to be used for future studies to determine phase of hepatitis B and possibly to predict disease outcomes and response to antiviral therapy.

5.4. Liver histology

Information about liver biopsies obtained as part of standard of care will be collected. For participants who have had a liver biopsy within 2 years of enrollment in the Cohort Study, unstained slides of the biopsy specimen will be requested from the institution where it was done, and shipped to the central pathology site for staining and ultimately central review. For participants in whom no liver biopsy has been done within 2 years of enrollment, sites may obtain biopsies when clinically indicated.

Biopsy slides will be sent for central pathology reading. In situations in which adequate tissue is obtained (greater than 2.5 cm), the residual tissue will be banked for future studies.

5.5. DNA for banking

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

6. Follow-up evaluation

6.1. Routine follow-up visits

Participants will be followed at predetermined intervals planned to coincide with accepted standard of care visits for managing patients with hepatitis B.

- Routine follow-up will be at weeks 12, 24, and 48 during the first year. The visit at week 12 will only consist of limited laboratory data.
- If the baseline evaluation is completed in one visit, or in two visits that occur before the window for the 12 week follow-up visit opens, then time 0 will be the day of the first (or only) baseline visit.
- If the baseline evaluation is completed at the time of the 12 week follow-up visit, or within the window for that visit, then that visit is considered to be the completion of the baseline visit as well as the 12 week follow-up visit. The next visit will be 24 weeks following the first baseline visit.
- Beyond week 48, follow-up evaluations will be conducted at 24 week intervals.
- Additional follow-up visits may be performed under special circumstances, such as during hepatitis flares or pregnancies (see section 6.2 below).

6.1.1. Routine scheduled follow-up data items

Follow up visits will consist of the following;

- Physical examination
- Blood tests (see Appendix D)
- Interim history
- Health behavior questionnaire (every 48 weeks)
- Brief symptom questionnaire
- Quality of Life (QOL) form (SF-36 version 2), (every 48 weeks)
- Fatigue questionnaire (at week 48 and then every 96 weeks)
- Medications (every 24 weeks only)
- Assessment for possible interim occurrence of any events related to disease progression (e.g., cirrhosis, hepatic decompensation, HCC, or liver transplantation)
- Interim antiviral treatment
- Liver biopsy data (if one was performed for clinical indication)
- For participants who consent, serum/plasma samples for storage will be collected at each follow-up visit. Fasting samples will be collected every 48 weeks.

6.2. Unscheduled follow-ups

Potential reasons for unscheduled visits may include abnormal liver tests including ALT flares, hepatic decompensation, suspicion of hepatocellular carcinoma, change in HBeAg or HBsAg status, development of significant non-hepatic condition that affects the management or prognosis of their hepatitis B, and pregnancy. Data items captured

at the unscheduled visits will be a subset of those items captured at the routine scheduled follow-up visits, unless specified otherwise in the sections below. Questionnaires will be administered to pregnant patients during pregnancy and following delivery. For patients who provided consent, blood specimens for storage may be collected at the time of unscheduled visits (e.g. liver biopsy, ALT flare, at time of diagnosis of hepatic decompensation). These specimens will be processed according to the same procedure as routine follow-up samples.

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

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

6.2.1.1. Evaluating participants experiencing an ALT flare

Flares are defined in section 3.2.1. Examples of data to be collected include:

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

6.2.1.2. Follow-up for participants experiencing an ALT flare

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

6.2.1.3. Evaluating participants achieving HBeAg or HBsAg loss

There are no specific data to be collected in participants who experience HBeAg/HBsAg loss beyond those data items captured at an unscheduled visit.

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

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

6.2.2. Participants who are pregnant

6.2.2.1. Evaluating participants who are pregnant

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

6.2.2.2. Follow-up in participants who are pregnant

Participants who are pregnant will return for visits that may coincide with a scheduled protocol visit or require extra visits. A visit will occur the first or second trimester and on or after 28 weeks gestation. After delivery, data will be collected 12, 24, and 72 weeks later. Should a woman become pregnant more than once during the study, special visits and data collection will be performed for each pregnancy as described in this section and section 6.2.2.1.

6.3. Follow-up in participants newly diagnosed with cirrhosis

There are no specific data to be collected in participants who are newly diagnosed with cirrhosis. The regular follow-up data collection adequately captures information of interest. The diagnosis of cirrhosis does not change the follow-up interval. However, all participants with cirrhosis will undergo HCC surveillance consisting of serum AFP measurement and liver ultrasound every 6 months.

6.4. Follow-up in participants experiencing clinical events

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

6.4.1. Participants experiencing hepatic decompensation

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

6.4.2. Participants newly diagnosed with HCC

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

6.5. Participants who have liver biopsy

Information will be collected regarding the technique and possible complications of the procedure.

6.6. Follow-up in participants undergoing liver transplantation

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

6.7. Participants who die

Date of death and cause of death will be collected.

7. Informed consent

In addition to a consent form for participation in the Cohort Study, subjects may be asked to sign a separate consent for genetics testing.

8. Statistical and design considerations

8.1. Statistical analyses

The primary aim of this study is to describe participants with hepatitis B virus (HBV) infection in a prospective cohort in the United States (US) and Canada and identify predictors of disease activation and progression. We provide analyses plans separately for both parts of the primary aim.

Describe participants with HBV infection

Baseline data will provide a snapshot of the characteristics of participants with HBV infection at the centers. The participants will be characterized by demographics, comorbid conditions, disease, and treatment history. Distribution of participants across different subgroups (e.g. race, ethnicity, HBV genotype, comorbidities) will be reported using group-specific proportions and their 95% exact or approximate confidence intervals as appropriate. Continuous participant characteristics such as age, HBV DNA levels and ALT will be summarized using mean and standard deviations or median and inter-quartile range depending on the distribution of the data. Distribution of ALT levels will also be investigated using the proposed cut-offs of ≤ 30 U/L for males and ≤ 20 U/L for females. Participant characteristics across subgroups will be graphically presented using histograms for categorical variables and box plots, histogram, and density plots for continuous variables.

Participants in different phases of HBV infection will be characterized by the distribution of demographic characteristics such as age, gender, race, ethnicity; medical history and sources of infection; and virological characteristics such as genotype. For continuous variables such as age, ALT and HBV DNA levels, group-specific summary statistics such as mean or median will be provided and uncertainty around such measures would be measured by standard deviations or inter-quartile range. Graphical procedures such as box plots and density plots will be used to visually examine the distribution across phases. Comparison of such variables across different phases will be done by analysis of variance or its nonparametric equivalent Kruskal-Wallis test. For categorical variables

such as race and genotype, contingency tables will be used for investigating distribution of such characteristics across participants in different phases of HBV infection. Chi-square or its exact equivalent will be used for formal test of association.

Predictors of disease activation and progression

Estimating event (disease progression, activation, HCC, decompensation) probabilities and identifying demographic, clinical, and histological factors associated with these events are of prime interest. Event probabilities, their variances, and confidence intervals will be calculated per cohort subset for dichotomous outcomes (e.g., ALT flare, HCC, death, fibrosis progression). Both rates per person-month and cumulative probabilities of event-free time, and their associated confidence intervals will be calculated. The cumulative incidence rate is preferred when there are competing risks (e.g., death removes participants from risk of other adverse events such as HCC) so this measure will also be calculated. These statistics will be calculated overall and for important subgroups (e.g., genotypes, people with high vs. low HBV DNA levels). Hypothesis tests will also be used to determine whether probabilities, rates, or cumulative probabilities differ between (or among) subgroups using chi-square tests for association, differences in Poisson parameters or log rank statistics, and the proportional hazards model, respectively. For all statistical tests, $p<0.05$ (two-sided) will indicate statistical significance.

Predictors of clinical outcomes will be assessed using regression models: logistic regression for event probabilities (e.g., proportion with ALT flare, HCC, death, fibrosis progression), Poisson regression for rates per person-month (HCC incidence rates), and proportional hazards models for time to event data (e.g. time to ALT flare, HCC, death, or fibrosis progression).

Distribution of variables over time will be analyzed using linear (for continuous variables such as HBV DNA), generalized linear (for binary or count, such as number of adverse events) models, as appropriate. These models will be used to assess the association of baseline and time varying participant characteristics with the longitudinal outcome data.

Statistical analysis plan for quantitative HBsAg and HBV DNA cut-offs as predictors of inactive carrier.

One of the goals of this study is to assess the accuracy of “baseline HBsAg below 1,000 IU/mL and HBV DNA below 1,000 IU/mL” as a predictor of inactive carriers, the definition of which is outlined in the secondary aims of this study, At baseline, each participant in the cohort study will be designated as “Inactive” or “Active” based on the following test. If the participant’s baseline HBsAg level is below 1,000 IU/mL and HBV DNA level is below 1,000 IU/mL, the participant will be labeled as “Inactive”; Otherwise, the participant will be labeled as “Active”, as long as there are HBsAg and HBV DNA levels. Participants will then be followed up routinely according to the cohort schedule to determine if they become a true inactive carrier.

The analysis will be centered around a 2X2 table, where each patient will belong to one of the four cells at the end of the study.

Table: Test result vs. true hepatitis B activity status. TA = True Active, FA = False Active, TI = True Inactive, FI = False Inactive, DA = Designated Active, OA = Observed Active, DI = Designated Inactive, OI = Observed Inactive

	Observed status		
Designated status at baseline	**Inactive**	**Active**	**Total**
Inactive	TI	FI	DI
Active	FA	TA	DA
Total	OI	OA	n

We will estimate the positive predictive value (PPV) and negative predictive value (NPV) of the test defined respectively as the proportion of participants who become inactive among those who were designated inactive at baseline, and the proportion who remain active among those who were labeled active at baseline. These are estimated as follows:

PPV = TI/DI
NPV = TA/DA.

We will also estimate retrospective sensitivity (SE) and specificity (SP) as follows:

SE = TI/OI
SP = TA/OA.

In addition, the false positive rate (FPR) and false negative rate (FNR) will be calculated as follows:

FPR = FI/OA
FNR = FA/OI

Standard errors of these quantities will be calculated using exact and approximate (asymptotic) methods for proportions. Exact and Wald 95% confidence intervals will be constructed for each of the above proportions.

We will also calculate Youden's index defined by J = SE – FPR = SE+SP-1, and the corresponding confidence interval. The Youden's index in this setting reflects the likelihood of being labeled as "inactive' among participants who will eventually become inactive versus those who will not.

Exploratory analysis will be performed with HBsAg level <2,000 IU/mL and HBV DNA <2,000 IU/mL as cutoffs to predict inactive carrier as defined in the cohort protocol. Exploratory analysis will also be performed using alternative definitions of inactive carriers as used in EASL and AASLD Practice guidelines.

Interim analysis will be performed on the first 1000 patients who have completed at least 6 months follow up in the cohort study.

8.2. Missing data

In general, missing outcome data will be handled as follows in statistical analyses. First, we will explore the pattern of missing data by comparing characteristics of participants with available data to those with missing data. For data missing completely at random (does not depend on observed outcome; MCAR) standard analytical techniques described earlier would produce unbiased results when the analysis is performed on the complete cases. For the time-to-event data analyses, analytical methods such as Kaplan-Meier estimation and Cox proportional hazards model account for MCAR drop-outs. However, in other cases where the dropout depends on observed outcome or covariates (missing at random, MAR) or unobserved outcome and/or covariates (missing not at random, MNAR), we will use selection models such as MNAR Dale model and Diggle-Kenward model. These models often require strong assumptions on the dropout mechanism which are primarily unverifiable based on the observed data. We will conduct sensitivity analyses to investigate the sensitivity of our conclusions to possible violation of such assumptions by fitting models under MCAR, MAR and MNAR assumptions and compare the model fits using log-likelihoods.

We will consider other approaches to analyzing longitudinal data with informative censoring including modeling the dropout process jointly with the fitted model of interest and weighted estimating equations using inverse probability of missingness to account for the potential bias that arises due to the missing outcomes.

8.3. Sample size / power considerations

Power of a study depends on the available sample size, desired effect size and type I error. The sample size for this study has been determined based on the practicability of recruitment, availability of resources, and adequacy of statistical power to test certain hypotheses that may be of interest. Since this is an observational study aimed to generate hypotheses, there will be additional hypotheses testing planned. For each planned analysis, we will compute the sample size required for a pre-determined level of statistical power (or the desired power for available sample size) and type I error. Here are two examples to provide an idea of the number of participants that will be required to test certain hypotheses with 80% power and 5% type I error using a two-sided test.

8.3.1. Testing the risk of cirrhosis across low and high HBV DNA group

Uchenna et al. (2006) reported that the incidence of cirrhosis in hepatitis B participants over a long period (mean = 11 years) of follow-up was 365 per 40,038 person-years-follow-up. If the same rate and a mean follow-up period of 4 years is assumed for the Hepatitis B Research Network cohort study, the incidence of cirrhosis will be approximately 3.65%. The null hypothesis being tested is that the risk of cirrhosis in high HBV DNA group (HBV DNA >10,000 IU/mL) is the same as that in low HBV DNA group (HBV DNA ≤ 10,000 IU/mL), while the alternative hypothesis is that the risk of cirrhosis in high HBV DNA group is RR times that in the low HBV DNA group B. The following table gives the number of participants required to detect different relative risks using a Cox proportional hazard model based on various overall incidence rates.

Table 1. Sample size estimates for comparing the risk of cirrhosis across low and high HBV DNA group

Covariate	Comparison Groups	Anticipated % of total study cohort*	Relative risk (RR)**	Total N Required Overall rate of incidence 3.65%	Total N Required Overall rate of incidence 5.0%	Total N Required Overall rate of incidence 6.0%	Total N Required Overall rate of incidence 7.0%
HBV DNA	>10,000 IU/mL	44%	2.5	4185	3055	2546	2182
	≤10,000 IU/mL	56%	1				
HBV DNA	>10,000 IU/mL	44%	3.0	2928	2137	1781	1527
	≤10,000 IU/mL	56%	1				

*From the distribution provided in Uchenna et al., Gastroenterology, 2006
**RR=1 is the reference group

8.3.2. Comparing the risk of HCC across different groups

Risk of HCC in HBV population is smaller compared to cirrhosis. Chen et al. (2006) reported that the incidence of HCC in hepatitis B participants over a long period (mean = 11.4 years) of follow-up was 164 per 41779 person-years-follow-up. If the same rate is expected and a mean follow-up period of 4 years is assumed for the longitudinal study, the incidence of HCC will be approximately 1.6%. Note that the study reported by Chen et al. had 85% HBeAg –ve and only 15% HBeAg +ve participants in the cohort. When adjusted for the expected HBeAg status in our participant population, the incidence rate increases to 3.1%. Therefore we provided two-sets of sample sizes required based on these two rates (1.6% and 3.1%). The null hypothesis being tested is that the risk of HCC in Group A (e.g. HBeAg +ve) is same as that in Group B (e.g. HBeAg –ve) while the alternative hypothesis is that the risk of HCC in group A is RR times that in group B. The following table gives the number of participants required to detect different relative risks using a Cox proportional hazard model based on various overall incidence rates.

Table 2. Sample size estimates for comparing the risk of HCC across different subgroups for varying expected relative risks

Comparison Groups	Anticipated % of total study cohort*	Relative risk (RR)**	Total N Required at 80% Power Overall incidence 1.6%	Total N Required at 80% Power Overall incidence 3.1%
HBeAg+ HBeAg-	40% 60%	3.0 1	6619	3633
HBeAg+ HBeAg-	40% 60%	3.5 1	5126	2814
ALT > ULN ALT ≤ ULN	47% 53%	3.0 1	6534	3373
ALT > ULN ALT ≤ ULN	47% 53%	3.5 1	5060	2612
ALT > ULN ALT ≤ ULN	47% 53%	4.0 1	4092	2112

* Based on site surveys
** A relative risk of 1 indicates the reference group.

9. Data management

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

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

DCC personnel meet weekly to discuss study status, recruitment, compliance, review data issues, clinical center participation, and other issues that arise during the course of the study.

10. Study Organization – Sites

This study will be conducted at approximately 21 clinical centers within the United States and Canada. A Data Coordinating Center (DCC) will coordinate operations, develop and implement data and other systems, maintain the database and perform data analyses. This study will use a biospecimen and a genetic repository. A central virology lab, immunology lab, pathology lab, and one or more central testing labs will be utilized to perform tests and to store specimens identified in the protocol.

11. Human subjects issues

11.1. Overview

Prototype protocols, consents and assents will be prepared for the study. Individual sites may modify material to comply with local regulatory requirements, but may not substantively alter the study. A copy of the approved site-specific consent form(s) and protocol must be submitted to the DCC for archive.

As required, the study protocol, consent forms, and data collection forms will be submitted to each clinical center's IRB, the DCC's IRB and the IRB for the central laboratory. Additionally, if required, each clinical center will submit to their IRB any recruitment materials to be used at their site. Sites must provide the DCC with copies of the initial IRB approval notice and subsequent renewals, as well as copies of the IRB approved protocols, and informed consent documents.

A signed consent form will be obtained from the subject (person with power of attorney for subjects who cannot consent for themselves). The subject's consent must be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject or legal representative, and this fact will be documented in the subject's record.

All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines. Given the epidemiology of HBV infection, subjects included in this study will include a large proportion of racial/ethnic minorities (Asian, American Indian/Alaska Native, Native Hawaiian or other Pacific Islander, Black or African American, Hispanics or Latinos) as well as non-Hispanic white subjects. We anticipate that the participants recruited from diverse sources, including community and tertiary referral populations, will capture the entire spectrum of HBV infection.

11.2. Standard of care

All participants will receive standard of care for HBV infection and identified associated medical problems outside of the study protocol. This will include providing health care, laboratory testing, HCC surveillance, counseling and educational materials at enrollment and on an ongoing basis during follow-up. Participants with acute hepatitis B may require additional epidemiologic inquiry and counseling to alert contacts and health authorities regarding possible unrecognized HBV infections.

11.3. Enrollment in other HBRN studies

The HBRN is planning to conduct several studies, both observational and interventional. Each of these studies will have separate protocols. Those participants found to be potentially eligible for enrollment in another study within the HBRN will be offered participation and asked to sign a separate consent form. Once a participant is enrolled in a HBRN clinical trial, he or she will no longer be active in the Cohort Study. Subsequently, when participation in the HBRN clinical trial is completed (either by virtue of study completion or early withdrawal), subjects will be asked to resume follow up according to the Cohort Study protocol.

11.4. Subject confidentiality

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

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

11.5. Participant withdrawal

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

11.6. Data and safety monitoring

Data and safety will be monitored by the NIDDK in conjunction with an NIDDK-appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the study. The description of DSMB activities is included in the DSMB Charter.

The DSMB charter will be developed by the NIDDK. It will monitor all aspects of the study (e.g., recruitment, protocol deviations, breeches of confidentiality, data quality,

attrition, descriptive characteristics), and recommend protocol modifications, including early study termination. Reports will be prepared by the DCC. Tables showing study progress will be presented by clinical center and overall. These will include recruitment, protocol deviations, attrition, breeches of confidentiality, and data quality. The DCC will maintain a cumulative summary of breeches of confidentiality to be forwarded to the DSMB for their meetings via conference call or in person. Based on the data presented, the DSMB will recommend continuation or termination of the study. A summary of the DSMB findings will be forwarded to all investigators for submission to their respective IRBs.

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Appendix A: Definitions

A.1. Acute Hepatitis B:

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

A.2. Phases or phenotypes of chronic hepatitis B

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

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

HBeAg-negative chronic hepatitis: Definite: presence of HBsAg without HBeAg but with abnormal serum ALT levels (at least twice the ULN) on two occasions or more at least 6 months apart. HBV DNA levels of greater than or equal to 1,000 IU/mL. **Probable:** Presence of HBsAg without HBeAg and HBV DNA greater than or equal to 1,000 IU/mL, but ALT levels between 1-2 times the ULN.

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

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

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

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

* ≥ 2 times ULN = definite, 1-2 times ULN = probable (ULN=30 U/L for males, 20 U/L for females)
** HBV DNA 1,000-10,000 IU/mL with normal ALT and no HBeAg-probable

Notes:

- The phase will be assigned based on HBeAg, ALT and serum HBV DNA level, regardless of the presence or absence of anti-HBe in serum
- Probable: 1) if all other criteria for chronic hepatitis B are met and serum ALT is elevated 1-2x ULN, participant will be assigned to “probable” chronic hepatitis B

(HBeAg positive or negative) or 2) if all other criteria for immune tolerant are met and serum HBV DNA is between 100,000 and 1,000,000 IU/mL, participant will be assigned to "probable" immune tolerant or 3) if all other criteria for inactive carrier are met and serum HBV DNA is between 1,000 and 10,000 IU/mL, participant will be assigned to "probable" inactive carrier

- Indeterminate: The phase or phenotype will be "indeterminate" if the participant does not clearly fall into any one of the above definite or probably categories or two or more sets of results at baseline are contradictory.

A.3. Normal ALT

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

A.4. ALT flare

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

A.5. Loss/Seroconversion/Seroreversion

A.5.1. HBeAg

In a participant who is previously HBeAg-positive and anti-HBe-negative, disappearance of HBeAg from the serum is HBeAg *loss*.

If this is accompanied by emergence of anti-HBe, it is HBeAg *seroconversion*.

Among HBeAg-negative participants, reappearance of HBeAg is considered HBeAg *seroreversion*.

A.5.2. HBsAg

By definition, a participant with HBV infection is HBsAg-positive. Anyone who is HBsAg-positive regardless of the presence or absence of anti-HBs is considered to be HBV-infected. A subsequent disappearance of HBsAg from the serum is HBsAg *loss*.

If this is accompanied by emergence of anti-HBs, it is referred to as HBsAg *seroconversion*.

A.6. Clinical events

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

A.6.1. Cirrhosis

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

- Any one of the following
    - Presence of ascites or hepatic hydrothorax

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

or in the absence of hepatic decompensation

- Any two of the following (in the absence of another explanation)
  - Splenomegaly
  - Nodular liver
  - Platelet count below 120,000/mm^3

A.6.2. Hepatic decompensation

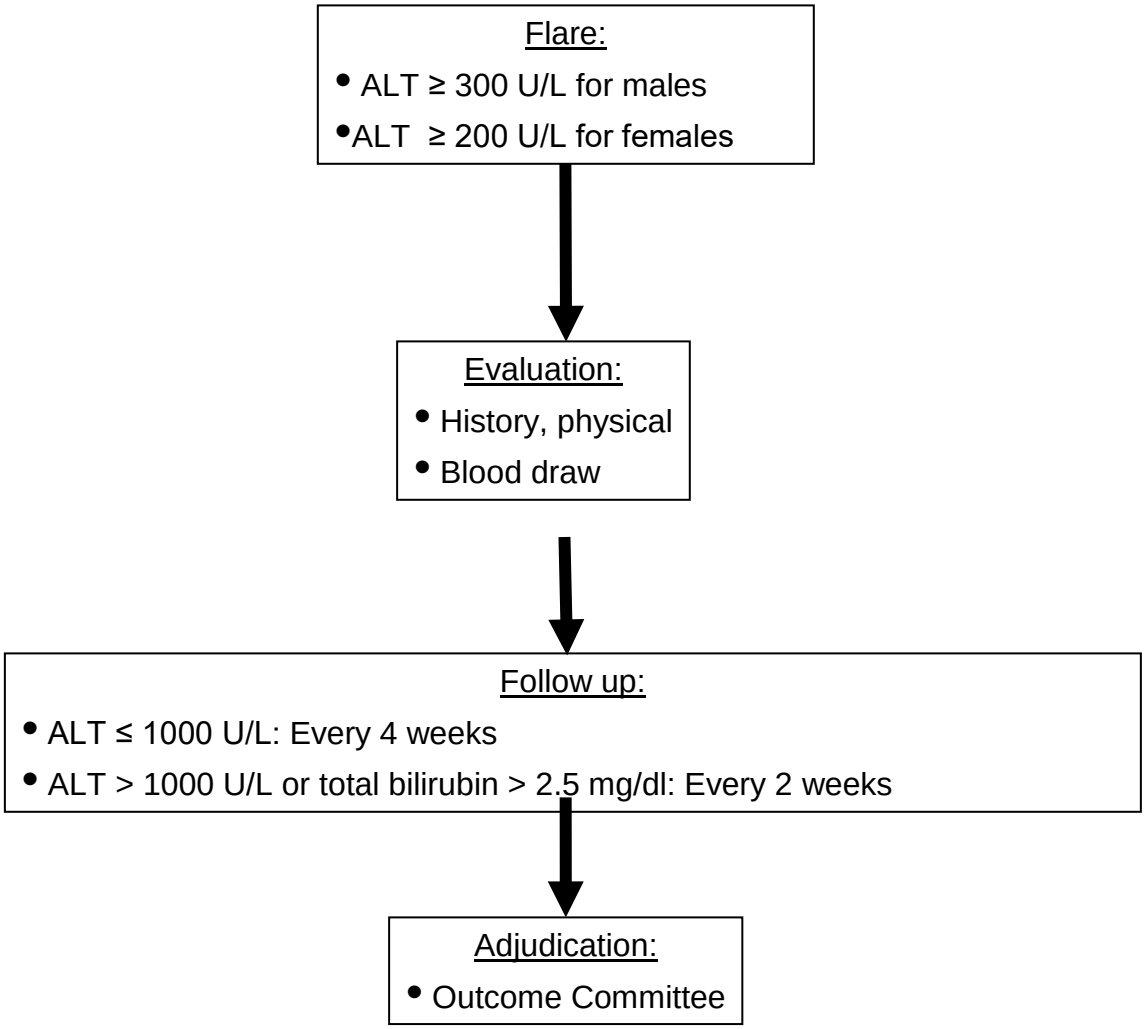
Development of hepatic decompensation will be defined by any of the following events:

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

A.6.3. Hepatocellular carcinoma (HCC)

The diagnosis of HCC will follow the AASLD guidelines.

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



Appendix C: Participating centers

Baltimore, MD: Johns Hopkins University

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, University of Hawaii/Hawaii Medical Center East

Minnesota: Mayo Clinic Rochester, University of Minnesota

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

Philadelphia, PA: University of Pennsylvania School of Medicine (Immunology 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

Appendix D

D.1: Data collection schedule

Item	Baseline	Scheduled	Scheduled	Scheduled	Beyond week 48
Cohort Calendar (weeks)		12	24	48	Repeat week 24 and 48 Alternately
Informed consent	X				
Demographics (year of birth, sex, race)	X				
Country of origin, education	X				
Family Hx, risk factors	X				
Health behaviors	X			X	X
SF-36 version 2	X			X	X
Fatigue questionnaire				X	X1
Symptoms	X	X	X	X	X
Medical history	X		X	X	X
Medication history (HBV Tx)	X		X	X	X
Medication history	X		X	X	X
Height	X				
Weight, Waist circumference	X			X	X
Blood pressure	X		X	X	X
Brief physical exam	X		X	X	X
CBC	X			X	X
ALT	X	X	X	X	X
Hepatic panel (AST, bilirubin, albumin)	X		X	X	X
Creatinine	X			X	X
Fasting glucose, insulin	Xa			X	X
Fasting total cholesterol, LDL, HDL, triglyceride	Xa			X	X
INR	X			X	X
QualitativeHBsAg	X			X	X
IgM anti-HBc	Xc				
QualitativeHBeAg	X		Xe	Xe	Xe
Anti-HBs	X			X	X
Anti-HBe	X		Xe	Xe	Xe
Quant HBV DNA	X	Y	X	X	X
HBV genotype and subtype	Xb				
HBV precore/BCP	Xb				
Anti-HCV	Xb				
Anti-HDV	X				
Anti-HIV	Xb				
Autoimmune markers	X				
Abdominal imaging	X		Xf	Xf	Xf
Liver biopsy	X		X	X	X
Non-fasting or fasting serum banking		X	X		X
Fasting serum/plasma banking	Xa			X	X
DNA banking	Xd				

X: Record available data, Y: From stored sample if necessary
1: Every 96 weeks
a: Can be obtained at follow-up week 12 if not at baseline
b: Results at any time
c: In new participants with uncertain diagnosis of hepatitis B
d: Can be obtained at any visit if not at baseline
e: May not be repeated in HBeAg- participants unless a flare
f: Participants who need HCC surveillance

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

D.2: Research whole blood draw schedule

Item				
	Baseline	**Week 12**	**Week 24 and every 48 weeks thereafter**	**Week 48 and every 48 weeks thereafter**
Research (ml)	**45**	**20**	**20**	**40**
Genetics (ml)	**5**			
Total (ml)	**50**	**20**	**20**	**40**

Additional samples may be collected at the time of a special visit (e.g. liver biopsy, ALT flare). The volume collected for research or storage may be adjusted to accommodate samples needed for approved ancillary studies.

Appendix E: Data collection for pregnant women