A Randomized, Double-Blind Comparison of the efficacy of Alpha-Interferon and Lamivudine combination therapy vs Alpha-Interferon monotherapy in the treatment of Chronic Hepatitis B

Teresa Everin

A. Purpose of Study

Chronic viral hepatitis is the principal cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma in the world. Worldwide, over 300 million people have chronic infection with hepatitis B virus (HBV). In the United States, chronic hepatitis B accounts for 5 to 10 percent of the cases of chronic liver disease and cirrhosis, with an estimated 200,000 to 300,000 cases of hepatitis B in the United States each year, leading to more than 10,000 hospitalizations and 5,000 hepatitis B-related deaths.

Chronic HBV infection is endemic in Africa and Asia where the virus is typically transmitted from mother to newborn or between close contacts in early childhood. High risk groups in the U.S. include intravenous drug users, persons with multiple sexual contacts, and health-care workers. The persistent presence of HBV DNA and HBeAg in serum correlate with ongoing viral replication and indicate infectivity. Ten percent of patients acquiring HBV as adults and 90% of those infected as neonates do not clear HBsAg from the serum within 6 months and, thus become chronically infected.

Patients with chronic hepatitis B and active viral replication are candidates for therapy with alpha-interferon. Alpha-interferon is currently the only treatment specifically approved by regulatory authorities throughout the world for chronic hepatitis B. It is given by injection and has potentially dose-limiting side effects.

The interferons are a family of glycoproteins produced primarily by monocytes and transformed lymphocytes, usually in response to viral infections. The interferons, appear to act through immunomodulatory mechanisms by binding to specific cell receptors in infected cells with activation of intracellular enzymes that have several antiviral actions. Interferon also enhances both T-cell-mediated and natural killer cell cytotoxicity.

The efficacy of alpha-interferon is variable. However, a recent meta-analysis of 15 clinical trials demonstrated an overall response rate (as measured by the number of patients in whom -there was a disappearance of HBeAg from serum) of 33 percent among patients receiving the drug compared with 12 percent of untreated controls.

Lamivudine is an oral nucleoside analogue which inhibits viral DNA replication, and has demonstrated strong antiviral activity against HBV and HIV. The drug is well absorbed after oral administration, and has been well tolerated in controlled studies in patients with human immunodeficiency virus (HIV) and those with chronic HBV infection for prolonged periods. In phase 2 studies, all doses studies (5 to 600 mg per day for up to six months) markedly reduced serum HBV DNA levels in Asians and whites. With doses of more than 100 mg per day the median suppression of serum HBV DNA was greater than 98 percent in most patients during treatment. However, when treatment was stopped, serum HBV DNA levels generally returned to pretreatment values.

In their recent multicenter one year double-blind study of lamivudine in 358 Chinese patients with chronic hepatitis B, Lai et al demonstrated that a 100mg dose of lamivudine daily was associated with substantial histologic improvement in many patients with chronic hepatitis B. Additionally, the researchers found that the 100mg dose was well tolerated, and when compared to placebo, was associated with a reduced progression of fibrosis, a 16 percent rate of hepatitis Be antigen seroconversion (loss of HBeAg, development of antibody to HBeAg, and undetectable HBV DNA), the suppression of HBV

DNA (98 percent reduction at week 52 as compared with the base-line value), and a 72 percent incidence of sustained normalization of alanine aminotransferase levels.

As alpha-interferon and lamivudine work through different antiviral mechanisms , one may postulate that greater treatment response rates may be obtained with a combination of the two therapies as opposed to either one as monotherapy.

Reducing markers of viral replication (HBeAg and HBV DNA) to undetectable levels has been shown to correlate with improved survival and fewer HBVrelated complications. The primary objective of this protocol is, therefore, to determine the efficacy and safety of combination therapy with oral lamivudine and the subcutaneously delivered alpha-interferon, as compared to therapy with alpha interferon monotherapy in patients with chronic Hepatitis B (HBV) infection.

Differential efficacy will be determined by comparing the proportion of 1) subjects in each treatment group who achieve undetectable serum HBeAg levels at the end of dosing and at 24 weeks following treatment and 2) subjects with undetectable serum HBV DNA defined as a level less than 1.6 pg/ml by the Abbott solution-hybridization assay and undetectable levels by PCR at the end of dosing and 24 weeks following treatment.

The secondary objectives are to determine safety as assessed by clinical adverse events and laboratory abnormalities, the proportion of subjects with undetectable serum HBsAg and appearance of anti-e antibody at the end of dosing and 24 weeks post dosing, the proportion of subjects with normalization of serum ALT at the end of dosing and 24 weeks post dosing, the proportion of subjects demonstrating improvement in histologic activity on liver biopsy 24 weeks post dosing.

B. Study Design and Statistical Analysis

Potentially eligible subjects will be identified through clinic visits at the CPMC Liver and GI Clinics, as well as University Medical Center affiliated GI clinics in collaborating centers throughout New York City. Once identified, subjects will be given the consent form and asked to participate.

Consenting subjects will undergo screening evaluations consisting mainly of medical history, physical examination and blood drawing.

Once the screening labs fulfill all entry criteria the subject will be asked to return to the GCRC for labs to count as the baseline visit. These tests will be sent to the central research lab. The subject will then be scheduled to begin dosing within 48 hours.

Subjects will be blindly randomized into one of two treatment groups to receive either 1) oral lamivudine 100mg daily for 16 weeks concurrent with subcutaneous (SC) alpha-interferon 3 million units three times per week for 16 weeks, followed by 36 weeks of oral lamivudine 100mg daily alone, or 2) SC alpha-interferon 3 million units three times per week for 16 weeks concurrent with oral placebo once daily for 16 weeks, followed by 36 weeks of continued once daily oral placebo.

Patients will be randomized with a block size of 8 (4 in the stratum with alphainterferon and lamivudine, 4 in the stratum with alpha-interferon alone). Each center will perform randomization for a full block of 8 patients before proceeding to the next block. The ratio of random assignments will be 1:1.

The study hypothesizes that subjects receiving lamivudine and alpha-interferon combination therapy will show a statistically significant increase in the loss of serum HBeAg and HBV DNA when compared to those subjects receiving alphainterferon monotherapy.

The null hypothesis presumes no difference in the response rate between the two treatment regimens.

With 102 patients in each arm, this study will have 80 percent power to detect a difference in serologic response between the treatment groups. This number is based on an estimated efficacy of combination treatment that is 20% above the accepted 30% estimate of efficacy of alpha-interferon mono-therapy.

This study will be performed under an intention to treat analysis.

Subjects will not be crossed over from one group to another.

Prolposed methods of statistical analysis We will employ a dichotomous analysis using chi-squared tests to: compare differences in the percentage of subjects in each study arm demonstrating HBeAg seroconversion at weeks 52 and 76 compare differences in the percentage of subjects in each study arm with undetectable levels of HBV DNA at weeks 52 and 76. compare differences in - the percentage of subjects in each study arm with improvement in Knodell necroinflammatory score at week 76. Base-line HBV DNA levels (log 10) in the two groups will be compared with the use of analysis of variance adjusted for the center. The Wilcoxon signed rank test will be used to compare differences among the two groups in the percentage change in HBV DNA levels at week 52 and 76.

C. Study Procedures

After the first clinic visit (at base line) patients will return during weeks 2 and 4 and every four weeks thereafter through week 52. Following 52 weeks of treatment, patients will return to the study sites every 4 weeks until week 76.

The likely duration of the entire study will depend largely on the rate of eligible patient identification and enrollment. At this point, we estimate a total study duration of approximately 3 years.

On the first day of dosing the subject will be required to have a set of labs, vital signs and a physical exam. Analysis of safety will include data for all patients randomly assigned to a treatment group receiving at least one dose of study medication.

For the first 16 weeks of the study, all subjects will be required to present to clinic sites three times per week for SC injection with alpha-interferon dose.

Serum will be assayed for HBV DNA (at base line and weeks 2,4,8,12,24,36,52, 64 and 76), HBeAg, antibody to HBeAg, and alanine aminotranferase levels at the same intervals, and HBsAg and antibody to HBsAg (at base line, week 52 and week 76). At each clinic visit, serum samples will be obtained through blood draws, and laboratory tests will be performed to determine the safety of the treatment, and adverse events sine the previous visit will be documented.

A liver biopsy will be performed at week 76 to compare results with those of the pre treatment biopsy. This second liver biopsy represents a procedure done solely for research purposes. Individual biopsy specimens will be scored with the use of the Knodell index, which grades the histologic activity of hepatitis on a scale from 0 to 22, with higher scores indicating more severe abnormalities. The overall Knodell score represents the sum of the scores for periportal bridging necrosis (0 to 10), intralobular degeneration and focal necrosis (0 to 4), portal inflammation (0 to 4) and fibrosis (0 to 4). Response rates will be based on the first three components of the score.

The histologic response will be defined as a decrease of at least two points on the Knodell necroinflammatory score at 76 weeks as compared with the base-line score. To reduce the potential for observer variability, biopsy specimens will be evaluated by a single independent histopathologist who is unaware of the treatment assignment and the sequence of the specimens. The degree of fibrosis and necroinflammatory activity will also be compared in each pair of biopsy specimens (with the investigator blinded with regard to treatment and biopsy sequence) to determine whether one specimen showed more severe necroinflammatory activity or more fibrosis (ranked response).

Staining for HBcAg will be performed with rabbit polyclonal antibody to HBcAg and indirect avidin-biotin-peroxidase immunohistochemical techniques.

Hepatic HBV DNA will be localized in hepatocytes with the use of a nonisotopic in situ hybridization technique and a digoxigenin-labeled full length human HBV DNA probe, prepared from a recombinant plasmid.

All virus will be assayed at CPMC research laboratories. Serum HBV DNA will be quantified with the use of a solution-hybridization assay that has a lower limit of detection of 1.6 pg per milliliter. HBeAg and antibody to HBeAg will be detected by a qualitative HBeAg enzyme immunoassay. HBsAg will be detected with a monoclonal qualitative third-generation enzyme immunoassay and antibody to HBsAg will be detected with a microparticle enzyme immunoassay.

D. Study Drugs

A112ha-interferon is currently the only treatment specifically approved by regulatory authorities throughout the world for chronic hepatitis B. The efficacy of alpha-interferon is variable, but a meta-analysis showed that 33 percent of patients receiving the drug had a loss of hepatitis Be antigen (HBeAg), as compared with 12 percent of untreated controls. Although clear dosing guidelines have yet to be established, most investigators agree that a dose approximating 3 million units delivered by subcutaneous injection 3 times weekly for 6 months is needed to obtain response (complete or partial). The standard dosing regimen will be used in this study.

Refer to section 12 of the IRB protocol for a discussion of potential side effects of therapy.

Lamivudine is an investigational drug for the treatment of chronic hepatitis B. In a recent multicenter one year double-blind trial of lamivudine in 358 Chinese patients with chronic hepatitis B, Lai et al demonstrated that a 100mg dose of lamivudine daily was associated with substantial histologic improvement in many patients with chronic hepatitis B. Additionally, the researchers found that the 100mg dose was well tolerated, and when compared to placebo, associated with a reduced progression of fibrosis, a 16 percent rate of HBeAg seroconversion (loss of HBeAg, development of antibody to HBeAg, and undetectable HBV DNA), the suppression of HBV DNA (98 percent reduction at week 52 as compared with the base-line value), and a 72 percent rate of sustained normalization of alanine aminotransferase levels. In this study, lamivudine will be administered at a dose of 100mg daily for a period of 12 months.

Refer to section 12 of the IRB protocol for a discussion of potential side effects of therapy.

E. Medical Devices

Not applicable in this study.

F. Study Questionnaires

This study will not involve the use of questionnaires.

G. Study Subjects

Eligible patients will include males and females, 18 years and older, with detectable HBsAg and HBeAg in serum at the time of screening and for at least the previous six months, serum HBV DNA levels of at least 5pg per milliliter (as determined with the use of a solution-hybridization assay; Abbott Diagnostics, Chicago) and alanine aminotransferase levels that are less than 10 times the upper limit of normal at screening and for at least the previous three months.

Inclusion in the study also requires pre-treatment liver biopsy demonstrating histopathology consistent with chronic hepatitis B within 6 months prior to enrollment.

Exclusion criteria are as follows:

- Hepatitis C or D or HIV infection.
- Decompensated liver disease (serum bilirubin level more than 2.5 times the upper limit of normal, a prothrombin time prolonged by more than 3 seconds and a serum albumin level lower than 3g per deciliter, or a history of ascites, variceal hemorrhage, or hepatic encephalopathy).
- Evidence of autoimmune hepatitis (antinuclear-antibody titer higher than 1:160). History of treatment with: an investigational drug within 30 days before enrollment; systemic antiviral therapy; immunomodulators; cytotoxic agents; corticosteroids within 6 months; lamivudine within 3 months prior to enrollment.

- Known history of hepatocellular carcinoma or physical or radiographic evidence of a hepatic mass.
- Known history of allergy to nucleoside analogs.
- Subjects who received less than 4 weeks of interferon therapy due to intolerance (acute hypersensitivity reactions, thyroid dysfunction, hypotension, arrhythmias, hepatotoxicity, severe flu-like symptoms unresponsive to symptomatic therapy or other evidence of serious intolerance documented by the investigator).
- Current alcohol or drug abuse. Patients unable to tolerate oral medication or subcutaneous injection.
- Sexually active subjects who are not surgically sterile and who are unwilling to practice a reliable method of contraception for the duration of the study.

Other serious conditions that might preclude completion of the study.

Additionally:

- All women of childbearing potential must have a negative pregnancy test within 24 hours prior to starting study medication.
- This study will not involve radiation exposure.
- This study will not involve the inclusion of vulnerable populations (minors, pregnant women, mental patients, prisoners, elderly persons, persons who are institutionalized, or persons unable to give informed consent).
- This study will not be restricted by gender or race.

H. Recruitment of Subjects

Potentially eligible subjects will be identified through routine clinic visits at the CPMC Liver and/or GI Clinics, as well as University Medical Center affiliated GI clinics in collaborating centers throughout the city. Once identified, subjects will be given the consent form and asked to participate.

As per CPMC policy, the patient's primary. physician will be asked to (1) agree that the patient is suitable for the study and (2) ascertain from the patient that he/she is willing to discuss the study with the research team before any approach may be attempted by the investigators.

I. Confidentiality of Study Data

All study data will be coded using unique code numbers for all study subjects. Personal identifiers will not be used as coding mechanisms. Data will be stored in a secure location on study site grounds, accessible only to the investigators.

J. Potential Conflict of Interest

Not applicable.

K. Location of the Study

Subjects will be studied at the GI/Liver clinics at CPMC, as well as several University Medical Center affiliated GI/Liver clinics in New York City.

L. Potential Risks

The potential risks of alpha-interferon therapy are influenza-like symptoms, prolonged fatigue, malaise, headache weight loss, mild leukopenia and thrombocytopenia, alopecia and in some cases clinical depression.

In contrast to alpha-interferon, lamivudine has been well tolerated in placebo controlled studies. Theoretically, the potential risks are thought to be similar to toxicities produced by other nucleoside analogs, including, hematologic (pancytopenia, anemia, leukopenia, thrombocytopenia), hepatic (hepatocellular damage, hypertriglyceridemia), neurologic (peripheral neuropathy coma seizures) renal, gastrointestinal (ulceration, mucositis, hemorrhage, pancreatitis), and pulmonary (necrosis). These potential risks will be monitored with the use of frequent laboratory tests checking for incidence of adverse events.

Sub-cutaneous injections and blood drawing may cause pain, bruising, or infection.

Liver biopsy may cause pain, bruising/bleeding, puncture of organ, or infection.

Although unlikely given the favorable side-effect profile of lamivudine, there is a chance that alpha-interferon/lamivudine combination therapy will not be tolerated as well, or be as effective as alpha-interferon monotherapy.

All study subjects will receive treatment.

M. Potential Benefits

There may be no direct benefit to the subject other than reduction of HBV DNA levels and/or HBeAg levels for a period of time. It is unclear how long this effect will last. Combination therapy with alpha-interferon and lamivudine may represent another potential therapy that can be used for the treatment of chronic hepatitis B unresponsive to alpha-interferon alone. Additionally, previous studies with lamivudine have suggested that prolonged suppression of viral replication by lamivudine can improve liver histology independent of HBeAg seroconversion. Alternatively, patients in the combination therapy arm of the study may enjoy no additional therapeutic benefit above those receiving standard alpha-interferon therapy.

N. Altemative Therapies

Currently, alpha-interferon is the only treatment specifically approved by regulatory authorities throughout the world for chronic hepatitis B.

O. Compensation to Subjects

No form of compensation. will be provided to patients enrolled in the study.

P. Costs to Subjects

Study participants will not incur any additional costs as a result of participation in the study. Subjects will not be responsible for any of the costs of laboratory and diagnostic tests, clinic visits and study medication.

Q. Minors as Research Subjects

This study will not involve the participation of minors.

R. Radiation or Radioactive Substances

This study will not involve the use of radiation or radioactive substances.