VIRAHEP- C STUDY PROTOCOL

VIRAHEP- C Summary

Major advances have been made over the last decade in the field of antiviral therapy for chronic hepatitis C. Combination therapy with interferon and ribavirin has improved virological sustained response rates to nearly 40% compared to only 10-15% for patients treated with interferon alone. Unfortunately, response to therapy is not uniformly favorable. Secondary analyses of large databases derived from clinical trials of various antiviral agents have suggested that there may be racial disparities in response to therapy for hepatitis C. Sustained response rates in African American patients appear to be significantly less than in Caucasian patients treated with the same regimens. However, these trials were not designed specifically to study race as a factor in response to therapy and very few African Americans have been included as study subjects, despite the high prevalence of hepatitis C in this population.

VIRAHEP-C is a multicenter, collaborative clinical trial, sponsored by NIDDK-NIH, designed to test the hypothesis that African Americans respond less well to antiviral therapy than Caucasian patients. A total of 400 patients, equally divided between African American and Caucasians, will be enrolled at eight clinical centers throughout the United States. All participants will be HCV genotype 1. Participants will be treated for 48 weeks with pegylated interferon alfa-2a 180 mcg/week plus ribavirin 1000-1200mg/day. Participants will be followed for an additional 48 weeks after cessation of therapy. Sustained virological response rates (undetectable HCV RNA in serum 24 weeks post-treatment) and durable sustained virological response rates (undetectable HCV RNA in serum 48 weeks post-treatment) between the two cohorts will be compared to determine differences in treatment response between the two racial groups.

Clinical and virological data from these treatment cohorts will be used to evaluate factors associated with resistance to antiviral therapy in African Americans and Caucasians with chronic hepatitis C. Pretreatment variables such as gender, history of alcohol use, HCV RNA levels, hepatic histology, among others, will be investigated to determine which factors may be associated with sustained virological response and how they may differ between racial groups.

Ancillary studies, using serum and peripheral blood mononuclear cells obtained from participants at various time points in this study, will be performed to investigate potential mechanisms of antiviral resistance. Thus, viral kinetics (the rate at which HCV RNA is cleared from serum during therapy), immunological responses, measures of interferon signaling pathways, and host and viral genetics will be evaluated in relationship to treatment response. This comprehensive study design that encompasses a clinical trial with prospective studies of mechanisms of antiviral resistance will be positioned to answer important clinical questions about the treatment of chronic hepatitis C.

1.0 Introduction

1.1 Background

Since the discovery of the hepatitis C virus (HCV) in 1989, there have been enormous advances in the understanding of the epidemiology, course, natural history and treatment of hepatitis C. In population-based surveys in the United States, 1.8% of Americans have been found to have antibody to hepatitis C (anti-HCV) and about three-fourths of these also have HCV RNA detectable in serum. [1] These findings indicate that approximately 3.2 million Americans have been infected with HCV and 2.5 million have chronic infection. Many if not most patients with chronic hepatitis C are unaware of the presence of this virus or liver disease; nevertheless, HCV infection is an important cause of cirrhosis, end-stage liver disease and hepatocellular carcinoma. Hepatitis C now accounts for more than half of newly diagnosed cases of chronic liver disease, and cirrhosis due to hepatitis C is now the chief cause of hepatocellular carcinoma and the single major reason for liver transplantation in adults in the United States and most Western countries. [2,3,4]

The course and consequences of chronic hepatitis C have been defined in many natural history studies. The best estimates are that cirrhosis develops in 10% of HCV infected persons per decade. Thus, the life-time risk of developing cirrhosis is probably 20 to 50% depending upon the age of acquisition. Progression to cirrhosis is more frequent in males, in older individuals, and in those who drink alcohol to excess or are immunosuppressed.

Therapy of hepatitis C continues to evolve and now is successful in 40 to 50% of treated patients. [5] The regimen of therapy that yields the highest rate of response is the combination of alpha interferon and ribavirin. [6,7] The recent introduction of pegylated, long-acting forms of alpha interferon (peginterferon) promise to provide an even higher rate of response than with standard interferon. [8-10] In two large, randomized controlled trials, the combination of peginterferon and ribavirin for 48 weeks yielded overall sustained response rates of 54% to 56%. [10-12] Retrospective analyses have repeatedly shown that responses to therapy are higher among persons infected with HCV genotypes 2 and 3 (66-82%) than those with genotype 1 (32-45%).

An important recent finding is that there are considerable racial differences in epidemiology, typical course, natural history and response to treatment of hepatitis C. [13] The same population based surveys mentioned above demonstrated that African Americans have a higher rate of anti-HCV than Caucasians (3.2% vs. 1.8%) and a higher proportion have detectable HCV RNA (86% vs. 68%).[1,13] These findings suggest that HCV infection is more common among African Americans than whites and that once infected, African Americans are also more likely to develop chronic infection. Findings from other cohort studies support these findings, but the reasons for these differences remain unknown.

The hepatitis C virus exists as at least six genotypes and several subtypes (1a, 1b, 2a, 2b, etc). Genotype 1 accounts for approximately 75% of HCV infections in the United States, genotype 2 for 10-15%, genotype 3 for 5-10%, and other genotypes and mixed infections for the remainder. The distribution of different genotypes in the United States also varies by race. Thus, genotype 1 affects 91% of African Americans compared to only 67% of whites. Genotype 1b is particularly more common among blacks (36%) than whites (18%) while genotype 3a is rare (<1% vs. 6%).[13] Again the reasons for these differences are not clear. Furthermore, other racial groups, such as Hispanic whites and Asians in the United States, more closely resemble whites than African Americans in rates of chronicity and in genotype distribution.

Perhaps more significant than differences in prevalence, genotype, and outcome of hepatitis C between African Americans and whites, have been differences in response to antiviral therapy. A retrospective analysis of a randomized controlled trial of consensus interferon showed that both end-of-treatment and sustained biochemical and virological responses were less frequent in African Americans than whites with chronic hepatitis C. [14] Specifically, only 5% (2 of 40) of black participants became HCV RNA negative by the end of therapy as compared to 33% (127 of 380) of white subjects. Sustained virological responses were also less common in African Americans, occurring in 12% (46 of 380) of white subjects, but in only 2% (1 of 40) of blacks. The differences appeared to be partially attributable to genotype differences, genotype 1 being more common among African American patients and also associated with a lower rate of virological response. Detailed analysis of changes in HCV RNA levels during therapy also showed racial differences. Median HCV RNA levels decreased to a lesser extent in black subjects than in whites (50% vs. 99.5%

reduction). Of note, in a logistic multiple regression model controlling for race, gender, baseline HCV RNA level and genotype (1 or not 1), the authors failed to demonstrate that either race or gender was associated with end of treatment or sustained virological response. The major reason for this lack of association in regression analyses may have been the few numbers of African Americans subjects, accounting for less than 5% of patients enrolled in this study.

Indeed, further analyses of several trials of antiviral therapy in chronic hepatitis C were limited because of the small number of African American subjects enrolled in these industry-sponsored studies. In large registration trials of interferon alfa-2a and alfa-2b, only 5% of enrolled subjects were black. [13] For comparison, population based surveys suggest that African Americans represent 22% of cases of chronic hepatitis C. In a review of results from large clinical trials of interferon therapy of hepatitis C, only one African American patient was identified who had a sustained virological response to alpha interferon monotherapy. [13]

An important advance in therapy of hepatitis C was the demonstration that the addition of ribavirin to interferon therapy increased the response rate 2 to 3 fold. [6,7] This increase in response was due to both a higher rate of HCV RNA negativity during treatment and a lower rate of relapse when therapy was stopped. Retrospective analyses of trials of combination therapy have shown a lesser difference in response rates between black and white subjects. Thus, two large, multicenter trials have been conducted comparing 24 and 48 week courses of alpha interferon alone to the combination of alpha interferon and ribavirin in patients with chronic hepatitis C. Among 1744 subjects randomized, only 53 (2.6%) were black (because the studies were partially international, some of the blacks were not American). [14] While 21% of white participants had a sustained virological response to alpha interferon monotherapy, none of 24 black subjects responded. With combination therapy, the overall response rate in blacks was 21% (6/28) which was considerably less than the 37% (346 of 925) response rate in whites. However, when controlled for by genotype, this difference was no longer statistically significant. Again, the small numbers of patients made it difficult to evaluate true differences. Repeated measures ANOVA were used to determine whether changes in serum HCV RNA levels during therapy varied by race. Compared to white subjects, black subjects had significantly less reduction in HCV RNA levels during therapy. This difference was most apparent in black subjects with genotype 1 infection who received interferon monotherapy. In multivariate analysis, race did not correlate with lack of virological response, but the few numbers of responses in black subjects (6 total) made the degree of accuracy poor.

The reasons for the lack of response to interferon therapy among African Americans are not clear. However, careful analyses of HCV RNA levels during therapy help define the degree of antiviral resistance. Monitoring HCV RNA during treatment shows several patterns of response including a “rapid” and “slow” responses in which levels fall to undetectable within a few weeks or months, as well as a “flat” and “nil” response in which HCV RNA levels fall by less than one $\log_{10}$ or not at all. [13,15,16] In studies of small numbers of patients, it appears that flat and nil responses are more common among African Americans than whites. These types of studies suggest that one can identify patients who are non-responders within a few weeks or months of starting therapy and do not have to wait for 12 months of treatment and 6 of follow-up to identify subjects as resistant to current modes of antiviral therapy. These types of analysis provide important insights into the nature of antiviral responses and suggest many avenues for future studies.

1.2 Study Rationale

The lack of adequate studies of antiviral therapy of hepatitis C in African Americans and the preliminary results suggesting a lower response rate make it difficult to recommend therapy and provide estimates of risk and benefit in this population. Larger, well-designed prospective trials are needed to provide reliable and accurate information on response and adverse event rates as well as the role of predictive factors for response. If there is a lower rate of response among African American patients, analysis of factors that correlate with a lack of response may well provide insights into the nature of viral resistance and provide a basis for improving response rates.

For these reasons, we propose a prospective analysis of optimal combination therapy of hepatitis C in 200 African Americans and 200 white Americans with careful prospective analysis of features that may predict and elucidate non-response to treatment. For this study, we have selected the combination of pegylated interferon and ribavirin to be given for 48 weeks and have focused solely on patients with genotype 1, the

genotype with the lowest rate of response. In preparing this protocol, we have met with representatives of industry and have formed an agreement (CRADA) with the Hoffmann-La Roche pharmaceutical company to use their products: peginterferon alfa-2a (Pegasys) in a dose of 180 mcg weekly for 48 weeks and ribavirin in a dose of either 1000 mg (body weight less than 75 kilograms) or 1200 mg (body weight equal to or greater than 75 kilograms). The trial will include at least four ancillary studies of antiviral resistance, including special studies of viral genetics (HCV sequence and polymerase function), immunology (CD4 and CD8 responses to HCV antigens), host genetics (candidate gene polymorphisms done on lymphocyte DNA preparations), and interferon-signaling (induction of interferon responsive gene products during therapy).

2.0 Study Objectives and Endpoints

2.1 Specific Aims

1. To evaluate racial differences in response to antiviral therapy for chronic hepatitis C. Our hypothesis is that African Americans respond poorly to therapy for hepatitis C when compared to Caucasians with similar disease characteristics. Well-defined cohorts of African American and Caucasian patients with chronic hepatitis C (all genotype 1) will be treated with combination therapy (pegylated interferon and ribavirin) to determine differences in virological response rates between the two races.

2. Evaluate factors associated with resistance to antiviral therapy in African Americans and Caucasians with chronic hepatitis C. Pretreatment variables such as gender, history of alcohol use, HCV RNA levels, hepatic histology, among others, will be investigated to determine which factors may be associated with sustained virological response and how they may differ between racial groups. On-treatment variables will include viral kinetics, immunological responses, host response to interferon therapy, and adherence to therapy.

3. Evaluate differences in HCV viral kinetics in response to antiviral therapy and its relationship to sustained virological response. The rate of clearance from serum of HCV RNA will be compared between a cohort of African Americans and Caucasians to determine if racial differences exist, and between responders and non-responders to determine if they are predictive of treatment failure or success and if they are useful in the management of patients with chronic HCV.

2.2 Efficacy Endpoints

The primary endpoint in this study will be a sustained virological response 24 weeks post treatment. The durability of this response will be reassessed 48 weeks after stopping therapy. Secondary endpoints will be changes in HCV RNA levels at 24, 48, and 72 hours, 4 weeks, 24 weeks, and at the end of treatment. Other secondary endpoints will be sustained biochemical responses and improvements of serum alanine aminotransferase levels at 4 weeks, 24 weeks, and at the end of treatment. The definitions of responses are given below.

Sustained virological response: Measured at post treatment week 24, is defined as lack of detectable HCV RNA in serum, by qualitative PCR with a lower limit of sensitivity of 50 IU/ml (e.g., Roche, Amplicor HCV assay).

Durable sustained virological response: Measured 48 weeks following discontinuation of therapy is defined as absence of detectable HCV RNA in serum by qualitative PCR.

Changes in HCV RNA: Measured at 24 hours, 4 weeks, 24 weeks, and at the end of treatment will be assessed by quantitative PCR (Roche, Monitor HCV assay). Changes will be categorized as decreases of <1 $\log_{10}$, 1-2 logs, >2 logs in IU, and negative (<50 IU) by qualitative PCR.

The primary endpoint of the study will be sustained virological response.

3.0 Study Design

3.1 Study Summary

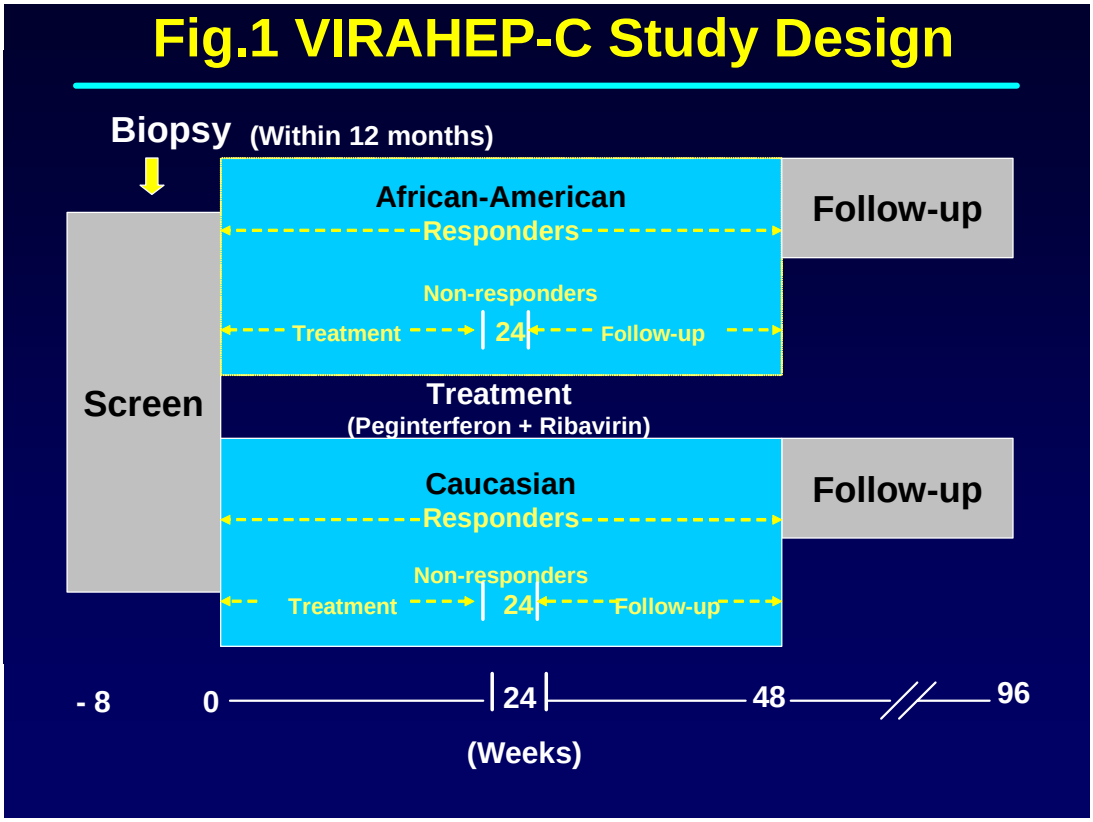
This will be a phase III, multicenter, open-label study to evaluate the relative efficacy of pegylated interferon and ribavirin in African American versus Caucasian patients with chronic hepatitis C and genotype 1. Participating centers and Principal Investigators in the study are shown in Table 1. A total of 200 African American and 200 Caucasian patients will be enrolled at 8 medical institutions. All patients will be genotype 1 and have quantifiable (600 IU/ml) HCV RNA. After careful evaluation, all patients will receive the same regimen of peginterferon alfa-2a and ribavirin (1000/1200 mg). Peginterferon will be administered once weekly by subcutaneous injection and ribavirin will be administered orally twice daily. After 24 weeks of treatment, HCV RNA will be tested by qualitative PCR in duplicate to identify responders (undetectable serum HCV RNA) and non-responders (HCV RNA positive in duplicate). Responders will continue on therapy for another 24 weeks whereas treatment for non-responders will cease. Follow-up will continue for 48 weeks after completing therapy for responders and 24 weeks after completing therapy for non-responders. Patients whose qualitative PCR results are indeterminate or discordant will follow the protocol for responders.

Sustained virological response (SVR, defined above) will be measured 24 weeks following completion of therapy. The durability of SVR will be measured at the end of the follow-up period, 48 weeks after discontinuing therapy.

Table 1
Location of Participating Centers

Institution	Location	Principal Investigator	Type of Center
Beth Israel Deaconess Medical Center	Boston, MA	Nezam Afdhal, MD	Clinical Center
New York-Presbyterian Medical Center	New York City, NY	Robert S. Brown, Jr. MD, MPH	Clinical Center
University of California, San Francisco	San Francisco, CA	Norah Terrault, MD, MPH	Clinical Center
Rush University	Chicago, IL	Thelma E. Wiley, MD	Clinical Center
University of Maryland	Baltimore, MD	Charles Howell, MD	Clinical Center
University of Miami	Miami, FL	Lennox Jeffers, MD	Clinical Center
University of Michigan	Ann Arbor, MI	Hari S. Conjeevaram, MD	Clinical Center
University of North Carolina	Chapel Hill, NC	Michael W. Fried, MD	Clinical Center
University of Pittsburgh	Pittsburgh, PA	Steven H. Belle, PhD	Data Coordinating Center

A schematic of the study design is shown in **Figure 1**. The required study visits and evaluations that will be performed at various time points are shown in subsequent tables.



3.2 Study Population

Definition of Race

For the purposes of classifying race, we will use self-identification. All participants must have been born in the United States. Racial and ethnic (Hispanic, non-Hispanic by self-identification) data will be collected in a self-report questionnaire completed by the patients (Appendix 1). A research coordinator will be available to answer questions about the form.

3.3 Entry Criteria

Inclusion Criteria

To be eligible for this trial, patients must meet the following inclusion criteria:

- Males or females; age between 18 and 70 years at screening
- Identify themselves as being Black/African American or White/Caucasian only, but not both
- Born in the United States (50 states)
- Serum HCV RNA above quantifiable level of detection (600 IU) (Roche HCV Monitor)
- HCV genotype 1 (alone or in combination with other genotypes)
- Liver biopsy obtained within 18 calendar months prior to screening. Patients with cirrhosis (Ishak Score 5-6) as assessed by Dr. Kleiner using a biopsy performed more than 18 months before screening will not need to undergo repeat biopsy.
- Patients with AFP above 100ng/ml and less than 500ng/ml, or patients with cirrhosis or transition to cirrhosis (Ishak score 3-6), must have an abdominal ultrasound, CT scan or MRI scan without evidence of hepatocellular carcinoma (within 8 months of treatment initiation), and the AFP level is shown to be stable (within 50ng/ml) or decreasing on repeat testing before enrollment.

- Negative urine pregnancy test (for women of childbearing potential) documented within the 24-hour period prior to the first dose of test drug. Additionally, all fertile male patients, male patients with female partners of childbearing age, and females of childbearing potential must be using two reliable forms of effective contraception during the study (while on drug and for 24 weeks following end of therapy).

Exclusion Criteria

Patients with any of the following will not be eligible for participation:

- Previous treatment with interferon or ribavirin
- Positive test at screening for anti-HIV
- Positive test for HBsAg done within the past 12 months
- Alcohol consumption of more than two drinks or equivalent (>20 grams) per day. Patients who consumed more than this in the past must have maintained a level of 20 grams or less per day of alcohol consumption for at least six months prior to screening.
- History of other chronic liver disease, including metabolic diseases, documented by appropriate test(s)
- Women with ongoing pregnancy or breast-feeding, or contemplating pregnancy
- Male partners of women who are pregnant or contemplating pregnancy
- Neutrophil count <1000 cells/mm^3, Hgb <11 g/dl in women or 12 g/dl in men, or platelet count <75,000 cells/mm^3. Any patient with a baseline increased risk for anemia (e.g. thalassemia, spherocytosis, history of GI bleeding) or for whom anemia would be medically problematic.
- Serum creatinine level >1.5 times the upper limit of normal at screening or CrCl < 75cc/min, or currently on dialysis
- Evidence of alcohol or drug abuse within 6 months prior to screening
- Any current (within past 6 months) severe psychiatric disorder such as depression, schizophrenia, bipolar illness, obsessive-compulsive disorder, severe anxiety, or personality disorder
- A prior suicide attempt, hospitalization for psychiatric disease, or a period of disability or impairment due to a psychiatric disease within the past 5 years. Patients whose psychiatric disease is controlled by medication and deemed psychologically stable by the investigator will be eligible for participation.
- History of immunologically mediated disease (e.g., inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hemolytic anemia, severe psoriasis, rheumatoid arthritis)
- History or other evidence of decompensated liver disease defined as any of the following: serum albumin <3 g/dl, direct bilirubin > 1.0 mg/dl, or PT/INR > 1.5 times normal, history or presence of ascites or encephalopathy, or bleeding from esophageal varices, or current jaundice
- History of cardiovascular or coronary artery disease, if, in the judgment of the investigator, an acute decrease in hemoglobin by up to 4 g/dl (as may be seen with ribavirin therapy) would not be well-tolerated
- History of a severe seizure disorder or patient has had seizures despite anticonvulsant therapy in the previous 2 years
- History of solid organ or bone marrow transplantation
- History of thyroid disease poorly controlled on prescribed medications
- History or other evidence of retinopathy on physical examination (history or fundoscopic evidence of retinal hemorrhage, retinal artery obstruction, retinal vein obstruction, cotton-wool spots)
- Chronic use of oral steroids
- Inability or unwillingness to provide informed consent or abide by the study protocol
- History or other evidence of severe illness or any other conditions that would make the patient, in the opinion of the investigator, unsuitable for the study

3.4 Study Treatment

All participants will receive identical treatment regimens consisting of peginterferon alfa-2a in pre-filled syringes in a dose of 180 μg administered once every 7 days by subcutaneous injection and ribavirin tablets administered twice daily at a dose of 1000 or 1200 mg/day (for body weights of < or ≥ 75 kg, respectively). The treatment period will be for 48 weeks except for those defined as "non-responders" to therapy (defined below) or in those with early discontinuation (see below).

Peginterferon has undergone extensive clinical evaluation and is in the process of licensure review by the FDA. An IND (BB-IND10355) has been filed for the current trial. Details of these agents are available in the attached investigators brochure**.** (Appendix 2)

3.4.1 Dose Adjustment Guidelines

Specific dose adjustment guidelines for peginterferon are provided in the tables below for treatment-related neutropenia, thrombocytopenia, and anemia. For other adverse effects considered to be possibly related to peginterferon, including laboratory abnormalities, adverse events, and vital signs changes, investigators should utilize the "General Dose Reduction Guidelines" (Section 3.4.2) below. When practical, abnormal laboratory results should be confirmed as soon as possible following notification of the investigator. If appropriate, downward adjustments in one level increments (see below) should be considered. The lowest dose of peginterferon that should be administered is 45 μg. It should be kept in mind that whereas these guidelines should be generally followed to promote consistency across centers, other responses by an investigator may be more appropriate in some circumstances. For laboratory and vital signs abnormalities, "severe" is defined as any value requiring intervention, further work-up, or more frequent follow-up.

3.4.2 General Dose Reduction Guidelines

The dose of study medications may be decreased due to adverse events or laboratory abnormalities. Dose reduction for peginterferon will be accomplished in graduated steps: Level 1 decrease from 180 μg to 135 μg; level 2 decrease from 135 μg to 90 μg; level 3 decrease from 90 μg to 45μg.

Peginterferon vials of 90 μg will be used for dose reduction requiring 90 μg or 45 μg. These will not be pre-filled syringes, and clinical center personnel will be instructed on the use of these vials.

Table 2
Peginterferon Dose Adjustments for Low Absolute Neutrophil and Platelet Counts

Neutrophils (cells/mm3)	Downward Dose Adjustment
< 500	Decrease dose stepwise by one level
< 250	Stop drug

Platelet Count (cells/mm3)	Downward Dose Adjustment
≥40,000	None
25,000 to < 40,000	Decrease dose stepwise by one level
< 25,000	Stop drug

For subjects who require a downward dose adjustment for neutropenia or thrombocytopenia, laboratory values should be repeated, if practical, prior to the next dose of peginterferon to confirm the result and dictate the course of action. Subjects who remain with a neutrophil count below 500 (but above 250) or a platelet count below 40,000 (but above 25,000) should receive stepwise dose reductions until levels of neutrophils and platelets are ≥ 500 or 40,000 cells/mm3, respectively, or until a dose of 45 μg of peginterferon is reached.

Table 3
Ribavirin Dose Adjustment for Anemia

Hemoglobin (g/dL)	Initial dose	Reduced dose
≤ 10 – 8.5	1000 mg q.d.	600 mg q.d.
	1200 mg q.d.	800 mg q.d.
< 8.5	Regardless of dose	Discontinue permanently

If laboratory abnormalities improve or resolve, the dose of peginterferon or ribavirin may be increased back to original dosing at the discretion of investigators with continued close monitoring. However, study participants must maintain blood counts consistent with the above schedule in order to remain on the original dose. If dose reduction is required for 4 consecutive weeks, reduced dose should be continued for the remainder of the study. Dose reduction for non-laboratory-related adverse events will be based upon severity ratings in accordance with modified WHO criteria (Appendix 3). For grade 1 toxicity, no dose reduction is needed. For toxicity grade 2 that persists over two consecutive safety visits and is not responsive to adjunctive symptomatic management, a level 1 dose decrease of peginterferon and/or a decrease in ribavirin may occur. For toxicity grade 3 not responsive to adjunctive measures, a level 1 dose decrease of peginterferon and/or a decrease in ribavirin will occur. Stepwise reduction will continue as noted above until symptoms abate or until the lowest allowable dose of peginterferon (45μg) is reached. Patients with grade 3 toxicity should be followed closely, possibly at more frequent intervals or via telephone contact to monitor in between regularly scheduled safety visits. If symptoms improve, return to previous dosing is allowed at the discretion of the investigator. For grade 4 toxicities, treatment will be discontinued. It should be noted that certain toxicities carry different levels of significance for each patient and therefore, the investigator should use these as guidelines only, within the context of clinical judgment.

For non-hematologic adverse events, ribavirin dose reduction will be defined as follows:
Level 1: decrease initial daily dose by 200mg (1200mg to 1000 mg or 1000mg to 800mg)
Level 2: decrease an additional 200 mg/day (1000mg to 800mg or 800 mg to 600mg)
Level 3: decrease an additional 200 mg/day (800 mg to 600 mg or 600mg to 400mg)

If adverse events improve, ribavirin may be increased to initial dosage at the discretion of the investigator provided that continued close monitoring is maintained. If adverse events continue at the same level of intensity despite maximal dose reduction, ribavirin may be discontinued at the discretion of the investigator.

Guidelines for controlling hepatic adverse events such as ALT flares, and abnormal total bilirubin and alkaline phosphatase are provided in Appendix 1.

Growth factors may not be used to maintain platelet counts, WBC, or hemoglobin levels.

3.4.3 Safety Measurements

Laboratory evaluations: Participants will undergo laboratory tests according to the time and event schedule to monitor for medication toxicities.

Adverse event monitoring: Participants will be queried by study personnel at each study visit about the incidence of adverse events. These will be documented in the study record and classified according to onset date, severity, relationship to study medication, action taken, and outcome. Serious adverse events will be reported to appropriate regulatory authorities (e.g., FDA), in addition to the Data Safety and Monitoring Board as described below, in a timely fashion.

Pregnancy: Women of child-bearing potential will have a urine pregnancy test performed within 24 hours of the first administration of study drugs, monthly for the duration of therapy, and at months 1, 3, and 6 following completion of therapy.

3.4.4 Depression Management Guidelines

Mood disorders (i.e., depression and anxiety) are common and problematic side effects of peginterferon. Mood disorders may develop during the first few months of treatment or later during therapy. Mood disorders can develop in individuals without a prior psychiatric history and present with subtle signs and symptoms such as fatigue, withdrawn behavior, poor appetite, irritability, or sleep disturbance. Although peginterferon dose reduction and anti-depressant medications have proven useful, severe and life-threatening neuropsychiatric toxicity including attempted and completed suicide have been reported. Therefore, a heightened awareness of depression and its potential impact on patient safety and compliance is necessary when prescribing peginterferon.

Detection of Depression

A local collaborative mental health professional (i.e. psychiatrist/ psychologist) will provide consultation and treatment for patients during screening and treatment. At each scheduled study visit, the patient will be assessed by the study coordinator for side effects, adverse events, and medication tolerance. At screen 1, baseline, weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48, and follow-up weeks 4, 12, 24, and 48, three questions regarding mood status will be asked.

1. Since your last visit, have you felt depressed, sad or blue most of the time?
   Yes or No
2. Since your last visit, have you often felt helpless about the future?
   Yes or No
3. Since your last visit, have you had thoughts about harming or killing yourself or others?
   Yes or No

If the patient responds Yes to any of the questions, the investigator will be informed and a management plan will be instituted after review of the patient.

Management of depression

Patients who answer affirmatively to any of the depression screening questions, appear to be clinically depressed, or suffering from other psychiatric side effects will be reviewed by the investigator. Recommended management steps include:

1. Reassurance with careful monitoring and close follow-up every 2-4 weeks for patients with minimal or mild symptoms.
2. Anti-depressant medications or referral to a mental health provider for patients with mild to moderate depressive symptoms.
3. Stepwise peginterferon dose reduction may be required as well as consultation with the mental health provider for patients with moderate to severe depressive symptoms (see dose adjustment guidelines).
4. For patients with severe depression or depression that is worsening despite peginterferon dose reduction, anti-depressants, or referral to a mental health professional, the study medications should be discontinued. These patients should be rapidly referred to the local collaborative mental health provider for further assessment and treatment.
5. Any patient who develops recurrent suicidal ideation, a suicide plan and/or makes a suicide attempt should have both peginterferon and ribavirin immediately discontinued and should be referred to the local mental health provider for further management.

3.5 Premature Discontinuation

Treatment may be discontinued prematurely for the following reasons:

1. Participant requests termination of the study medication;
2. Participant is intolerant of the study medication despite predetermined dose reductions and side effect management strategies;
3. Safety concerns at the discretion of the investigator. These concerns will be documented in the source documents.

Participants who discontinue treatment prematurely for the above reasons will be encouraged to remain in the post-treatment follow-up period for an additional 24 weeks, unless they withdraw consent. For those discontinued prematurely, blood samples will be obtained as listed for the week 48 end-of-treatment visit.

4.0 Study Visits

4.1 Screening Visit 1: The first screening visit will take place no more than eight weeks prior to anticipated dose initiation. Written informed consent will be obtained prior to any screening evaluations. This initial screening visit will be used to determine patient eligibility as per the inclusion and exclusion criteria.

Table 4
Screening Visit 1

Informed Consent	Main study (See Appendix 4)
Race Determination	Self-administered questionnaire
Medical History and Complete Physical	Include waist and hip circumference measurement
Chest X-ray	Patients with pre-existing pulmonary disease
EKG	Patients ≥ 40 years of age
CBC/Differential	
Chemistry	ALT, AST, AlkPO4, total and direct bilirubin, albumin, cholesterol, uric acid, BUN, creatinine, glucose (non-fasting), alpha-fetoprotein
Coagulation Tests	PT/PTT
Thyroid Test	TSH
Pregnancy Test	Urine
Urinalysis	Routine chemistry and microscopic exam
Viral Genetics	HCV genotype
Viral Kinetics	HCV RNA quantitative
Other Viral Serology	Anti HIV, anti-HCV Documented within 12 months: HBsAg, HBcAb
Other Serology and Chemistry (if not previously verified as normal)	Fe, TIBC, ferritin, iron saturation, ceruloplasmin (if clinically indicated), alpha-1-antitrypsin, anti-nuclear antibody
Liver Biopsy	Adequate tissue must be available within 18 months of screening visit
Serum Storage	Serum 3 ml
Education Video	"General Hepatitis C"

Diabetics will be required to have a fundoscopic examination within one year before the end of screening.

4.2 Screening Visit 2: A second visit, to occur anytime in the 8-week period after the initial screening, is required prior to dosing initiation to perform baseline studies (blood drawing, measurement instruments) that will be used in analyses of therapeutic response.

Table 5
Screening Visit 2

Informed Consent	Genetics consent (See Appendix 4)
Immunology (CD4/CD8)	
Questionnaires	Quality of Life, CES-D, Social Support Questionnaire, Self-efficacy Questionnaire, Sexual Function Questionnaire
Education Video	"Virahep-C Participation"
Other	Aberrant laboratory studies requiring confirmation

4.3 Baseline Visit (Day 0): Once the patient has completed the two screening visits and has been confirmed to be eligible, he/she will return for additional laboratory studies and comprehensive instruction in self-injection techniques by study personnel. These three visits, screening 1, screening 2, and baseline (day 0) will occur within 56 days.

Table 6
Baseline (Day 0) visit

Interim Medical History	
CBC/Differential	
Chemistry	ALT, AST, AlkPO4, total and direct bilirubin, fasting triglycerides, glucose, insulin
Serum Storage	Serum 3ml
Viral Genetics	
Patient Genetics	
Viral Kinetics	HCV RNA quantitative
Pharmacokinetics	Peginterferon alfa-2a levels, 2',5'-OAS serum activity
Interferon Signaling	
Pregnancy Test	Urine
Adherence MATI	
Medication Education	Self-injection techniques (including video), use of MEMS caps, adherence materials
Study Medication Administration	Supervised by research coordinator

4.4 Treatment Period: Pegylated interferon will be administered as a weekly subcutaneous injection and ribavirin will be administered as tablets twice daily. Treatment will be continued for 48 weeks (except in the absence of virological response as defined below). During the treatment period, participants will return for safety and efficacy evaluations at weeks 1, 2, 4, 6 (safety labs only), and 8, and then monthly until treatment is completed.

Efficacy assessment at week 24 will determine if participants are eligible to continue the full 48 weeks of treatment. Participants who have a positive qualitative test result, in duplicate, will be discontinued from study medication at week 24. These participants will then enter the follow-up period and be followed for an additional 24 weeks (post-treatment weeks 4, 12, 24). If they begin other antiviral therapy, they will be discontinued from follow-up.

Table 7
Safety and Efficacy Visits

Interim Medical History	
Symptom-directed Physical Exam	Vital signs and weight at each visit; physical exam every 3 months or as needed
Adverse Event Assessment	
Concurrent Medication Assessment	
CBC/Differential	Each visit (except days 1 and 2)
Chemistry	Each visit except week 1; ALT, AST, AlkPO4, total and direct bilirubin, albumin, uric acid, BUN, creatinine, glucose. Fasting triglycerides, glucose, and insulin at week 24, and week 48 for responders only.
Pregnancy Test	Urine, each visit (except days 1and 2)
TSH	Weeks 12, 24, 36, 48
PT/PTT	Weeks 24 and 48
Viral Genetics	Days 1, 2, 7, 14, 28, and weeks 8, 12, and 24. For responders, at end of treatment (48 weeks)
Viral Kinetics	Days 1, 2, 7, 14, 28, and weeks 8, 12, 24, and 36. For responders, at end of treatment (48 weeks)
Pharmacokinetics (PEG-IFN alfa-2a levels 2',5'-OAS serum activity)	Days 1, 2, 7, 14, 28, and weeks 8, 12, and 24. For responders, at end of treatment (48 weeks)
Serum Storage	Each visit (except weeks 6, 16, 20, 28, 32, 40, 44)
Interferon Signaling	Days 1, 2, 7, 14, 28

Immunology (CD4/CD8)	Weeks 8, 24, and end of treatment (week 48 for responders), or at early termination visit
CES_D*	Weeks 4, 12, 24, 48
Symptom Assessment Questionnaire*	Each visit (except days 1 and 2)
Brief MATI	Weeks 4, 12, 20, 28, 36
Gratitude Letter	Weeks 12, 36
Telephone Call	Week 24-26 (pending viral studies)
MEMS Use Questionnaire*	Weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48
Adherence Questionnaire*	Weeks 4, 12, 24, 36, 48
Social Support and Self-efficacy Questionnaire*	Week 24
Sexual Function Questionnaire*	Weeks 4, 12, 24, 48
Adherence Videos	Weeks 1, 4, 12, 24, 36
Data Collection from Electronic Monitors	Weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48

*Also assessed at the time of early termination for patients who discontinue prior to Week 24.

4.5 Follow-up Period: Participants will be followed for an additional 48 weeks following end of treatment. Participants who responded to therapy at week 24 will be treated for 48 weeks. They will return for post-treatment follow-up visits at follow-up weeks 4, 12, 24, 36, and 48. Non-responders will be followed for 24 weeks only. During these visits, participants will be evaluated for resolution of adverse events and laboratory studies will be drawn to measure efficacy. Participants will be contacted by telephone on one occasion between follow-up visits in order to foster continuity and encourage adherence with study follow-up.

Table 8
Follow-up Visit Procedures

Interim Medical History	
Symptom-directed Physical Exam	Vital signs and weight at each visit; physical exam at weeks 12, 24, and 48, and as needed
Adverse Event Assessment	
Concurrent Medication Assessment	
CBC/Differential	Each visit
Chemistry	Each visit: ALT, AST, AlkPO4, direct bilirubin Follow-up weeks 12, 24, 48: albumin, uric acid, BUN, creatinine, glucose Follow-up week 24: fasting triglycerides, glucose, insulin
Pregnancy Test	Urine, every visit
TSH	Follow-up weeks 12, 24, 48
PT/PTT	Follow-up weeks 12, 24, 48
Serum Storage	Each visit
Interferon Signaling	Any follow-up timepoint; at least n=72
Viral Genetics	Each visit
Viral Kinetics	Each visit
Pharmacokinetics (PEG-IFN alfa-2a only)	Follow-up week12 for responders only
Immunology (CD4)	Follow-up week 24
Immunology (CD8)	Follow-up week 24; n=100
CES_D	Follow-up week 24
Symptom Assessment Questionnaire	Each visit
Quality of Life	Follow-up week 24
Sexual Function Questionnaire	Follow-up week 24

4.6 Unscheduled Visits: Participants who have changes in severity or initiation of new symptoms, or who need to have laboratory tests repeated, will be asked to return to the study site for unscheduled evaluations. All additional evaluations will be included in the study record.

5.0 Adherence

Few studies have examined the relationship between medication adherence and sustained virological response (SVR) in treatment of chronic hepatitis C. In post-hoc analyses, McHutchison et al. [17] showed non-statistical differences in SVR according to adherence as defined by pharmacy records and pill counts. Among individuals who received 80% or more of their total interferon dose, with 80% or more of the ribavirin dose, for 80% or more of the expected duration of therapy (80-80-80), 48% achieved SVR as compared to 41% from the intent-to-treat analysis of the original study. Similar increases in SVR were observed in subgroups defined according to genotype. In addition, the dose of ribavirin administered appears to be critical in achieving SVR. Only 28% of individuals who received ribavirin at a dose of less than 10.6 mg/kg/day (800 mg/day) achieved SVR as compared to 44% when greater than 10.6 mg/kg/day was taken. Taken together, these findings suggest that poor adherence, particularly to ribavirin, will lead to lower rates of SVR. McHutchison et al. [6] also showed the effect of adherence on SVR with pegylated interferon and ribavirin. In another post-hoc analysis, the investigators showed that 63% of participants with 80-80-80 adherence, taking a dose of 10.6 mg/kg of ribavirin or more, achieved SVR as compared with 54% from the intent-to-treat analysis of the original study.

Nevertheless, it remains unclear if adherence is a factor that explains the low SVR observed in African Americans as compared to Caucasians. In the studies by McHutchison et al., [6,17] adherence differences between African Americans and whites were not reported. However, the number of African Americans enrolled in these and other studies was very small and therefore it is difficult to draw definitive conclusions about adherence in African Americans and effects on SVR. Moreover, previous studies in HCV have used relatively crude measures of medication adherence or not measured adherence at all (e.g., Younossi et al.) [18]; as a result, it is unclear whether measurement bias may be a factor in explaining the apparent effects of poor adherence. Therefore, the relationship of adherence and SRV needs to be studied in greater detail using a prospective study design.

5.1 Adherence Measures

Electronic Monitoring

Microprocessor technology is now being used in a high-technology approach to electronically monitor and measure adherence. When used correctly, electronic monitoring is considered the consummate technology for assessing adherence. [19-21] The most common type of electronic monitor is known as the Medication Event Management System (MEMS). MEMS uses a computer chip in the cap of a medication vial that records the precise date and time the vial was opened and presumably when the drug was taken. Electronic monitoring provides information about drug intake behavior that cannot be obtained from medical histories or from clinical laboratory examination. In addition, when used correctly, electronic monitoring supplies a more reliable measure of adherence than any other measure because it provides a time series of the adherence pattern over the total treatment time. As a measure of drug exposure, electronic monitoring has been shown to be valid, sensitive, specific, and representative of adherence behavior. [22]

5.2 Procedures

Patient Education and Adherence Program

The adherence program for the study will consist of three central components: (a) informational-exchange, (b) skills development, and (c) social support enlistment. One of the principle tools utilized in this study will be the Medication Adherence Training Interview (i.e., MATI), a structured interview for medication education and adherence training. The MATI has been empirically tested in behavioral medical research in an HIV population at one of the Virahep-C clinical sites and now has been modified for use in patients with chronic hepatitis C. *A copy of the MATI appears in Appendix 5.*

5.3 Baseline (Day 0). Regarding adherence, the main purpose of the baseline visit is for skills training. Participants will be encouraged to bring along a supportive partner or friend to the baseline visit. During this visit, participants will be taught: a) techniques for self-injection of peginterferon; b) self-management of side

effects; and c) basic problem solving skills. Participants will be shown a video that will explain the medication regimen including instructions on drug dosing and self-injection, scheduling of dosing, management of side effects, storage recommendations, and tips on remembering doses. After viewing the video, a clinician will briefly review the contents of the video with the participant, demonstrate proper self-injection technique, and then allow the participant to practice the technique on pads that simulate human skin and fat tissue.

During this visit the study coordinator will also engage the participant in goal setting. Participants will be asked by the coordinator to set short-term goals (e.g., achieving a near-perfect adherence rate for the next 30 days) that may be important to the efficacy and safety of antiviral therapy. Participants will also be asked to create self-incentives for attaining the stated goals. The rationale is that individuals achieve greater self-directed change if they reward their successful efforts than if they provide no incentives for themselves.

Participants will be asked to self-monitor their own adherence behaviors. Participants will be given a diary and asked to keep track of non-adherence and the events that foster it. The clinician will assist the participant with planning a dosing schedule and integrating the medication times into lifestyle patterns. Written information and a dosing card summarizing the information from the session will be provided

5.4 Study Visits. At subsequent study visits, participants will be asked about tolerance to medications and adherence will be stressed. A brief version of the MATI protocol will be followed during weeks 4, 12, 20, 28, and 36 to review how medications are taken and encourage adherence. The content of the adherence interview will include discussion of non-adherence events shown in the diary, assistance with side effect management, and support for remaining in the trial.

Structured Phone Calls/Periodic Telephone Contact

In addition to the clinic-based patient education, participants will receive a telephone call by a trained study coordinator between weeks 24 to 26. The intent is to identify problems or concerns participants may have between visits that can be addressed with study coordinators. During the call the coordinator will inquire whether the participant is having any difficulty taking medicines regularly and as prescribed. If the participant is not having difficulties, the coordinator will commend the participant's efforts, inquire if the participant has any questions, and conclude the call. If the participant is having difficulties, the study coordinator will inquire about the nature of the difficulties and record participant responses and any recommendations made.

Periodic Acknowledgements and Expressions of Gratitude

Patients will be periodically thanked for their participation through a card or letter mailed from each site. This is a low cost method that may encourage continued participation and adherence. A card or letter will be sent at weeks 12 and 36.

Adherence Data Collection

In the study, the MEMS cap will record extensive information about medication-taking behavior among participants in the clinical trial. This information will be supplemented with self-reported information collected during clinic visits. This will require, however, that the participants always take the ribavirin capsules and peginterferon syringes directly from bottles fitted with the electronic monitoring caps (explained below).

Data Collection Procedures

At the beginning of the study, the study coordinator at each of the participating sites will be shipped a supply of the electronic compliance monitoring caps. Study coordinators will be trained to provide standardized instruction on electronic monitoring system use and participants will be given printed materials that explain the system.

At day 0, the pharmacy will dispense 180 ribavirin capsules in the original bottles supplied by the pharmaceutical manufacturer. In addition, the pharmacy will also dispense five pre-filled peginterferon syringes placed inside of opaque prescription bottles that accommodate MEMS caps. Lastly, pharmacies will provide needles for injection that fit the prefilled peginterferon syringes. Lids from both the ribavirin and peginterferon bottles will be replaced by the study coordinator with child-resistant MEMS caps. The opening and closing of the MEMS caps by the participant will electronically record dosing dates and times. Monitoring caps will be color coded to avoid inadvertently switching the two caps.

Electronic monitoring system follow-up will occur during the regularly scheduled monthly study visits. The follow-up will ensure that the monitors are placed on the refilled bottles of both medications and the MEMS caps are being used properly. During the visits, participants will complete a short four-item questionnaire that asks how the electronic monitors are being used. In addition, data collected from the monitors will be downloaded at each visit by the coordinator and sent electronically through a modem connection to a central database.

At completion of the study (i.e., weeks 24 or 48), the electronic monitoring system caps will be collected. Study sites will provide participants with $25 for returning both caps undamaged.

Adherence Questionnaire. A detailed assessment of adherence is needed to understand whether variation in clinical outcomes is related to varying levels of adherence. This assessment is particularly important in individuals who fail maintenance therapy because failure may be related to behavioral variation subsequent to a change in therapy, characteristics of the new therapy, or low treatment efficacy. To distinguish between these potential factors, multiple measures of adherence and patient attitudes will also be assessed.

A self-reported adherence questionnaire will be administered at weeks 4, 12, 24, 36, and 48. The questionnaire will ask participants about any doses missed during each of the previous four days and how medications are being taken. In addition, nine "yes/no" questions regarding compliance with the medication schedule will be asked as well as reasons for missing doses.

Other measurement tools: Social support questionnaire, self-efficacy questionnaire (see Appendix 6) will also be administered at Screen 2 visit, and at Week 24, or at early termination visit for patients who discontinue prior to Week 24.

6.0 Data Analysis/Statistical Power

The study is designed to have 400 patients; 200 White/Caucasian, 200 Black/African American. There will be 80% power to detect a difference in the percentages of undetectable viral RNA 6 months following treatment (sustained virological response, SVR) between racial groups if the percentage of White/Caucasian patients with a SVR is 40% and the percentage in Black/African-American patients is 26% using a two-sided test with $\alpha = 0.05$. In multivariable analyses, there will be 80% power to detect a difference if the percentages of SVR are 40% for Whites/Caucasians and 25% for Blacks/African Americans, assuming a multiple correlation coefficient of 0.4 between race and the other independent variables using a two-sided test with $\alpha = 0.05$.

Primary analyses will be performed using all enrolled patients. Patients will be considered enrolled in the study upon taking the first dose of study medication. Participants who do not have outcome data will be included as treatment failures. We will also perform analyses that include only those participants with known outcome data.

Summary statistics will be generated to describe the study sample at baseline. Descriptive statistics, including measures of central tendency (e.g. means, medians) and estimates of spread (e.g., standard deviations) will be used for continuous variables such as age. Frequency distributions will be used for categorical variables such as education level. Ninety-five percent confidence intervals will be calculated for all point estimates of continuous data. Graphical displays (e.g. histograms, box plots) will also be used to describe the data. The baseline demographic information and viral characteristics of the racial groups will be compared using the chi-square test for association or exact tests for differences in proportions or trend in ordinal data. Continuous variables may be transformed, where appropriate, to meet the assumptions of parametric tests. The Student's t-test or a non-parametric alternative, the Wilcoxon rank-sum test, will be used to determine differences in the distribution of continuous data between the two racial groups.

The proportion of African Americans and Caucasians with SVR will be compared using the chi-square test of association. The association between race and SVR after adjusting for potential confounders such as adherence, HCV sub-genotype, initial viral level, ALT, body mass index, and baseline histology will be assessed through multiple logistic regression analyses. The Hosmer-Lemeshow statistic will be calculated to determine if there is evidence that the final multiple logistic regression model does not fit. [23] These

analyses will be repeated for durable viral response, i.e., having undetectable viral RNA 12 months following treatment discontinuation.

Generalized estimating equation (GEE) methods of Zeger and Liang. [24,25] will be used to assess the differences between the racial groups with respect to changes in serum HCV RNA levels during therapy. This approach models both the regression of the response variable on the independent variables and accounts for the correlated nature of the responses. Analyses using conventional regression methods could provide erroneous variance estimates and loss of statistical efficiency because of the conventional assumption that each response is statistically independent. As a result, invalid inferences could be made if the conventional approach were applied to these longitudinal data.

The models examining response to treatment will include measures of adherence. As an initial step in examining adherence, graphical displays of the dosing history of each participant will be plotted and compared to the prescribed dosing regimen. Graphs include chronological plots showing the date and time of dose intake and frequency histograms of inter-dose intervals. The inter-dose interval representing the length of time between two successive doses will be calculated. For ribavirin, the optimal inter-dose interval is 12 hours and for peginterferon it is 7 days. Longer dosing intervals represent non-adherence and times in which the participant theoretically did not have adequate inhibitory drug concentration. In addition, summary statistics of adherence such as the percentage of prescribed doses taken and the percentage of drug holidays will be derived from the MEMS cap data. GEEs with multiple responses and adherence as a time-dependent covariate will be examined.

We will also examine adherence as an outcome variable. Mutivariable analyses, using GEE methodology, will be employed to identify factors independently associated with adherence. The primary response variable examined in the analyses will be the dose intake per week for the 48 weeks of the study. Dose intake refers to the weekly doses of ribavirin and peginterferon consumed as recorded by the MEMS cap. Separate regression analyses will be performed to examine the effect of each medication.

We are also interested in examining the relationship between viral kinetics and outcome. Neumann et. al[26] and Zeuzem[27], discuss the two-phase decline in serum HCV RNA levels following the initiation of therapy consisting of the daily administration of interferon-2b. After an initial 8 to 10 hour pharmacokinetic period, there is an approximate 2.0 log (base 10) decline in serum HCV RNA levels during the next 48 hours by inhibiting viral production. From day 2 on, viral decline is markedly slower, which reflects the clearance of HCV infected liver cells. The viral kinetics following therapy with pegylated interferon and ribavirin may follow a different pattern, possibly reflecting a three-phase, rather than a two-phase, decline. The viral kinetics will be described using nonlinear regression methods to estimate parameters of models of viral infection for each patient. [28] Then, the distributions of these parameters will be compared between racial groups using, for example, the Wilcoxon Rank Sum test. We will use generalized linear models to determine whether the parameters differ by race after adjusting for confounding factors that, ideally, would be specified in advance. Models may be fit for early phase (e.g., days 0-3) to see whether there are race differences in viral production, and for the later time period to see whether there are differences in virion clearance.

For all analyses, a p-value of less than 0.05 will be used to indicate statistical significance.

7.0 Study Organization

7.1 Sites (See Table 1)

This study will be conducted at eight clinical centers geographically distributed across the United States. The clinical centers are all tertiary care institutions and academic medical centers with extensive experience in the conduct of clinical trials of antiviral agents for the treatment of chronic hepatitis C. A Data Coordinating Center will coordinate and oversee operations for the study, maintain the database and perform data analyses. Four ancillary studies investigating potential determinants of treatment response, were selected at four additional sites. There is a central pathologist (David Kleiner, MD, PhD) at the National Cancer Institute. A central virology lab and serum and tissue repository (BBI Biotech), and a drug distribution center (Clinical Trial Services) have been selected.

Ancillary Studies, Study Sites and Principal Investigators

Patient genetics - Cedars-Sinai Medical Center, Los Angeles, CA – Huiying Yang, MD, PhD
Viral genetics - St. Louis University, St. Louis, MO – John E. Tavis, PhD
Patient metabolic responses to interferon - Indiana University, Bloomington, IN – Milton W. Taylor, PhD
Patient immune responses to the Hepatitis C virus (HCV) and to treatment - Portland Veterans Administration Medical Center, Portland, OR – Hugo R. Rosen, MD

Central Pathology and Pathologist

Pathology Laboratory, National Cancer Institute, Bethesda, MD – David E. Kleiner, MD, PhD

7.2 NIDDK

Project Scientist – Patricia Robuck, PhD, MPH

7.3 Committees

A steering committee and an executive committee were formed to set policy and govern the conduct of the study.

Steering Committee: Serves as the primary governing body of the study; responsible for policy decisions; provides oversight in planning the overall study design, facilitates the conduct and monitoring of the study, and reporting study results; votes on and approves all major decisions, final protocol and subsequent amendments. Members consist of principal investigators of the clinical centers, ancillary studies, the coordinating center, and the NIDDK project scientist. A chairperson and co-chair are appointed by the NIDDK from among the clinical center PIs.

Executive Committee: Manages day-to-day issues of the study; makes decisions required between the Steering Committee meetings, as needed for efficient progress of the study, and reports its actions to the Steering Committee on a regular basis; organizes and sets agendas for Steering Committee meetings. Members consist of the Steering Committee chair and co-chair, the Coordinating Center PI, NIDDK Project Scientist, and the DDDN Division Director.

7.4 Subcommittees

Subcommittees were formed to work on specific areas of the study and to make recommendations to the Steering Committee. Members consist of investigators from the clinical sites, ancillary studies, Coordinating Center, and NIDDK, including individuals with expertise in the relevant areas. The subcommittees and their missions are:

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Adherence Subcommittee: Develop methods and questionnaires used to assess adherence and compliance in this study; define criteria for inclusion and exclusion based upon preliminary assessment of likelihood of compliance; develop plans to analyze and quantify compliance and its correlation with response. The final decision of which instruments and methods to use to measure adherence and compliance will be made by the Steering Committee upon the recommendations of the Adherence Committee.

Ancillary Subcommittee: Make recommendations on ancillary studies and prepare guidance for conduct of the ancillary studies and interactions and communications with the Clinical Centers and Data Coordinating Center; review proposals for ancillary studies and make detailed recommendations to the Steering Committee regarding which studies should be supported as well as how they impact on the overall clinical study; make recommendations to the Steering Committee and NIDDK regarding opportunities for other ancillary studies not initially planned in the grant applications that might be included in the Virahep C study; prepare detailed protocol for the timing, amounts, and characteristics of the clinical material to be gathered from patients for the ancillary studies. These protocols should be coordinated with the clinical protocol and must address issues of practicality and safety of the collection, with assurances that the ancillary studies do not interfere with clinical care of patients or responses. Prepare sections of the consent documents dealing with gathering of ancillary study materials, or prepare separate consent forms as necessary. Provide an

interface between the principal investigators on the ancillary studies and the Data Coordinating and Clinical Centers.

Coordinators Subcommittee: Attend to the day-to-day operations of the study including recruitment, protocol adherence, consistent and complete data collection at each clinical center. Make recommendations to the Steering Committee regarding any study issues that may require modification or resolution.

Exemption Subcommittee: Review on a case by case basis, protocol exemption petitions for subjects who do not meet one or more of the eligibility criteria (inclusion and exclusion).

Measurement Subcommittee: Develop parts of the protocol that require special forms and assessments that go beyond typical clinical assessment; develop means of assessing race and ethnic background of patients, with careful and detailed description of how the assessment will be performed and interpreted; develop or select other instruments to be used to assess and monitor patients, such as socioeconomic questionnaires, risk-factor questionnaires, depression and anxiety scales, symptom scales and quality of life questionnaires. The final decision on adoption of the specific measure instrument will be made by the Steering Committee after recommendations from the Measurements Subcommittee.

Pathology Subcommittee: Develop the policy for liver biopsies, the handling and mailing of samples to a central pathologist, the definition of an adequate sample (in ml and quality, numbers of stains or slides available, timeliness) and develop the scale and grading system to be used in the study; help in the analysis of data as relates to histological features. A central pathologist will read all slides but will be assisted by two or three pathologists from the individual clinical centers.

Protocol Subcommittee: Prepare the final written protocol for the study and thus prepare summary, background information, study design, inclusion and exclusion criteria, treatment regimen, monitoring schedule, adverse event grading and criteria for dose modification and drug discontinuation, statistical analysis, patient protection, and references sections of the protocol; develop details of the protocol and study design, but major decisions in the design and the final protocol will be approved by the Steering Committee. A subcommittee of the Protocol Committee will prepare the master consent form for the study.

Publications/Presentations Subcommittee: Develop the policy for publications in regards to preparation of manuscripts, assignment of tasks in analysis and writing, policy as regards to requesting data analysis, authorship policy and other issues related to publications. Prepare a formal publication policy for both full manuscripts and abstracts; prepare a list of possible publications that will arise from this study and prepare paragraphs regarding the scope of each and how they intersect with the probably final major manuscript to arise from this study.

Safety Subcommittee: Develop policy regarding reporting of adverse events; review all serious adverse events with appropriate timeliness; prepare annual reports as necessary.

Virology Subcommittee: Prepare the details of how viral kinetics will be assessed and what specialized virological testing will be performed, including timing of samples and assays to be done on all patients as well as a more carefully assessed subset of patients; prepare detailed document of how to perform the viral kinetic study, prepare and handle the blood samples and ship to the central virology laboratory; recommend the virological assays to be performed on specimens including conventional commercial assays such as quantitative and qualitative PCR for HCV RNA as well as special virological testing such as genotyping, quasispecies determination, and genome sequencing. Prepare the part of the protocol dealing with virological testing; provide oversight and monitoring of accuracy of the virology testing laboratory; aid in the analysis and interpretation of the virological testing results. The final decision on which virological assays will be used in the study will be made by the Steering Committee.

Website Subcommittee: Create and maintain a website for public consumption. The website will include general information about hepatitis C; a description of the Virahep-C study, including the goals of the study and its ancillary projects; and contact information at the clinical sites for persons interested in enrolling the study.

7.5 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) consisting of five individuals who are independent of the institutions and investigators participating in the clinical study, who have no financial ties to the outcome of the trial has been appointed by the NIDDK in consultation with the Steering Committee. Written documentation attesting to the absence of conflict of interest will be required of all DSMB members on an annual basis. The ongoing review of the data by this independent committee assures the investigators and the NIDDK that the trial can continue without jeopardizing patient safety. The roster and charter of the DSMB members will be given to each investigator participating in the study for submission to their IRB.

The DSMB is comprised of experts in the fields of hepatology, clinical trial methodology, biostatistics and ethics. The members are: John Vierling, MD (Cedars-Sinai Medical Center), Daryl Lau, MD (University of Texas Medical Branch, Galveston), Raj Reddy, MD (University of Pennsylvania), Andrea Reid, MD (Massachusetts General Hospital), and Chris Gennings, PhD (Virginia Commonwealth University). A DSMB chairperson (John Vierling, MD) and a Medical Safety Officer (Geoffrey Block, MD, Co-Investigator, Coordinating Center) have been appointed. The Medical Safety Officer will serve as the contact person for adverse event reporting. An NIDDK executive secretary (Jay Everhart, MD) will be present for all DSMB meetings.

The DSMB will review the study protocol, recommend recruitment initiation, monitor all aspects of the study (e.g., recruitment, protocol deviations, adherence, adverse events, site visit summaries, data quality, attrition, descriptive characteristics, efficacy), and recommend protocol modifications, including early study termination.

Quarterly reports will be prepared by the Coordinating Center. Tables showing study progress will be presented by clinical center and overall. These will include recruitment, protocol deviations, adherence, attrition, adverse events, data quality, descriptive characteristics of the study sample, and efficacy.

All adverse events will be reported to the Medical Safety Officer, Dr. Block for review. Dr. Block will distribute reports of serious adverse events (SAE), which are both unexpected and related to the study medication, to the DSMB, the FDA, and all participating clinical centers so that they may submit the report to their local Institutional Review Board. Note that an adverse event is classified as serious if it results in one of the following: hospitalization or prolonged hospitalization; life-threatening; death; significant or permanent disability; congenital anomaly/birth defect (in the case of an unanticipated birth to a study participant); or requires intervention to prevent permanent damage.

The Coordinating Center will work with Dr. Block and the Steering Committee to maintain a cumulative summary of adverse events overall and stratified by serious/non-serious status that will be forwarded to the DSMB every three months.

The DSMB will meet twice yearly, once in person and once via teleconference to review the cumulative data. A closed session will be held to review safety and data quality. Based on the data presented, the DSMB will recommend continuation or termination of the study. For example, the DSMB may recommend stopping the trial prematurely if the incidence of SAE is excessive. A summary of the DSMB findings will be forwarded to all investigators for submission to their respective IRBs.

8.0 Ancillary Studies

In order to reach specific aims 2 and 3 described in this protocol, a number of ancillary studies are planned utilizing the data and specimens derived from the treatment cohorts. These studies will include investigations of hepatitis C viral kinetics, immunology, genetic studies (patient and virus), and interferon signaling. These studies are described in detail below:

8.1 Viral Kinetics

The kinetics of HCV response to interferon have been recently described to undergo a biphasic response with the 1st phase being rapid, dose-dependent and accounting for a 0.5 to 2.0-log drop in HCV RNA levels

within 24 hours. This phase reflects the effectiveness of interferon in blocking viral production. A 2nd slower phase of viral decline follows which is not interferon dose dependent, reflects the death of HCV infected liver cells and correlates with the serum half-life of interferon. Its decline slope is an excellent predictor of SVR with interferon monotherapy or combination therapy. In preliminary results, it has been shown that SVR may be predicted as early as 24 hrs of initiating interferon based on 1st phase kinetic parameters. Therefore, the first specific aim of this study is to assess the best and earliest predictor(s) of SVR using viral kinetic modeling principles. In preliminary studies, it has been shown that African American patients treated with interferon alone have a blunted 1st phase viral decline but more importantly, a significantly slower 2nd phase viral decline. Based on preliminary findings that 2nd phase decline correlates directly with interferon half-life and that SVR may be improved in African American patients treated with peginterferon. Peginterferon in combination with ribavirin will likely optimize response.

Rationale for Viral Kinetics and Peginterferon alfa-2a Pharmacokinetics and Pharmacodynamics - Treatment Design

The purpose of this study is to:

1. Optimize treatment for patients who are infected with genotype 1 virus and are inherently more resistant to standard interferon treatment protocols.
2. Provide a clinical framework to understand why certain patients or races are inherently more resistant to interferon.
3. Understand the dynamics of HCV response in order to determine whether therapy can be discontinued earlier in patients who are resistant to current therapy and unlikely to respond. In order to assess the earliest time point(s) that can predict SVR we propose performing dynamic kinetic studies to assess viral kinetic parameters and their relationship to SVR. Serum sampling will occur at various time points (see Table 9 for measurement of HCV RNA, peginterferon alfa-2a levels, and 2',5'-OAS serum activity (a marker of interferon induction).

Table 9
Blood sampling schedule for viral kinetic studies

	Baseline (Day 0)	Day					Month						
		1	2	7	14	28	2 (Wk8)	3 (Wk12)	6 (Wk24)	12 (Wk48)	15 (Wk 60)	18 (Wk 72)	24 (Wk96)
Viral Kinetics	X	X	X	X	X	X	X	X	X	X		X	X
Pharmaco -kinetics	X	X	X	X	X	X	X	X	X	X	X PEG-IFN alfa-2a only		
ALT	X	X	X	X	X	X	X	X	X	X		X	X

Blood will be drawn for HCV RNA determination at baseline, days 1, 2, 7, 14, 28 and months 2, 3, 6, 12, 18 and 24 (see Table 9). The frequency of this sampling is to assess the early and late declines with peginterferon alfa-2a and whether there are differences in interferon effectiveness, intrinsic viral clearance, and viral production. Blood for peginterferon alfa-2a serum levels will be obtained at each point where we measure RNA in the first week and selected later time points (see Table 9). 2',5'-OAS, a marker of interferon responsiveness, will also be measured at these time points.

8.2 Ancillary Studies of Mechanisms of Antiviral Resistance

The following ancillary studies focus on determining the possible mechanisms underlying response/non-response to anti-viral therapy among African Americans, specifically, and among HCV-infected individuals, in general. Crucial to each of these studies is the availability of a large sample size of African Americans and Caucasian participants, detailed clinical information including, but not limited to, histological severity, viral load and HCV genotype and response to therapy, and access to optimally collected serum, PBMCs and liver tissue at specified timepoints. These four studies will evaluate virological factors, host genetics, interferon signaling pathways, and immunologic response in African American and Caucasian participants treated with peginterferon and ribavirin.

All of the ancillary studies will be collecting samples on all Virahep-C study participants (n=400) at baseline. Each of the ancillary studies, except for the Patient Genetics study, will continue to collect samples on all subjects up to and including the visit occurring at 4 weeks after the initiation of treatment. Beyond 4 weeks, specimens will be collected on all patients for Viral Genetics, Viral Kinetics, Pharmacokinetics and Immunology (CD4+) studies, and on a subset of patients for Interferon Signaling (at least n=72) and Immunology (CD8+, n=100) studies.

Consent for participation in the ancillary studies will be included in the main protocol consent with the exception of the larger volume blood draw needed at the end of follow-up (post treatment week 24) in 100 participants for the CD8+ Immunology study. A separate consent document will be created for this study. The ancillary studies require whole blood or plasma. Liver tissue will be used for pathology (minimum length of 1 cm if Trucut needle and 2 cm if Jamashedi or other skinny needle) and any additional tissue collected will be banked for future studies using a standardized method which is optimal for RNA preservation.

A summary of the specimens required and the timepoints for obtaining these specimens are summarized in Tables 13-14.

8.2.1 Viral Genetics Ancillary Study

Principal Investigator: John Tavis, PhD
St. Louis University, St. Louis

Background and Rationale:
HCV replicates as a collection of quasispecies, and this underlies much of its biology, especially in immune evasion and in antiviral resistance. We will therefore study the impact of therapy for HCV on quasispecies development and on RNA replication in African Americans and Caucasians. We will sequence the complete viral genomes from a minimum of 96 patients undergoing therapy and then clone, express, and analyze the HCV RNA polymerase that replicates the viral genome from patients with representative RNA polymerase sequences. These studies will provide a comprehensive view of the HCV genome during therapy that may allow us to identify viral factors underlying the difference in response to antiviral therapy between African Americans and Caucasians. They will also provide data about RNA replication that may guide development of novel antiviral therapies.

Study Aim:
To characterize HCV genetic heterogeneity by sequence and quasispecies in patients with flat, slow, and rapid early responses to antiviral therapy. The functional consequences of variation in the NS5B RdRp gene will be determined.

Study Endpoints:
(a) Quasispecies distribution and complexity in African Americans and Caucasians.
(b) Quasispecies distribution and complexity as a predictor of non-response, early and late response.
(c) Specific HCV sequences (within entire genome) predictive of non-response, early and late response.
(d) Correlate HCV RdRp activity with genetic drift and clinical response.

Study Population:
Minimum of n=96 with 6 groups of no less than 16 each (Groups: flat, rapid and slow responders for both African American and Caucasian groups). Note that serum will be obtained from all 400 patients for four

weeks for two reasons. First, to be able to increase the statistical power of the Viral Genetics study by sequencing short regions of genomes derived from additional patients. Second, to be able to expand the specific regions of the genome sequenced to identify individual viral variations that may affect or aid in the interpretation of the results of the other ancillary studies (e.g., sequence the NS3 region of all immunology patients so that the epitopes present in each patient are known).

Method for Analysis/Power Calculations:
Note: The power calculations are based on 96 patients, or the smallest sample size that would be used for this study.

Comparing HCV genetic sequences within **race** groups:
A test for proportions will be used to compare the proportion of Caucasians to that of African Americans who have (or don't have) a particular HCV genetic sequence. Given a sample size of 96 subjects with equal numbers of Caucasians and African Americans (48 in each group), and using a two-tailed test with $\alpha = 0.05$, we will have 80% power to detect that 35% of one race have the particular sequence versus 10% in the other racial group. This corresponds to an odds ratio of approximately 4.8.

Comparing HCV genetic sequences within **responder** groups:
Data will be analyzed using a chi-square test to compare the proportions among the three responder groups (defined above) that have (or don't have) a particular genetic sequence.

Given a sample size of 96 subjects, divided into 3 evenly-sized responder groups, there will be 80% power to detect an effect size of 0.32 at $\alpha = 0.05$ using a two-sided test. An effect size for a chi-square test of 0.32 is considered to be small to medium.[29] An example of proportions across 3 groups yielding an effect size of approximately 0.32 is 10%, 27%, 44% (or conversely, 90%, 73%, 56%).

HCV RdRp

Logistic regression models will be fit to: (1) analyze the association between RdRp activity and particular sequence patterns in the viral genomic sequence; and (2) analyze the association between RdRp activity and response (e.g., null responder vs. responder). RdRp can be classified as both a categorical variable (e.g., activity greater than twice the normal levels) and continuous variable (i.e., the percentage of activity that is consistent with a standard RdRp).

RdRp activity will be analyzed as a categorical and continuous predictor variable with response as the outcome variable. Given that by study design one-third (n=32) of the patients will be null responders, there will be 80% power to detect an odds ratio of 3.8 for being a null responder if the RdRp activity is greater than twice the normal level at $\alpha = 0.05$ and using a two-tailed test. Keeping RdRp continuous (and performing transformations to ensure normality), we will have 80% power to detect an odds ratio of 2.2 for a standard deviation increase from the mean of the transformed RdRp level using a two-sided test with an alpha of 0.05.

8.2.2 Patient Genetics Ancillary Study

Principal Investigator: Huiying Yang, MD, PhD
Cedars-Sinai Medical Center, Los Angeles

Background and Rationale:
The outcome of HCV infection is determined by the interaction between the virus and the host immune response. The administration of interferon alpha in the treatment of chronic hepatitis C can have a direct antiviral effect and activate the immune system, resulting in elimination of the virus. Although response to interferon treatment differs depending on the viral load and viral genotype, it is not yet clear why different responses are observed in individuals with the same viral load and same genotype. It is likely that genetic constituents of the host may play an important role in viral clearance, disease outcome, and treatment response. However, the data on patient genetic studies are sparse, mostly focused on the HLA class I and class II genes, and the results have been inconsistent. We will test the hypothesis that host genetic factors contribute to treatment response and disease progression of HCV infected individuals. We will investigate the role of selected immunological genes involved in innate and adaptive, humoral and cellular immune

responses in treatment response, while considering the potential confounding effect of viral load, viral genotype, disease duration, and other non-genetic risk factors.

Study Aim:
To determine the contribution of patient genetic factors underlying responsiveness/non-responsiveness to antiviral therapy in African Americans and Caucasians. A lymphoblastoid cell line will be established to facilitate the proposed studies. We will test at least 22 candidate genes chosen on the basis of their role in interferon response and CD4+ (TH) type 1 and 2 responses using markers of 3.5KB density. Based on the significance level of associations, a minimum of 5 candidate genes will be further studied by fine mapping.

Study Endpoints:
(a) Evaluate associations between the 22 (or more) candidate genes and non-response to anti-viral therapy by comparing gene frequencies in responders and non-responders.
(b) Fine mapping of 5 (or more) candidate genes to identify specific genetic variants in the gene that determine therapeutic response/non-response.

Study Population:
N=400, all enrolled study participants
Additional 400 anti-HCV positive viremic patients from an external (to Virahep-C) NIDDK source. To further increase the study's power, and serve as an important control group, 50 anti-HCV positive non-viremic (resolved infection) patients obtained from Hugo Rosen (Principal Investigator, Immunology Study, Portland VA Medical Center) will be tested. A separate consent form for genetic studies will be obtained from participants (See Appendix 4). Refusal to participate in the genetics study will not exclude patients from participating in the treatment trial or other ancillary studies.

Method for Analysis/Power Calculations:
To test the association between response to therapy and genetic markers, we will compare the response rate among different genotypic groups or between carriers and non-carriers. A Chi-square or exact test, or a Mantel-Haenszel test when data are stratified across levels of a potentially confounding factor, will be used to test association between genotype and phenotype. For quantitative phenotype (e.g. reduction of viral levels since the treatment), we will compare the mean difference or percent change between each genotype group (homozygous for allele 1, heterozygous, and homozygous for allele 2) or the allele carrier and the non-carrier groups. A one-way ANOVA or two-sample t-test (or a non-parametric equivalent, e.g., Wilcoxon's rank sum test) will be used to test the associations between genotype and phenotype. The tests we use will depend on the allele frequencies, trait distribution, and sample size in each group. If an allele's frequency is low, e.g. 10%, we may not have enough homozygotes for that allele, and as a result the comparison will be between two groups (carrier and non-carrier). If preliminary analysis indicates potential confounding, analysis of covariance, Mantel-Haenszel Chi-square statistics, or multiple logistic regression will be used to control for confounding. Since Caucasians and African Americans have different response rates to interferon/ribavirin therapy, the effect of each specific gene may be not be the same in these racial groups. Thus, we will first analyze data separately within each racial group. Subsequent analyses will be based on all subjects by either combining results from stratified analysis (Mantel-Haenszel Chi-square test) or through adjustment for race in multivariable modeling (e.g., multiple logistic regression).

Using Mantel-Haenszel statistics with strata defined by race, we estimated power for detecting various differences in the response rate between disease allele carriers and non-carriers in the 400 samples (200 Caucasians and 200 African Americans). The table below lists the power under three different situations: at 0.05 significance level without adjustment for multiple comparisons, at the 0.0005 level (comparison-wise type I error rate of 0.05 divided by 100, i.e., adjusting for 100 tests), and at the 0.00025 level (comparison-wise type I error rate of 0.05 divided by 200, i.e., adjusting for 200 tests). The disease allele frequency is assumed to be 10% in the HCV patient population. Overall, the power is >80% except when the difference of response rates between carriers and non-carriers is less than 20% in both Caucasians and African Americans and the Bonferroni correction is applied. These power calculations were based on assessing a qualitative variable of response to therapy (viral clearance). But we may also test response to therapy by using quantitative measures, such as reduction of viral levels since therapy.

Table 10

Power to detect genetic association between a genetic marker and response to therapy when assuming a dominant genetic model, disease allele frequency =0.1, and 400 samples (200 Caucasians and 200 African Americans)

Response Rate				Power1[a]	Power2[b]	Power3[c]
Caucasian		African American				
Carrier	Non-carrier	Carrier	Non-carrier			
0.5	0.2	0.2	0.0	0.999	0.954	0.933
		0.3	0.0	1.000	0.996	0.994
		0.3	0.1	0.994	0.833	0.784
		0.4	0.1	0.999	0.971	0.957
0.5	0.3	0.2	0.0	0.960	0.590	0.519
		0.3	0.0	0.997	0.889	0.851
		0.3	0.1	0.905	0.417	0.348
		0.4	0.1	0.987	0.761	0.701
0.6	0.3	0.2	0.0	0.998	0.909	0.875
		0.3	0.0	1.000	0.989	0.982
		0.3	0.1	0.989	0.776	0.718
		0.4	0.1	0.999	0.950	0.929
0.6	0.4	0.2	0.0	0.951	0.552	0.480
		0.3	0.0	0.996	0.863	0.819
		0.3	0.1	0.896	0.397	0.329
		0.4	0.1	0.984	0.736	0.674

Notes: [a] power at 0.05 significance level; [b] power at 0.0005 significance level (adjust for 100 tests); [c] power at 0.00025 significance level (adjust for 200 tests).

8.2.3 Interferon Signaling Ancillary Study

Principal Investigator: Milton Taylor, PhD
Indiana University, Bloomington

Background and Rationale:

Interferons are known to induce a large number of genes, including transcription factors (STATs), kinases, anti-viral activities, and nucleases. It has been estimated from DNA microarrays that there may be as many as 300 genes whose expression is modified following interferon treatment. Many of these changes occur within 4-6 hours of treatment in vitro. Likewise changes in serum cytokines have been shown to occur in humans following interferon treatment. These cytokines in turn may induce other factors. Utilizing the approach of DNA microarrays, where we can examine a large number of changes in gene expression at the one time, we will look at patterns of changes in expression following treatment of patients with the combination of PEG-interferon and ribavirin. This will be done using RNA extracted from PBMC at early time points after the initiation of treatment. We are seeking to determine if there are major changes in responders, which are not seen in non-responders, and whether such changes can be linked to outcome of treatment. Additionally, we will examine whether there are any genetic differences in patterns between African Americans and Caucasian patients, and whether there are anti-viral mechanisms (e.g. PKR) that are non-functioning in some patients. The DNA array is only a first stage in this type of analysis, since any data received from the array has to be further analyzed by Northern analysis and quantitative PCR. We shall also examine the induction of cytokine genes as targets in DNA arrays. If we find increases in cytokine production, we will follow-up by measuring the levels of that cytokine in the serum. This should give a very comprehensive picture of the molecular changes that occur following interferon administration.

Study Aim:

To determine if unique cytokines induced by interferon-based therapy (early and late responses) are expressed differently in African Americans and Caucasians and responders and non-responders.

Study Endpoints:

(a) Differences in cytokine gene expression (using DNA arrays) in flat, early and late responders, African Americans versus Caucasians
(b) Differences in specific cytokine levels identified in (a) in serum for African Americans versus Caucasians and among the 3 response patterns
(c) Differences in measured cytokine levels in cultured PBMCs from African Americans and Caucasians before and after interferon treatment (in vitro)
(d) Differences in the early (12-24 hours following the start of antiviral therapy) expression of interferon-inducible genes (PKR, oligo A synthetas, Mx protein, indole amine 2,3 dioxygenase or others identified by DNA array analyses) in African Americans and Caucasians

Study Population:

Minimum of n=72 with 6 groups of no less than 12 each (Groups: flat, rapid and slow response types for both African Americans and Caucasian groups).

Note: Since the response pattern will only be determined by week 4 of the study (based upon viral kinetic data), blood be obtained from all 400 patients up to and including week 4. However, only the 72 (or more) patients randomly selected for study (within groups defined by response pattern and race) will require blood draws beyond week 4.

Method for Analysis/Power Calculations:

Note: The power calculations for this study were done assuming either 2 or 3 responder groups (i.e., for some analyses the slow and fast responder groups will be combined) and with cytokine response treated as either a continuous or categorical variable. The power calculations are based on n=72, or the smallest sample size that will be used for this study.

Method 1: A chi-square test will be used to compare the proportions of treatment non-responders, treatment slow responders and treatment fast responders (i.e., 3 responder groups) that show a defined level (established *a priori*) of cytokine response. A sample size of 72 will yield an effect size of 0.37 (small to medium effect size). An example of proportions that could be found in the non-responders, slow-responders and fast responders, respectively, at an effect size of 0.37 is 10%, 30%, and 50%.

Method 2: A test for proportions will be employed to compare the proportion of treatment responders (slow and fast responders combined) who show a defined level of cytokine response compared to that of non-responders. Thus, these analyses are based on 2 responder groups. Given a sample size of 72 subjects and using a two-tailed test with $\alpha = 0.05$, we will have 80% power to detect that 42% of responders have the cytokine response if 10% of the non-responders have the cytokine response.

Method 3: Using a one-way analysis of variance (ANOVA) test to compare the distributions of the level of cytokine response to treatment among slow responders, fast responders and non-responders (i.e., 3 responder groups). Given a sample size of 72 subjects and using an alpha of 0.05, two-tailed test, we will have 80% power to detect an effect size of 0.38, corresponding to a large effect size for ANOVA. [29]

Method 4: A t-test to compare the distributions of the level of cytokine responses between treatment responders (slow and fast responders combined) versus non-responders. Thus, these analyses are based on 2 responder groups. Given a sample of 72 subjects and using a two-tailed test with $\alpha = 0.05$, we will have 80% power to detect a difference in the mean level of cytokine response between treatment responders and non-responders equal to an effect size of 0.68 (approximately two-thirds of a standard deviation). According to Cohen, an effect size for a t-test of 0.7 would be in the medium-large range.

8.2.4 Immunology Study

Principal Investigator: Hugo Rosen, MD
VA Medical Center, Portland

Background and Rationale:
We propose to study the specific cellular immune responses underlying resistance to antiviral therapy in chronic hepatitis C virus infection. For HCV infections, significant differences have been found in the strength and pattern of CD4+ T-cell responses during different stages of disease and in different patient subsets. Missale *et al* [30i] demonstrated that the anti-viral CD4+ T cell response during *acute* HCV infection was a critical determinant of disease resolution and correlated with a favorable course of infection, i.e., acute self-limited disease. Patients who became HCV-RNA negative and normalized serum aminotransferases had more frequently detectable and significantly stronger CD4+ T cell responses to HCV antigens E2, NS (nonstructural protein) 3, NS4 and NS5 as compared to patients who developed persistent viremia and biochemical evidence of chronic liver injury. Our understanding of the contribution of virus-specific CD8+ cytotoxic T lymphocyte (CTL) responses to viral clearance following antiviral therapy is also incomplete. Nelson and colleagues demonstrated that the presence of detectable HCV-specific CTL activity in liver-infiltrating lymphocytes was associated with a sustained response to subsequent interferon treatment, whereas virus-specific CTLs were not detectable in the liver of interferon nonresponders. [30] Using a TNF-α based ELISPOT assay, Lohr et al [31] serially analyzed the peripheral blood CTL precursor (CTLp) frequencies specific for HLA A2-restricted T cell epitopes in 11 interferon-α treated patients. Marked increases in CTLp frequencies were observed among therapy responders as compared to non-responders, suggesting that these quantitative multispecific responses could contribute to the reduction of the viral load in treated patients. In fact, the CTL induction observed in non-responders was associated with only transient reductions of the high viral load; therefore, it may be hypothesized that quantitatively insufficient CTL induction could favor resistance to antiviral therapy.

Using highly sensitive assays, we will focus on qualitative, quantitative and kinetic aspects of successful HCV-specific CD4+ and CD8+ T cell responses to create a conceptual paradigm that can be examined by further experiments to facilitate development of vaccines and immunotherapies.

Study Aim:
To study the specific cellular immune responses underlying resistance to antiviral therapy in chronic hepatitis C virus infection, with focus on the qualitative, quantitative and kinetic aspects of successful HCV-specific CD4+ and CD8+ T cell responses in peripheral blood to distinguish responders/non-responders in African Americans and Caucasians.

Study Endpoints:
(a) Effector frequencies of HCV-specific CD4+ Th1 –producing cells (determined by γ-Interferon ELISPOT). We will use whole proteins for core, E2, NS3, NS4, NS5 and overlapping mer-peptides for regions for which proteins are not available, e.g., p7/NS2, in all patients pre-treatment. We will determine if the magnitude and breadth of CD4+ T cell responses are predictive of response (both early viral kinetics response and long term clinical response).
(b) <u>*Sub-aim*</u>, presuming Dr. Yang finds cytokine genetic polymorphisms correlate with treatment outcome: The frequency of other cytokine-producing cells (i.e., IL-4, IL-10, TNF-alpha) will be determined using a multiparameter, flow cytometric intracellular cytokine staining assay following PMA (mitogen) stimulation to define the functional relevance of these polymorphisms.
(c) The kinetics of CD 4+ T cell responses, i.e., the expansion of the number of proteins/peptides that elicit cytokine production (as assessed by Interferon-γ ELISPOT) and the rate at which this occurs after initiation of therapy.
(d) Frequency of CD8+ T cells specific to NS3 region in 100 patients (see below) will be determined. Using 15-mer-peptides and autologous dendritic cells as antigen presenting cells in the Interferon ELISPOT assay, we will test the hypothesis that HCV-specific CTL specificities and magnitude expand in responders but not in non-responders.

Study Population:

<u>CD4+ studies (N= 400):</u> All 400 patients will be studied to attain adequate power to detect a smaller effect size and because of the lack of data in African Americans (on the one hand, African Americans have less fibrosis and therefore we would expect higher Th1 frequencies; on the other hand, African Americans have lower response rates to treatment and therefore one might predict a lower pre-treatment frequency of Th1 effector cells).

CD8+ studies (N = 100): 100 subjects with genotype 1a [20] will be consented later depending on their response to therapy. These power calculations are based upon unadjusted analyses, and fibrosis and viral load will be controlled for as potential confounding variables using logistic regression modeling.

Method for Analysis/Power Calculations:

CD4 Analyses

Method 1: A two-sample t-test will be utilized to compare the distributions of the number of cells that react between the responders and non-responders. The table below shows the power to detect various effect sizes with $\alpha = 0.05$, using a two-sided test, and assuming that 33% of the Caucasians will respond to therapy and either 25% or 15% of the African Americans will respond to therapy. Note that effect sizes of 0.4, 0.5, and 0.6 would be considered to be small to medium. [29]

Table 11

Racial Group	Power	Proportion Responding To Therapy	Effect Size
Caucasians	0.76	0.33	0.4
African Americans	0.86	0.25	0.5
African Americans	0.86	0.15	0.6

Method 2: Logistic regression models with responder/non-responder as the outcome variable will be fit to test the detectable increase in the odds of responding for every standard deviation increase from the mean number of cells which show some reaction. These models will be based on both unadjusted and adjusted analyses.

The table below shows the detectable odds ratios from logistic regression analyses, based on 80% power and assuming that 33% of the Caucasians will respond, either 25% or 15% of the African Americans will respond, and a multiple correlation coefficient of 0.4 (i.e., the correlation between the independent variable of interest, the number of cells that react, with all the other independent variables). [32]

Table 12

Racial Group	Proportion Responding	Odds Ratio (unadjusted)	Odds Ratio (adjusted)
Caucasians	0.33	1.6	1.7
African Americans	0.25	1.7	1.8
African Americans	0.15	1.85	2.0

Example of Method 2: Given a sample size of 200 Caucasians patients and assuming that one-third will be responders, we will have 80% power to detect an odds ratio of 1.6 for every one standard deviation increase from the mean number of cells that react, using a two-tailed test and $\alpha = 0.05$. This can be interpreted as saying that the odds of being a responder increases by 60% for every one standard deviation increase in the number of cells that react. Adjusting for potential confounders, and assuming a multiple correlation coefficient of 0.4 between the number of cells that react and the other independent variables, we will have 80% power to detect an odds ratio of 1.7.

CD8 Analyses

The number of CD8 cells showing response will be categorized using a clinically meaningful cutpoint. Then the proportion of responders showing greater than the cut-off value of cell responses will be compared to that of the non-responders. This comparison will be done within the racial groups.

Assuming $\alpha = 0.05$, two-sided test, there will be 80% power to detect a difference if 60% of the responders show greater than the cut-off value of cell responses and 20%, or fewer of the non-responders do. This corresponds to an odds ratio of approximately 6.4. In multivariable analyses, there will be 80% power to detect a difference in proportions between 60% in the responders and 15% or less in the non-responders, using a two-tailed test with alpha equal to 0.05, and assuming a multiple correlation coefficient between the covariate of interest (e.g., responder) and the other independent variables. This corresponds to an odds ratio of about 8.5. These effects are large, however, it is expected that the differences will be substantial and that these are reasonable estimates.

Table 13

Ancillary Studies and Viral Kinetics: Number of Patients, Specimen Type

Study	Number of Patients	Patient Specifications	Specimen	Minimum Volume per Timepoint
Viral Genetics (Tavis)	400	Initially, the Viral Genetics study will include at least 96 patients based upon early response pattern and race	Plasma	2 ml (5 ml whole blood)
Viral Kinetics	400		Plasma	2 ml (5 ml whole blood)
Pharmacokinetics	400		Serum	3 ml (7 ml whole blood)
Patient Genetics (Yang)	400	NA	Whole blood	15 ml
Interferon Signaling (Taylor)	All up to and including 4 wks, at least 72 at post-treatment	For 72 (or more), equal number with each early response pattern and race	Whole blood	16 ml
Immunology (Rosen)	400 100 of 400 above	All patients will have CD4+ T cell analysis CD8 studies: Genotype 1a; 25 responders, 25 nonresponders in each of the Caucasian and African American racial groups	Whole blood Whole blood	56 ml at pre for all; 32 ml at 8, 24 wks for all; 48 wks for responders; 56 ml (24 wks post treatment for 300 patients (not assigned to CD8 studies below) same schedule as above, but will have 136 ml (instead of 56 ml) drawn at 24 wks post treatment)
Liver Tissue (future studies)	Varies	NA	Liver	Residual from pathology requirements

Table 14

Ancillary Studies and Viral Kinetics: Specimen Timepoints and Total Volumes

	Pre-Treatment	**Treatment**										**Post-Treatment**		
	Screen 2	BL Time 0	Day 1	Day 2	Wk 1 (Day 7)	Wk 2 (Day 14)	Wk 4 (Day 28)	Wk 8	Wk 12	Wk 24	Wk 48 **Respond-ers only**	Wk 24	Additional Follow-Up (4,12,36, 48 Wk) **Responders Only**	Any Follow-Up Visit***
Viral Genetics (Tavis)		5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml at each time	
Viral Kinetics		5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	
Pharmacokinetics		7ml	7ml	7ml	7ml	7ml	7ml	7ml	7ml	7ml	7ml		7ml §	
Patient Genetics (Yang)		15ml												
IFN Signaling (Taylor)		16ml	16ml	16ml	16ml	16ml	16ml							16ml (at least n=72)
Immunology CD4+ (Rosen)	56ml							32ml		32ml	32ml	56ml		
Immunology CD8+ (Rosen)	56ml*							32ml*		32ml*	32ml*	136ml**		
TOTAL Volume	56ml	48ml	33ml	33ml	33ml	33ml	33ml	49ml	17ml	49ml	49ml	66ml (n=300) 146ml (n=100)	10ml at each time + 7ml at follow-up wk 12	16ml (at least n=72)
TOTAL Liver (mm)	Varies											Varies		

* Will use blood from that taken for the CD4 study (i.e., additional blood will NOT be taken for CD8 at these time points). 56 ml (= 7 CPT tubes); 32 ml (= 4 CPT tubes)

** 136 ml (= 17 CPT tubes) at post treatment week 24

*** Any post treatment visit is suitable, preferably week 24

§ Follow-up week 12 for peginterferon alfa-2a only

APPENDIX 1

Proposed Action Criteria for Hepatic Adverse Events (ALT Flares)

1) ALT

Baseline Serum [ALT]	On-Treatment Serum [ALT]	Action
≤100	<200	None
	200 - 300	Repeat test in 1 week. If ALT decreased or stable (≤10% increase), continue at present dose and follow every 1-2 weeks to assure stability. If increased by >10%, decrease dose by 1 Level and follow with weekly testing until ALT is stable or decreased.
	301 - 500	Repeat test prior to administering dose. If ALT decreased or stable (≤10% increase), decrease by 1 Level and follow weekly to assure stability. If increased by >10%, hold dose until ALT decreases to <300 then resume study medication at 2 Level decrease and follow every week until stable. If a further 10% increase occurs, stop study medication and complete SAE documentation.
	>500	Hold study medication until ALT decreased to <300 then resume study medication at 2 Level decrease and follow every week. If ALT >300, stop study medication and complete SAE documentation.
101 - 200	≤300	None
	301 - 500	Repeat test prior to administering dose. If ALT decreased or stable (≤10% increase), decrease by 1 Level and follow weekly to assure stability. If increased by >10%, hold dose until ALT decreases to <300 then resume study medication at 2 Level decrease and follow every week until stable. If a further 10% increase occurs, stop study medication and complete SAE documentation.
	>500	Hold study medication until ALT decreased to <300 then resume study medication at 2 Level decrease and follow every week. If ALT >300, stop study medication and complete SAE documentation.
201 - 300	≤400	None
	401 - 500	Repeat test prior to administering dose. If ALT decreased or stable (≤10% increase), decrease by 1 Level and follow weekly to assure stability. If increased by >10%, hold dose until ALT decreases to <300 then resume study medication at 2 Level decrease and follow every week until stable. If a further 10% increase occurs, stop study medication and complete SAE documentation.
	>500	Hold study medication until ALT decreased to <300 then resume study medication at 2 Level decrease and follow every week. If ALT >300, stop study medication and complete SAE documentation.
301 - 500	≤500	None
	>500	Repeat test prior to administering dose. If ALT decreased or stable (≤10% increase), decrease by 1 Level and follow weekly to assure stability. If increased by >10%, hold dose until ALT decreases to less than baseline then resume study medication at 2 Level decrease and follow every week until stable. If a further 10% increase occurs, stop study medication and complete SAE documentation.
> 500	≤25% Increase	None
	>25% Increase	Repeat test prior to administering dose. If ALT decreased or stable (≤10% increase), decrease by 1 Level and follow weekly to assure stability. If increased by >10%, hold dose until ALT decreases to less than baseline then resume study medication at 2 Level decrease and follow every week until stable. If a further 10% increase occurs, stop study medication and complete SAE documentation.

Note: This table is based on an upper limit of normal (ULN) serum ALT of 43 U/L for men and 34 U/L for women. The ULN at the investigator's laboratory should be considered when employing this table.

APPENDIX 1 (CONTINUED)

2) Total Bilirubin and Alkaline Phosphatase

If total bilirubin is less than 5 mg/dl, no study medication changes are indicated.

If total bilirubin is between 5mg/dL and 8mg/dL, refer to the Virahep-C protocol dose reduction guidelines for hemoglobin after excluding other causes of elevated bilirubin.

If total bilirubin is >8mg/dL, withhold both ribavirin and Pegasys until the underlying cause is determined. Do not resume study medication until total bilirubin is <5mg/dL

If alkaline phosphatase is < 2 X ULN, no changes in study medication dosing are indicated.

If alkaline phosphatase is between 2 to 3 X ULN, repeat alkaline phosphatase in 1 week. If alkaline phosphatase decreases or is stable (< 10% increase), continue at present dose and follow every 2 weeks to assure stability. If increased by > 10%, decrease dose by 1 level and follow with weekly testing until alkaline phosphatase is stable or decreased.

If alkaline phosphatase is > 3X ULN, withhold both ribavirin and Pegasys until the underlying cause is identified. Do not resume study medications until alkaline phosphatase is < 2 X ULN.

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