Technical Protocol

The Second Multicenter Hemophilia Cohort Study (MHCS-II)

James J. Goedert, M.D.*
Principal Investigator

Philip Rosenberg, Ph.D.†
Eric Engels, M.D.*
Thomas O’Brien, M.D., M.P.H.*
Charles Rabkin, M.D., M.Sc.*
Denise Whitby, Ph.D.‡
National Cancer Institute Co-Investigators

* Viral Epidemiology Branch, National Cancer Institute
† Biostatistics Branch, National Cancer Institute
‡ Viral Epidemiology Section, AIDS Vaccine Program, Scientific Applications
International Corporation, Frederick Cancer Research and Development Center, National Cancer Institute.

*Viral Epidemiology Branch, National Cancer Institute
6120 Executive Boulevard, Room 8012
Rockville, Maryland 20852
Telephone: 301-435-4724
Facsimile: 301-402-0817
Email: GoedertJ@mail.nih.gov

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The Second Multicenter Hemophilia Cohort Study (MHCS-II) will evaluate and prospectively follow approximately 4500 persons with hemophilia who were exposed to hepatitis C virus (HCV). The vast majority will have been infected with HCV, and approximately 1/3 will have been infected with human immunodeficiency virus (HIV). Primary objectives are to quantify the rates of liver decompensation, hepatocellular carcinoma, and non-Hodgkin lymphoma and to evaluate candidate clinical, genetic, virologic, serologic and immunologic markers that are likely to be on the causal pathway for these conditions. Candidate clinical and laboratory markers will be examined longitudinally to define changes over time and their relationships to one another. Collaborative studies will focus on genome scanning and evaluation of candidate genetic loci for susceptibility or resistance to HCV and HIV infections or to the diseases that result from these infections. Additional studies will identify response and complication rates of various anti-HCV and anti-HIV regimens in the setting of comprehensive clinical care of persons with hemophilia.

1.0 Background and Rationale

Persons with hemophilia (PWH) are at high risk for spontaneous or prolonged bleeding, which is often disabling and may be life threatening. Episodes of bleeding can be reduced or shortened by replacement of plasma clotting factors, most often factor VIII (for hemophilia A) or factor IX (for hemophilia B). Highly effective, commercially prepared concentrated clotting factors were licensed for clinical use in 1973 and were in widespread use in the United States by 1975. These commercial factor concentrates were prepared from up to 20,000 units of donated plasma per batch. In recent years, up to 60,000 donors have been included per batch. As a consequence of exposure to many thousands of donors, PWH were at very high risk of infection with blood borne viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).\textsuperscript{2,3} Vaccination against HBV reduced this risk during the 1980s. Safe and effective vaccines against HCV and HIV have not been developed, but the risk of these two infections were reduced in the late 1980s through sensitive assays for screening donated plasma and through heat-treatment and other procedures to inactivate known and unknown viruses in the concentrate products.\textsuperscript{4} Nonetheless, approximately 50% of PWH born before 1985 were infected with HIV and 80% of those born before 1990 were infected with HCV.\textsuperscript{2,3} This is a population that can provide enormous insight on the natural history and factors that affect the risk of disease complications of these infections.

About half of those infected with HIV have died of the acquired immunodeficiency syndrome (AIDS) or related conditions,\textsuperscript{5} including a 10- to 200-fold increased risk for AIDS-associated non-Hodgkin lymphoma (NHL).\textsuperscript{6} Studies of NHL include the discovery of clonal 8;14 chromosomal rearrangement in circulating peripheral blood lymphocytes that are pathognomonic of Burkitt’s lymphoma.\textsuperscript{7} A substantially increased risk of NHL
has been found with genetic polymorphisms in CCR5 and SDF1 and most recently in the IL-6 promoter.\textsuperscript{8,9}

HIV-related mortality rate fell dramatically following the development of highly active anti-retroviral therapy (HAART) regimens in 1996. Most of this reduction can be attributed to a sharp drop in the incidence of \textit{Pneumocystis carinii} pneumonia and other life-threatening opportunistic infections.\textsuperscript{10} The incidence of AIDS-associated non-Hodgkin lymphoma may have declined as well, although this appears to be limited to large-cell immunoblastic lymphomas, particularly of the brain.\textsuperscript{10,11}

Among PWH without HIV, complications related to HCV, including hepatocellular carcinoma and particularly liver decompensation resulting from end-stage cirrhosis, have been increasing slowly but steadily.\textsuperscript{3,12,13} Liver decompensation includes persistent ascites, bleeding esophageal varices, hepatic encephalopathy, or receipt of a liver transplant. As illustrated by “icebergs” in Figure 1, about 20\% of HCV-infected people successfully clear the virus. Of the other 80\% in whom HCV can be detected in their blood, the vast majority have subclinical conditions and laboratory abnormalities, such as elevations in transaminase levels or bilirubin or decreases in albumin. Most of these people have various degrees of inflammation on liver biopsy, and an unknown fraction have cirrhosis (hepatic fibrosis) which is clearly on the causal pathway to HCV-related liver decompensation and hepatocellular carcinoma. Between years 2000 and 2005, the number of HCV-infected PWH without HIV will remain approximately stable, but the proportion with clinically evident HCV-related conditions, such as liver decompensation and hepatocellular carcinoma, is expected to increase substantially (Figure 1 below).

Various disorders of B lymphocytes, including non-Hodgkin lymphoma, have been associated with HCV infection, but these are heterogeneous, complex, and controversial.\textsuperscript{14} One B-cell disorder, mixed cryoglobulinemia, is clearly linked to HCV infection and appears to be a precursor of non-Hodgkin lymphoma.\textsuperscript{15} Unlike for HIV, anti-HCV therapy is still at an early stage of development. Thus, the prognosis for HCV-infected PWH is unknown.
In many respects, PWH are highly suitable for prospective cohort studies. They have an inherited defect in a single gene that manifests as an inability of the blood to coagulate appropriately (a “coagulopathy”). These genes are transmitted as classical X-linked or autosomal recessive Mendelian traits, with no known relationship to other genes or diseases. Their coagulopathy leads to persistent bleeding with trauma and often spontaneous bleeding into large joints, muscles, and occasionally other organs. Thus, they require regular contact with health care professionals, typically at comprehensive centers that have developed expertise in the care of PWH. Because of their close contact with the health care system, losses to follow-up are very low. The hemophilia population includes all socioeconomic strata, and it has a very wide age range, permitting analyses of the effects of age on various exposures and diseases. Generalizability is limited largely to males, as the most frequent genetic mutations are X-linked. Blacks are under-represented among the hemophilia populations in Europe and the Americas, perhaps reflecting adverse selection during the slave era.

The Viral Epidemiology Branch (VEB) of the U.S. National Cancer Institute initiated the Multicenter Hemophilia Cohort Study (MHCS) in 1982 and, between 1985 and 1992, expanded it to identify and quantify risk factors for HIV/AIDS. VEB now presents the current protocol, for a Second MHCS (MHCS-II), to better understand the natural history of HCV and to identify later consequences of HIV and its therapy.

2.0 Objectives

2.1 Estimate the prevalence and incidence of three major events -- liver decompensation, hepatocellular carcinoma, and non-Hodgkin lymphoma -- in persons with hemophilia (PWH) who are infected with HCV.

2.11 Estimate dates of initial HCV infections for all individuals with hemophilia and to apply these dates to clarify how age at HCV infection and duration of HCV infection each affect the events in Objective 2.1.

2.2 Estimate the magnitude of the effects of co-infection with HIV, co-infection with other viruses, and alcohol consumption on the HCV-related events in Objective 2.1.

2.3 Identify virologic, serologic or immunologic predictive markers of the HCV-related events in Objective 2.1, and define the longitudinal relationships of these markers to one another.

2.4 Identify host genes that confer substantial susceptibility or resistance to HCV infection and to the events in Objective 2.1.

2.5 Identify host genes that confer substantial susceptibility or resistance to HIV infection, to AIDS following HIV infection, and to major AIDS-related clinical conditions, particularly NHL.
2.6 Identify virologic, serologic or immunologic predictive markers of AIDS-related NHL, and define the longitudinal relationships of these markers to one another.

3.0 Design overview

3.1 We anticipate enrolling approximately 4560 subjects from approximately 50 comprehensive hemophilia centers into the MHCS-II. The “HCV cohort” will include approximately 4500 subjects with HCV, of whom 1500 will also have HIV. The “HIV cohort” will include the same 1500 subjects with HCV and HIV mentioned above, but they will be analyzed from the date of HIV infection, rather than from HCV infection. And a few subjects will be enrolled (approximately 60) with HIV but not HCV, to be evaluated for genetic resistance to HCV infection.

3.2 HCV cohort

The HCV cohort will include all enrolled subjects who have been infected with HCV. Based on MHCS-I, the cohort is expected to be 4% female and to have the following distribution of birth years:

<table>
<thead>
<tr>
<th>Birth Years</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Before 1930</td>
<td>2%</td>
</tr>
<tr>
<td>1930-39</td>
<td>5%</td>
</tr>
<tr>
<td>1940-49</td>
<td>12%</td>
</tr>
<tr>
<td>1950-59</td>
<td>19%</td>
</tr>
<tr>
<td>1960-69</td>
<td>23%</td>
</tr>
<tr>
<td>1970-79</td>
<td>23%</td>
</tr>
<tr>
<td>1980-89</td>
<td>16%</td>
</tr>
</tbody>
</table>

The natural time scale will be used, which is from the estimated date of HCV infection to an event of interest, death, or censoring (Figure 2, lower panel below). Time begins on the estimated date of HCV infection, but follow-up begins on the date of the first blood sample collected for the MHCS-I or -II.

Timelines for HIV (upper) and HCV (lower) cohorts in the MHCS-II *

* For each subject: Left arrow head = first blood sample; Right arrow head = end-of-followup
The anticipated overlap in the HCV cohort between MHCS-I and -II is shown in Figure 3 (Venn diagram, below). Person-years will be counted only from the date of follow-up (the first blood sample) to the date of an event of interest, death, or censoring. Survivorship bias should be low, as the incidence of disease is low during the first 20 years with HCV. The 15 years of data from MHCS-I serve to further reduce survivorship bias. The main outcomes are:

- liver decompensation (130 without HIV and 200 with HIV expected),
- hepatocellular carcinoma (10-15 without HIV and 5-10 with HIV expected), and
- non-Hodgkin lymphoma (10 without HIV and 65 with HIV expected).

Correlation analyses will focus on relationships among HCV viral load, anti-HCV levels, and other postulated markers of the main outcomes, such as serum cholesterol and levels of inflammatory cytokines. Fixed covariates will include age at HCV infection and host genetic polymorphisms. Time-dependent covariates will include HIV, highly active anti-retroviral treatment (HAART), alcohol consumption, and the postulated markers.

3.3 HIV cohort

The HIV cohort will include all enrolled subjects who have been infected with HIV. Based on MHCS-I, the cohort is expected to be 1% female and to have the following distribution of birth years:

<table>
<thead>
<tr>
<th>Birth Year Range</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1930</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>1930-39</td>
<td>1%</td>
</tr>
<tr>
<td>1940-49</td>
<td>10%</td>
</tr>
<tr>
<td>1950-59</td>
<td>23%</td>
</tr>
<tr>
<td>1960-69</td>
<td>35%</td>
</tr>
<tr>
<td>1970-79</td>
<td>28%</td>
</tr>
<tr>
<td>1980-89</td>
<td>3%</td>
</tr>
</tbody>
</table>

The natural time scale will be used, which is from the estimated date of HIV infection to an event of interest, death, or censoring (Figure 2, upper panel, page 5). Time begins on the estimated date of HIV infection, but follow-up begins on the date of the first blood
sample collected for the MHCS-I or -II. The anticipated overlap in the HIV cohort between MHCS-I and -II is shown in Figure 3 (Venn diagram, page 6). Person-years will be counted only from the date of follow-up (the first blood sample) to the date of an event of interest, death, or censoring. Survivorship bias is an important consideration, as the subjects who will enter in 2001 are HIV survivors. The 15 years of data from MHCS-I can be used to estimate the magnitude of survivorship bias. The primary interests, however, are late outcomes of HIV. There should not be substantial informative censoring, as subjects with one AIDS condition are still at risk for others, such as the main outcomes of interest in AIDS-associated non-Hodgkin lymphoma (100 expected).

Correlation analyses will focus on relationships among HIV viral load, CD4 counts, and other postulated markers of the main outcomes, such as serum cholesterol and levels of inflammatory cytokines. Fixed covariates will include age at HIV infection and host genetic polymorphisms. Time-dependent covariates will include HCV, highly active anti-retroviral treatment, alcohol consumption, and the postulated markers, such as plasma levels of interleukin 6 (IL-6). Nested case-control studies will be used for some analyses, such as detection of clonal 8;14 translocations and its relationship to non-Hodgkin lymphoma.

3.4 Genetic susceptibility to HIV or HCV infection
Rare but highly informative PWHs who were exposed to contaminated clotting factor concentrates but did not become infected will be enrolled and followed. Risk of infection will be calculated from exposure histories. Those whose probability of infection was greater than 90% will be defined as highly exposed negatives, for HCV, HIV, or possibly both. The effects on infection risk of candidate genes (CCR5, CD81, LDL) will be calculated, and genome scanning techniques will be used to identify new genes associated with the highly exposed negative status.

Hypothesis generating analyses will be undertaken to detect genes that affect control of HCV or HIV or their resulting diseases, using hemophilic siblings who are enrolled in the HCV or HIV cohorts and who are discordant on quantitative (e.g., viral load) or categorical outcomes (e.g., infection, AIDS, liver decompensation).

3.5 Central Laboratory Analyses and Special Studies
Anticoagulated peripheral venous blood from each participant will be collected annually. These will be separated into plasma and mononuclear leukocytes and cryopreserved in the central repository if the samples can be delivered overnight. Otherwise, particularly for foreign sites, the blood samples will be separated and frozen locally and subsequently shipped to the repository. HCV and HIV viral load and selected other assays will be performed by a central testing laboratory. Other uses of the blood samples will be approved upon review of scientific concepts (Attachment 1) for “Special Studies” as described in Subsection 6.23, below. These are expected to include a wide range of virologic, immunologic, chemical, and human genetic assays as required to achieve the Objectives stipulated in Section 2.0, above.
4.0 Eligibility

4.1 Must be registered patient with an inherited coagulation disorder at a participating MHCS-II center. Disorders include hemophilia A or B (congenital factor VIII or IX deficiency), deficiencies in other factors such as V or XI, and vonWillebrand’s disease. (Unless noted otherwise, all of the disorders will collectively be referred to as “hemophilia”.) All such hemophilia and vonWillebrand’s disease patients are to be recruited as study participants.

4.2 Since January 1, 1993, must have had at least one positive result on a licensed assay for HCV antibodies, HIV antibodies, or HIV RNA.

4.3 Must be at least 13 years of age at enrollment.

4.4 Must provide signed informed consent or, for minors, signed assent plus signed informed consent from the parent or guardian. Consent indicates understanding of the MHCS-II and, specifically, agreement to provide the data and blood samples as described in the consent form and below in the protocol.

5.0 Exclusion criteria

5.1 Is not a patient with an inherited coagulation disorder.

5.2 Does not have a positive test for HCV antibodies, HIV antibodies, or HIV RNA on a licensed assay performed since January 1, 1993.

5.3 Is less than 13 years of age.

5.4 Lacks informed consent/assent.

6.0 Operational overview

6.1 Overview and Centers
The MHCS-II is a prospective study of cohorts of all patients with inherited coagulation disorders who were infected with HCV or HIV and who are alive and registered at a participating comprehensive hemophilia treatment center between July 1 and December 31, 2000. The anticipated centers are listed in Attachment 2.

6.2 Organization, Responsibilities, and Scientific Concepts

6.21 The Principal Investigator (Dr. Goedert) and the Project Manager of Research Triangle Institute (RTI, Dr. Kroner), which is the prime contractor, have primary responsibility for the conduct of the MHCS-II.

6.22 Drs. Goedert and Kroner, 2-4 senior investigators of their choosing, and a lay advocate from the hemophilia community, will constitute the Executive Committee, which will have the responsibility for determining the policies and procedures of the MHCS-II.

6.23 The Executive Committee will invite 10-15 specialists in hemophilia, infectious diseases, epidemiology, and hepatology research to participate in the Steering Committee. The Steering Committee will have two responsibilities:
• to provide expert advice to the Executive Committee; and
• to review and recommend approval or disapproval of concepts submitted for particular analyses or special studies within the MHCS-II. Submitted concepts must be consistent with the objectives of the MHCS-II, should include multiple MHCS-II centers whenever feasible, should be proportional in length to the complexity and cost of the proposed analysis, and should include a preliminary list of first, senior (last), and co-authors of the anticipated manuscript(s).

6.24 The Co-Investigators will include a scientific or clinical collaborator from each participating hemophilia center, as well as specialists in infectious diseases, epidemiology, genetics, or hepatology, as approved by the Executive Committee.

6.25 The Executive Committee will invite 6-8 Co-Investigators to serve on the Publications Committee, which will be chaired by a member of the Executive Committee. The Publications Committee’s responsibility will be to review and approve scientific abstracts and manuscripts prior to submission for publication. This review will focus primarily on the validity of the work, the appropriateness of the named authors, inclusion of “... for the Second Multicenter Hemophilia Cohort Study” as a co-author, and inclusion of an Appendix with the names and affiliations of all MHCS-II co-investigators.

6.26 Other than the Principal Investigator and Project Manager (Drs. Goedert and Kroner) the members of the Executive Committee, the Steering Committee, and the Publications Committee will serve one-year terms which may be renewed.

6.3 Meetings

6.31 The Executive Committee will have a one-day in person semi-annual meeting, probably in the Washington/Rockville area, and will have monthly or ad hoc conference calls to review concepts or other matters arising.

6.32 The Steering Committee will have a one- or two-day in person annual meeting, probably in the Washington/Rockville area, or more frequently if summoned by the Executive Committee.

6.33 All Committee members and Co-Investigators will be invited to an initial two-day meeting in the Washington/Rockville area to discuss the objectives of the MHCS-II, the protocol, data and specimen collection, and other issues arising. One session will be devoted to the policies and procedures of the Publications Committee. Follow-up two-day meetings to discuss progress and new developments relevant to the MHCS-II objectives will be scheduled every 1 to 1½ years thereafter.
7.0 Recruitment and field operations

7.1 Data and Specimen Collection

7.11 Core data and a blood sample will be obtained from each participant at enrollment and (contingent upon sufficient funds) annually for three to four years thereafter. The core data will include abstracted items from the participant’s medical history (including factor concentrate use, Attachment 3), a targeted physical examination (Attachment 4), a self-administered questionnaire focused primarily on initial exposures to plasma and plasma products and on signs and symptoms of liver disease (Attachment 5), and a form for the results of locally performed hematology and serum chemistry tests (Attachment 6).

7.12 A 30mL peripheral venous blood sample will be drawn, of which 10mL will be used at the participating center to perform a complete blood count and platelet count; a prothrombin time and International Normalized Ratio (INR); and serum chemistry analysis for alanine aminotransferase; aspartate aminotransferase; total, direct, and indirect bilirubin; total cholesterol; albumin; and globulins. A (-glutamyl transeptidase ((-GTP) may be included if there are sufficient funds. The other 20mL will be drawn into two 10mL vacutainer tubes with acid-citrate dextrose (ACD) anticoagulant. Within the United States, the two ACD tubes must be packaged and shipped by overnight express carrier to a central repository. Outside of the United States, the ACD-anticoagulated blood must be separated and stored on site as 0.25mL aliquots of rapidly frozen plasma and viably cryopreserved mononuclear leukocytes (as many aliquots as possible with a minimum of 5x10^6 cells/aliquot). These aliquots will be shipped in a liquid-nitrogen dry shipper (to be provided by VEB) on a specified schedule to the central repository. All specimen shipments, domestic and international, will be packaged and shipped according to ICAO/IATA regulations and by a person trained in these procedures.

7.13 Data collection and phlebotomy generally will be performed during the course of a routine clinic visit, although this is not required. Local staff will enter the data (Attachments 3, 4, and 6) into a secure Web site established by the coordinating center. The Participant Questionnaire (Attachment 5) will be mailed to the coordinating center for data entry.

7.14 The annual follow-up visits will include the same blood sample (with shipping or local cryopreservation), laboratory data, physical examination, and abbreviated versions of Attachments 3 and 5. Participants who transfer between MHCS-II centers will be followed at the receiving center and will not be re-enrolled.

7.15 Formalin-fixed, paraffin-embedded tissue blocks will be obtained from all biopsies or resections of the liver, lymph node, and known or possible malignant tissue. Unstained microscopic slides will be sought if blocks are not available. The blocks and slides will be obtained only after the requisite diagnostic procedures for clinical care have been completed. They will be shipped to RTI and stored in the central repository for
future laboratory investigations of virus-host interactions, potential markers of cancer or cirrhosis, and the like.

7.16 A routine urine sample will be collected at enrollment and at each follow-up visit. The fresh urine will be tested by the MHCS-II nurse or coordinator with a Dipstix to detect and semi-quantify blood or protein that would be indicative of HCV-associated, type II mixed cryoglobulinemia.

7.2 Training, Data Management, Quality Control, and Corrective Actions

7.21 A designated MHCS-II coordinator, usually a nurse, for each center will be trained by Research Triangle Institute over the course of approximately two days at a central location in the Washington/Rockville area. Training will include:

• non-coercive recruitment of study participants;
• obtaining informed consent/assent;
• responsibility to assure the integrity and confidentiality of MHCS-II research records;
• review and clarification of all items in Attachments 3-6;
• phlebotomy procedures including universal biosafety precautions;
• required hematology and chemistry data;
• hard-copy data recording;
• storage of MHCS-II research records in secure, locked files at the center, particularly the linkage between participants’ names and their subject identification (ID) numbers;
• data entry into the secure Web site via the internet; and
• packaging and shipping of biospecimens according to ICAO/IATA regulations.

7.22 RTI will electronically edit the data transmitted by the coordinators for out-of-range or other invalid data. RTI will promptly return invalid data to the coordinator for correction, and they will tally and track error and response rates for each coordinator and center. The Executive Committee will determine rates of unsatisfactory performance that will lead to re-training of a coordinator, site visiting a center, replacement of a coordinator, or cancellation of a center’s participation in the MHCS-II.

7.23 RTI will monitor the yield and the quality of the fresh and frozen specimens received from each MHCS-II center, using standardized data provided by the central repository. The Executive Committee will review these data quarterly and will determine rates of unsatisfactory specimen yield or quality that would result in corrective actions. Such actions could include re-training of a coordinator, formal training of laboratory technical staff (for deficiencies in frozen samples), site visiting a center, replacement of a coordinator, requiring changes in laboratory procedures or responsible laboratory technical staff at the center, or cancellation of a center’s participation in the MHCS-II.

7.24 RTI will query every MHCS-II center semi-annually to assure that the security of the linkage between the participants’ names and their subject ID numbers has not been
compromised. Any suspected breach in the security of the research data or of the name-ID linkage will prompt an immediate site visit and whatever corrective action is deemed necessary.
8.0 Statistical analyses and power

8.1 Statistical Analysis

8.11 HCV cohort

Means [and 95% confidence intervals (CI)] for continuous data and frequency tables for categorical data will be used to describe the cohort. Contingency tables will be used with \( \Pi^2 \) or exact tests for comparisons of groups.

For most individuals in MHCS-II, the date of HCV infection is uncertain. However, an exposure window can be defined for each individual that incorporates information about each individual’s age at exposure, types of exposures, and dates of exposures. Imputation methods can be used to estimate the most likely date of infection within the window. The uncertainty of the imputation, and the consequent impact of this uncertainty on the conclusions, can also be assessed.

There are two types of exposures. The first type of exposure resulted from the use of whole-blood transfusion (pre-1950) or cryoprecipitate (1950-present) for the treatment of a bleeding episode. A whole blood transfusion reflects exposure to a single donor. In contrast, cryoprecipitate is derived by pooling blood from approximately 20 donors. These therapies were the treatment of choice for bleeding episodes that occurred prior to 1966/1968.

In 1966, Factor VIII concentrate was licensed for use in the United States. Factor IX concentrate was licensed in 1968. Neither type of concentrate was in widespread use until the early 1970s. Factor concentrates are derived from pooled donations of 20,000 or more donors. In 1990, HCV antibody testing and methods to inactivate viruses eliminated HCV contaminated blood or blood products from the blood supply. Until that time, however, each exposure to whole-blood or cryoprecipitate was potentially infectious because HCV infection had become established at relatively low levels in the general population. It is reasonable to assume that each treatment with cryoprecipitate carried on the order of a 1-2% chance of exposure to HCV (20 donors times 0.05-1.0% historical prevalence in the donor population). Furthermore, between 1966/1968 and 1990, any exposure to factor concentrate was remarkably infectious, and even a single exposure to concentrate was almost certain to result in infection.

The MHCS-II entry questionnaire will obtain a detailed exposure history for each subject. From these data, we will construct an exposure window \((F,L,X)\). The first quantity, \(F\), is the age at first exposure to whole blood or cryoprecipitate. The second quantity, \(L\), is the smaller of the following: age at last exposure to cryoprecipitate, age at first exposure to factor concentrates, or age in 1990). The last quantity, \(X\), is an indicator variable equal to 1 if the subject was ever exposed to factor concentrate between 1966/1968 and 1990, 0 otherwise.
The naïve imputation for the age at infection \( \hat{a} \) is defined by the following rule: \( \hat{a} = L \) if \( X = 1 \); \( \hat{a} = (F + L)/2 \) if \( X = 0 \). In words, the imputed age of infection is the age at first exposure to concentrate, if the subject ever used concentrate, otherwise is the midpoint age of potentially infectious exposures to whole blood or cryoprecipitate.

We will also construct a more refined assessment using data on exposures by age. In this more refined approach, we will incorporate information on the amount of use of whole blood or cryoprecipitate between ages \( F \) and \( L \) inclusive. With this additional information, we will derive maximum likelihood estimates of the age at exposure under the assumption that the hazard of infection due to whole blood or cryoprecipitate is directly proportional to the amount of exposure.

With either approach, estimation of the age at exposure will be subject to uncertainty. We will evaluate the impact of this uncertainty using sensitivity analyses. For subjects unexposed to concentrate, these sensitivity analyses will set all imputed values to the earliest and latest possible dates for each subject, e.g., to \( L \) and to \( F \). If the results are sensitive to these perturbations, we will evaluate whether sophisticated multiple imputation methods can lead to more informative analysis.

It should be noted that objectives 2.2–2.6 do not involve age-at-infection per se, although in the analysis we may wish to control for age-at-infection. It is reasonable to assume that hemophilic subjects who were born in the same year and who had a similar degree of severity of their hemophilia (i.e., need for cryoprecipitate or concentrates) probably faced similar hazards of infection. This assumes that much of the risk of infection reflects common secular trends in prevalence of HCV in the donor pool, uniform access to factor concentrates once these become widely available in the early 1970s, and a consistent age-dependent peak in treatment use during middle childhood. Therefore, for these objectives, in addition to analysis based on imputed dates, we will also perform stratified analyses based on strata defined by the intersection of birth cohort and severity of hemophilia.

The three main events of interest are liver decompensation, hepatocellular carcinoma, and non-Hodgkin lymphoma. Liver decompensation and hepatocellular carcinoma involve the same organ and, given the lack of effective therapy, both are highly lethal events. Thus, a subject is very unlikely to have both conditions diagnosed. Therefore, standard methods for censoring follow-up will be used when either of these events occurs. In contrast, non-Hodgkin lymphoma infrequently involves the liver and may be effectively treated. Thus, follow-up for possible liver decompensation or hepatocellular carcinoma will not be censored for subjects who develop a non-Hodgkin lymphoma. No informative censoring among these events is anticipated, but alternative events, such as death from any cause, also will be evaluated. Primary analysis of each event will use the Kaplan-Meier product-limit method, modified to begin follow-up on the date of the first blood sample collected for the MHCS-I or -II. Time will be measured from the estimated date of HCV infection to the earliest of the date of the event, the date of last follow-up, or the date of death. Cumulative incidence rates (95% CI) during follow-up of each outcome, comparing groups who were infected with HCV at 10, 20, 30, and 40 years previously. For this analysis, we will use the Nelson-Aalen estimator, which is preferred
over Kaplan-Meier for extreme patterns of late entry. Differences in incidence rates among n discrete categorical groups, such as HCV genotypes, will be determined with a n-1 degree-of-freedom (df) log-rank test. Ordered groups, such as age group at infection, will be compared with a 1 df log-rank trend test.

Cox proportional hazards models will be developed using the same time scale to evaluate and compare the contributions of multiple variables. Log-log plots from the Kaplan-Meier output will be examined for each variable to check for any violation of the proportional hazards assumption of the model. For any variable that does violate the assumption, models will be developed piecewise in time to evaluate differing effects of the covariates on early compared to late hazards. Variables that do not change over time, such as age at HCV infection and genetic polymorphisms such as in IL-6-174C and SDF1-3'A, will be treated as fixed covariates.

Variables that change over time will be evaluated as time-dependent covariates. The most important of these are HIV seroconversion status (0 until HIV seroconversion, 1 thereafter), the date of detection of clonal c-myc (8;14) translocation in peripheral blood lymphocytes (for non-Hodgkin lymphoma), and the dates and values of potential markers of the events, such as HCV antibody levels (for liver decompensation or hepatocellular carcinoma). Sensitivity analyses will be performed, particularly with multiple imputation of the HCV infection dates because of they may be imprecise in some subjects.

8.12 HIV cohort

Similar methods to those described immediately above for the HCV cohort will be used for the HIV cohort, albeit with the following important differences. The main event of interest is AIDS-associated non-Hodgkin lymphoma. Other AIDS-defining diseases and death from any cause also will be evaluated, but no informative censoring among these events is anticipated. As for Kaplan-Meier and proportional hazards analyses of the HCV cohort, follow-up will begin on the date of the first blood sample collected for the MHCS-I or -II. However, for the HIV cohort, time will be measured from the estimated date of HIV seroconversion to the earliest of the date of lymphoma, the date of last follow-up, or the date of death. The most important fixed covariates will be genetic polymorphisms, such as IL-6-174C, CCR5(32 and SDF1-3'A, and age at HIV seroconversion. Duration of HCV infection prior to HIV seroconversion will be considered as a fixed covariate, but it is not expected to contribute to the hazard of AIDS-associated non-Hodgkin lymphoma. The most important time-dependent covariates will be the dates and values of plasma levels of IL-6 and of chemokine ligands of CXCR4 such as SDF-1 and RANTES, and especially the dates of initiation of HAART and of HAART failure (defined as recurrent HIV viremia), each of which may have substantial effects on the risk of AIDS-associated non-Hodgkin lymphoma. Nested case-control studies will be done for certain markers, such as the risk of non-Hodgkin’s lymphoma with detection of clonal c-myc (8;14) translocation in peripheral blood lymphocytes.
8.13 Genetic susceptibility to HIV or HCV infection

For HIV, we have defined high exposure as use of plasma products associated with a greater than 90% probability of infection. Studies of PWHs who escaped HIV infection despite such exposure led to the discovery of the CCR5\(32\) polymorphism, the critical role of CCR5 as the primary co-receptor for HIV and the consequences of HIV infection despite CCR5\(32\) homozygosity.\(^{18-20}\) Proposals for collaborative laboratory studies will be encouraged and performed selectively to elucidate postulated mechanisms of resistance to HIV infection, including mechanisms other than CCR5\(32.\)^{21,22}

For HCV, studies have shown an almost 100% incidence of infection after the first infusion of unsterilized clotting factor concentrates.\(^{23,24}\) Thus, any PWH who used one of these plasma products will be defined as highly exposed to HCV. Proposals for collaborative laboratory studies such as those performed for HIV will be encouraged and performed selectively. Polymorphic candidate genes that are likely to affect resistance to HCV infection or the probability of HCV clearance, such as candidate HCV receptors (CD81 and LDL) are examples.

We previously demonstrated concordance between HLA haplotypes and AIDS incidence in HIV-infected sibling pairs.\(^{25}\) With HCV but not HIV, the incidence of liver decompensation is expected to be low. Thus, the primary emphasis will be a hypothesis generating effort to detect genes associated with discordant HCV viral loads in sibling pairs. Linkage analysis to candidate and anonymous (snp) genetic markers will be evaluated with discordant sibpair linkage analyses or with the discordant sibling transmission disequilibrium test.\(^{26,27}\) With an anticipated 180 sibling pairs with HCV but not HIV, we would have modest power for a discrete outcome but more substantial power on a continuous “trait”, that is, difference in HCV viral load.

8.2 Statistical Power

8.21 HCV cohort

The main outcomes are:
- liver decompensation (130 without HIV and 200 with HIV expected),
- hepatocellular carcinoma (10-15 without HIV and 5-10 with HIV expected), and
- non-Hodgkin lymphoma (10 without HIV and 65 with HIV expected).

Relevant to the first part of Objective 2.3, “Identify virologic, serologic or immunologic predictive markers of these HCV-related events…” the following table indicates the minimum relative risks that can be detected among the subjects with HCV but not HIV. We assume that 2400 (80%) of the 3000 subjects will be at risk by virtue of persistent HCV infection (i.e., detectable HCV viremia) and that loss to follow-up will be 10% (240) over four years, leaving an effective cohort size of 2160 subjects followed for four years.

<table>
<thead>
<tr>
<th>Probability of event</th>
<th>Minimum relative risk detectable with (\forall=0.05), (\exists=0.80)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

16
This table indicates that the size of the HCV cohort (without HIV) is sufficient to detect moderate associations (about 2-fold) with liver decompensation, particularly at the likely cumulative rate (0.06) or at the conservative rate (0.03) for relatively common exposures. Only stronger associations can be detected for less common exposures (2.6- to 3.5-fold) at the conservative rate. For the two malignancies, (hepatocellular carcinoma and non-Hodgkin lymphoma without HIV/AIDS), strong associations (3.5- to 4-fold) can be detected for common exposures, but only extremely strong effects (>6-fold) can be detected if the prevalence of the exposure is 10% or less.

For the second part of Objective 2.3, to “… define the longitudinal relationships of these markers to one another”, differences in candidate markers that are on a continuous scale (such as HCV viral load) will be tested with a comparison of means. The example in the table below presents the power to detect a difference in HCV viral load (either at entry or in change over four years) by fixed covariates that are present in 10% or 2% of the cohort (such as black race, female sex, or an autosomal dominant gene). As above, of the 3000 subjects in the HCV cohort without HIV, 20% (600) are assumed to have cleared the infection, leaving 2400 for comparison of viral load among those with persistent infection. With persistent HCV infection HCV viral load is assumed to have mean 5.0 log₁₀ equivalents/mL, standard deviation (SD) 0.5 log₁₀ equivalents/mL in the population. For change in viral load over four years, SD=0.71 log₁₀ equivalents/mL/yr, and an 8% loss-to-follow-up is assumed.

<table>
<thead>
<tr>
<th>HCV load</th>
<th>Group</th>
<th>No.</th>
<th>Postulated mean</th>
<th>Power (α=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>at enrollment</td>
<td>wild-type</td>
<td>2160</td>
<td>5.0 log₁₀ equivalents/mL</td>
<td>0.84</td>
</tr>
<tr>
<td>at enrollment</td>
<td>candidate</td>
<td>240</td>
<td>5.1 log₁₀ equivalents/mL</td>
<td></td>
</tr>
<tr>
<td>change over 1 year</td>
<td>wild-type</td>
<td>2160</td>
<td>0 log₁₀ equivalents/mL/yr</td>
<td>0.54</td>
</tr>
<tr>
<td>change over 1 year</td>
<td>candidate</td>
<td>240</td>
<td>0.1 log₁₀ equivalents/mL/yr</td>
<td></td>
</tr>
<tr>
<td>change over 1 year</td>
<td>wild-type</td>
<td>2160</td>
<td>0 log₁₀ equivalents/mL/yr</td>
<td>0.87</td>
</tr>
<tr>
<td>change over 1 year</td>
<td>candidate</td>
<td>48</td>
<td>0.15 log₁₀ equivalents/mL/yr</td>
<td>0.16</td>
</tr>
<tr>
<td>change over 1 year</td>
<td>wild-type</td>
<td>2160</td>
<td>0 log₁₀ equivalents/mL/yr</td>
<td>0.50</td>
</tr>
<tr>
<td>change over 1 year</td>
<td>candidate</td>
<td>48</td>
<td>0.2 log₁₀ equivalents/mL/yr</td>
<td></td>
</tr>
<tr>
<td>change over 1 year</td>
<td>wild-type</td>
<td>2160</td>
<td>0 log₁₀ equivalents/mL/yr</td>
<td>0.83</td>
</tr>
<tr>
<td>change over 1 year</td>
<td>candidate</td>
<td>48</td>
<td>0.3 log₁₀ equivalents/mL/yr</td>
<td></td>
</tr>
</tbody>
</table>
The table indicates that the cohort of subjects with HCV but not HIV has excellent power to detect small differences in baseline HCV viral load (0.1 log\textsubscript{10} equivalents/mL). For longitudinal changes in HCV viral load, the cohort is sufficiently large to detect relatively large changes (0.15 log\textsubscript{10} equivalents/mL/yr) for a candidate marker with 10% prevalence. For rarer markers (2%), only extremely large changes (0.3 log\textsubscript{10} equivalents/mL/yr) will be detected.

8.22 HIV cohort

In the cohort of subjects with HIV infection, the main outcome of interest is AIDS-associated non-Hodgkin lymphoma (65 cases expected). Relevant to Objective 2.6, to “Identify virologic, serologic or immunologic predictive markers of AIDS-related NHL….”, the following table indicates the minimum relative risks that can be detected among the subjects with HIV. We assume that all subjects are at risk and that loss to follow-up will be 10% (150) over four years, leaving an effective cohort size of 1350 subjects followed for four years.

<table>
<thead>
<tr>
<th>Probability of event in unexposed subjects over four years</th>
<th>Minimum relative risk detectable with ( \forall=0.05 ), ( \exists=0.80 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probability of exposure</td>
</tr>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>0.02 (NHL, likely)</td>
<td>2.44</td>
</tr>
<tr>
<td>0.01 (NHL, conservative)</td>
<td>3.17</td>
</tr>
</tbody>
</table>

For continuous markers split at quartiles, the following table shows the power to detect a significant association (with \( \forall=0.05 \), \( \exists=0.80 \)) in the cohort of 1350 HIV-infected subjects at two cumulative event rates (0.01 and 0.02) and at four relative risks (\( e^\exists \), ranging from \( e^{0.2} \) to \( e^{0.5} \)) per quartile.

<table>
<thead>
<tr>
<th>Baseline Rate</th>
<th>Relative risk per quartile</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1.22</td>
<td>0.15</td>
</tr>
<tr>
<td>0.01</td>
<td>1.35</td>
<td>0.33</td>
</tr>
<tr>
<td>0.01</td>
<td>1.49</td>
<td>0.60</td>
</tr>
<tr>
<td>0.01</td>
<td>1.65</td>
<td>0.86</td>
</tr>
<tr>
<td>0.02</td>
<td>1.22</td>
<td>0.26</td>
</tr>
<tr>
<td>0.02</td>
<td>1.35</td>
<td>0.57</td>
</tr>
<tr>
<td>0.02</td>
<td>1.49</td>
<td>0.88</td>
</tr>
<tr>
<td>0.02</td>
<td>1.65</td>
<td>0.99</td>
</tr>
</tbody>
</table>
The first table indicates that the size of the HIV cohort is sufficient to detect moderately strong associations (2.5-fold to 2.7-fold) for relatively common exposures that may be associated with AIDS-associated NHL, provided that the baseline (“unexposed”) event rate is at least 0.02 (27 cases). In the unlikely event that the baseline rate is half as large (0.01), only stronger associations (3.2-fold to 3.6-fold) with AIDS-NHL will be detected. Likewise, only very strong associations (>3.8-fold) can be detected with uncommon exposures.

8.23 Genetic susceptibility to HCV infection

For power, we considered two outcomes of interest among subjects with HCV but not HIV. First, we postulate a co-dominant and then a recessive trait associated with clearance of (i.e., recovery from) HCV viremia. This is expected in 600 (20%) of the 3000 subjects without HIV in the HCV cohort. The following table (with $\forall=0.05$, $\exists=0.80$) indicates the power to detect associations at various allele frequencies and relative risks ($e^{0.3}$ to $e^{1.0}$).

<table>
<thead>
<tr>
<th>Genetic effect</th>
<th>Allele frequency</th>
<th>Relative risk</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-dominant</td>
<td>0.3</td>
<td>1.65</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.65</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>1.35</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.35</td>
<td>0.85</td>
</tr>
<tr>
<td>Recessive</td>
<td>0.2</td>
<td>1.65</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>2.72</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.65</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.35</td>
<td>0.79</td>
</tr>
</tbody>
</table>

This indicates that the study has excellent power to detect an effect on HCV clearance of dominant genes with an allele frequency of at least 10% and of recessive genes with an allele frequency of 50%. The power is low for detecting associations with recessive genes with an allele frequencies of 20% or less (that is, 4% homozygote recessives) unless the association is very strong.

The second outcome of interest is genetic resistance to HCV infection. A very strong recessive association is postulated, as seen with CCR532 homozygosity and resistance to HIV infection. We postulate that 2% of HCV-exposed hemophiliacs escaped HCV infection because of genetic resistance. Thus, we will compare 60 HCV-seronegative and 1500 HCV-seropositive subjects, all of whom have HIV infection. As shown in the following table (with $\forall=0.05$, $\exists=0.80$), even with relatively common alleles (10% to 20%) we only expect to have sufficient power to detect recessive genes that have tremendously large relative risks ($e^{2.0}$ to $e^{4.0}$).
<table>
<thead>
<tr>
<th>Genetic effect</th>
<th>Allele frequency</th>
<th>Relative risk</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive</td>
<td>0.2</td>
<td>7.4</td>
<td>0.71</td>
</tr>
<tr>
<td>“</td>
<td>0.2</td>
<td>12.2</td>
<td>0.78</td>
</tr>
<tr>
<td>“</td>
<td>0.2</td>
<td>20.1</td>
<td>0.82</td>
</tr>
<tr>
<td>“</td>
<td>0.2</td>
<td>33.1</td>
<td>0.85</td>
</tr>
<tr>
<td>“</td>
<td>0.2</td>
<td>54.6</td>
<td>0.88</td>
</tr>
<tr>
<td>“</td>
<td>0.1</td>
<td>12.2</td>
<td>0.52</td>
</tr>
<tr>
<td>“</td>
<td>0.1</td>
<td>20.1</td>
<td>0.57</td>
</tr>
<tr>
<td>“</td>
<td>0.1</td>
<td>33.1</td>
<td>0.60</td>
</tr>
<tr>
<td>“</td>
<td>0.1</td>
<td>54.6</td>
<td>0.62</td>
</tr>
</tbody>
</table>

This indicates that the study will have sufficient power to detect extremely strong associations with resistance to HCV infection at allele frequencies similar to CCR532.

9.0 Personnel and Authorship

This project includes collaborating investigators from the National Cancer Institute, Research Triangle Institute, the MHCS-II centers, and other groups. Prior to submission for publication, scientific abstracts and manuscripts must be provided by FAX or email to RTI for immediate forwarding to the Publications Committee and to the National Cancer Institute for review and approval. The review by both entities will include the validity of the work, the appropriateness of the named authors, and the attribution of the work to the MHCS-II as well as to the authors’ institutions.

10.0 Human Participants Protection

10.1 Multiple or Single Project Assurance and Institutional Review Board (IRB) Approval

The MHCS-II does not administer treatment or conduct interventions. The MHCS-II is an observational study and thus of low risk to the study participants. Nonetheless, an institution may not enroll patients as study participants until the protocol and consent/assent forms have been reviewed and approved by its Institutional Review Board (IRB) which is operating under a Multiple Project Assurance document or Single Project Assurance document approved from the U.S. Office of Human Research Protection (OHRP).

10.2 Role of the National Cancer Institute and RTI IRBs

The National Cancer Institute’s Special Studies Institutional Review Board (SSIRB) and the RTI IRB will review and approve the protocol and template consent/assent forms
The SSIRB and the NIH Office of Human Subjects Research will facilitate Single Project Assurance documentation for those institutions (mostly non-U.S.) that have not been approved by OHRP for a Multiple Project Assurance.

10.3 Retention, Protection, and Use of Identifying Information

Names and other identifying information will be retained only by the MHCS-II center, not by RTI or the National Cancer Institute. Participants’ data and blood samples will be labeled with unique subject identification (ID) code numbers that can be linked to names only by the MHCS-II center (as described in section 7.21, above). For participants who are lost-to-follow-up by the center (defined as unable to locate by telephone or mail), names and other identifying information will be provided to RTI (but not to the National Cancer Institute) for ascertainment of vital status and cause of death through the National Death Index, for ascertainment of cancer through population-based cancer registries, and for possible tracing for future studies. Identifying information will not be provided to RTI for participants who continue to be followed by the MHCS-II center.

10.4 Confidentiality of Data

All collected data will be kept confidential to the extent permitted by law, as described in section 7.24, above. As described in section 7.21, above, the coordinator at each MHCS-II center will be trained in the requirement for maintaining confidentiality of the data and in the consequences of a breach in confidentiality. In addition, a Certificate of Confidentiality to limit the possibility of forced disclosure under subpoena or other legal action will be requested through the National Cancer Institute.

10.5 Access to Laboratory Assay Results by Participants

10.51 The results of hematology, coagulation, and chemistry assays performed by a participant’s own center are of potential clinical value for individual participants. These locally generated test results will be linked to subject ID numbers and will be reported back to the MHCS-II center through the secure Web site.

10.52 The results of other assays, excluding human genetic testing, that are of potential clinical value also will be reported back to the MHCS-II center through the secure Web site, but only if the assays were performed in a laboratory certified under the Clinical Laboratory Improvement Amendments (CLIA). Such results generally will not be available for at least 8 weeks.

10.53 The locally and centrally generated laboratory data reported to the MHCS-II center may be reported to individual participants at the discretion of the local co-investigator.
10.54 Assay results on individuals that will not be reported back to the MHCS-II center include all of the following:
I. assay results generated by laboratories that are not CLIA certified;
II. assay results with no demonstrated clinical application; and
III. human genetic (DNA) results.

Thus, the MHCS-II center will not be able to link such data to individual identifying information.

10.55 Because the MHCS-II data base will constitute a “Federal System of Records” as defined by the Federal Privacy Act, a participant does not relinquish his right to obtain all of his own test results, including those in section 10.54, above. To obtain all of his own laboratory test results, a participant (or his parents or guardian) will be required to submit a written request to the MHCS-II center. The center will send a copy of this written request to the Principal Investigator (Dr. Goedert) after the participant’s subject ID number has been added and his name and other identifying information redacted. Dr. Goedert will instruct RTI to generate a report of all of the participant’s test results, linked to the subject ID number. Dr. Goedert will send this report to the co-investigator at the MHCS-II center, who will verify the linkage to the requesting participant’s name and will verify that the request has been fulfilled.

10.6 Unbiased Recruitment

All eligible participants will be recruited without bias. Because hemophilia A and hemophilia B are X-linked congenital disorders, the vast majority of participants will be male. Approximately equal numbers of male and female participants with vonWillebrand’s disease are anticipated. Adolescent children will be included, but very few children under age 15 are expected because there were extremely few HIV or HCV transmissions through factor concentrates after 1985.

10.7 Rights of Refusal and Withdrawal

Patients will be free to refuse enrollment, and participants will be free to withdraw at any time. Participants will be allowed to skip individual questions on the self-administered questionnaire (Attachment 5), such as sexual activity or alcohol use. However, partial consent will not be permitted. Thus, unwillingness to have assays performed that are critical to achieving the objectives of the MHCS-II, including testing for HCV, HIV, and human genetic polymorphisms that are postulated to affect the events of interest, will result in a patient being refused enrollment and in the administrative withdrawal of an enrolled participant.

10.8 Risk/benefit assessment for minor participants
Risk of participation is minimal for both minor and adult participants. Benefits are modest, including:

- annual HIV viral load on each individual HIV infected participant (retail cost $50 per assay);
- annual HCV viral load on each individual participant (retail cost $75 per assay);
- consolidated on-line summary of clinically performed liver function tests on each individual participant;
- consolidated on-line summary of all physical examination and medication data on each individual participant;
- immediate access to state-of-the-art information on HCV, HIV, and their related clinical conditions for each participant’s clinician, providing a context for considering each individual participant’s situation.

11.0 Protocol Attachments

1) Special Studies Concept Form
2) List of anticipated participating centers
3) Core Abstract Form
4) Physical Exam Form
5) Participant Questionnaire
6) Laboratory Form
7) Template Consent Forms
8) Template Assent Form

12.0 References


11 International Collaboration on HIV and Cancer. The impact of highly active anti-retroviral therapy on the incidence of cancer in adults infected with the human immunodeficiency virus: collaborative reanalysis of individual data on 49,228 HIV-infected adults from 11 cohort studies in developed countries. J National Cancer Inst (In press).


