Clinical and Translational Science Institute Prospective Longitudinal Assessment of Coinfected Subjects with HIV/Hepatitis C for Endothelial Function Study (CTSI-PLACE Study)

A Prospective, Longitudinal Study of Endothelial Function in HIV/HCV Coinfected Subjects

Sponsored by:

UCLA AIDS Institute
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STUDY MANAGEMENT

All questions concerning this protocol should be sent to the study Co-Chairs (Kara Chew, Debika Bhattacharya, David Hardy, Eric Daar, and Wilbert Jordan) via e-mail. The team members should generally respond within 48 hours.

Clinical Management
For questions concerning entry criteria, toxicity management, concomitant medications, and coenrollment, contact the protocol co-chairs via email. Include the protocol name, patient identification number (PID), and a brief relevant history.

Laboratory/Peripheral arterial tonometry
For questions specifically related to the protocol-specific assays or procedures, investigators should contact the co-chairs via email.

Data Management
For nonclinical questions about inclusion/exclusion criteria, case report forms (CRF), the CRF schedule of events, delinquencies, REDCap data entry, and other data management issues, contact Kara Chew (kchew@mednet.ucla.edu).

- Include the PID and a detailed question.

Subject Registration
For subject registration questions or problems:

- Send an e-mail message to Kara Chew (kchew@mednet.ucla.edu) and Debika Bhattacharya (debikab@mednet.ucla.edu).

REDCap/Database/Data Entry Problems
Contact Kara Chew (kchew@mednet.ucla.edu) or Martin Lai, Informatics Core Manager, UCLA Clinical & Translational Research Center (email mylai@mednet.ucla.edu, phone 310-794-9396).

Protocol Document Questions
For questions concerning the protocol document, contact the study Co-Chairs.

Phone Calls
Sites are responsible for documenting any phone calls made to protocol team members.

PROTOCOL-SPECIFIC GLOSSARY OF TERMS

ACE-I	angiotensin-converting enzyme inhibitor
ANC	absolute neutrophil count
AI	AIDS Institute
AE	adverse events
ALT	alanine aminotransferase
ARB	angiotensin-receptor blocker
ART	antiretroviral therapy
AST	aspartate aminotransferase
CAD	coronary artery disease
CAP	College of American Pathologists
CCB	calcium-channel blocker
CDU	Charles Drew University
CFAR	Center for AIDS Research
CHD	coronary heart disease
CI	confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case Report Form
CTSI	Clinical and Translational Science Institute
CVD	cardiovascular disease
DAA	direct acting agent (ie antiviral drug)
EAE	expedited adverse event
EC	ethics committee
EOT	end of treatment
FLP	fasting lipid panel
GLP	good laboratory practice
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HDL	high-density lipoprotein cholesterol
HOMA-IR	Homeostasis Model of Assessment - Insulin Resistance
hsCRP	high-sensitivity C-reactive protein
IATA	International Air Transport Association
ICF	informed consent form
ICH	International Conference on Harmonisation
IFN	interferon
IL-6	interleukin-6
INR	international normalized ratio
IRB	institutional review board
LAB	LA Biomed/Harbor-UCLA
LDL	low-density lipoprotein cholesterol
LPC	Laboratory Processing Chart
Lp-PLA2	lipoprotein-associated phospholipase A2
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OI	opportunistic infection
PAT	peripheral arterial tonometry
PEG-IFN	pegylated interferon

RBV	ribavirin
RHI	reactive hyperemia index
RNA	ribonucleic acid
SAE	serious adverse event
sICAM-1	soluble ICAM-1
sP-selectin	soluble P-selectin
SVR	sustained virologic response
TC	total cholesterol
TG	triglycerides
WBC	white blood cell
UCLA-WW	UCLA-Westwood

SCHEMA

Clinical and Translational Science Institute Prospective Longitudinal Assessment of Coinfected Subjects with HIV/Hepatitis C for Endothelial Function Study (CTSI-PLACE Study)

DESIGN — Prospective, observational, longitudinal cohort of HIV/HCV coinfected subjects matched to HIV monoinfected controls with primary cross-sectional analysis of baseline endothelial function and circulating cardiovascular biomarkers.

DURATION — Subjects will be on study for a minimum of 52 weeks. Those who initiate HCV treatment will be on study for at least 24 weeks after end of HCV treatment.

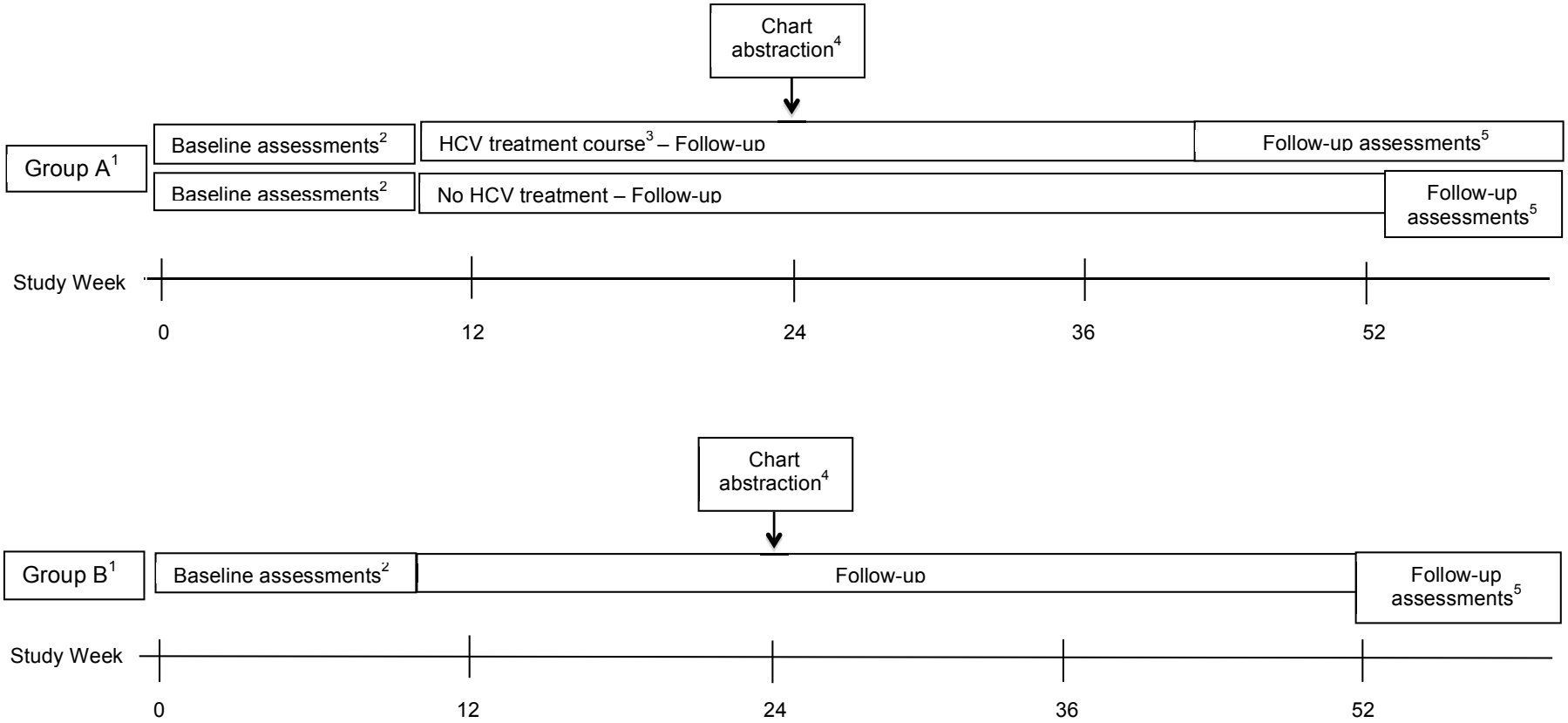
SAMPLE SIZE — A total of 120 subjects will be enrolled in the study (80 HIV/HCV coinfected and 40 HIV monoinfected subjects).

POPULATION — Group A: 80 HIV/HCV coinfected subjects
Group B: 40 HIV monoinfected subjects

All subjects ≥ 18 years of age without known CVD at baseline with CD4 cell count ≥200 cells/mm^3 and HIV-1 RNA <50 copies/mL within 120 days of study entry and on stable antiretroviral therapy (ART) since the time of last CD4 and HIV-1 RNA measurement. Those with a HCV infection must have HCV RNA ≥ 10,000 IU/mL. The two groups will be frequency matched based upon age, race, sex, diabetes, smoking status, hormonal therapy and statin use, and CD4 cell count at baseline.

PROCEDURES — Group A (HIV/HCV coinfected)
Baseline: All subjects will undergo baseline endothelial function testing and laboratory assays on study entry.
Follow-up: Subjects who do not initiate HCV treatment will have follow-up endothelial function and laboratory testing at 1 year. Subjects who initiate HCV treatment will have follow-up endothelial function and laboratory testing at 4 weeks after completing HCV treatment.

Group B (HIV monoinfected)
Baseline: All subjects will undergo baseline endothelial function testing and laboratory assays on study entry.
Follow-up: All subjects will have follow-up endothelial function and laboratory testing at 1 year.



[1] Group A, HIV/HCV coinfected; Group B, HIV monoinfected
[2] Baseline assessments as described in section 6.1, including vital signs, medical history, medications, medical record abstraction, serum/plasma collection and processing for laboratories and storage, and PAT testing
[3] HCV treatment may be initiated at any point following study entry
[4] The chart abstraction visit will consist of data abstraction by study staff from the medical record for the preceding 6 months (from time of study entry).
[5] Follow-up assessments as described in section 6.1. PAT and biomarker testing at 1 year for all subjects except those that initiate HCV treatment. For those that initiate HCV treatment, PAT and biomarker testing should occur at 4 weeks after end of HCV treatment. HCV-treated subjects should then continue to be followed until 24 weeks after end of HCV treatment and HCV viral load at 12 and 24 weeks after end of HCV treatment recorded (through medical record abstraction).

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Hypotheses:

1.1.1 HCV is additive with HIV in promoting CVD risk through increased immune activation and inflammation.

1.1.2 Markers of endothelial function, inflammation, and thrombosis that are not produced by the liver will provide more accurate and novel biomarkers for assessment of CVD risk in HIV/HCV coinfected persons.

1.1.3 Treatment of HCV in HIV/HCV coinfected persons will improve endothelial function and reduce chronic immune activation and inflammation.

1.2 Primary Objectives

1.2.1 To establish a UCLA CTSI-wide cohort of demographically diverse HIV/HCV coinfected and matched HIV monoinfected men and women with HIV suppressed on stable antiretroviral therapy (ART).

1.2.2. To determine the effect of HCV coinfection on endothelial function in the HIV-1-infected cohort at baseline.

1.2.3 To assess whether non-hepatic biomarkers of endothelial dysfunction, inflammation, and thrombosis (sICAM-1, sE-selectin, Lp-PLA2, IL-6, and D-dimer) are better correlates of endothelial function than traditional hepatic-derived biomarkers (hsCRP and lipids) in HIV/HCV coinfected in cross-sectional assessment at baseline.

1.3 Secondary Objectives

1.3.1 To determine the effect of HCV coinfection on endothelial function in the HIV-1-infected cohort over one year of follow-up.

1.3.2 To assess whether non-hepatic biomarkers of endothelial dysfunction, inflammation, and thrombosis (sICAM-1, sE-selectin, Lp-PLA2, IL-6, and D-dimer) are better correlates of longitudinal endothelial function than traditional hepatic-derived biomarkers (hsCRP and lipids) in HIV/HCV coinfected persons

1.3.3 To determine the effect of HCV coinfection on metabolic parameters (insulin resistance, cholesterol) in the HIV-1 infected cohort at baseline.

1.3.4 To determine the effect of HCV coinfection on metabolic parameters (insulin resistance, cholesterol) in the HIV-1 infected cohort over one year of follow-up.

1.4 Exploratory Objectives

1.4.1 To explore the effect of HCV viremia on endothelial function in HIV/HCV coinfected persons.

1.4.2 To explore the effect of HCV viremia on circulating CVD biomarkers in HIV/HCV coinfected persons.

1.4.3 To explore the effect of HCV viremia on metabolic parameters (insulin resistance, cholesterol) in HIV/HCV coinfected persons.

1.4.4 To explore the effect of HCV coinfection on circulating plasma levels of endothelial, platelet, and T-cell microparticles in HIV-infected persons.

2.0 INTRODUCTION

2.1 Background

Hepatitis C virus (HCV) infection is a leading cause of morbidity and mortality among those with HIV-1. Non-liver related morbidity and mortality, including cardiovascular outcomes, are poorly characterized in HIV/HCV coinfection. All-cause mortality is increased in HIV patients with HCV coinfection, independent of HIV disease progression.[1] Not only liver-related, but non-liver related deaths may be reduced with HCV treatment.[2,3] However, non-liver related outcomes in HIV/HCV coinfection remain poorly characterized. Large retrospective observational studies have suggested an increased risk of CVD events in HCV-infected individuals, both with and without HIV infection.[4-6] The contribution of HCV to elevated CVD risk in HIV patients is unknown. Multiple potential mechanisms for increased CVD risk with HCV infection exist, including hepatic steatosis, insulin resistance, and diabetes mellitus.[7,8] Chronic immune activation, implicated in HIV-associated accelerated atherosclerosis, is further increased with HCV coinfection.[9,10]

Endothelial function, a predictor of CVD risk, has not been adequately characterized in HIV/HCV coinfection. Endothelial dysfunction mediates development and progression of atherosclerosis.[11] HIV-infected patients have impaired endothelial function compared to non-HIV-infected patients.[12] In a single small cross-sectional analysis by Masia et al, no difference was seen in endothelial function measured by brachial flow-mediated dilation (FMD) in HIV/HCV coinfected versus HIV monoinfected persons, but interpretation of their findings is limited by confounding related to inconsistent HIV suppression, which has a clear impact on these measures.[13] Peripheral arterial tonometry (PAT) is an established, non-invasive method for assessment of endothelial function that has been shown to both correlate with known CVD risk factors and predict CVD risk in multiple non-HIV infected populations.[14-16] Its characteristics of reproducibility and reduced inter-operator variability in comparison to FMD make it an attractive tool for the assessment of CVD risk in HIV-infected persons.[17]

Routine CVD risk markers are altered with HCV infection and non-hepatically produced markers of endothelial dysfunction and inflammation may be better predictors of CVD risk in this patient population. Lipid and hsCRP levels are widely used for CVD risk

assessment.[18,19] However, hsCRP and lipids are reduced in the setting of HCV-associated chronic liver disease,[20,21] related to impaired hepatic function and an incompletely understood interaction between HCV and host lipid metabolism.[22] Thus, routine markers may underestimate CVD risk in HCV coinfected persons. Non-hepatic CVD biomarkers which may have improved predictive utility in HIV/HCV coinfection include: soluble ICAM-1 (sICAM-1), soluble E-selectin (sE-selectin), lipoprotein-associated phospholipase A2 (Lp-PLA2), D-dimer, and interleukin-6 (IL-6). In large prospective studies, levels of these biomarkers predicted future CVD events and were consistently associated with classical risk factors.[23-34] Lp-PLA2 has never been described in HCV and minimal data exist for HIV-infected individuals.[35] A few studies have compared sICAM-1 and sE-selectin levels in HIV/HCV versus HIV- or HCV-monoinfected controls and found higher baseline levels with HIV/HCV coinfection,[29,30,36] but none have appropriately controlled for key confounders such as HIV viremia or traditional CVD risk factors. One study attempted to correlate levels of vascular adhesion molecules with endothelial dysfunction by FMD, but interpretation of their findings is similarly limited by the heterogeneity of their cohort and significant confounding.[13] Thus, the predictive utility of these markers remains unknown.

Microparticles (MPs) are a novel marker of endothelial dysfunction. MPs are cell membrane blebs produced by activated and injured cells that retain the cellular hallmarks of their parent cells.[37] While a stimulus such as infection or tissue injury is believed to trigger MP formation, MPs stimulate a "sterile," self-propagating, inflammatory cascade. MPs are understudied in HIV infection, but T cell MP numbers correlate with severity of hepatic disease in HCV mono-infection,[38] and endothelial cell MP numbers correlate strongly with degree of endothelial dysfunction in the general population.[39,40] Similar to peripheral blood mononuclear cells (PBMCs), MP phenotyping and quantification via flow cytometry allows for assessment of immune activation and cellular dysfunction, but MPs can be measured on frozen plasma and are not subject to the same viability concerns as PBMCs.

Successful HCV therapy may improve CVD outcomes. CVD risk factors such as hepatic steatosis and insulin resistance improve with HCV clearance.[41-44] A growing, though still small, body of literature describes reduction of levels of endothelial markers and inflammation in subjects who responded to HCV treatment as compared to those who did not.[29,30,36] These studies did not include an accompanying, more direct surrogate vascular measure of CVD risk such as endothelial dysfunction by PAT or FMD. The clinical significance of the observed change in circulating biomarkers is unknown. Our study will be the first to address both gaps.

2.1 Rationale

In this era of aging coinfected persons on effective ART, CVD and metabolic outcomes must be better characterized. There are no existing prospective, longitudinal cohorts designed to investigate endothelial function in this population. It is not widely recognized by clinicians that the hepatically-produced CVD risk markers used in routine clinical practice may be unreliable in coinfected persons, and data characterizing non-hepatic markers in this population are lacking. Through a unique, multi-disciplinary collaboration between the four UCLA CTSI institutions, we will establish a HIV/HCV coinfected cohort

diverse with respect to race/ethnicity, gender, socioeconomic class, and mode of HCV transmission, in a large catchment area, and for whom outcomes have not been described. The HIV/HCV cohort will be matched with HIV monoinfected controls, all with HIV suppression on stable ART, for cross-sectional and longitudinal evaluation of endothelial function, as well as improved characterization of both novel and traditional CVD risk biomarkers. We will conduct novel exploratory analysis of the effect of HCV treatment and virologic suppression on endothelial function. The project is intended to generate preliminary data for application for R01 or program project funding to continue the cohort and improve our understanding of CVD and metabolic outcomes with chronic HIV/HCV coinfection.

2.2 Study Population

There will be two study groups: Group A will be HIV/HCV coinfected subjects and Group B will be HIV monoinfected subjects. All HCV coinfected subjects will have evidence of ongoing HCV replication on study entry. The HIV/HCV group may include both HCV treatment-naïve subjects and treatment-experienced subjects who have failed prior HCV therapy, as ongoing HCV replication, irrespective of prior HCV treatment, is believed to be the primary driver of HCV-associated chronic inflammation and immune activation and end-organ disease. To control for the effect of HIV infection on the outcomes of interest, all subjects are to have undetectable HIV RNA (<50 copies/mL) and CD4 cell count ≥ 200 cells/mm^3. All HCV genotypes are permitted in the study. Subjects who initiate HCV treatment following study entry are included to allow exploratory analysis of the potential benefit of HCV treatment and virologic clearance on CVD.

2.3 Selection of PAT as measure of endothelial function and correlate for CVD risk.

Endothelial dysfunction represents an early stage of coronary artery disease (CAD).[45] The presence of endothelial dysfunction in coronary or peripheral vessels constitutes an independent predictor of cardiovascular events.[46] Noninvasive measures of peripheral vascular endothelial function have been developed, but most are limited by operator dependency or complexity.[47-49]

PAT is a noninvasive technique to assess peripheral microvascular endothelial function by measuring changes in digital pulse volume during reactive hyperemia. PAT has favorable characteristics when compared to other noninvasive measures of peripheral vascular endothelial function with respect to reduced complexity, operator independence, and reproducibility.[17,49]

Endothelial dysfunction is reversible and early detection of endothelial dysfunction may have therapeutic and prognostic implications. Multiple studies have demonstrated changes in endothelial function measured by PAT within 1 year or less, including with interventions.[49-51]

3.0 STUDY DESIGN

This is a prospective, observational, longitudinal study of two groups, Group A, HIV/HCV coinfected subjects and Group B, HIV monoinfected subjects. The HIV/HCV subjects

will be frequency-matched to the HIV monoinfected subjects by age (<40, 40-49, 50-54, and ≥55 years), race (white, black, other), sex at birth, diabetes (yes/no), smoking status (current or not), HMG Co-A reductase inhibitor (statin) and hormonal therapy use, and CD4 cell count (200-349 and ≥350 cells/mm3) at baseline. All subjects will be followed for a minimum of 1 year. A subset of the HIV/HCV coinfected group are expected to initiate HCV treatment during this time and will be followed through up to 24 weeks following end of HCV treatment. All subjects will be included in baseline cross-sectional analysis of endothelial function, measured by peripheral arterial tonometry (PAT) and laboratory testing for circulating CVD biomarkers. All subjects will have follow-up PAT and laboratory testing and clinical follow-up. Those HIV/HCV coinfected that do not begin HCV treatment and all HIV moninfected will have follow-up PAT and laboratory testing at 1 year. Those HIV/HCV coinfected who begin HCV treatment will have follow-up PAT and laboratory testing at 4 weeks after end of HCV treatment, but will be followed clinically for up to 24 weeks after end of treatment to capture complete HCV treatment response data.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 Men and women ≥ 18 years of age.

4.1.2 Hepatitis C status:

4.1.2.1 For HIV/HCV coinfected participants:

4.1.2.1.1 Presence of chronic HCV infection, defined by presence of serum HCV RNA in a subject with HCV antibody for ≥ 180 days, or two documented HCV RNA positive results >180 days apart, or positive HCV RNA with biopsy demonstrating chronic hepatitis.

NOTE: Two separate antibody tests > 180 days apart are not required; only that the positive HCV antibody test should be ≥ 180 days ago, with current serum HCV viremia as outlined in section 4.1.2.1.2.

4.1.2.1.2 Serum or plasma HCV RNA level ≥ 10,000 IU/mL at any point prior to study entry and in the absence of intervening HCV treatment, by any laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent.

NOTE: This must be a quantitative HCV RNA test, not a qualitative HCV RNA test.

4.1.2.2 For HIV monoinfected participants:
Absence of chronic HCV infection, defined as a negative HCV screening antibody (+/- undetectable HCV RNA) within 1 year prior to

study entry.

4.1.3 HIV infection, documented by any licensed rapid HIV test or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit at any time prior to study entry and confirmed by a licensed Western blot or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 p24 antigen, or plasma HIV-1 RNA viral load.

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (eg, indirect versus competitive), or a Western blot or a plasma HIV-1 RNA.

4.1.4 CD4+ T-cell count > 200 cells/mm^3, and none less than 200 cells/mm^3, during the 12 weeks prior to study entry, at any laboratory that has CLIA certification or its equivalent.

4.1.5 Plasma HIV-1 RNA < 50 copies/mL at screening, and none > 200 copies/mL, during the 12 weeks prior to study entry, at any laboratory that has CLIA certification or its equivalent.

4.1.6 On continuous and stable ART for at least 12 weeks with CD4+ T cell count and plasma HIV-1 RNA measurements as in 4.1.4 and 4.1.5. Changes in regimen prior to study entry are allowed as long as they were not for treatment failure, in which case subjects should be on the same ART regimen for at least 8 weeks prior to study entry with documented HIV RNA <50 copies/mL on this regimen. Subjects should have no plans to change ART in the next 52 weeks.

4.1.7 Ability and willingness of subject to provide written informed consent.

4.2 Exclusion Criteria

4.2.1 Known cardiovascular disease, as defined by a self-reported history or medical record documentation of any of the following: myocardial infarction, angina pectoris, prior revascularization (coronary artery bypass graft, percutaneous coronary intervention), peripheral vascular disease, arrhythmias, congestive heart failure, congenital heart disease, cerebrovascular disease (stroke, transient ischemic attack).

4.2.2 Diabetes requiring insulin therapy or hemoglobin A1c > 8% within 6 months prior to study entry.

4.2.3 Inability to conform to the following drug interruptions for PAT testing, whether due to safety (determined by the investigator) or willingness: No caffeine for 24 hours prior; no nicotine for 4 hours prior; no recreational or prescription stimulant use for 24 hours prior; stopping of beta blockers, short-acting calcium channel blockers (CCBs), nitrates, angiotensin-converting enzyme inhibitors (ACE-Is), angiotensin-receptor blockers (ARBs), and renin-inhibitors for 24 hours prior; and stopping of long acting CCBs 48 hours prior to testing.

4.2.4 Evidence of decompensated liver disease manifested by presence of or history of ascites, variceal bleeding, or hepatic encephalopathy.

4.2.5 Other known causes of significant liver disease including chronic or acute hepatitis B, acute hepatitis A, hemochromatosis, or homozygote alpha-1 antitrypsin deficiency.

4.2.6 Serious illness including acute liver-related disease and malignancy requiring systemic treatment or hospitalization within 12 weeks prior to study entry.

4.2.7 Presence of active or acute AIDS-defining opportunistic infections (OIs) within 12 weeks prior to study entry, which may affect CVD biomarkers. A list of AIDS-defining opportunistic infections as defined by the CDC, can be found in Appendix B of the following document:
http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

4.2.8 History of major organ transplantation with an existing functional graft and on immunosuppressive therapy.

4.2.9 History of known vascular disorder or autoimmune processes including Crohn's disease, ulcerative colitis, severe psoriasis, rheumatoid arthritis, and cryoglobulinemia that may affect vascular studies.

4.2.10 Pregnancy.

4.2.11 HCV treatment (any approved or investigational agents) within 24 weeks prior to study entry.

4.2.12 Use of immune-based therapies or systemic corticosteroids which may affect vascular studies or inflammatory/endothelial biomarkers within 12 weeks prior to study entry.

NOTE: Routine standard of care, including hepatitis A and/or B, human papilloma virus, influenza, pneumococcal, tetanus, and meningococcal vaccines are permitted if administered at least 14 days before biomarker blood collections and peripheral arterial tonometry examinations.

4.2.13 Advanced renal insufficiency as defined by glomerular filtration rate (GFR) < 30 mL/min/1.73 m^2 or treatment by dialysis.

4.3 Study Enrollment Procedures

4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit proof of IRB approval and site-specific informed consent forms (ICFs) to Kara Chew (kchew@mednet.ucla.edu) and UCLA-Westwood (UCLA-WW) regulatory staff (Irma Franco-Gonzalez, ifranco-gonzalez@mednet.ucla.edu). They will ensure that all of the required documents have been received.

All site-specific and primary UCLA-WW IRB documents should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment, sites should implement the amendment immediately. Sites should submit proof of approved amendments for their site via email to Kara Chew and UCLA-WW regulatory staff (Irma Franco-Gonzalez). A copy of the amendment should be retained in the site's regulatory files.

Once a candidate for study entry has been identified, details will be carefully discussed with the subject. The subject (or, when necessary, legal guardian if the subject is under guardianship) will be asked to read and sign the approved protocol consent form.

For subjects from whom a signed informed consent has been obtained, a CTSI-PLACE Study Screening Checklist must be entered through REDCap, the study database.

4.3.2 Subject Registration

For subjects from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, a CTSI-PLACE Study Screening Failure Result form must be completed and keyed into the database.

4.4 Coenrollment Guidelines. Coenrollment into any study requires study team approval.

5.0 Study Medications. No medications are being given as part of this study, but medications are relevant to the study questions and should be documented at each study visit.

5.0.1 Concomitant Medications

Whenever a concomitant medication is initiated or a dose changed, investigators must document the change in source documents and CRFs.

5.0.2 Required Medications

All antiretroviral regimens are acceptable.

5.0.3 Prohibited Medications

There are no prohibited medications specific to this study.

6.0 CLINICAL, LABORATORY, AND ENDOTHELIAL FUNCTION EVALUATIONS

6.1 Schedule of Events

Evaluation	Screening	Entry Week 0	Chart abstraction	Study Visit	Premature Study Discontinuation[1]
Week			Week 24	Week 52[2]	
Window			±28 days	±28 days	
Documentation of HCV Status*	X			X	X
Documentation of HIV-1 Infection*	X				
Medical History/Medication History	X	X	X	X	X
Clinical Assessment[3]	X	X		X	X
Concomitant Medications		X	X	X	X
Hematology/Chemistry/Liver Function Tests*		X		X	X
INR*		X		X	X
CD4+/CD8+ T-Cell Counts*	X		X	X	X
Plasma HIV-1 RNA*	X		X	X	X
Serum HCV RNA (screening)*	X				
Serum HCV RNA (on-study)*			X	X	X
HCV Genotype Test*		X			
Hemoglobin A1c*	X			X	X
Pregnancy test	X			X	X
Liver biopsy results*		X		X	X
Fasting Stored Serum/Plasma for Batched CVD Biomarkers[4]		X		X	X
Stored Serum/Plasma for Future Studies		X		X	X
Peripheral Arterial Tonometry		X		X	X

*Longitudinal values of these laboratory tests, if available, will be obtained via chart abstraction from the participants' medical records. Specific tests will be performed in real-time only as indicated in the instructions for evaluations. INR should be collected for HCV coinfected subjects only. Hemoglobin A1c should be collected for diabetic subjects only. Availability of liver biopsy results will be assessed and, if available, results recorded from the medical record. Liver biopsy will not be performed for this study. For HIV monoinfected subjects, screening HCV antibody should be performed at the week 52 visit.

[1]All subjects enrolled in the study for a minimum of 3 months but less than 1 year should have a premature study discontinuation visit, including repeat PAT and biomarker testing. If this testing occurs while on HCV treatment, concurrent treatment status should be documented in source documents and on the CRF. For subjects enrolled in the study for fewer than 3 months, telephone interview and chart abstraction should be conducted for clinical data from the time of study entry to study discontinuation.
[2]For coinfected subjects who initiate HCV treatment, week 52 assessments should occur at 4 weeks after end of treatment (EOT), but no earlier than 3 weeks and no later than 8 weeks after EOT. If not available in the medical record, a serum HCV RNA should be performed at 4 weeks after EOT. The medical record should be reviewed and HCV RNA at 12 and 24 weeks after EOT should be recorded.
[3]Required elements for clinical assessments are described in section 6.3.
[4]Batched biomarkers will include fasting lipid panel, fasting insulin, fasting glucose, hsCRP, IL-6, D-dimer, sICAM-1, sE-selectin, and Lp-PLA2. Fasting insulin and fasting glucose should only be performed on non-diabetic subjects. Plasma will also be tested for microparticles.

6.2 Timing of Evaluations

6.2.1 Screening Evaluations

Screening evaluations must occur within 4 weeks prior to the subject starting any study procedures.

6.2.2 Entry Evaluations

Entry evaluations must occur within 4 weeks of the screening visit.

6.2.3 Post-Entry Evaluations

On-Study Evaluations
Evaluations should occur after registration. The week 24 visit consists only of review of the medical record and abstraction of data from the time since study entry. The week 52 study visit should be scheduled within the visit window. For HCV-infected subjects who initiate HCV treatment, the week 52 visit should occur at 4 weeks after end of HCV treatment, but not earlier than 3 weeks and no later than 8 weeks after EOT.

Study Completion Evaluations
Clinical assessment and laboratory evaluation, as outlined in section 6.1, will be performed at study completion (week 52 visit).

6.2.4 Discontinuation Evaluations

Evaluations for Registered Subjects Who Do Not Start Study Procedures
All CRFs must be completed and keyed for the period up to and including week 0.

Subjects who discontinue the study prior to 1 year
Subjects who permanently discontinue study prior to 1 year and have been enrolled for at least 3 months will have a study discontinuation visit including

repeat biomarker and PAT testing. Subjects on study for < 3 months should be contacted by phone for a telephone interview and have medical record review and data abstraction for clinical events, including new diagnoses and change in medications, since the time of study entry.

Pregnancy
Female subjects who conceive after study entry will be discontinued from the study.

6.3 Instructions for Evaluations

All clinical, laboratory, and tonometry information required by this protocol is to be present in the source documents. All stated evaluations are to be recorded on the CRF and keyed into the database unless otherwise specified. This includes events that meet the following International Conference on Harmonisation (ICH) definitions for a serious adverse event (AE):

- Results in death
- Life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other important medical event (may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent one of the events listed above).

6.3.1 Documentation of HIV-1 Infection

Please refer to section 4.0 regarding assay requirements for HIV-1 documentation. Year of HIV diagnosis and nadir CD4 cell count should be recorded on the CRF.

6.3.2 Documentation of HCV Infection

Please refer to section 4.0 regarding requirements for HCV documentation for both HIV/HCV coinfected and HIV monoinfected subjects. Year of HCV diagnosis and mode of HCV transmission should be recorded on the CRF.

6.3.3 Documentation of Liver Biopsy

If a liver biopsy has been performed within 5 years prior to study entry or is performed during the course of the study, documentation of date of biopsy, liver fibrosis and activity scores, steatosis measurement, and scoring system (e.g. METAVIR, Knodell, etc.) should be recorded on the CRF.

6.3.4 Medical History

The medical history must include all diagnoses, year of initial diagnosis, and status of diagnosis (ongoing condition or not). Any allergies to medications and their formulations must be documented. Record tobacco, alcohol, and drug use history on the CRF. Record menopause status on the CRF.

6.3.5 Medication History

A medication history must be present, including start and stop dates. All current medications should be recorded. The table below lists the medications that must be included in the history.

Medication Category	Complete History or Timeframe	Record on CRFs
HIV treatment	Within 120 days prior to entry	Yes
HCV treatment	Complete History	Yes
Other prescription drugs	Within 42 days prior to entry	Yes
Non-prescription drugs	Within 42 days prior to entry and taken daily for at least 7 days	Yes
Complementary and alternative medicines	Within 42 days prior to entry and taken daily for at least 7 days	Yes

6.3.6 Clinical Assessments

Vital Signs
Blood pressure and heart rate will be recorded at screening and entry.

Height
Height will be measured at entry.

Weight
Weight will be measured at entry and week 52 visit.

Waist Circumference
Waist circumference will be measured at entry and week 52/study discontinuation visit. Measure waist circumference at level of iliac crest in centimeters (cm).

Signs and Symptoms
At entry, all signs and symptoms that occurred within 42 days before entry must be recorded. At week 52/study discontinuation visit, all signs and symptoms that occurred within 42 days prior must be recorded.

6.3.7 Concomitant Medications

Initiation and discontinuation of all concomitant medications should be recorded on the CRF with start and stop dates. All ART (including interruption of ART for at least 7 days), HCV treatment (approved or experimental), immune-based and

anti-inflammatory therapy, prescription drugs, vaccines, alternative therapies, dietary supplements, and any experimental therapies taken daily for at least 7 days must be recorded. Changes only in dose or formulation do not need to be recorded.

At the premature study discontinuation visit, record all concomitant medications and ART per above guidelines, including ongoing medications.

6.3.8 Laboratory Evaluations (Note: the majority of these will be collected from the medical records and will not be drawn for the study)

The following must be recorded on CRFs and keyed into the database **unless otherwise indicated**:

Hematology
Hemoglobin, hematocrit, white blood cell count (WBC), absolute neutrophil count (ANC), and platelets within 120 days prior to study entry and **within 120 days prior to the** week 52/study discontinuation visit.

Chemistry
Sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, and glucose within 120 days prior to study entry and **within 120 days prior to the** week 52/study discontinuation visit.

Liver Function Tests
Total bilirubin, indirect bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase, and albumin within 120 days prior to study entry and **within 120 days prior to the** week 52/study discontinuation visit.

INR
INR obtained at a local laboratory within 120 days prior to study and **within 120 days prior to the** week 52/study discontinuation visit.

Pregnancy Test
For women with reproductive potential: Serum or urine beta-human chorionic gonadotropin (β-HCG [urine test must have a sensitivity of at least 50 mIU/mL]) within 28 days prior to study entry and at week 52/study discontinuation visit.

Hepatitis C Antibody Test
For HIV-monoinfected subjects only, screening HCV antibody within 1 year prior to study entry and within 120 days prior to the week 52/study discontinuation visit.

Hemoglobin A1c
For diabetic subjects only, hemoglobin A1c obtained within 180 days prior to study entry and within 120 days prior to the week 52/study discontinuation visit.

6.3.9 Immunologic Studies

CD4+/CD8+ T-cell counts
Absolute CD4+/CD8+ T-cell count and percentages within 12 weeks prior to entry from a laboratory that possesses CLIA certification or an equivalent. CD4 nadir should be recorded on the CRF. At the week 24 and week 52 visits, the medical record should be reviewed and all post-entry values obtained since the last study visit should be recorded.

6.3.10 Virologic Studies

Plasma HIV-1 RNA
Plasma HIV-1 RNA level within 12 weeks prior to study entry and within 28 days of week 52**/study discontinuation** visit, at any laboratory that has CLIA certification or its equivalent.

Subjects who switch their ART regimen prior to study entry (see 4.1.6) should have an HIV-1 RNA level performed while on the new regimen, prior to study entry. Record post-entry values obtained as part of routine care.

The requirement of plasma HIV-1 RNA level within 28 days of the week 52/study discontinuation visit is waived for subjects that meet ALL of the following criteria:

1) HIV-1 RNA <50 copies/mL on all laboratory testing in the preceding 2 years
2) HIV-1 RNA <50 copies/mL in the preceding 90 days
3) No change in ART regimen since study entry
4) No ART interruption of more than 7 consecutive days or 14 total days in the preceding 90 days
5) No HCV treatment since study entry

For subjects who meet ALL of the above criteria, the most recent HIV-1 RNA level from the medical record, closest to the time of the week 52/study discontinuation visit, is acceptable.

Serum HCV RNA (screening)
At screening, the HCV RNA result must be obtained by any FDA-approved test for quantifying HCV RNA at any local laboratory that has a CLIA certification or its equivalent. Screening HCV RNA at any point prior to study entry is acceptable, in the absence of intervening treatment.

Serum HCV RNA (on-study evaluations)
At the week 24 and week 52 visits, the medical record should be reviewed and all post-entry values obtained since the last study visit should be recorded.

Coinfected subjects who begin HCV treatment should have HCV RNA testing at 4 weeks after EOT ("week 52" visit), but no earlier than 3 weeks and and no later than 8 weeks after EOT. This may be abstracted from the medical record and if not done, should be performed using a CLIA-certified or equivalent laboratory. The medical record should be reviewed and HCV RNA at weeks 12 and 24 after EOT should be documented.

HCV Genotype Test
A documented result (serum or plasma) is a source document from a CAP/CLIA-approved laboratory at any time prior to entry. For those subjects lacking a documented result, HCV genotyping should be performed by a local CAP or CLIA-approved (or its equivalent) laboratory.

6.3.11 Stored Plasma and Serum for batched testing for biomarkers

Stored plasma and serum will be collected at the indicated visits (section 6.1) for centralized, batched testing at the UCLA Clinical and Translational Research Laboratory (CTRL) for the following biomarkers: hsCRP, fasting glucose, fasting insulin, fasting lipid panel, hsCRP, sICAM-1, sE-selectin, Lp-PLA2, IL-6, and D-dimer. Fasting glucose and fasting insulin will be performed for non-diabetic subjects only. Subjects must be fasting at least 8 hours (nothing by mouth except medications and water). If subjects indicate a non-fasting state, they should be asked to return in a fasting state within the required visit window (please refer to section 6.1 for more information regarding visit window). If that is not possible, a non-fasting specimen should be obtained the same day, and the non-fasting state should be recorded on the CRF. Stored plasma will be collected at the indicated visits (section 6.1) for centralized testing for endothelial, monocyte, and T-cell microparticles in the laboratory of Dr. Otto Yang (UCLA Division of Infectious Diseases). Samples should be collected, processed, and stored on-site frozen at -70°C until shipped to UCLA-WW in accordance with the CTSI-PLACE Study Lab Processing Chart (LPC) (Appendix II). **Subjects should not have received vaccines within 14 days prior to plasma and serum collection.**

6.3.12 Stored Plasma and Serum for future studies

Stored plasma and serum will be collected and processed at the indicated visits (section 6.1) for future studies. Samples will be temporarily stored on site at -70°C until transfer to UCLA-WW for central long-term storage. Samples should be transferred to UCLA-WW every 1-2 months.

6.3.13 Peripheral Arterial Tonometry testing

Endothelial function will be measured by the FDA-approved method of Endo PAT 2000 (Itamar® Medical Ltd, Caesarea, Israel) at baseline and 52 weeks in all subjects except those undergoing HCV treatment. Those undergoing HCV treatment will have follow-up PAT at 4 weeks after end of HCV treatment. PAT will be done at two sites: at CSMC by Dr. C. Noel Bairey Merz and at the UCLA Center for Human Nutrition by Dr. Zhaoping Li. The device consists of plethysmographically-based probes to assess digital volume changes

accompanying pulse waves.[17,46] A blood pressure cuff will be placed on one upper arm (study arm), while the other arm will serve as a control (control arm). Peripheral arterial tonometry probes will be placed on one finger of each hand for continuous recording of the PAT signal (baseline arterial pulse wave amplitude, PWA). After a 5-minute equilibration period, the blood pressure cuff will be inflated to suprasystolic pressures for 5 minutes (the ischemic stimulus). The cuff will then be deflated while PAT recording is continued for 5 minutes (Figure 1). Reactive hyperemia index (RHI) is the primary measure of endothelial function. The RHI is automatically derived using Endo PAT version 3.0.4 software, as the ratio of the average PWA over a 1 minute interval starting 1 minute following cuff release to the pre-occlusion PWA (average amplitude over 3.5 minutes before cuff inflation), normalized to the control arm.

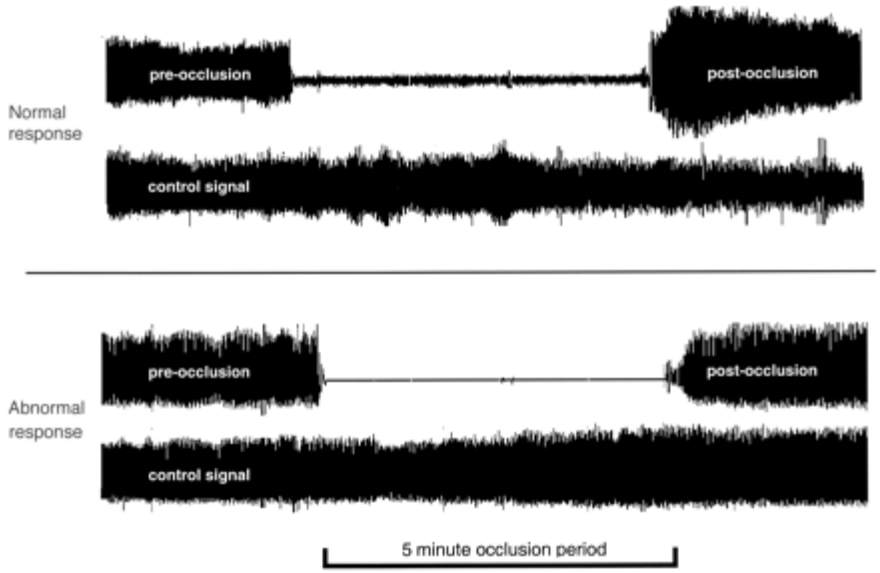


Figure 1 Representative reactive hyperemia peripheral arterial tonometry recordings of subjects with normal and abnormal reactive hyperemic response. Normal response is characterized by a distinct increase in the signal amplitude after cuff release compared with baseline.

The choice to use the average 1-min PAT signal starting 1 min after cuff deflation to describe the magnitude of reactive hyperemia is based on the observation that this time interval provides the best information regarding detection of coronary endothelial dysfunction as determined by receiver operating characteristic (ROC) curve analysis.

Subjects will be required to be fasting for 12 hours and to abstain from the following prior to PAT: caffeine for 24 hours; nicotine for 4 hours; beta blockers, calcium-channel blockers (CCBs), nitrates, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, renin-inhibitors for 24 hours, long acting CCBs for 48 hours;

prescribed or recreational stimulant use for 24 hours; vigorous exercise for 12 hours. **Subjects should not have received vaccines within 14 days prior to PAT testing.**

7.0 CLINICAL MANAGEMENT ISSUES

No clinical management issues are expected as no treatment is being provided in this study.

7.1 Toxicity

No toxicities are anticipated as no treatment or invasive procedures are being performed as part of this study.

7.2 Pregnancy

Pregnancy is not a contraindication to PAT or laboratory testing, but may affect measurements of endothelial function. Pregnancy status should be documented at each PAT visit for female subjects (week 0 and week 52 or 4 weeks after EOT for those with treated HCV).

8.0 CRITERIA FOR DISCONTINUATION

8.1 Premature/Permanent Study Discontinuation

- Request by the subject to withdraw.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to interfere with the validity of the study results.
- At the discretion of the institutional review board (IRB), Office for Human Research Protections (OHRP), investigator, or sponsors.

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The CTSI-PLACE Study consists of single-arm evaluation to assess endothelial function cross-sectionally and longitudinally in two study populations: HIV/HCV coinfected (Group A), a subset of which will undergo HCV treatment, and HIV monoinfected (Group B). The primary endpoint is endothelial function measured by peripheral arterial tonometry reactive hyperemia index (RHI) in both groups.

9.2 Endpoints

9.2.1 Primary Endpoints

9.2.1.1 Reactive hyperemia index (RHI) by PAT at baseline.

9.2.2 Secondary Endpoints

9.2.2.1 Insulin resistance by HOMA-IR (fasting insulin (μU/mL) x fasting glucose (mg/dl)/405) at baseline and week 52.

9.2.2.2 Framingham risk score for 10-year CHD and 10-year CVD risk at baseline and week 52.

9.2.2.3 Cardiovascular biomarkers (fasting lipid panel, hsCRP, IL-6, D-dimer, sICAM-1, sE-selectin, Lp-PLA2) at baseline and week 52.

9.2.2.4 RHI by PAT at week 52

9.2.2.5 Change in RHI , biomarkers, HOMA-IR, and FRS from baseline to week 52.

9.3 Sample Size and Accrual

9.3.1 The primary comparison is of baseline RHI between the HIV/HCV coinfected and HIV monoinfected groups. The estimated difference in RHI between the 2 groups is assumed to be the difference between high-risk CVD (HIV/HCV) and moderate-risk CVD (HIV). RHI is expected then to be 1.6 in HIV/HCV and 1.3 in HIV, with standard deviation (SD) of 0.37.[52] With a sample size of 80 HIV/HCV and 40 HIV, we have an estimated power of 99% for detection of the expected difference in RHI between the HIV/HCV and HIV groups. However, if we conservatively estimate the difference in baseline RHI to be 0.2 between the 2 groups, we will have 80% power with this sample size. For select secondary analyses of differences in the CVD biomarkers of sICAM-1 (expected difference of 779 vs1284 ng/mL, SD=526) and sE-selectin (118 vs 223 ng/mL, SD=190),[30] we will have 99% and 81% power, respectively, with this sample size.

Accrual of about 20 subjects per month is expected, to complete enrollment in 6 months.

9.3.2 With 80 HCV/HIV subjects, we will have 80% power to detect a correlation between any one marker and RHI of > 0.31. With 40 HIV subjects, we will have 80% power to detect a correlation >0.42, correlations that have been seen in other populations.[53,54]

9.3.3 Among 80 HIV/HCV subjects, we expect about 40 (50%) will initiate HCV treatment. Out of 40 who initiate HCV treatment, we expect about 27 (67%) will be responders and 13 will be non-responders. To compare responders (n=27) with subjects without treatment (n=40), we will have 80% power to detect a difference of 0.7 in effect size. To compare HIV monoinfected controls (n=40)

and untreated HIV/HCV subjects, we will have 80% power to detect a difference of 0.63 in effect size.

9.4 Data Analysis Plan

9.4.1 Objective: To determine the effect of HCV coinfection on endothelial function in the HIV-1-infected cohort at baseline.

Descriptive statistics, including mean, SD, median, minimum, maximum, and interquartile range, will be provided for the overall cohort, by HCV status, and by PAT performance site for baseline patient characteristics, RHI, CVD biomarkers, HOMA-IR, hemoglobin A1c, and FRS. Histograms and scatter plots will be produced to visually inspect the distributions and pairwise association. Log-transformation as necessary will be done for RHI, if its distribution is found to be skewed. ANOVA (analysis of variance) will be used to compare continuous variables by HCV status and site and Chi-square test will be used to compare categorical variables. All tests will be 2-sided and a p-value less than 0.05 will be considered statistically significant.

Two sample t-test or Wilcoxon rank sum will be performed to compare RHI in the HIV/HCV vs HIV groups. Multiple linear regression analysis will also be carried out to compare RHI in HIV/HCV vs HIV, adjusting for PAT performance site and any differences in baseline characteristics including CD4 nadir and ART type. Subgroup analyses by race/ethnicity and gender will be conducted. Similar analyses will be carried out to compare biomarkers between the HIV/HCV and HIV groups. Given multiple comparisons, in addition to unadjusted p-values, we will report significance according to Benjamini-Hochberg false discovery rate method.[55]

9.4.2 Objective: To assess whether non-hepatic biomarkers of endothelial dysfunction, inflammation, and thrombosis (sICAM-1, sE-selectin, Lp-PLA2, IL-6, and D-dimer) are better correlates of endothelial function than traditional hepatic-derived biomarkers (hsCRP and lipids) in HIV/HCV-coinfected in cross-sectional assessment.

Correlation analysis (Pearson or Spearman) will be conducted to evaluate the association of each circulating biomarker as the predictor and RHI as a continuous outcome variable. Further correlation of biomarkers with each other and with RHI will be performed by use of correlation matrices. Multiple linear regression models will be used while controlling for confounders as in Aim 1 analysis, but with each biomarker as the primary independent variable to determine the contribution of each biomarker as a predictor of RHI. A final model will be constructed by including all significant biomarkers to predict RHI. The adjusted R-squared value will be used to indicate the predictive ability of the model. Similar analysis will be carried out in HIV-monoinfected subjects only to derive the RHI prediction model. The models for each cohort will be compared to each other for their predictive ability and differences in individual biomarker

significance in each cohort. This analysis will allow us to define whether non-hepatically-derived biomarkers have stronger correlation with RHI than hepatically-derived biomarkers.

9.4.3 Objective: To determine the effect of HCV co-infection and HCV virologic suppression post treatment on endothelial function and CVD biomarkers in the HIV-1-infected cohort over one year of follow-up.

Change in RHI and change in biomarkers from baseline to follow-up will be compared among 4 groups of subjects (HCV treatment responders and non-responders, untreated HIV/HCV subjects, and HIV controls). Boxplots will be used to visually display the difference among 4 groups. Pair-wise comparison will be carried out based on 2-sample t-test or Wilcoxon test. ANCOVA will be carried out to compare among 4 groups adjusting for baseline values and demographic characteristics and other baseline confounders. Adjusted comparison between any 2 groups will be estimated based on appropriate contrast. Correlation analysis will be carried out between change in biomarkers and change in RHI.

10.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

10.1 Records to Be Kept

Case report forms (CRF) will be provided for each subject. Subjects must not be identified by name on any CRFs. Subjects will be identified by an assigned patient identification number (PID) in the REDCap database. Data must be keyed in to the study database, REDCap.

No personally identifiable information (PII) may be entered into REDCap. A code key must be maintained at each site for all subjects who sign the study informed consent form.

Users of REDcap must have completed HIPAA training or access will not be assigned. All REDCap operations will be tracked through audit trail. REDCap is a web-based data management system. The REDCap web server uses SSL encryption and system updates are automatically applied when they are available. For added security, the REDCap database server sits within a firewall. Both the web and database servers are backed up daily.

10.2 Role of Data Management

10.2.1 Instructions concerning the recording of study data on CRFs will be provided. Each CTSI site is responsible for keying the data in a timely fashion.

10.2.2 It is the responsibility of the CTSI-PLACE Study Co-PIs to assure the quality of computerized data for the study. This role extends from protocol development to generation of the final study databases.

10.3 Clinical Site Monitoring and Record Availability

10.3.1 UCLA-WW investigators will serve as monitors for the other sites and at UCLA-WW the CTU coordinator (Cara O'Connor) will serve as the site monitor. Monitors will review the individual subject records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' laboratories and freezers to review specimen storage and management.

10.3.2 The site investigator will make study documents (eg, consent forms, code key, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the OHRP, and the sponsors for confirmation of the study data.

11.0 HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from the subject (or legally guardian, or person with power of attorney for subjects who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject's legal guardian, and this fact will be documented in the subject's record.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by coded number to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, OHRP, or sponsors.

11.3 Study Discontinuation

The study may be discontinued at any time by the IRB, OHRP, or government agencies as part of their duties to ensure that research subjects are protected.

12.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this study will be determined by the study co-chairs.

13.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

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