



Protocol for the Heart Failure Clinical Research Network

Characterizing HIV-related Diastolic Dysfunction

A Cross Sectional Study Leveraging the NHLBI Heart Failure Clinical Research Network

NCT03170219

Compiled by:
The Heart Failure Network Research Group
Version 1.0

Distributed by:
Heart Failure Network Coordinating Center
Duke Clinical Research Institute
Duke University
P.O. Box 17969
Durham, NC27715

Funding Sponsor: National Heart, Lung, and Blood Institute
National Institutes of Health

Project Officer: Patrice Desvigne-Nickens, MD

Network Chair: Eugene Braunwald, MD

**Sponsor/
Coordinating Center:** Adrian Hernandez, MD
Kevin Anstrom, PhD
Duke Clinical Research Institute
Durham, NC

Protocol Principal Investigator: Javed Butler, MD
Stony Brook University Medical Center

Protocol Development Team: Eric Velazquez, MD (CC Lead Investigator)
Priscilla Hsue, MD
Rebecca Scherzer
Sanjiv Shah, MD
Svati Shah, MD
Christopher DeFillipi

Biomarker Core Laboratory: Russell Tracy, PhD
University of Vermont
Burlington, VT

MRI Core Laboratory: Raymond Kim, MD
Duke University Medical Center
Durham, NC

Echo Core Laboratory: Sanjiv Shah, MD
Northwestern University
Chicago, IL

Metabolomics Core Laboratory: Svati Shah, MD
Duke University Medical Center
Durham, NC

1 LIST OF ABBREVIATIONS

Abbreviation	Definition
ACC	American College of Cardiology
ACTG	AIDS Clinical Trials Group
AE	Adverse event
AHA	American Heart Association
ART	Anti-Retroviral Therapy
CC	Coordinating Center
CD	Cluster of Differentiation
CFAR	Center for AIDS Research
CI	Cardiac index
CMR	Cardiac magnetic resonance
cMRI	Cardiac magnetic resonance imaging
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive Protein
CTSA	Clinical Translational Science Award
CV	Cardiovascular
CVD	Cardiovascular disease
DD	Diastolic Dysfunction
DCRI	Duke Clinical Research Institute
DMPI	Duke Molecular Physiology Institute
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EDC	Electronic data capture
EKG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EF	Ejection fraction
FDR	False discovery rate
GCP	Good clinical practice
HAART	Highly active antiretroviral therapy
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrfEF	Heart failure with reduced ejection fraction
HFN	Heart Failure Clinical Research Network
HHS	Department of Health and Human Services
HIV	Human Immunodeficiency Virus
HR	Heart rate
hsCRP	High sensitivity c-reactive protein
ICF	Informed consent form
IEC	Independent Ethics Committee
IL-1	Interleukin-1
IMT	Intima-Media Thickness
IRB	Institutional Review Board
ITT	Intention to treat
LA	Left Atrium
LV	Left ventricular
LVSD	Left ventricular systolic dysfunction
MACS	Multicenter AIDS Cohort Study

MESA	Multi-Ethnic Study of Atherosclerosis
MRI	Magnetic Resonance Imaging
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NHLBI	National Heart, Lung, and Blood Institute
NYHA	New York Heart Association
PCA	Principle components analysis
PE	Physical examination
PMI	Precision Medicine Institute
QOL	Quality of life
RA	Rheumatoid Arthritis
RCC	Regional Coordinating Center
RCT	Randomized clinical trial
ROS	Reactive oxygen species
SAE	Serious adverse event
SAR	Suspected adverse reaction
SBP	Systolic blood pressure
SD	Standard deviation
SLE	Systemic Lupus Erythematosus
SV	Stroke volume
VO ₂	Volume of oxygen

TABLE OF CONTENTS

1	List of Abbreviations.....	3
2	EXECUTIVE SUMMARY.....	7
3	INTRODUCTION.....	8
3.1	Background and Significance.....	8
3.2	Preliminary Studies.....	8
4	OBJECTIVES and HYPOTHESIS.....	9
4.1	Exploratory Objectives	11
5	BASIC STUDY DESIGN.....	11
5.1	Screening Phase	11
5.2	Baseline Evaluation Phase.....	12
5.3	Follow-up Phase	12
6	Study Flow Diagram.....	12
7	STUDY POPULATION AND ELIGIBILITY CRITERIA.....	12
7.1	Inclusion Criteria.....	13
7.2	Exclusion Criteria	13
8	SCREENING PROCEDURES	13
8.1	Pre-screening.....	13
8.2	Screening Phase	13
9	BASELINE EVALUATION.....	14
9.1	Baseline Evaluation Phase.....	14
10	FOLLOW-UP EVALUATIONS.....	14
11	OUTCOME DETERMINATIONS	14
11.1	Biomarkers	14
11.1.1	Inflammation and Immune Activation	16
11.2	Myocardial Fibrosis and Inflammation.....	16
11.3	Proteomics/Metabolomics.....	16
11.4	Left Atrial Reserve	16
11.5	Phenomapping	17
12	PARTICIPANT SAFETY.....	17
12.1	Institutional Review Boards.....	17

12.2	Recording and Reporting of AEs/SAEs.....	17
13	STATISTICAL CONSIDERATIONS	17
13.1	Overview	17
13.2	Power Calculations and Statistical Analysis for Biomarkers.....	18
13.3	Phenomapping Statistical Analysis.....	18
13.4	cMRI Power Calculation and Statistical Analysis.....	19
13.5	Protein / Metabolite Statistical Analyses	19
13.6	Integrated Molecular Statistical Analysis	19
13.7	Echo Power Calculation and Statistical Analysis	20
14	DATA MANAGEMENT PROCEDURES	20
14.1	Overview of Data Management	20
14.2	Publication Policy	21
15	STUDY ADMINISTRATION	21
15.1	Data and Safety Monitoring Board (DSMB)	21
15.2	Coordinating Center	21
15.3	Core Laboratories	21
15.3.1	Biomarker Core Laboratory	21
15.3.2	Echocardiography Core Laboratory.....	21
15.3.3	Cardiac Magnetic Resonance Imaging Core Laboratory	22
16	Ethical and REGULATORY considerations.....	22
16.1	Ethics and Good Clinical Practice	22
16.2	Institutional Review Board/Independent Ethics Committee	22
16.3	Informed Consent Procedures	22
16.3.1	Informed Consent.....	22
16.3.2	Confidentiality and HIPAA Requirements	23
16.3.3	Protections of Human Subjects	23
16.3.4	Summary of the Risks and Benefits.....	23
17	Remote MONITORING	24
18	ReferenNCES	24
19	APPENDICES.....	30
19.1	Schedule of Assessments	30

2 EXECUTIVE SUMMARY

Title	Characterizing HIV-related Diastolic Dysfunction
Indication	HIV+ patients
Location	Approximately 12 clinical centers in the United States
Brief Rationale	<p>With the advent of highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV) type 1 infection has become a chronic disease. The proportion of patients expected to survive 5, 10, and 15 years after conversion in the HAART era are 99%, 93% and 89% respectively. With increased life expectancy and decreased morbidity from opportunistic infections, the importance of chronic complications associated with HIV-1 infection, including HF is becoming more evident. The advent of HAART has altered the epidemiology of HIV associated cardiomyopathy evolving from a primarily left ventricular systolic dysfunction to the growing recognition of left ventricular DD. DD is associated with the development of atrial fibrillation and heart failure (HF), and portends higher risk for all-cause mortality. Thus there is a widespread prevalence of cardiac abnormalities in HIV infected individuals that are associated with HF development and may represent a sub-clinical abnormality that may be potentially intervened upon to reduce the risk of subsequent HF. There are little data to understand the natural history and pathogenesis of cardiac abnormalities, specifically DD in HIV+ individuals, which may adversely affect the longevity and quality of life of these individuals.</p>
Objective	To characterize the determinants, mechanisms, and consequences of diastolic dysfunction in patients with HIV infection
Patient Population	Approximately 200 HAART-treated virally suppressed HIV+ subjects (100 HIV+/DD+ & 100 HIV+/DD-)
Study Design	Multi-center, cross-sectional study

3 INTRODUCTION

3.1 Background and Significance

Heart failure (HF) patients have $\approx 50\%$ mortality risk within 5 year of diagnosis, underscoring the need to better understand determinants of HF risk in order to develop effective screening and prevention interventions. An emerging risk factor for HF is human immunodeficiency virus (HIV) infection. HIV-related HF was identified as a major priority by the 2015 NHLBI AIDS Working Group. Since the early reports from the 1980s, the pattern of left ventricular (LV) dysfunction in HIV has evolved. Studies in the modern anti-retroviral therapy (ART) era have demonstrated lower rates of LV systolic dysfunction¹ but strikingly higher rates of diastolic dysfunction (DD), ranging from 26-50% as compared to 6-30% in the general population.² The mean age of HIV+ subjects with DD ranged from 38-49 years,¹ which is at least a decade younger than DD onset in the general population.³ DD is associated with atrial fibrillation, HF, and mortality.⁴⁻⁶ Thus there is a concern that the next epidemic in HIV+ patients will be HF with preserved ejection fraction (HFpEF). There is an unmet need to study DD in HIV+ patients to understand why they develop more prevalent and early onset DD.

Diastolic Dysfunction and Heart Failure

Left ventricular (LV) diastolic dysfunction (DD) is frequently present in older adults, with a prevalence ranging from 6-30% depending on the methodology, definition, and population studied. DD is associated with the development of atrial fibrillation and heart failure (HF), and portends higher risk for all-cause mortality. Previous studies have shown that DD is associated with obesity, hypertension, and metabolic syndrome, conditions that are associated with elevated inflammatory cytokines and endothelial dysfunction. It is important to note that DD is not the same as diastolic heart failure or HF with preserved ejection fraction (HFpEF). DD (also known as subclinical DD or preclinical DD) is an asymptomatic pathophysiological abnormality that precedes overt, symptomatic HF. As such, it is a clear representation of ACC/AHA Stage B HF (asymptomatic cardiac structural and functional abnormalities). It is critically important to understand DD prevalence, pathogenesis, and sequelae given its association with significant morbidity and mortality.

HIV-Related Diastolic Dysfunction and Heart Failure

With the advent of highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV) infection has become a chronic disease. The proportion of patients expected to survive 5, 10, and 15 years after seroconversion in the current era are 99%, 93% and 89%, respectively. With this increased life expectancy, chronic complications including HF with preserved ejection fraction (HFpEF) are emerging. Contemporary HAART has altered the epidemiology of HIV-related cardiomyopathy from primarily LV systolic dysfunction (LVSD), reported in 7-20% of patients in earlier studies, to predominantly DD, reported in up to 50% of HIV patients. There are few data to understand the natural history and pathogenesis of the growing prevalence of DD in HIV+ subjects who are virally suppressed on optimal HAART therapy. The growing DD prevalence in HIV, and the link of DD with HFpEF and mortality, raise significant health concerns for these patients.

3.2 Preliminary Studies

Earlier studies reported a prevalence of left ventricular systolic dysfunction between 7.5% and 20%.¹ A recent study showed that 32% of well controlled HIV infected patients had a left ventricular ejection fraction of 45-55% and only 2.3% had an EF<45%, but DD was present in

48%.¹ Hsue⁹ et al found only 4% of patients had ejection fraction <50%, whereas 49% had DD, and Schuster⁷² et al found that 13% of patients had an ejection fraction <50% but 64% had DD. A meta-analysis of 11 studies in the HAART era assessed 2242 well-controlled, asymptomatic HIV-1 infected patients, and reported a prevalence of left ventricular systolic dysfunction of 8.3% and left ventricular DD of 43.4%.² Recent studies using MRI in small cohorts of HIV+ patients suggest that treated and suppressed HIV infection is associated with myocardial inflammation, fibrosis and LV dysfunction.^{7,8} Furthermore, the editorial accompanying these studies called for studies that correlate biomarkers, imaging and tissue samples to understand the underlying mechanism of the findings.⁹

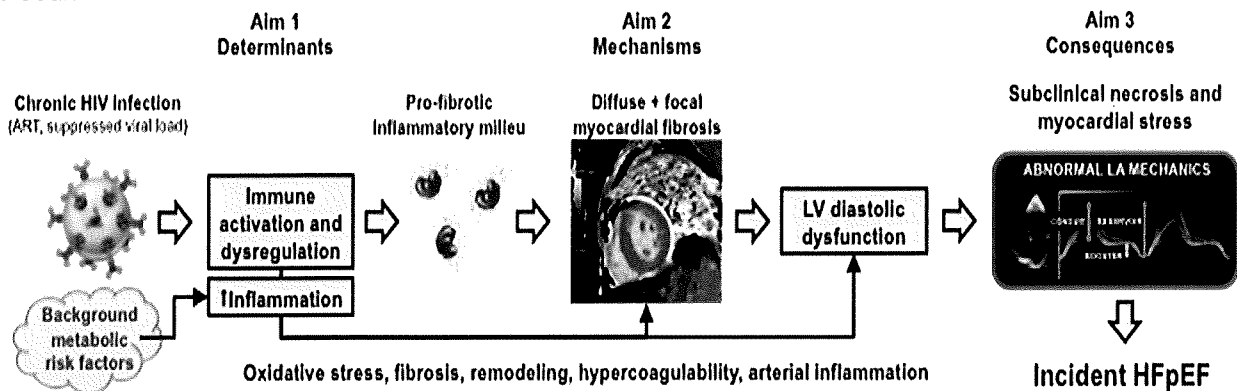
Thus there is a widespread prevalence of cardiac abnormalities in HIV infected individuals that are associated with HF development and may represent a sub-clinical abnormality that may be potentially intervened upon to reduce the risk of subsequent HF. There are little data to understand the natural history and pathogenesis of cardiac abnormalities, specifically DD in HIV+ individuals that may adversely affect their longevity.

A new theoretical framework for understanding diastolic dysfunction and HFpEF postulates a central role for inflammation in the progression of disease.¹⁰ It has been hypothesized that many of the cardiovascular abnormalities in HFpEF can be understood as the downstream consequence of the pro-inflammatory state associated with diseases such as obesity, hypertension, diabetes, chronic obstructive pulmonary disease, anemia, and chronic kidney disease.¹⁰ Because of their heightened state of inflammation that begins at the time of infection¹¹, HIV-infected people may present a unique model to better understand the inflammation hypothesis in HFpEF, and provide insight on the pathogenesis and progression of diastolic dysfunction and HFpEF in all populations. Because there are no evidence-based therapies for HFpEF, novel studies examining HFpEF in the HIV population may provide critical knowledge that helps solve the puzzle of how to treat this perplexing disease.

4 OBJECTIVES AND HYPOTHESIS

Multiple domains of biomarkers (fibrosis and remodeling, arterial inflammation, hypercoagulability and oxidative stress), proteomic and metabolomics analyses, and other cardiac structural and functional changes will be harnessed to elucidate the mechanistic pathways leading to DD in HIV. Exploratory analyses will assess if DD in HIV is associated with higher wall stress and the potential microvascular dysfunction in these patients leads to subclinical myocardial necrosis.

FIGURE 1



The overarching objective of this protocol is to study the determinants, mechanisms, and consequences of DD in HIV infected individuals.

Objective 1: Understand the determinants of diastolic dysfunction

Aim 1a: To compare persistent inflammation between HIV+/DD- and HIV+/DD+ subjects.

Hypothesis: HIV+/DD+ subjects will have greater levels of pro-inflammatory cytokines (IL-6, hsCRP) than HIV+/DD- subjects despite ART and viral suppression

Aim 1b: To compare immune activation between HIV+/DD- and HIV+/DD+ subjects.

Hypothesis: HIV+/DD+ persons will have greater immune activation (sCD163; sCD14; CD14 and CD16 expression; and T cell activation and subsets: Th1, Threg, CD4+, CD8+ T cell activation) than HIV+/DD- subjects.

Aim 1c: To compare inflammation between HIV+/DD+ and HIV-/DD+ subjects.

Hypothesis: HIV+/DD+ persons will have greater inflammation (IL-6, hsCRP) than HIV-/DD+ subjects.

Aim 1d: Perform phenomics of aggregate demographic, clinical, biomarker, electrocardiogram, and imaging data to define risk factor phenotype signatures, and relate these to HIV+/DD+ vs. HIV-/DD+.

Hypothesis: Specific pheno-groups will be associated with HIV+/DD+ and will differ from HIV-/DD+.

Objective 2: Understand the mechanisms of diastolic dysfunction

Aim 2a: To compare myocardial fibrosis by magnetic resonance imaging between HIV+/DD+ and HIV+/DD- subjects.

Hypothesis: HIV+/DD+ persons will have greater fibrosis burden than HIV+/DD- persons.

Aim 2b: To identify systemic determinants of DD in HIV+ persons.

Hypothesis: HIV+/DD+ persons will have higher serum levels of biomarkers of fibrosis and remodeling, arterial inflammation, oxidative stress, and hypercoagulability than HIV+/DD- or HIV-/DD+ subjects.

Aim 2c: To study the proteomic and metabolomic profile of HIV+/DD+ subjects.

Hypothesis: The broad representation of biological pathways included on the proteomic and metabolomic panels will enable identification of novel mechanisms underlying DD in HIV+ subjects.

Objective 3: Understand the consequences of diastolic dysfunction.

Aim 3a: To study the effect of DD on mechanics of the left atrium in HIV.

Hypothesis: HIV+/DD+ subjects will have blunted augmentation of left atrial strain during passive leg raise compared to HIV+/DD- and HIV-/DD+ subjects.

Aim 3b: To study the sub-clinical necrosis and myocardial stress in HIV+/DD+ subjects.

Hypothesis: HIV+/DD+ subjects will have higher troponin and NTproBNP levels than HIV+/DD- and HIV-/DD+ subjects.

4.1 Exploratory Objectives

Exploratory analyses will assess if DD in HIV is associated with higher wall stress and the potential microvascular dysfunction in these patients leads to subclinical myocardial necrosis.

A common pool of uncommitted resources will be used to solicit further ancillary sub-studies. These studies may be related to focus on other populations (e.g. HIV+ subjects without viral suppression), assessment of other novel immune activation markers or viral DNA or residual replication, gut microbiome and its association with DD, arterial stiffness and endothelial function, and epicardial, myocardial, and visceral fat distribution, and skeletal muscle function in HIV patients with and without DD.

5 BASIC STUDY DESIGN

This is a multicenter clinical trial of a cross section of HIV+ patients with and without diastolic dysfunction. Sites currently participating in the Heart Failure Clinical Research Network (HFN) will collaborate with their HIV clinics to identify eligible patients. Approximately 200 HAART-treated virally suppressed HIV+ subjects (100 HIV+/DD+ & 100 HIV+/DD-) will be enrolled. Data will be managed by the HFN Coordinating Center (CC) at Duke University. This study will evaluate biomarkers, phenomapping, metabolomics, cMRI, echocardiography to determine characteristics unique to this patient population. This patient population will then be compared to the MESA (Multi-Ethnic Study of Atherosclerosis) population providing subjects who are HIV-/DD+ (see section 7.0).

5.1 Screening Phase

Subjects who are HIV positive, have been on HAART for ≥ 6 months and are virally suppressed will be screened for participation in the study using the inclusion and exclusion criteria listed below. Eligible patients will be approached to consent to the study. Those who agree to participate will be screened with an echo to determine if they are EF eligible and classified as DD+ or DD-. Diastolic dysfunction criteria are:

- (1) LVEF $> 50\%$
- (2) Evidence of impaired LV relaxation (septal $e' < 7\text{cm/s}$ or lateral $e' < 10\text{cm/s}$)
- (3) Evidence of chronically elevated LV filling pressure or LVH (or concentric remodeling)
 - (a) LA volume index $> 28\text{ml/m}^2$ or

- (b) LV hypertrophy (LV mass index $>95\text{g/m}^2$ in women, $>115\text{g/m}^2$ in men or
- (c) Concentric LV remodeling (relative to wall thickness >0.42)

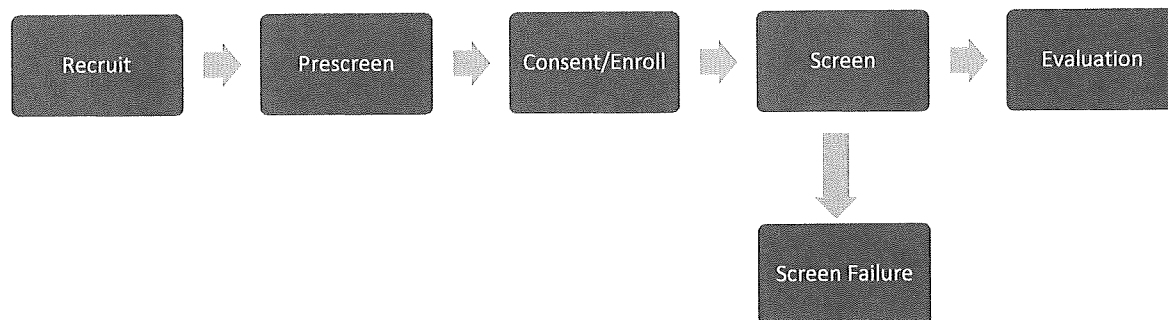
5.2 Baseline Evaluation Phase

Subjects who qualify for the study will have a detailed medical history, physical exam, blood drawn, and cMRI tests acquired.

5.3 Follow-up Phase

There is no follow-up phase in this study. Study participation will conclude after the Baseline Visit.

6 STUDY FLOW DIAGRAM



7 STUDY POPULATION AND ELIGIBILITY CRITERIA

Study Population

Subjects who are receiving care at a site participating in the Heart Failure Clinical Research Network program, are HIV positive, have been on HAART for ≥ 6 months and are virally suppressed will be screened for participation in the study using the inclusion and exclusion criteria listed below.

Enrolled subjects will be compared to non-HIV subjects with DD (HIV-/DD+) from MESA.

MESA Longitudinal Cohort Study

MESA is an NIH-funded longitudinal cohort study that recruited 6,814 participants from 6 centers (Winston-Salem, Los Angeles, Baltimore, Chicago, New York, Minneapolis/St Paul). At enrollment during 2000-2002, all participants were aged 45-84 and free of CV disease (CVD). There has been continuous surveillance for CVD events including HF. MESA Exam 6 will enroll

3500+ participants and will be conducted from September 2016 to March 2018. MESA Exam 6 will include comprehensive echocardiography, arterial stiffness measures, and blood collection for biomarkers. There is a concurrent echocardiographic study in MESA exam 6 that has been funded (R01 HL127028, PI Sanjiv Shah) that will recruit 3500+ participants from 2016-17 and will form the cohort for HIV-/DD+ for the present study; the HIV study and MESA will have an identical dynamic echocardiography protocol, with a single reading center at Northwestern University for both studies.

7.1 Inclusion Criteria

1. Age ≥ 40 years
2. Willingness and ability to provide informed consent
3. HIV antibody positive
4. On HAART for ≥ 6 months (HIV positive cohort only)
5. History of adequate viral suppression as defined by most recent HIV RNA level < 200 copies/mL performed in the past 6 months
6. LVEF $> 50\%$

7.2 Exclusion Criteria

1. Past EF $< 50\%$
2. Moderate or severe valve stenosis or regurgitation, or past repair or replacement
3. Percutaneous or surgical revascularization or active angina
4. Persistent atrial fibrillation
5. BP > 160 mmHg SBP or > 100 mmHg DBP
6. Comorbid inflammatory disease (e.g. RA or SLE)
7. Active cancer or cancer chemotherapy treatment in the prior year (except skin cancer that did not require chemotherapy or radiation)
8. Chronic use of steroids or anti-inflammatory therapy
9. GFR < 30 mL/min
10. Active in a clinical trial with investigational product
11. Pregnant or lactating females
12. Contraindication to cMR or gadolinium injection (such as severe claustrophobia, metal implants, etc.)

7.2.1 Exclusion Criteria for T1 mapping substudy

1. Atrial Fibrillation at time of MRI

8 SCREENING PROCEDURES

8.1 Pre-screening

Patients meeting eligibility criteria will be approached regarding participation in the study.

8.2 Screening Phase

Subjects who sign the informed consent document will have an echocardiogram (echo). If the subject continues to meet the inclusion criteria, they will proceed to the baseline

procedures. Women of child bearing potential who are not post-menopausal will receive a pregnancy test (urine or serum) within 48 hours prior to the MRI.

9 BASELINE EVALUATION

9.1 Baseline Evaluation Phase

Detailed data will be collected to clinically characterize patients including:

- Demographics
- Risk factors and duration of HIV
- Illicit drug and tobacco use
- Alcohol use
- Co-infections including hepatitis B and C
- Comorbidities including diabetes mellitus, hypertension, chronic kidney disease, body mass index and dyslipidemia
- Medications, including previous antiretroviral therapy
- Physical examination
- Medical history
- Collection of historical bloodwork (chemistry and hematology) values and HIV specific laboratory testing including CD4 count and HIV RNA levels (no blood will be drawn for this purpose)
- Past cardiac procedures
- Past imaging study results
- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- EKG
- Blood draw for biomarkers, -omics, and biorepository (if consented to biorepository)
- cMRI
- cMRI T-1 mapping substudy, if available at the center and subject consented to additional testing, including a hematocrit lab draw to calculate extracellular volume

10 FOLLOW-UP EVALUATIONS

There are no follow-up visits nor follow-up procedures in this protocol.

11 OUTCOME DETERMINATIONS

Understanding DD pathogenesis in HIV will allow for the identification of treatment paradigms for these patients at risk of HF, which can be very challenging to treat once HF is clinically overt.

11.1 Biomarkers

The biomarkers selected for the various pathophysiologic domains represent biomarkers that have either an established evidence base in HIV, or domains applicable to the hypothesized cardiovascular pathophysiology in HIV DD. We propose to study biomarkers related to

inflammation, immune activation, fibrosis and remodeling, coagulation, and oxidative stress.

TABLE 1

Biomarkers			
Inflammation	IL-6, hsCRP	Arterial inflammation	Oxidized LDL, LpPLA2
Immune activation	sCD163, sCD14, monocytes expression of CD14 and CD16, T cell activation and subsets: Th1, Threg, CD4+, CD8+ T cell activation	Fibrosis and remodeling	ST2, GDF-15, C1P:MMP-1 ratio, Gal 3
Coagulation	D-dimer, fibrinogen	Oxidative stress	Myeloperoxidase
Necrosis	hs troponin I	Stress	NTproBNP

In HIV infection, biomarkers of immunity and inflammation are elevated even in the setting of treated suppressed HIV compared to non-infected controls, and higher levels are associated with adverse events and mortality.¹²⁻¹⁸ **IL-6** is associated with an increased risk of CVD events and mortality in HIV+ subjects. Highest quartile of IL-6 is associated with 8-fold increased risk of mortality in HIV. IL-6 and **CRP** have been shown to be associated with 4 and 2-fold increased risk of CVD events, respectively. Higher levels of inflammation markers in HIV+ subjects are associated with higher viral load. IL-6 and **D-dimer** levels may mediate the relationship between HAART interruption and risk for mortality. T cell activation has been associated with carotid IMT and arterial stiffness in HIV-infected individuals.¹⁹⁻²¹ Monocytes are classified on the basis of their **CD14 and CD16** surface expression into *classic* (CD14++/CD16-), *intermediate* (CD14++/CD16+) and *non-classic* (CD14low/+CD16++) subsets.²² In HIV, expansion of intermediate and non-classical monocytes has been shown, however only the expansion of non-classical monocyte persists after starting HAART.²³ Intermediate monocytes²⁴ and CD16+ monocytes expressing CX3CR1²⁵ have been associated with subclinical atherosclerosis. Which features of peripheral monocytes are related to the increased DD and HF risk in treated HIV is not known. Circulating soluble CD163 (**sCD163**) is a scavenger receptor found on monocytes and shed by proteolytic cleavage after pro-inflammatory stimulation.^{26, 27} HAART can reduce cCD163 in HIV.²⁸ Elevated levels of sCD163 are associated with coronary plaque¹⁶ and shortened telomere length²⁹ in HIV.

Soluble ST2 (**sST2**), predominantly released by endothelial cells,^{30,31} along with being a marker of inflammation, promotes cardiac fibrosis by acting as a decoy receptor for IL-33, preventing IL-33 binding with its ligand, the myocyte bound ST2 receptor. Elevated concentrations of sST2 are associated with mortality and DD in the general and in those with HIV.^{32,33} **Oxidized LDL** is associated with non-calcified plaque in HIV.³⁴ **Galectin-3** is released by activated macrophages and has been shown in animal models to be associated with cardiac fibrosis.³⁵ Galectin-3 levels may be upregulated in HIV.³⁶ Galectin-3 is associated with cardiovascular events and death.³⁷ Galectin-3 is associated with carotid intima-media thickening in women with HIV.³⁸ **GDF-15**, a biomarker produced by both myocytes and endothelial cells, is an independent prognostic marker of death in HIV and adverse CV outcomes in multiple non-HIV cohorts.^{32,33,39}

Biomarkers representing myocardial injury (**hs-cTnI**) and strain (**NT-proBNP**) inform the role of inflammation on preclinical myocardial disease. hs-cTnT is a powerful prognosticator of CVD events, including HF.⁴⁰⁻⁴² Studies have shown a strong association of hs-cTnT levels with LV mass and function in general.^{40,43} NT-proBNP levels also predict the risk of incident HF.^{44,45} NT-

proBNP may predict CVD events in HIV.⁴⁶ NT-proBNP levels are inversely associated with decreased radial strain, a subtle measure of systolic dysfunction.⁴⁷ In HIV NT-proBNP remains a cardiac specific marker of strain that is likely preceded by preclinical evidence of myocardial injury and potentially fibrosis.

11.1.1 Inflammation and Immune Activation

Biomarkers of immunity and inflammation are elevated in HIV and higher levels are associated with adverse cardiac events and mortality. Although HAART reduces immune activation and inflammation, studies show persistent T cell activation and inflammation despite therapy. This may be related to residual HIV replication, gut mucosal injury and microbial translocation, co-infections, and homeostatic drive, which in turn may be determined by when HAART is initiated, degree of CD4 T cell restoration, age, comorbidities, and genetic background. This proposal seeks to study the importance of persistent inflammation and immune activation despite HAART and viral suppression on development of DD, its associated pathophysiologic perturbations, and its consequences on cardiac function.

11.2 Myocardial Fibrosis and Inflammation

Advances in cardiac magnetic resonance (CMR) imaging now allow for the detection of novel structural and functional characteristics that inform the pathophysiology of cardiac dysfunction. Along with assessing standard clinical and subclinical structural and functional derangements, this study will use CMR to study focal and diffuse myocardial fibrosis, and myocardial inflammation and edema in HIV+ subjects with and without DD. A sub study assessing for diffuse interstitial fibrosis in a subset of patients using T1 mapping CMR to index ECV will be conducted.

11.3 Proteomics/Metabolomics

There remains a knowledge gap about factors that predict risk beyond traditional risk factors and the mechanisms underlying the increased risk for DD and HF in HIV. Immune dysregulation with persistence of HIV DNA and the resultant inflammation have been implicated.^{48,49} While biomarker studies have been conducted in HIV, these have focused primarily on individual inflammatory biomarkers. Many nucleoside reverse transcriptase inhibitors may result in mitochondrial toxicity⁵⁰ and some protease inhibitors can induce endoplasmic reticulum stress,⁵¹ both pathways with metabolic byproducts proposed herein. The broad representation of pathways included in the proteomics and metabolomics panels enables testing of many known pathways and identification of novel mechanisms. High throughput -omics approach has identified novel markers and mechanisms in general, but has not been specifically applied to investigation of DD and HF pathogenesis in HIV. This study therefore has a strong likelihood of identifying novel molecular pathways of DD and HF pathogenesis in HIV, and aims to identify drug targets and a deeper understanding of the relationship between HIV, DD, and HF.

11.4 Left Atrial Reserve

Left atrial (LA) dysfunction is associated with development of HFpEF. In patients with prevalent HFpEF, LA dysfunction leads to pulmonary hypertension, reduced exercise capacity, and ultimately poor clinical outcomes. This protocol will use novel speckle-tracking image analysis in order to understand subclinical functional abnormalities in the LA. The passive leg raise test will determine the ability of the LA to augment its reservoir and booster function in response to

increased preload. Dynamic changes in LA strain will identify differences between HIV+/DD+, HIV+/DD-, and HIV-/DD+ subjects.

11.5 Phenomapping

DD represents the end result of multiple risk factors. Understanding this heterogeneity of DD may allow for development of targeted prevention and treatment interventions. An ideal classification would group together pathophysiologically homogeneous individuals who may behave in a similar manner. Machine learning, the process of using data to learn relationships between subjects, is ideally suited for this. Machine learning approaches can be supervised or unsupervised. Supervised learning aim to predict specific outcomes. Unsupervised learning attempts detection of the intrinsic structure within data, independent of outcomes, and is seen as an initial strategy to derive a set of features for novel classification, which can subsequently be used for supervised learning. With sophisticated tools ranging from biomarkers to imaging, “deep phenotyping” is possible to better classify heterogeneous syndromes like DD. Applying machine learning algorithms to dense phenotyping may enable detection of novel patterns in dense, multi-dimensional data obtained from patients with DD. Prior work suggests that these “phenogroups” have unique pathophysiologic profiles and outcomes.

12 PARTICIPANT SAFETY

12.1 Institutional Review Boards

All HFN sites will submit the study protocol, informed consent form, and other study documents to their IRB for approval—the approval letter for each clinical center will be stored at the CC. Any amendments to the protocol, other than minor administrative changes, must be approved by each IRB before they are implemented.

12.2 Recording and Reporting of AEs/SAEs

General collection of AEs and SAEs will not be included for this observational study, based on the assumption that this protocol is exempt from investigational new drug regulations under FDA Code of Federal Regulations (CFR) Title 21, 312.2(b). Research sites should abide by their local IRB policies and procedures and any post marketed/spontaneous reporting regulatory guidelines for any approved drugs.

13 STATISTICAL CONSIDERATIONS

13.1 Overview

A statistical analysis plan will be completed before the data are analyzed in a blinded fashion. Statistical tests with p-value <0.05 will be considered statistically significant. The trial results will be reported according to guidelines specified in the CONSORT statement. A flow diagram describing screening, recruitment, dropout, and vital status will be included in the primary manuscript. Analyses will be performed using SAS software (SAS Institute, Inc, Cary, NC).

13.2 Power Calculations and Statistical Analysis for Biomarkers

The primary statistical comparison for Aim 1a is the between group (the HIV+/DD+ and HIV+/DD- cohorts) difference in log-transformed IL-6 biomarker levels. Log-transformed IL-6 (log pg/ml) is expected to have a standard deviation of approximately 0.75.⁵² Sample sizes of 100 subjects in each group will provide 80% and 90% power to detect differences of 0.30 and 0.35 (log pg/ml), respectively. These sample size calculations were based on a two-sample t-test with a two-sided type I error of 0.05. Additionally these calculations suggest that any comparison of (properly transformed) continuous variables between the HIV+/DD- and HIV+/DD+ cohorts will have 80% and 90% power to detect differences of 0.40 and 0.46 standard deviations, respectively. Comparisons between the HIV+/DD+ or HIV+/DD- cohorts and the HIV-/DD+ cohort will have greater power due to the larger number of subjects in the HIV-/DD+ cohort. Statistical summaries will use median (25th, 75th percentiles) and mean (standard deviation) for continuous measures and frequencies (percentages) for categorical variables. Because many biomarkers are expected to be right-skewed, we will log-transform each marker or use another appropriate transformation to normalize its distribution. Unadjusted comparisons between groups will be based on t-tests after the appropriate transformations for continuous measures and chi-square tests for categorical measures. For Aim 1a, propensity scores will be the primary tool used to obtain balanced comparisons between cohorts. The propensity score is defined as the probability of having group membership (e.g. HIV+/DD+) conditional on observed covariates and is a commonly used balancing score used in observational studies.⁵³ Three different methods of propensity score adjustment will be applied. The primary analysis method will be based on inverse probability weightings. Secondary approaches will be based on full propensity score matching and doubly-robust estimation.^{54, 55} Distributional overlap and covariate balance will be assessed using the recently developed best practices for propensity score analyses.⁵⁶ The primary adjusted analysis of IL-6 levels between cohorts will be conducted at the two-sided 0.05 level.

13.3 Phenomapping Statistical Analysis

For machine learning analyses, quantitative phenotypic data collected at clinic visits or at study enrollment for HFN cohort, and from prior and current MESA visits, will be used. These data include clinical, demographic, medication, laboratory, electrocardiogram, echocardiogram, and CMR phenotypes. We will standardize individual variables (mean 0, SD 1) so that biomarkers with larger variances will not have a greater influence on cluster assignment. We will then use gene expression hierarchical clustering software to create an unbiased phenotype heat map (phenomap). Next, in R software, we will use statistical learning approaches to refine our phenomics analysis and algorithms by using penalized model-based clustering, principle components analysis (PCA), multinomial logistic regression with lasso penalty, and cross-validation with model fitting to determine the ideal number of phenogroups.⁵⁷⁻⁶⁶ Dr. Sanjiv Shah has developed a generalizable logistic regression model to define membership for each pheno-group in order to permit robust classification of subjects with minimal set of phenotypic features. His model was created using a penalized form of statistical learning, specifically multinomial logistic regression with L1 norm (lasso), which determines regression coefficients by minimizing the deviance of the model, subject to a penalty for the sum of the absolute value of the coefficients. The penalty results in inclusion of only a subset of variables into the final model. The weight of the penalty (λ) was determined using 10-fold cross-validation. Cross-validation and model fitting will be performed using the *cv.glmnet* and *glmnet* functions, respectively, in the *glmnet* package within R statistical software. Using the multinomial logistic regression with lasso approach, a limited subset of variables will be used for classifying study participants into pheno-groups. Alternate

supervised and unsupervised machine learning techniques (e.g., support vector machines, tensor factorization, and neural networks) will also be explored to determine whether they are superior to unsupervised model-based clustering approaches.

13.4 cMRI Power Calculation and Statistical Analysis

The primary statistical comparison for Aim 2a is the between group (the HIV+/DD- and HIV+/DD+ cohorts) difference in myocardial fibrosis levels. The sample sizes of 100 subjects in each cohort will provide 80% and 90% power to detect differences of 0.40 and 0.46 standard deviations, respectively. For Aim 2a, propensity scores will be the primary tool used to obtain balanced comparisons between cohorts. Three different methods of propensity score adjustment will be applied. The primary analysis method will be based on inverse probability weightings. Secondary approaches will be based on full propensity score matching and doubly-robust estimation. Assessment of distributional overlap and covariate balance will be assessed using the recently developed best practices for propensity score analyses. The primary adjusted analysis of fibrosis levels between cohorts will be conducted at that two-sided 0.05 level.

13.5 Protein / Metabolite Statistical Analyses

Primary analyses will be of individual proteins/ metabolites of interest proposed in Aims 1-3. Non-normal levels will be log transformed. Multivariable generalized linear regression adjusted for potential confounders will be used to test for differences in mean values of analytes between HIV+/DD+ and HIV+/DD- subjects. Analytes with >25% values as "0" (e.g. below the lower limits of quantification) will be analyzed as binary traits. Analytes that do not attain normality will be analyzed using non-parametric tests. Benjamini and Hochberg false discovery rate (FDR) will be used for multiple comparisons. To identify novel proteins or metabolites that differentiate HIV+/DD+ and HIV+/DD- subjects, we will analyze all other proteins available on the SomaScan platform and the full array of metabolomic data. FDR will be used for adjustment for multiple comparisons. Box-and-whisker plots will be used to visually assess data, and mean metabolite/protein levels and effect sizes will be compared to those from our previous work in non-HIV cohorts.

SomaLogic's SomaScan platform is a relatively new platform but has been used in many studies including population based studies. There are some existing data that SomaLogic has shared with us including binding specificity for each protein, comparison for some proteins to more standard assays, CVs and lower limits of quantification. For proteins that are found to be significant in this proposal that do not have comparison data to standard assays (and showing high correlation), standard assays (e.g. ELISAs) will also be performed on all samples to validate the results on the SomaLogic platform.

13.6 Integrated Molecular Statistical Analysis

Analytic methods will be used for integration of the multi-dimensional data. Principle components analysis (PCA) with varimax rotation and use of the Kaiser criterion for identification of PCA factors will be tested for discrimination. Models will be further tested with random forests and penalized regression,⁶⁷ either with or without dimension reduction.⁶⁸ To control for flexibility, we will test accuracy of prediction with cross-validation. Although high-throughput -omic technologies are used to predict outcomes, they present challenges related to high-dimensional data spaces created where modest number of samples are often defined by

thousands of measurements. We will utilize Bayesian Factor Regression Models^{69,70} to reduce the effective dimension of the data prior to model building. We will utilize a two-step factor regression approach to building predictive models. First, factor models will be used to aggregate subsets of metabolomics or proteomics data into single meta-genes based on high levels of co-expression.⁷¹ Second, the factors will be used collectively as independent variables to build predictive model, either penalized regression or random forests. Model accuracy for discrimination of DD will be assessed by area under the receiver operating characteristic curves. We will also utilize pathway-based models, assessing for over-representation of the most significant predictors in pathways using Ingenuity Software for Pathway Analysis and biochemical pathway analysis using UniPathway annotation (www.grenoble.prabi.fr/obiwarehouse/unipathway).

13.7 Echo Power Calculation and Statistical Analysis

The primary statistical comparison for echo is between group (the HIV+/DD- and HIV+/DD- cohorts) difference in left atrial strain. The sample sizes of 100 subjects in each cohort will provide 80% and 90% power to detect differences of 0.40 and 0.46 standard deviations, respectively. Propensity scores will be the primary tool used to obtain balanced comparisons between cohorts. Three different methods of propensity score adjustment will be applied. The primary analysis method will be based on inverse probability weighting. Secondary approaches will be based on full propensity score matching and doubly-robust estimation. Assessment of distributional overlap and covariate balance will be assessed using the recently developed best practices for propensity score analyses. The primary adjusted analysis of LA strain levels between cohorts will be conducted at that two-sided 0.05 level.

14 DATA MANAGEMENT PROCEDURES

14.1 Overview of Data Management

The CC will have primary responsibility for data management, including the development of data collection systems, data monitoring processes, and data storage and back-up. State-of-the-art technology will be used for the management of the network's data.

Data Management Process: The EDC system will be used for data entry and simple reports. All data will be entered into the eCRF by personnel at the clinical sites. Any out-of-range values and missing key variables will be flagged and addressed in real-time at the site during data entry. When a query is generated on a particular variable, a flag is raised in a database field; the system tracks the queries and produces reports of outstanding queries. Queries can also be generated from manual or statistical review of the data forms.

The CC will create reports to identify trends in the data that may require additional clarification and training. These reports will be available to the sites and to the study leadership as we work with the sites to correct negative trends and eliminate future data errors. The CC will perform internal database quality-control checks during the study to identify systematic deviations requiring corrections.

14.2 Publication Policy

Dissemination of preliminary information can adversely affect the objectivity of study data. For this reason, Investigators will be prohibited from performing subset analyses at any point prior to the conclusion of the study, and any data, other than safety data, cannot be used for publication or reporting outside of this study until the study is completed or discontinued by the DSMB or HFN Steering Committee.

15 STUDY ADMINISTRATION

15.1 Data and Safety Monitoring Board (DSMB)

A DSMB has been appointed and vetted by the NHLBI for the HFN, and will function as the DSMB for this trial. This committee consists of a group of highly experienced individuals with extensive pertinent expertise in HF and clinical trials. Ad hoc members with expertise in imaging and HIV have been identified to review this protocol. The DSMB will advise the HFN Steering Committee regarding the scientific merit of the trial. There will be no ongoing safety data for the DSMB to review.

15.2 Coordinating Center

The DCRI will function as the CC for this trial as specified by the National Institute of Health and NHLBI HFN grant.

15.3 Core Laboratories

15.3.1 Biomarker Core Laboratory

The University of Vermont will serve as the core laboratory for measurement of HFN biomarkers. Plasma specimens will be collected at the baseline study visit. Samples will be processed at the clinical centers according to the procedures provided by the core laboratory. Samples will be shipped to the core laboratory on dry ice (Refer to Biomarker Core Laboratory Manual of Procedures). Processing and analysis of biomarker samples will be performed under the oversight of Dr. Russell Tracy.

15.3.2 Echocardiography Core Laboratory

Northwestern University will serve as the core laboratory for measurement of HFN echoes. Echo images will be relayed to the core lab from the clinical centers via HeartIT (Refer to Echo Core Laboratory Manual of Procedures). Heart Imaging Technologies (Heart IT) is an image management solution software to encrypt and securely transfer images. Evaluation of echocardiograms will be performed under the oversight of Dr. Sanjiv Shah.

Echocardiography will be performed at the clinical sites and reviewed at Northwestern University echocardiographic core laboratory using the techniques described in the Echocardiography Manual of Operations for the HIV study. All clinical sites will be required to submit sample echocardiographic studies to the core laboratory for site certification prior to commencing enrollment of patients in the trial.

15.3.3 Cardiac Magnetic Resonance Imaging Core Laboratory

Duke University Medical Center will serve as the core laboratory for measurement of HFN cMRIs (Refer to cMRI Core Laboratory Manual of Procedures). cMRI images will be relayed to the core lab from the clinical centers via HeartIT (Refer to cMRI Core Laboratory Manual of Procedures). Heart Imaging Technologies (Heart IT) is an image management solution software to encrypt and securely transfer images. Processing and review of cMRIs will be performed under the oversight of Dr. Raymond Kim.

MRI will be performed at the clinical sites and reviewed at Duke University MRI core laboratory using the techniques described in the MRI Manual of Operations for the HIV study. All clinical sites will be required to submit sample MRI studies to the core laboratory for site certification prior to commencing enrollment of patients in the trial.

15.3.4 Metabolomics and Proteomics Core Laboratory

Duke University Medical Center will serve as the core laboratory for measurement of Metabolomics and Proteomics. Metabolomic and proteomic profiling will be conducted at the Duke Molecular Physiology Institute (DMPI) under the oversight of Dr. Svati Shah.

16 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol. These procedures are designed to ensure adherence to Good Clinical Practice, as described in the ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.

16.2 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be provided to the CC before study initiation. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

16.3 Informed Consent Procedures

16.3.1 Informed Consent

The Investigator or designee must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. If a patient agrees to participate in the study, they will review and sign the site-specific Internal Review Board (IRB) approved informed consent form (ICF). The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If written consent is not possible, oral consent can be obtained if witnessed by a signed statement from one or more persons not involved in the study, mentioning why the patient was unable to sign the form. No patient can enter the study before his/her informed consent has been obtained.

The ICFs are part of the protocol, and must be submitted by the investigator with it for IRB/IEC approval. The CC will supply proposed informed consent forms, which comply with regulatory requirements, and are considered appropriate for the study. Any changes to the proposed consent form suggested by the Investigator must be agreed to by the CC before submission to the IRB/IEC, and a copy of the approved version must be provided to the CC after IRB/IEC approval.

16.3.2 Confidentiality and HIPAA Requirements

All information collected on study participants will be stored in a confidential manner using the procedures in place at each participating center. Only approved study personnel will have access to data collected as part of the study. Study participants will be identified by a participant ID number on all study documents. Data will be transmitted to the CC in a secure manner, and stored securely at the CC using standard Duke Clinical Research Institute (DCRI) operating procedures. Data will be transmitted to the core labs in a secure manner and stored securely.

16.3.3 Protections of Human Subjects

Protections for human subjects of research are required under Department of Health and Human Services (HHS) regulations at 45 CFR 46.

Each institution engaged in (non-exempt) HHS-supported human subjects research must provide a written Assurance of Compliance, satisfactory to the Office for Protection from Research Risks, that it will comply with the HHS human subjects regulations—45 CFR46.103(a).

16.3.4 Summary of the Risks and Benefits

Risks associated with a blood draw: The risks of drawing blood include bleeding at the puncture site, bruising and pain. These occur in a very small portion of the population.

Risks associated with an Echocardiogram or Echo: The risks of associated with an echo include pressure on the chest with a machine to obtain the pictures of the heart. Rarely, the pressure can be uncomfortable.

Risks associated with a cMRI: Serious reactions to the contrast agent used during some MRI tests are very rare. However, side effects are possible and include headache, nausea, dizziness, changes in taste, and allergic reactions. Rarely, the contrast agent can harm people who have severe kidney or liver disease. The substance may cause nephrogenic systemic fibrosis.

17 REMOTE MONITORING

The study will be monitored remotely by representatives of the DCRI or its designee according to the prospective clinical monitoring plan for the following purposes:

- To enable real-time monitoring of compliance with study protocol inclusion and exclusion criteria is enabled via triggers and range checks programmed in the InForm database.
- To assist site personnel who will verify data identified within query reports against source documents through frequent telephone and email contact.
- To verify that written informed consent was obtained before initiation of any screening procedures that are performed solely for the purpose of determining eligibility for the clinical study and/or prior to the participant's randomization to a procedure.

18 REFERENCES

1. Remick J, Georgiopoulou V, Marti C, Ofotokun I, Kalogeropoulos A, Lewis W, Butler J. Heart failure in patients with human immunodeficiency virus infection: epidemiology, pathophysiology, treatment, and future research. *Circulation*. 2014 Apr 29;129(17):1781-9. doi: 10.1161/CIRCULATIONAHA.113.004574.
2. Cerrato E, D'Ascenzo F, Biondi-Zoccai G, Calcagno A, Frea S, Grosso Marra W, Castagno D, Omedè P, Quadri G, Sciuto F, Presutti D, Frati G, Bonora S, Moretti C, Gaita F. Cardiac dysfunction in pauci symptomatic human immunodeficiency virus patients: a meta-analysis in the highly active antiretroviral therapy era. *Eur Heart J*. 2013;34:1432–1436.
3. Fischer M, Baessler A, Hense HW, Hengstenberg C, Muscholl M, Holmer S, Döring A, Broeckel U, Riegger G, Schunkert H. Prevalence of left ventricular diastolic dysfunction in the community. Results from a Doppler echocardiographic-based survey of a population sample. *Eur Heart J* 2003;24:320–328.
4. Achong N, Wahi S, Marwick TH. Evolution and outcome of diastolic dysfunction. *Heart* 2009;95:813–818.
5. Aurigemma GP, Gottdiener JS, Shemanski L, Gardin J, Kitzman D. Predictive value of systolic and diastolic function for incident congestive heart failure in the elderly: the cardiovascular health study. *J Am Coll Cardiol* 2001;37:1042–1048.
6. Rosenberg MA, Manning WJ. Diastolic dysfunction and risk of atrial fibrillation: a mechanistic appraisal. *Circulation* 2012;126:2353–2362.
7. Ntusi N, O'Dwyer E, Dorrell L, Wainwright E, Piechnik S, Clutton G, Hancock G, Ferreira V, Cox P, Badri M, Karamitsos T, Emmanuel S, Clarke K, Neubauer S, Holloway C. HIV-1-Related Cardiovascular Disease Is Associated With Chronic Inflammation, Frequent Pericardial Effusions, and Probable Myocardial Edema. *Circ Cardiovasc Imaging*. 2016 Mar 9: e004427.
8. Luetkens JA, Doerner J, Schwarze-Zander C, Wasmuth JC, Boesecke C, Sprinkart AM, Schmeel FC, Homsí R, Gieseke J, Schile HH, Rockstroh JK, Naehle CP. Cardiac Magnetic Resonance Reveals Signs of Subclinical Myocardial Inflammation in Asymptomatic HIV-Infected Patients. *Circ Cardiovasc Imaging*. 2016 Mar 9: e004091.
9. Hsue PY, Tawakol A. Inflammation and Fibrosis in HIV: Getting to the Heart of the Matter. *Circ Cardiovasc Imaging*. 2016 Mar 9: e004427.
10. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013 Jul 23;62(4):263-71