Losartan to reduce Inflammation and Fibrosis Endpoints in HIV (LIFE-HIV) Trial

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NOTE: This protocol was developed consistent with 'Standard Protocol Items: Recommendations for Intervention Trials' (the SPIRIT initiative).[1]

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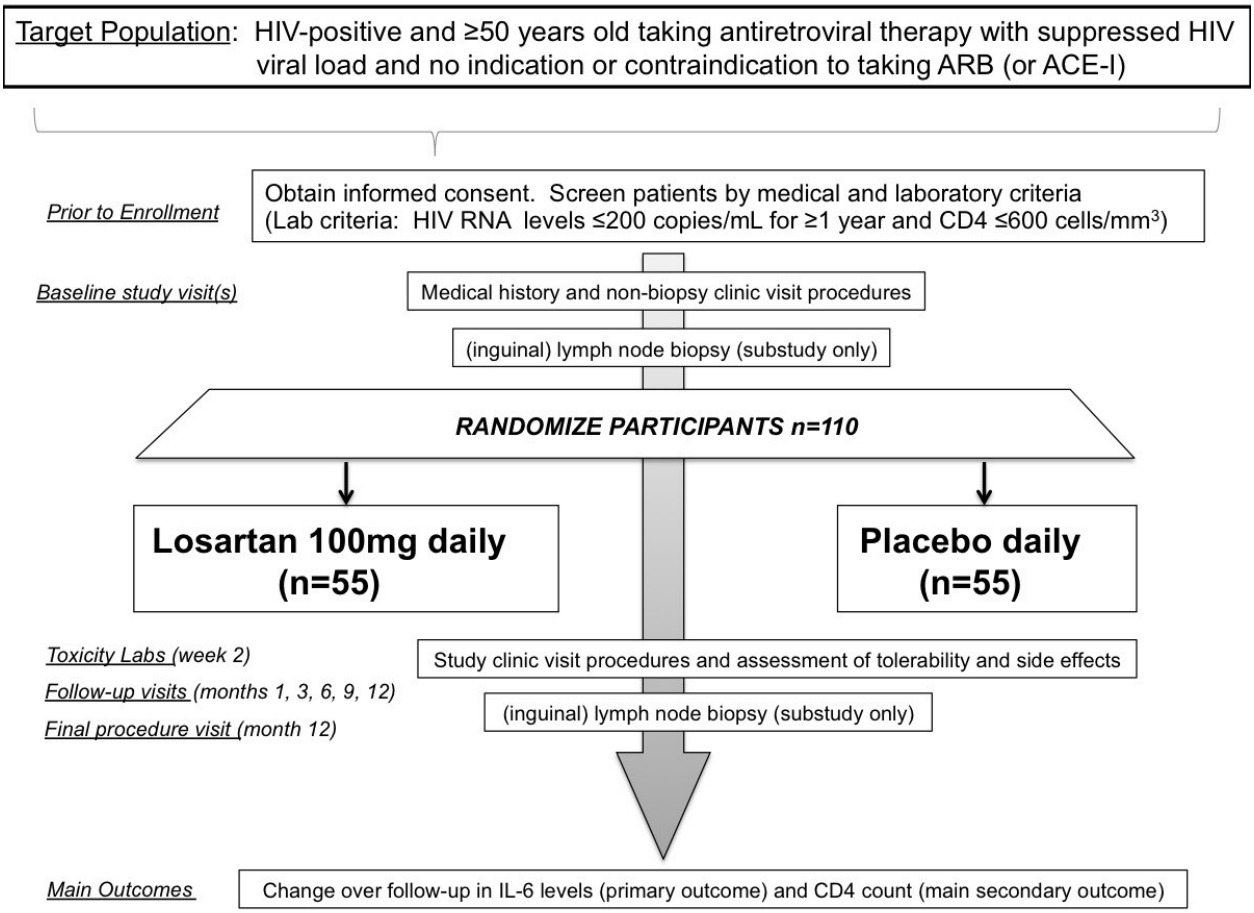
List of Abbreviations

This list should be modified to include protocol-specific terms.

ACE-I	Angiotensin Converting Enzyme Inhibitor
AE	Adverse Event/Adverse Experience
AIDS	Acquired Immune Deficiency Syndrome
ARB	Angiotensin Receptor Blocker
ART	Antiretroviral Therapy
BMP	Basic Metabolic Panel
BP	Blood Pressure
CCC	Clinical Coordinating Center
CLIA	Clinical Laboratory Improvement Amendments
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CVD	Cardiovascular Disease
DCC	Data and Coordinating Center
DM	Diabetes Mellitus
DSMB	Data and Safety Monitoring Board
ESPRIT	Evaluation of Subcutaneous Proleukin® in a Randomized International Trial
FDA	Food and Drug Administration
FRCn	Fibroblastic Reticular Cell Network
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
IHC	Immunohistochemistry
IL-6	Interleukin-6
IND	Investigational New Drug
IRB	Institutional Review Board
LN	Lymph Node
LT	Lymphatic Tissue
N	Number (typically refers to participants)
NIA	National Institute of Aging, NIH
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PBMC	Peripheral Blood Mononuclear Cells
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QIA	Quantitative Image Analysis
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SD	Standard Deviation
SILCAAT	Subcutaneous, Recombinant, Human Interleukin-2 in HIV-Infected Patients with Low CD4+ Counts Under Active Antiretroviral Therapy
SMART	Strategic Management of Anti-Retroviral Therapy
TGF-β	Transforming growth factor-beta
TF	Tissue Factor

	Protocol Summary
Full Title	Losartan to reduce Inflammation and Fibrosis Endpoints in HIV
Short Title	LIFE-HIV
Clinical Trial Phase	Phase 2
IND Sponsor	*NA*
Conducted By	Minneapolis Medical Research Foundation and the University of Minnesota
Principal Investigator	Jason Baker, MD, MS
Lead Statistician	Julian Wolfson, PhD
Sample Size	110 participants
Study Population	HIV positive patients ≥50 years on stable ART with HIV RNA level ≤200 copies/mL, CD4+ count ≤600 cells/mm^3, and no indication for angiotensin receptor blocker (or ACE-inhibitor)
Accrual Period	Anticipated enrollment 1 year
Study Design	Randomize 110 participants 1:1 to receive losartan 100mg or matched placebo daily (double-blind).
Study Duration	12 months
Intervention	Losartan 100mg versus matching placebo taken daily
Primary Objective	To evaluate the effects of losartan on systemic inflammation, as reflected in plasma levels of IL-6
(Main) Secondary Objective	To evaluate the effects of losartan on immune recovery, as reflected in peripheral blood CD4+ T-cells
Additional Secondary Objectives	Evaluating the effects of losartan on monocyte and T-cell activation in blood, coagulation activation, recovery of T-cell subsets in blood, adherence, tolerability, durability of findings, and lymphatic tissue assessments (in a subset of n≥30) of fibrosis, cellular activation, and T-cell homeostasis

Schematic of Study Design



Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), and the applicable regulatory requirements including U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR 50, 56 and 21 CFR 312), directive 2001/20/EC on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use as amended by Commission Directive 2005/28/EC and NIAID Clinical Terms of Award. All key personnel (all individuals responsible for the design and conduct of this study) have completed appropriate Human Subjects Protection Training.

1 INTRODUCTION

1.1 Background and Rationale

Premise

Contemporary antiretroviral therapy (ART)-treated HIV-infected patients are at increased risk for cardiovascular disease (CVD), cancer, and other HIV-associated non-AIDS conditions. This excess risk is due to multiple factors, including a higher burden of traditional risk factors, persistent inflammation and sub-optimal immune recovery after starting ART. Independent of HIV infection, older age increases risk for non-AIDS conditions and is itself associated with greater inflammation, reduced immune recovery after starting ART, and declining immune function (so called immune senescence). Safe treatments that target this pathology, beyond HIV treatment with ART, represent a major unmet need for older HIV positive patients.

The LIFE-HIV trial is a critical step to advance this field, prior to conducting a clinical outcome trial, by testing the effects of losartan treatment in a translational clinical trial that will improve our understanding of non-AIDS disease pathogenesis. Ongoing inflammation contributes to excess CVD, cancer and disease in multiple organ systems. Specific to HIV pathogenesis, immune activation and associated inflammation also drives collagen deposition (i.e., fibrosis) in lymphatic tissue (LT), disrupting T-cell homeostasis and limiting immune recovery, which further increases non-AIDS risk. Losartan is a safe, inexpensive, medication with immunomodulatory and anti-fibrotic properties, quite apart from lowering blood pressure. We will test the hypothesis that losartan will reduce inflammation and improve tissue fibrosis leading to additional immunologic recovery. These treatment effects have been demonstrated in uninfected populations (e.g., those with renal or connective tissue diseases), but have not been tested among HIV positive patients where the mechanisms driving ongoing immune activation are somewhat unique to this disease.

Scientific Background

The Changing Spectrum of Morbidity and Mortality Among HIV Positive Patients:
Antiretroviral therapy (ART) effectively and durably suppresses HIV replication, leads to immune recovery (increasing CD4+ T-cell counts) and prolonged life expectancy, and has changed the spectrum of morbidity and mortality among HIV positive persons.[2-8] Among well-treated patients with levels of HIV RNA below the level of detection, non-AIDS-related conditions are now a more common cause of morbidity and mortality than AIDS.[6, 9-11] The most relevant serious non-AIDS-related diseases in current clinical practice include atherosclerotic cardiovascular disease (CVD), cancer, liver disease, end-stage renal disease, bone disease and subclinical neurocognitive dysfunction. Of these, CVD and cancer constitute the vast majority of clinical events.[5, 10-13]

What Contributes to Persistent Inflammation in Persons with ART-treated HIV Infection?
HIV treatment with ART reduces activation of the immune response, including levels of inflammatory cytokines, though most immunologic abnormalities persist compared to

HIV uninfected persons.[14, 15] The level of T-cell activation (e.g., CD38/HLA-DR expression), reflecting adaptive immunity, predicts risk for AIDS[16] and has been associated with CVD risk in cross-sectional studies.[17, 18] More recent data demonstrate that innate immune activation also contributes to chronic inflammation.[19-21] Activated monocytes (e.g., CD16+) are more frequent with HIV infection.[22, 23] The CD16+ monocyte subset may be preferentially infected by HIV, demonstrate greater production of cytokines, and is independently predictive of coronary artery calcium (CAC) among HIV+ patients.[24-26]

The precise mechanisms driving high level immune activation are not entirely clear, but appear to involve both a persistent anti-HIV response (even with HIV RNA at low levels) and a more generalized immune activation (e.g., cytokine release).[15, 27-29] Potential factors specific to HIV infection that may account for excess inflammation include: a) destruction of gut epithelium, which leads to chronic translocation of bacterial products, b) dysregulated inflammatory responses to co-pathogens (e.g., cytomegalovirus), and c) loss of key regulatory response. Given that drivers of HIV-related inflammation are multi-factorial and diverse, a treatment that down regulates the inflammatory response more broadly irrespective of the cause, like losartan, may be more likely to show efficacy.

<u>Pathologic Consequences of Ongoing Immune Activation For Immune Recovery:</u>
An important pathologic consequence of persistent immune activation is fibrosis of lymph node (LN) structures necessary for T-cell homeostasis.[30] Ongoing immune activation stimulates production of TGF-ß that leads to fibrotic changes in the parafollicular T-cell zone of lymph nodes. LN fibrosis damages the fibroblastic reticular cell network (FRCn) that forms the skeletal anatomy of the T-cell zone, produces IL-7, and directs movement of T-cells within the LN. The loss of naïve T-cells further perpetuates the process through declines in lymphotoxin-β (from T-cells) that is essential for FRCn vitality. The vicious cycle leads to more reductions in IL-7 and T-cell depletion. LN collagen is therefore not only a cause of T-cell depletion but also contributes to incomplete immune recovery with ART via impaired survival of naïve T-cells. Losartan has been shown, both in vitro and in vivo, to inhibit TGF-ß and reverse existing fibrosis.[31-35] These treatment effects may be particularly important for older HIV patients, given that normal aging leads to thymic involution and increased apoptosis, reducing circulating naïve T-cells.[36, 37]

<u>Study Outcomes: IL-6 and CD4 count Predict Non-AIDS Risk:</u>
Excess risk for non-AIDS conditions is due to multiple factors, including a higher burden of traditional risk factors, but persistent immune depletion and ongoing inflammation despite effective ART are key risk factors specific to HIV and are the focus of this trial. We have previously reported that CD4+ T-cell count, including changes after starting ART, predict risk for a composite of non-AIDS conditions (including CVD and cancer).[38, 39] Although risk for both AIDS and non-AIDS conditions decline with higher CD4+ counts, this is more drastic for AIDS events.[39, 40] Non-AIDS conditions are therefore much more common than AIDS events at moderate to higher (e.g., >500 cells/mm3) CD4+ counts.[41, 42] Mechanisms differ between distinct non-AIDS conditions, but

associations between CD4+ count and risk for these conditions has been reproduced in multiple cohorts.[6, 43-47]

Recent data from a number of epidemiologic studies have shown that inflammatory biomarkers well validated in the aging and cardiovascular fields[22, 48-51] are elevated with untreated and (to a lesser degree) treated HIV infection,[14, 52, 53] and predict risk for CVD, cancer, and non-AIDS mortality.[54-60] Our group conducted a series of nested case-control and cohort studies, using data from 3 large HIV trials (SMART, ESPRIT, and SILCAAT), and determined that associations for risk of CVD, cancer, a composite of serious non-AIDS events, and all-cause mortality was most robust for levels of interleukin-6 (IL-6).[61-63] These associations are not attenuated by adjusting for HIV or traditional CVD risk factors, are several-fold higher than reported in HIV-negative studies, and persist over a median of 5.5 years of follow-up, making it an ideal risk marker for a broad composite of non-AIDS conditions.[54, 62, 64, 65]

Rationale for Losartan

Losartan has well-established off-target, or non-blood pressure lowering, effects that may benefit HIV positive patients. Angiotensin-2 (AT-2), a potent vasoconstrictor, is a key regulator of the renin-angiotensin-aldosterone system (RAAS) that is also pro-inflammatory (through Nuclear Factor-Kappa B [NF-κB] pathways) and pro-fibrotic (via transforming growth factor beta [TGFβ1] induced connective tissue growth factor [CTGF] expression).[31-35] ARBs (angiotensin receptor blockers) selectively block AT receptor-1, mitigating the consequences for inflammation and fibrosis, but importantly, have fewer side effects than ACE-I. Tolerability to losartan (100mg) is considered excellent, with self-reported side effects no different than for placebo in clinical trials.[66]

There exists a large body of evidence that ARBs (e.g., losartan) down-regulate immune activation. Angiotensin-2 induces activity of NF-κB,[67, 68] a transcription factor that, in part, regulates immune activation and cytokine release (e.g., IL-6 and TNF-α).[67, 69, 70] Numerous (HIV uninfected) studies of ARBs or ACE-Is demonstrate reductions in inflammatory markers (e.g., IL-6, TNF-α, and CRP) via mechanisms both related to and independent of angiotensin receptor blockade.[71-75] In hemodialysis patients, losartan also decreased monocyte activation (CD16+) *in vivo* (to levels seen in healthy controls) and prevented monocyte activation (or CD16 expression) *in vitro*.[76] In animal models, ARBs suppress transcription and release of IL-6 and other cytokines by monocytes.[77-79]

Independent of reducing immune activation, losartan may improve T-cell homeostasis and immune recovery by reversing existing tissue fibrosis through a well characterized mechanism of blocking TGF-ß at the level of phosphorylation of SMAD-2,3.[31-35, 80-83] In animal models (of vascular, renal and cardiac fibrosis), losartan therapy inhibits TGF-ß activity and improves histologic abnormalities.[84, 85] In humans, losartan reduces urinary levels of TGF-ß among those with transplant related kidney fibrosis,[86, 87] reversed fibrosis and improved in cardiac function among those with myocardial fibrosis.[88] Losartan's net effect is also to shift the balance of matrix metalloproteases and their inhibitors in favor of tissue remodeling, which then reduces fibrosis.[89-91]

Finally, the 100mg dose of losartan was chosen: a) to maximize the treatment effect on inflammation and fibrosis,[76, 86, 92, 93] b) due to the excellent safety profile and lack of difference in adverse events between 50mg and 100mg doses (data from 20 clinical trials),[66] and c) due to the predictable CVD risk reduction through BP lowering.

1.2 Objectives

Our general goal is to evaluate the potential effectiveness of losartan (100mg daily) for reducing inflammation and improving immune recovery, given the potential for these treatment effects to reduce risk for long-term non-AIDS-defining complications among older HIV positive participants. Prior to conducting a clinical outcome trial, candidate treatments must be studied among HIV positive patients given the unique pathogenesis driving inflammation and disease risk. Study objectives then include:

- Primary Objective: Evaluate the treatment effects of losartan on systemic inflammation

- (Main) Secondary Objective: Evaluate the treatment effects of losartan on T-cell homeostasis and immune recovery of T-cell population

2 METHODOLOGY

2.1 Trial Design

The potential benefits of losartan (100mg daily) will be studied among HIV positive individuals over age 50 years whose CD4 counts remain ≤600 cells/mm^3. Participants (n=120, 60 per group) will be randomized to receive losartan or matching placebo daily. After randomization, participants will start losartan (or placebo) at a dose of 50mg once daily, increasing to 100mg once daily at the 2-week study visit pending results of a week 2 toxicity lab evaluation (see 2.4 below for criteria). Following month 1, participants will return for follow-up study visit procedures at months 3, 6, 9, and 12. A lymph node biopsy will also be performed at baseline and month 12 from a subset of participants (n≥30).

Changes from baseline in measures of inflammation, immune activation, immune recovery and fibrosis within lymphatic tissues will be studied. The primary outcome will be the average of IL-6 levels over 12 months, and the main secondary outcome will be change in CD4 count in blood over 12 months.

2.2 Study Population

The target population is HIV positive patients receiving effective treatment with ART and at low risk for AIDS defining complications. For these participants, non-AIDS defining long-term complications (e.g., CVD and cancer) dominate the spectrum of morbidity and mortality. We will also focus on participants over age 50, as advanced age is

associated with inflammation, poor immune recovery, and also increased risk for long-term complications such as CVD and cancer. Any antiretroviral medications can be used. Detailed inclusion and exclusion criteria are listed below. There are no restrictions on the use of concurrent mediations other than ARB or ACE-I, but participants with common diseases known to be pro-inflammatory will be excluded.

2.3 Eligibility Criteria

Inclusion Criteria

- HIV infection (verified by previous positive antibody or detectable HIV RNA level)
- Age ≥50 years
- Receiving continuous ART for ≥2 years; regimen changes acceptable if >4 weeks prior to screening when there is an ART class change (i.e., corresponding to either a deletion or addition of an ART class), or at anytime for an antiretroviral change that is within an ART class.
- HIV RNA level ≤200 copies/mL for ≥1 year (1 measure ≥200 allowed if also <500 and preceded and followed by one or more undetectable values)
- Blood CD4+ T-cell count ≤600 cells/mm^3
- Systolic blood pressure ≥110 mmHg (mean value, if ≥2 measures obtained)
- Estimated GFR ≥30 mL/min/1.73m^2
- Do not anticipate starting (or stopping) statin or aspirin therapy during the study
- For women of child bearing potential, agrees to use a reliable form of birth control

Exclusion Criteria

- Pregnancy or breast feeding
- A contra-indication to taking an ARB (e.g., cirrhosis, prior angioedema with ACE-I, or use of drug with potential drug-interaction [e.g., rifaximin])
- A clinical indication for ARB or ACEi therapy
- Current treatment with ARB or medication with overlapping mechanism (e.g., ACE-I or aldosterone antagonist)
- Treatment with immune-modulatory drugs within the past 6 months
- Hepatitis C treatments (e.g., interferon, ribavirin) within the past 6 months
- Serum potassium >5.0 mmol/L within the past 3 months
- Grade 3/4 lab abnormality (from BMP, CBC or liver function panel)
- Invasive cancer in the prior year or receiving cancer treatment (not including carcinoma-in-situ or basal cell cancer of the skin)
- Cirrhosis or end-stage liver disease
- Rheumatologic or chronic inflammatory disease (e.g., systemic lupus erythematosus, psoriasis, rheumatoid arthritis, vasculitis, sarcoidosis, Crohn's disease)
- Assessment by the clinical investigator that enrollment into the study could entail excess risk to the participant, beyond what is intended or expected.
- BMI >40mg/kg^2 for participants co-enrolled into the LN biopsy substudy.

NOTE: For participants consented to co-enroll into the LN biopsy substudy, the following approach based on BMI at screening visit will be applied:

i. BMI ≤30: participants can be co-enrolled into LN substudy. A LN assessment should done upon consent (by MD, NP, or RN) to determine if the participant has palpable LN in the inguinal region. Findings will be documented on the LN Biopsy Referral Form.
ii. BMI 30-40: participants must have documentation of palpable LN in the inguinal region at the screening visit.
iii. BMI >40: participants are not eligible for LN biopsy substudy.

2.4 Study Medication Intervention

At baseline, participants will start losartan or matching placebo at 50mg daily. At 2 weeks, side effects and blood chemistries (BMP) will be assessed. The dose of losartan/placebo will increase to 100mg daily at the week 2 assessment if no toxicities or side effects are present that are attributable to study medication (see section 3.2 below for specific study drug dose modification criteria). A similar repeat assessment will then be performed at month 1 to verify that the 100mg dose is tolerated.

Randomization and Blinding

Eligible participants who consent to study procedures will be randomized 1:1 to receive losartan or matching placebo, with stratification by clinical site and by whether the participant consents to undergo a LN biopsy. Treatment assignments will be generated using a permuted block randomization scheme. Sites will use a secure, on-line program to obtain treatment assignments. The program will also verify eligibility and the existence of key baseline data. Treatment assignments will be verified and recorded in a dataset blinded to view by site staff.

A bottle ID number (BID) unique to each bottle will be assigned to participants, and used by study staff to obtain the correct blinded study drug. Sites will not have access to the treatment assignment linked to each bottle number, and bottle numbers will not be assigned in sequence.

Blinding will occur at 3 levels: 1) treatment assignment will be blinded, 2) interim data summaries will blinded to all but the unblinded statistician and 3) core lab staff will be blinded to treatment group. In the rare event that the blind must be broken, every effort will be made to minimize the extent of the unblinding (for staff and participants) and an assessment of blinding will occur at the end of the study.

Study Drug Production and Distribution

Active losartan 50mg tablets and matching placebo will be provided and distributed by Merck Pharmaceuticals. From baseline to 1-month visit, participants will take one 50mg tablet daily. When/if the study drug dose is increased to 100mg, participants will take 2x 50mg tabs once daily (for 100mg). Study drugs will be provided in 100 count bottles. One bottle will be dispensed at baseline, and one additional bottle at the 1-month visit. Following this, 2 bottles will be dispensed at all subsequent visits (months 3, 6, and 9). Sites will order study drug from the DCC, which will communicate with Merck to ship

drug to sites. Merck will provide the DCC with the key linking the BID (unique to each bottle) with whether the contents are active losartan or matching placebo. The DCC will then instruct sites which bottle(s) to dispense to patients based on the BID.

Risks Associated with Study Drug

Losartan is approved by the FDA and used to treat high blood pressure. Possible side effects of losartan include dizziness, lightheadedness, headache, fatigue, cough, upset stomach, vomiting, diarrhea, sore throat, fever, sweating, fast heart rate, chest pain, weakness, anemia, allergic reaction and kidney damage. In clinical studies, most of these side effects were no more common than what was experienced with placebo medication. Participants will undergo a side effect assessment at each visit (see case report form and protocol implementation manual for 'symptom check list').

Losartan should not be used during pregnancy. If a developing fetus is exposed to losartan, injury or even death may result. For this reason, pregnancy is an exclusion criterion and any pre-menopausal women of child bearing potential must be willing to use birth control during the study.

2.5 Outcomes

Primary Outcome

- IL-6 plasma levels: change from baseline over 12 months

Main Secondary Outcome

- CD4+ T-cell count in blood: change from baseline over 12 months

Additional Secondary Outcomes

- Adherence
- Tolerability and side effects
- Monocyte activation immune-phenotyping (e.g., CD14+/CD16+)
- Systemic inflammatory biomarkers (e.g., sCD14, sCD163, TNF-α, sTNFr-1, IL-6r, high sensitivity C-reactive protein)
- Coagulation activity (e.g., D-dimer levels)
- T-cell activation and senescence phenotypes in blood
- Recovery of CD4+ T-cell subsets (e.g., naïve) in blood
- HIV-specific T-cell responses
- Plasma biomarkers of fibrosis (e.g., hyaluronic acid)
- Lymphatic Tissue fibrosis (LN biopsy substudy)
- Lymphatic Tissue cellular immune activation (LN biopsy substudy)
- Lymphatic Tissue T-cell homeostasis (LN biopsy substudy)
- Frailty assessment (including 5 components: unintentional weight loss, physical inactivity, exhaustion, weak grip strength and slow walk)

2.6 Study Visit Schedule

Participants will be screened and, if eligibility criteria are met, will be randomized at the baseline visit to start active study drug or matched placebo (blinded). Randomization must occur within 90 days of all screening labs. *NOTE: the 90-day screening to randomization window applies to all participants, both those co-enrolled into the LN biopsy substudy and those not co-enrolled.*

Baseline laboratory evaluation must occur within 30 days of randomization. For participants enrolled in the LN biopsy substudy, baseline labs cannot be drawn within the 7 days following the biopsy procedure. A LN biopsy specimen must be obtained prior to randomizing the participant into the LN substudy. If a biopsy procedure is attempted but a LN specimen is not obtained at baseline, the participant will still be eligible to be randomized into the main study (but not the LN substudy). *NOTE: the biopsy procedure may occur after the baseline labs are obtained but still prior to randomization.*

After screening and baseline visits, participants will start losartan 50mg daily (or placebo) and return for a lab draw to assess toxicity (BMP only) at week 2. At the week 2 visit (after toxicity lab results return), participants will increase to losartan 100mg daily (or placebo) if they meet criteria based on results from the 2-week assessment (see section 2.4 above). Subsequent study visit procedures will occur in the context of visits at months 1, 3, 6, 9, and 12. For participants enrolled in the LN biopsy substudy, month 12 visit labs cannot be drawn within the 7 days following the month 12 biopsy procedure (NOTE: the biopsy procedure may occur after the month 12 labs are obtained). Participant should continue to take study medication until all study visits are completed; for participants enrolled in the LN biopsy substudy this includes BOTH the month 12 biopsy as well as the month 12 labs.

The target visit window for each study visit is guided by anticipated study drug quantity that the participant will have at the time. For week 2 and month 1, this corresponds to a 14-day target window (or +/- 7 days). Corresponding target windows for month 3, 6, 9, and 12 study visits are 32 days (or +/- 16 days).

NOTE: the 'actual' visit window for months 3, 6, 9, and 12 will be +/- 42 days (i.e., no gaps between visits where participants would not be in a visit window), but if >16days additional study drug should be dispensed to the participant—consistent with making every effort to capture study visit data while keeping participants on study drug.

The visit schedule is outlined in the table below. Patients will be fasting for all blood draws (except screening).

Toxicity labs and a clinical assessment will be ascertained as part of follow-up study visits. If clinical labs (e.g., HIV RNA level, CD4 count, BMP, hepatic panel, and CBC) are available as part of routine clinical monitoring during the study visit window, these results may be used in place of repeating clinic labs at the study visit. If new symptoms develop that may be related to study medications, the site study investigator is to be

notified within 24 hours and a clinical determination is to be made whether additional toxicity labs and/or adherence requires assessing.

Table 2: Study outcomes and visit timeline

	Screening	Baseline	Week 2	Month 1	Month 3	Month 6	Month 9	Month 12
Informed consent and eligibility criteria	X	--	--	--	--	--	--	--
Randomization	--	X	--	--	--	--	--	--
Clinical Assessments								
Adherence	--	--	--	X	X	X	X	X
Side Effects (subjective including symptom check list)	--	X	X	X	X	X	X	X
Toxicity labs (BMP)	X	X	X	X	X	X	X	X
Toxicity labs (CBC and hepatic panel)	X	--	--	--	X	X	X	X
HIV RNA level	X	--	--	--	X	X	X	X
Hep Bs Ag & Hep C Ab (results within 1 year acceptable)*	--	X	--	--	--	--	--	--
Lipid panel (results within 1 year acceptable)	--	X	--	--	--	--	--	--
Pregnancy test (during f/u for women of childbearing potential)	X	X	--	--	X	X	X	X
Blood Outcome Measures								
IL-6 levels (plasma)	X	X	--	X	X	X	X	X
CD4+ T-cell count (blood)	X	X	--	X	X	X	X	X
Inflammatory, coagulation and fibrosis plasma biomarkers	--	X	--	--	X	X	--	X
Monocyte and T-cell Immunophenotyping	--	X	--	--	--	X	--	X
Functional Assessment								
Frailty criteria: unintentional weight loss, exhaustion, physical-inactivity, weak-grip and slow-walk	--	X	--	--	--	X	--	X
Tissue Outcome Measures								
LN fibrosis	--	X	--	--	--	--	--	X
LN cellular immune activation	--	X	--	--	--	--	--	X
Stored plasma	X	X	--	X	X	X	X	X
Stored urine	--	X	--	--	--	X	--	X
Stored PBMCs	--	X	--	--	--	X	--	X

**Prior results that do not need repeating include: HCV Ab positive, HBsAg positive, or HBsAb positive along with HBsAg negative*

2.7 Sample Size Considerations

Power for Reduced Inflammation (IL-6 levels)

- The primary analysis will compare baseline-adjusted log IL-6 measurements at 1, 3, 6, 9 and 12 months between treatment groups.
- Standard deviation (SD) data from SMART (n=122; baseline and month 1) and ESPRIT (n=147; baseline and month 12) on participants who were on continuous ART between both time points were used to estimate a log IL-6 SD of 0.74 pg/mL.
- We assumed a 28% reduction in IL-6 with losartan was plausible based on our pilot data and data from HIV negative studies in the general population.[73, 74, 94]

Based on assumptions above, assuming 5% missing data, and considering the potential

clinical risk reduction associated with IL-6 changes of this magnitude (see epidemiologic data, following paragraph), a total sample size of n=110 will be enrolled. With this sample we will have 80% power to detect an absolute reduction of 0.3 log(IL-6), corresponding to a 26% reduction in IL-6, with a two-sided Type I error of 0.05.

Pooled data from 3,766 participants in SMART, ESPRIT and SILCAAT trials who were taking continuous ART with an HIV RNA level <400 copies/mL at entry—a group that resembles our target population—indicate that a 26% lower baseline IL-6 level is associated with a 34% lower risk of serious non-AIDS events or death from any cause.[62]

Power for Immune Recovery (CD4+ T-cell count)

- The primary analysis will consider average changes in log10 blood CD4 levels (total and naïve) over 12 months. Total CD4 count will be measured at 1, 3, 6, 9, and 12 months, and 4 pre-treatment baseline values (2 historical within prior year, 1 screening, 1 baseline) will be averaged to compute baseline CD4+ count for analyses. Averaging multiple baseline measurements will reduce variability and thereby increase statistical power. The log10 naïve CD4+ subset count will be measured at baseline and months 6 and 12.
- Based on participants ≥ age 50 in the SMART and ESPRIT studies (n=510 total), the estimated SD of yearly changes in log10 total CD4+ count is 0.11. Based on preliminary data from Dr. Sereti's lab, the estimated SD of yearly changes in the log10 naïve CD4 subset is 0.24.

With our sample size of n=110, we estimate that we will have 80% power to detect a 9.6% (19.8%) difference in total (naïve) CD4 count. Assuming a baseline total CD4 level of ~300-400 cells/mm^3, a 9.6% increase in CD4 count corresponds to an approximate average annual increase of 30-40 cells per year. From our prior epidemiologic work studying clinical risk based on total CD4+ count in blood after treatment with ART, a difference in this range would correspond to 5-8% difference in risk for a composite of non-AIDS events.[38, 39]

3 CLINICAL MANAGEMENT

3.1 Administration of Study Procedures

Study nurses at each clinical site will be trained prior to study implementation and will perform all study visit procedures during the conduct of the study. The lead physician investigator at each site will conduct clinical and drug toxicity assessments as indicated during the study.

3.2 Study Drug Dose Modification

At baseline, participants will start losartan or matching placebo at 50mg daily. At 2 weeks, side effects and blood chemistries (basic metabolic panel [BMP]) will be assessed. The dose of losartan/placebo will increase to 100mg daily at this week 2 visit (i.e., once lab results return) if all of the following criteria are met:

a) Systolic blood pressure (BP) ≥90 mmHg

b) Potassium ≤5.0 mmol/L
c) eGFR ≥30 mL/min/1.73m^2
d) Lack of a grade ≥3 side effect since the last evaluation deemed possibly/probably related to the study drug.
e) Lack of significant side effects deemed related to study drug (e.g., lightheaded upon standing)
f) Lack of other concerns to increase study drug dosing, as determined by site investigator

If participants are increased to 100mg dose at the week 2 visit and continue to tolerate this dose at the month 1 visit, they will be contacted by phone to verify tolerability 2-4 weeks following the month 1 visit. Participants may be evaluated by clinical labs and/or have side effects assessed at any point during the study, per the discretion of the site investigator. If participants are increased to 100mg daily (or matching placebo) they will maintain this dose for the duration of the 12-month study unless new side effects or adverse events necessitate re-evaluation (see below criteria).

If participants do NOT increase dose to 100mg daily at the week 2 visit (e.g., if symptomatic or if systolic BP is not ≥90 mmHg), the criteria for dose escalation may be reassessed at the 1-month visit. If participants do not meet criteria for dose increase (from 50mg to 100mg daily) after the 1-month visit, they will be maintained on 50mg dose until the month 3 visit, at which time the dose may be increased to 100mg daily if the five criteria above are met (a-e). At any point when participants have dose increased from 50mg to 100mg, they will be contacted by phone within 2 weeks to assess sides effects and have clinical labs drawn to assess toxicity; a full study visit for side effect and adverse event documentation can occur at any point, per the discretion of the site investigator.

Dose Reduction: Tolerability, side effects, and clinical laboratory monitoring will be assessed at each study visit (via participant report and clinical labs), and at any other time point at the discretion of the clinical site investigator. Adverse events that are assessed as related to study drug will prompt re-evaluation via phone or as an adverse event visit, per clinical discretion of the site investigator. If this event worsens or does not resolve after re-evaluation, then study medication dose should be decreased (i.e., from 100mg to 50mg) or stopped (if taking 50mg daily). If signs and symptoms resolve after 2 weeks, then the participant may resume or increase dose (i.e., back to 100mg if previously reduced to 50mg daily), per the clinical discretion of the site investigator.

Stopping Study Medication: If an adverse event of grade ≥3 is assessed as related to study drug, study medication should be stopped and the participant should be re-evaluated in 2 weeks. If a grade 2 rash is persistent, study medication may also be stopped at the discretion of the site investigator and the participant should be re-evaluated in 2 weeks. If signs and symptoms resolve after 2 weeks, then the participant may be re-challenged at a lower dose (i.e., at 50mg if previously taking 100mg daily) per the clinical discretion of the site investigator. If study medication is stopped for an adverse event that is later determined NOT to be related to study medication, then the

medication may be resumed at 50mg and increased to 100mg per initial criteria for dose escalation and at the discretion of the clinical site investigator.

3.3 Adherence and Tolerability

Adherence will be assessed via participant self-report and pill count (by study nurse) during each follow-up visit. Tolerability will also be assessed via self-report, with additional laboratory assessments performed for toxicity.

3.4 Data Collection

All data will be entered into web-based case report forms and transferred to servers at the data coordinating center (DCC) at University of Minnesota School of Public Health using REDCap software. Paper source documentation will also be kept in a secure, locked location at each clinical site. Study nurses and/or other qualified research technicians will perform all blood draws and blood specimen processing. The blood specimen collection tubes and processing protocols are outlined in the study laboratory manual.

3.5 Blood Specimen Processing

See the LIFE-HIV study laboratory manual for details on blood specimen processing. Participants will be fasting for all study visit blood draws (optional for screening visit). Plasma and serum specimens will need to be processed from whole blood within 4 hours of collection.

3.6 Clinical Labs

All clinical labs will be measured on fresh blood specimens the day of the study visit, at the site clinical lab using CLIA approved standards. Plasma HIV RNA level will be estimated using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

The following clinical labs will be assessed to monitor for toxicity per Table 2 above:

i. Complete Blood Count (CBC, with platelet count and differential)
ii. Basic Metabolic Panel (BMP)
iii. Hepatic panel (AST, ALT, total bilirubin, alkaline phosphatase)

3.7 Plasma Biomarkers

Plasma specimens will be analyzed at the Laboratory for Clinical Biochemistry Research (U of Vermont), under the direction of Dr. Russell Tracy. IL-6 will be measured with Chemiluminescent Sandwich ELISA (R&D Systems). A high degree of repeatability (low analytic variability) has been reported by Dr. Tracy's lab for assessing IL-6 and other plasma biomarkers.[95-101]

3.8 Immunologic Phenotypes

Immunophenotyping will be performed on cryopreserved PBMCs using multi-color flow cytometry at an intramural NIAID/NIH laboratory under the direction of Dr. Irini Sereti.

Panels of fluorochrome-conjugated antibodies for cell surface markers not affected by cryopreservation (e.g., CD3, CD4, CD8, CD38, HLA-DR, CD14, CD16, CD142 or tissue factor (TF) and viability dye to exclude non-viable cells) have been validated previously by Dr. Sereti.[102] The prevalence of classical (CD14+/CD16-), intermediate (CD14+/CD16+), and non-classical ($CD14^{dim}$/CD16+) monocyte phenotypes and T cells phenotypes reflecting activation (HLA-DR+/ CD38+) with be characterized; and with these methods additional phenotypes may be easily characterized (e.g. TF expression on monocytes, T-regulatory cells, or senescent T-cells). Only live cells will be included; samples with low (<75%) viability will not be processed.

3.9 Lymphatic Tissue Assessment

Lymph node (LN) biopsy will be performed in a subset of at least 30 participants at baseline and month 12. Criteria for enrollment into the LN substudy include: a) participant consent, b) lack of contraindications to the LN biopsy procedure, and c) palpable lymph nodes on exam by site investigator or research coordinator. All participants at NIH will be offered participation into the LN substudy, and participants at HCMC and Allina/Abbott Northwestern (in Minneapolis) will be offered participation until/if 25 patients are enrolled. Planned analyses will be exploratory in nature focused on assessment of: a) collagen deposition reflecting fibrosis in T-cell zone, b) changes in CD4 T-cell populations, c) changes in the structure of the FRCn, and d) changes in cellular immune activation in lymphatic tissue.

Biopsies will be performed at the University of Minnesota (for HCMC and Abbot Northwestern sites) and the NIH clinical center, by general surgeons using a research facility equipped with inpatient rooms. Tissue specimens are placed into fresh 4% paraformaldehyde for 24 hours and then transferred to 80% ethanol for paraffin embedding. LN tissue can also be divided at the bedside prior to processing to accommodate additional methods that requiring non-fixed tissue.

Changes in LN T-cell zone collagen, the size of T-cell populations, and the FRCn structure will be measured using established methods of IHC with antibody staining and quantitative image analysis (QIA) developed by Dr. Schacker's lab at the University of Minnesota.[80, 81, 91, 103-107] A similar approach will be used to assess cellular immune activation within lymphatic tissue.

4 SAFETY AND ADVERSE EVENTS

4.1 Measures to reduce risk for adverse events

Risks related to low blood pressure, or hypotension, are minimal but may increase with dehydration/hypovolemia. To reduce this risk, participants will be counseled on maintaining good hydration and diet, and on the potential effects of orthostatic hypotension.

4.2 Adverse Event Definitions

Adverse Event (AE): Any untoward medical occurrence in a clinical research participant administered an investigational product, which may or may not have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product.

If a preexisting condition worsens post-enrollment (frequency increases and/or severity grade increases), it should be reported as an adverse event.

A symptom check-list will be included in the CRF (detailed in the protocol implementation manual) that includes common symptoms or side effects typically related to losartan therapy.

Appendix D provides a table of adverse events, with grading criteria, adopted from the DAIDS 2009 Table for Grading the Severity of Adult and Pediatric Adverse Events

Serious Adverse Event (SAE): Any adverse event occurring at any dose that results in any of the following outcomes:

i. Death,
ii. A life-threatening condition,
iii. A congenital anomaly/birth defect,
iv. Inpatient hospitalization or prolongation of existing hospitalization,
v. Persistent or significant disability/incapacity,
vi. An important medical event that, based upon appropriate medical judgment, may jeopardize the patient or subject and may require intervention to prevent one of the outcomes listed above.

Unanticipated Problem (UP) is any event that is:

i. Unexpected, AND
ii. Related to study participation in this study, AND
iii. Increase the risk of harm to the participant or others (or be an SAE)

4.3 Documenting Adverse Events

All new adverse events of grade ≥3 or deemed related to study drug will be ascertained and recorded on study visit CRFs (through the online REDCAP interface). Criteria for assigning a grade of severity of adverse events are summarized in Appendix D.
NOTE: adverse events based on laboratory criteria will only be ascertained/recorded if ≥ grade 3, and no adverse events will be ascertained/recorded based on CD4+ T-cell count criteria.

There are five severity grades that can be assigned to adverse events, which are defined as follows:

i. Grade 1 = Mild

ii. Grade 2 = Moderate
iii. Grade 3 = Severe
iv. Grade 4 = Potentially life-threatening
v. Grade 5 = Death

Additional information ascertained for each adverse event includes: a) date of onset, b) relationship to study drug (see section 4.4 below), c) any action taken on study drug, and d) whether the adverse event qualifies as a serious adverse event (including the SAE criteria fulfilled).

Source documentation (in addition to the CRF variables for AEs) will collected at clinical sites for all serious adverse events, as well as any other problems involving risks to participants that are unanticipated or unexpected and are reasonably believed to be related to research participation.

4.4 Adverse Event Relationship to Study Drug

For all adverse events grade ≥3 that occur after randomization, study investigator(s) will assess the potential relationship of the event to the study medication. One of the following designations will be used:

i. Related: There is a reasonable possibility that the AE may be related to the study agent(s).

ii. Not related: There is not a reasonable possibility that the AE may be related to the study agent(s). Alternative etiology, diagnosis or, explanation for the AE should be provided.

4.5 Reporting Adverse Events

The IRB (or Human Subjects Committee) for the site where the event occurred will receive immediate notification of all serious adverse events (SAE) that are ALSO deemed protocol related, OR for any an unanticipated problem (UP; which are by definition protocol related). ***NOTE:*** *an adverse event (of any grade) that is unexpected, is reasonably believed to be related to study participation, and involves risk to the participant or others should be captured as a UP (per definition above 4.2).*

The site will enter this information into the adverse event log and SAE/UP case report form (via REDCap reporting). The data coordinating center (DCC) will distribute a summary of each SAE/UP event to the remaining clinical sites and participating institutions for submission to their respective IRBs.

The site will also complete FDA Form 3500 (MedWatch; see below), and submit this form to the FDA (via online reporting) and also to the DCC. Within 2 business days of learning of the event, clinical sites must:

a) Inform local IRB per institutional guidelines and timelines

b) fill out an SAE/UP case report form, via REDCap online reporting
c) fill out an MedWatch Form FDA 3500
d) either submit MedWatch Form FDA 3500 to the FDA or complete voluntary reporting online via FDA MedWatch (see below for link)
e) email the completed MedWatch Form FDA 3500 to: study PI (Jason Baker; baker@umn.edu), DCC (Gary Collins; gary-c@ccbr.umn.edu)

The DCC will submit FDA Form 3500 and any other information on the SAE/UP from the REDCap CRF, to a representative for Merck Pharmaceuticals. Reporting of all SAE/UP events will be made to FDA and Merck Pharmaceuticals using FDA Form 3500.

FDA MedWatch reporting forms may be found at:
http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm

FDA MedWatch voluntary reporting can be performed at:
https://www.accessdata.fda.gov/scripts/medwatch/

4.6 Participant Withdrawal

Participants may withdraw from the study at any time at their request, as described in the consent. All randomized participants will be encouraged to complete a final study visit, including all visit procedures.

5 Data and Safety Monitoring Plan

The Principal Investigator (PI) will be responsible for ensuring participants' safety on a daily basis. A Data and Safety Monitoring Board (DSMB) will be formed with Dr. Wolfson as the un-blinded statistician; other members will not be from institutions involved in the study and will be approved by NIA. Committee members will have experience with HIV/AIDS clinical research studies. Prior to enrollment the DSMB will review the protocol and data analysis plan.

The DSMB will act in an advisory capacity to the NIA Director to monitor participant safety, evaluate the progress of the study, and review procedures for maintaining the confidentiality of data, the quality of data collection, management, and analyses. During the study the DSMB will meet at least yearly.

Refer to the DSMB Charter and data and safety monitoring plan for details.

5.1 Interim Analysis

Most of the primary blood and tissue outcomes will not be available until after visits are completed, as samples will be batch processed at our participating laboratory site. Hence, there will be no formal interim analyses for efficacy or futility related to the main study outcomes.

However, the lead study statistician will prepare and submit reports to the DSMB every 3 months documenting study enrollment totals and adverse events. While we do not anticipate significant issues with safety in this short-term study, a recommendation by the DSMB to stop the study would likely be based on an adverse event rate that is deemed unacceptably high.

Recommendations from the DSMB (e.g., continue the study as planned), and a letter summarizing the findings at the end of the study, will be transmitted to each site for submission to their IRB.

5.2 Frequency and Content of Data and Safety Monitoring

To ensure compliance and data are reported correctly and completely, CRF data will be monitored monthly for completeness and compliance with the study protocol. Queries for clarification or data completion will be posted to a site-specific and password protected section of the LIFE-HIV website.

The PI will be informed of serious adverse events as soon as they occur and will notify the NIA and DSMB within 24 hours of notification. During the study the DSMB will meet at least yearly either in-person or by teleconference call to review study progress, data quality, and participant safety. An initial DSMB meeting will be scheduled as soon as any of the following occurs:

i. Six months have elapsed since enrollment of the first patient
ii. 50% of goal enrollment is achieved
iii. 30 person-years of follow-up is achieved

The data and safety monitoring report will include data on: enrollment (including progress), visit attendance, completeness of data, completeness and quality of specimen collection, baseline characteristics, adherence and discontinuation of medication, and side effects and adverse events.

5.3 DSMB Membership and Affiliation

The following individual(s) has/have accepted position(s) as part of the DSMB. DSMB membership will be reviewed and approved by the NIA. Should there be any questions regarding the independence of the DSMB, it will be addressed and corrected if necessary at that time:

- Biostatistician: Bryan Shepherd, PhD (bryan.shepherd@vanderbilt.edu)
- HIV/ID medicine: Kevin High, MD (khigh@wakehealth.edu)
- Cardiology medicine: Michael Kiernan, MD (mkiernan@tuftsmedicalcenter.org)

5.4 DSMB Conflict of Interest and Protection of Confidentiality

DSMB members should have no direct involvement with the study investigators or intervention. Each DSMB member will sign a Conflict of Interest Statement which

includes current affiliations, if any, with pharmaceutical and biotechnology companies (e.g., stockholder, consultant), and any other relationship that could be perceived as a conflict of interest related to the study and / or associated with commercial interests pertinent to study objectives.

Data will be presented in a blinded manner during the open sessions of the DSMB. At DSMB meetings, data and discussion are confidential. Participant identities will not be known to the DSMB members.

5.5 DSMB Responsibilities

The DSMB Charter provides a detailed list of the DSMB responsibilities. They include:

- Review the research protocol, informed consent documents and plans for data safety and monitoring;
- Recommend subject recruitment be initiated after receipt of a satisfactory protocol;
- Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- Review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
- Protect the safety of the study participants;
- Report to NIA on the safety and progress of the trial;
- Make recommendations to the NIA and the Principal Investigator concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- Ensure the confidentiality of the study data and the results of monitoring; and,
- Assist the NIA by commenting on any problems with study conduct, enrollment, sample size, and/or data collection.

6 ANALYSIS PLAN

The general goal of this study is to evaluate the potential treatment effects of losartan for reducing systemic inflammation and improving immune recovery. For the effect of losartan on systemic inflammation, the outcome will be the average of log IL-6 levels (plasma) from samples obtained at months, 1, 3, 6, 9, and 12; two pre-treatment log IL-6 measurements will be averaged to estimate baseline log IL-6 level. The primary

analysis will be intention to treat using a linear model for the aforementioned outcomes with treatment group indicator as the main predictor, fitted using the Generalized Estimating Equations approach with an independence working correlation structure. The model will be adjusted for baseline log IL-6 and clinical center. The hypothesis of no treatment effect will be assessed by considering the statistical significance of the treatment group indicator. Additional analyses will consider change in IL-6 at specific visit time points, as well as the percent of participants that achieve an IL-6 level <1.1 pg/mL (i.e., the 25th percentile among HIV+ patients with viral suppression in the SMART/ESPRIT/SILCAAT trials)

Immune recovery will be assessed by modeling $\log_{10}$ CD4+ T-cell counts in blood, using a linear mixed effects model that, in addition to the model components described for the systemic inflammation analysis above, incorporates a main effect for time (in months), a treatment-by-time interaction, and individual-specific intercepts and slopes (for time). The hypothesis of no treatment effect on rate of yearly change in $\log_{10}$ CD4 counts will be evaluated by considering the statistical significance of the treatment-by-time interaction term.

Subgroup analyses will be performed to explore potential for differential treatment effects (e.g., entry CD4 count [including ≥ vs. < 350], CD4:CD8 ratio, use of statins, aspirin, or other drugs with potential anti-inflammatory or anti-fibrotic properties, and co-morbidities or polymorbidities). Trends in treatment effects on the primary outcome (log IL-6) over time will be assessed using linear mixed effects models similar to those described for the modeling of $\log_{10}$ CD4+.

The main analyses for primary and secondary outcomes will be intention to treat. As secondary analyses, we will perform per-protocol (‘on treatment’) and compliance-adjusted analyses, and will also carry out analyses using all available data by multiply imputing missing longitudinal outcome measurements via the chained equations method, using linear mixed effects models incorporating baseline covariates, the treatment indicator, and non-missing outcome measurements.

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APPENDIX A: BLOOD/URINE SPECIMEN COLLECTION AND STORAGE

Updated: August 2014

		Screening	Baseline	Follow-up* →				
Month (M) of Study			M 0	M 1	M 3	M 6	M 9	M 12
Clinical	**Tube Type (for draw)**	mL (#tubes)	mL (#tubes)	mL (#tubes)	mL (#tubes)	mL (#tubes)	mL (#tubes)	mL (#tubes)
CD4 count +/- CBC	4mL (EDTA) lavender	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)
BMP, hepatic panel, and/or lipid panel	5mL green	5 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)
Pregnancy, urine/serum		X	X	--	X	X	X	X
HBsAg, HCV Ab	4mL (serum) red	--	2 (1)	--	--	--	--	--
HIV RNA level	10mL (EDTA) lavender	5 (1)	--	--	5 (1)	5 (1)	5 (1)	5 (1)
Blood for Storage	**Tube Type (for draw)**							
PBMC	8-10mL ACD tube	--	40-50 (5)	--	--	40-50 (5)	--	40-50 (5)
Serum	4.5mL SST	--	4 (1)	--	4 (1)	--	4 (1)	--
Platelet Poor Plasma	10mL (EDTA) lavender	8-10 (1)	24 (3)	24 (3)	24 (3)	24 (3)	24 (3)	24 (3)
Platelet Poor Plasma	4.5mL (NaCitrate) blue	--	8 (2)	8 (2)	--	8 (2)	--	8 (2)
Whole Blood Storage	[take from NaCitrate tube prior to processing]	--	□	□	--	□	--	□
Daily Blood Volume (mL)		≤25	≤100	≤40	≤50	≤110	≤40	≤110
Cryovials for Storage	**(Tube Type for Draw)**	# vials	# vials	# vials	# vials	# vials	# vials	# vials
Serum	SST		2		2		2	
PP Plasma	EDTA	4	8	8	8	8	8	8
PP Plasma	NaCitrate		2	2		2		2
Whole blood	NaCitrate		2	1		1		1
Urine	Urine Collection		1			1		1
Cryovials Totals (74)		**4**	**15**	**11**	**10**	**12**	**10**	**12**

*NOTE: week 2 visit will collect BMP (5mL green tube) for clinical toxicity assessment, but no additional blood collection for processing or storage

APPENDIX B: INFORMED CONSENT FORM (ICF) – WITH LN BIOPSY

Protocol Name: **Losartan to reduce Inflammation and Fibrosis Endpoints in HIV**

Short Title: **LIFE-HIV**

Funding: **National Institute of Aging / National Institutes of Health**
Merck Pharmaceuticals (study drug)

Site: **[SITE]**

Investigators: **[SITE INVESTIGATOR(S)]**

CONSENT FOR PARTICIPATING IN AN NIA/NIH-FUNDED RESEARCH TRIAL

INTRODUCTION AND PURPOSE: WHY IS THIS STUDY BEING DONE?
You are invited to be in a research study that looks at the use of a medication that may improve the health of HIV positive people who are already on HIV medicines. The medication (losartan, or COZAAR) is approved by the Food and Drug Administration (FDA), but not for treatment of HIV infection; it is commonly used to treat high blood pressure. However, this medication may help address some of the damage that HIV causes in the body. One hundred HIV positive patients will be enrolled in this study, at 5 clinical sites in the U.S.

Damage caused by HIV infection results in problems with the immune system. These problems cannot be fully corrected, even with effective treatment using antiretroviral medications. One of these problems is inflammation. 'Inflammation' occurs when the body's immune system is responding to injury or infection. Inflammation can be helpful in the short term, but when it is persistent it can also cause more damage to the body over time. HIV also damages the immune system by causing scarring in tissues such as lymph nodes. This scarring limits recovery of the immune system after treatment with antiretroviral therapy is started.

Ongoing inflammation and damage to the immune system is thought to contribute to risk for heart disease and cancer among HIV positive persons. This study will determine if losartan, a commonly used medication for blood pressure, reduces inflammation and improves immune recovery (possibly by reducing scarring in lymph nodes).

YOUR PARTICIPATION IS VOLUNTARY
This consent form gives you information about the clinical research study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign this consent form. You will be given a copy to keep.

Before you learn about the study, it is important that you know the following:
Your participation is entirely voluntary.

You may decide not to take part or to withdraw from the study at any time without losing the benefits of your routine medical care.

Eligibility: Who is being asked to be part of this research study?
You may be eligible for this study if you are over age 50, you have HIV infection, you are doing well with taking your antiretroviral medications, and you do not have contraindications to study procedures. Your HIV 'viral load' must be undetectable. If your doctor or the study investigators feel that it would not be safe to take the study medication then you will not be eligible to participate. Some factors that could make you ineligible for this study include low blood pressure, chronic inflammatory disease, and liver or kidney disease.

HOW LONG WILL YOU BE IN THE STUDY?
The study will last approximately 12 months. After the screening visit, if you qualify, you will come in for a 'baseline' study visit, receive a blood draw after 2 weeks and then a follow-up visit at 1, 3, 6, 9, and 12 months.

HOW WILL THE STUDY WORK?
If you agree to participate in this study, you will be randomly assigned (like flipping a coin) to start either losartan or a placebo (inactive pill) for 12 months. Neither you, nor the study investigators, will know which study medication you will take. You will start out taking 50mg of losartan or matching placebo. If you are not having any problems after 2 weeks, the dose will be increased to 100mg of losartan or matching placebo for the duration of the study.

PROCEDURES: WHAT DO YOU HAVE TO DO IF YOU ARE IN THIS STUDY?

Screening visit:
You will be asked to come in for a screening visit, where your study investigators will review the study procedures and this consent form. If you agree to participate, you will have your blood drawn (approximately 2 tablespoons) and medical history reviewed (similar to a routine clinic visit) to determine if you are eligible to participate. If you meet study criteria, you will then return within 2 months to begin the study.

Baseline and follow-up (month 1, 3, 6, 9, 12) clinic study visits:
The baseline and follow-up clinical visit will last approximately 1-2 hours, and will consist of:

Medical History: You will be interviewed to review your medical history and assess your risk for developing heart disease. We will also access your medical chart to obtain the results of recent lab tests and medications you are taking.

Blood Draw: We will obtain a blood sample (up to 8 tablespoons) from a vein in your arm. The total blood draw volumes per visit will be less than 100 mL. <u>You must be fasting for at least 6 hours</u> prior to having your blood drawn at study visits. Blood samples will be used to measure markers in the blood related to inflammation and other markers that may contribute to heart disease and other complications of HIV infection. These samples may be stored for up to 20 years.

At the baseline visit, you will start taking losartan (or placebo). You will take 1 tablet (50 mg) every day, until a member of the study team tells you to stop or changes your dose. The nurses will give you the study medicine during your clinic visits. At follow-up visits you will be asked to bring in all of your study medication, and adherence and tolerability to the treatment will be assessed.

Blood draw at week 2:
Two weeks after you start taking study medication (at baseline), you will be asked to have your blood drawn to make sure you are not having significant toxicity from the study medication. You will also be asked several questions to determine if you are experiencing any side effects. If the study investigators determine that you are not having any toxicity or side effects, the dose of the study medication will be increased from 50mg to 100mg at (or immediately after) this visit.

Biopsy procedure visits (baseline and month 12):
If you agree to a lymph node biopsy and study investigators can palpate a candidate lymph node to biopsy, a surgeon will collect a lymph node from your groin twice during the study. This procedure will occur after or very close to the baseline and month 12 study visits. The purpose of this biopsy is to see if losartan reduces scarring in immune system tissue, that in-turn improves recovery of immune cells (such as T-cells).

Lymph node biopsies of your groin will be done at [the University of Minnesota or NIH Clinical Center]. The actual surgery will only take 30-40 minutes but the visit will take approximately 6 hours. You should not eat or drink anything for at least 6 hours before surgery. You will be given one dose of an oral antibiotic 30 minutes prior to the biopsy. The groin area will be scrubbed with an antiseptic solution. Local anesthetics (similar to Novocain) will be injected to numb the area. An incision between 1 and 3 inches will be made. The lymph node will be uncovered and removed. A lymph node is about the size of a peanut. The surgeon will close the wound with stitches, and then a bandage will be put over the wound.

After a period of about 4 hours you will be allowed to leave the biopsy appointment. You will be asked to remain inactive until the next morning. You and the surgeon will discuss the use of a drug to relieve any pain. The entire procedure from the time you enter the hospital until you are discharged should be no longer than approximately 6 hours.

Three days after the procedure you will be examined in the clinic. The wound will be examined and you will be asked questions about pain, drainage from the wound, or discomfort. If there is any sign of infection you will be referred back to the surgeon for another examination and a prescription of antibiotics or another appropriate treatment. The exact antibiotic will be chosen by the surgeon or investigator. The surgeon will advise you as to the best way to manage stitches that are in place.

Physical function test (baseline, month 6 and month 12):

We will also ask you to perform a short physical function test at three times during the study. The first test is grip strength. You will be asked to squeeze a hand held device for a few seconds that measures the force you can generate. The second test involves having you walk a short distance of four meters at your usual pace. This will be done twice. These physical functions tests will take 5 minutes.

STORED SAMPLES

During your participation in this study, blood will be collected by standard blood drawing techniques. Lymph node tissue will be collected by biopsy.

Samples will be used to evaluate the potential benefits of losartan. Additional samples will be stored up to 20 years for future research. These samples can help us learn more about HIV, AIDS, immune function, inflammation, fibrosis (scarring), or other related diseases. In general, the research tests we perform are not like routine medical tests and may not relate directly to your medical care. For this reason, we may not put future test results in your medical record or share these test results with your medical provider

Genetic Testing

Future research on stored samples might involve genetic testing. Genetic testing may tell researchers something about how health or illness is passed on to you by your parents or from you to your children. Some genetic information, such as the ability to make certain proteins in the body, has been associated with an increased risk of certain diseases like arthritis.

Any genetic information collected or discovered about you or your family will be confidential. Genetic information about you will not be revealed to others, including your relatives, without your permission. We will not release any information about you or your family to any insurance company or employer unless you sign a document allowing release of information.

HOW WILL YOU GET MEDICINES FOR THE STUDY?

Study medications will be provided to you by the study and will be distributed by study nurses during study visits. You will be provided with sufficient supply to last the duration of the study. You will be asked to return any unused study medication, including empty containers.

WHAT ARE THE RISKS AND/OR DISCOMFORTS OF THIS STUDY?

You will be monitored for side effects at each visit, and your lab tests will include an evaluation for signs of medication toxicity.

Risks of Study Procedures

You will have your blood drawn at each study visit. This is identical to having your blood drawn at a medical clinic, and can involve discomfort, light-headedness and/or minor bruising. Physical examination of the groin may be uncomfortable and cause embarrassment. Discussion of past medical history or risk factors for HIV infection may be

stressful and cause anxiety. You may decline to answer any questions that you do not feel comfortable answering.

Study Medication: Losartan

This medication is approved by the FDA. Losartan is used to treat high blood pressure. The possible side effects of Losartan include dizziness, lightheadedness, headache, fatigue, cough, upset stomach, vomiting, diarrhea, sore throat, fever, sweating, fast heart rate, chest pain, weakness, anemia (low red blood cell count), allergic reaction and kidney damage. We do not expect that losartan will interact with any of your HIV medicines. In clinical studies, most of these side effects were no more common than what was experienced with placebo medication.

This medication should not be used during pregnancy. If a developing fetus is exposed to losartan, injury or even death may result. For this reason, if you are a woman of childbearing potential, you will be asked whether or not you are sexually active and if you are, what type of birth control you use. You must be willing to use a reliable form of birth control for the duration of the study period, such as a barrier method or spermicide. Condoms cannot be the only form of birth control you use. If you become pregnant during the study or think you may be pregnant, you should inform either the site investigator or a study nurse immediately. We will ask that you return any study medication. We will ask permission to contact you at the end of the pregnancy to check on the health of you and the baby.

Lymph Node Biopsies

If you have agreed to a lymph node biopsy, the biopsy procedure may cause pain, even though you have been given an anesthetic. There may be bleeding associated with the procedure. There is the risk of infection; however, it is less than 1-2 percent. There is the possibility you might develop a seroma, which is a collection of fluid under the skin and around the wound. This is also very rare. There is the possibility that you may develop a scar at the site of your lymph node biopsy. You will most likely have pain after the lymph node biopsy. Occasionally, when we do a lymph node biopsy we cannot find the lymph node that was felt prior to the procedure. This is unlikely; however, it does happen on occasion.

Physical Function Tests

You may fall during the short 4-meter walk. If you feel unable to perform any of the walk test or grip strength test, you will not need to perform them. We will not ask you to perform any activities that put you at more than a minimal risk of falling or injuring yourself.

WHAT ABOUT PREGNANCY AND BREASTFEEDING?

If you are pregnant, breastfeeding or planning to become pregnant, you will not be eligible for this study. If you become pregnant during the study, you will be asked to stop study medications and withdraw. Therefore, if it is possible that you could become pregnant, we ask that you use a reliable form of birth control for the duration of the study (listed above in ‘Study Medication’).

WHAT ARE THE BENEFITS OF THIS STUDY?
If you take part in this study, there are no anticipated benefits to your health. The research has the potential to benefit people with HIV infection, after the study is completed and the findings are analyzed.

COMPENSATION
You will be paid $XX for each study visit you attend after the screening visit. You will receive $XXX for each biopsy of a groin lymph node you complete. If you complete two study-related lymph node biopsies, you may receive a total of $XXX for participating in the study. Study investigators may ask for your social security number as part of the monitoring process for this compensation.

WHAT IF THERE ARE NEW FINDINGS?
We will not be analyzing data during the study. However, if during the course of this research study, there are significant new findings discovered which might influence your willingness to continue, the researchers will inform you of those developments. You may request your own results after the study by contacting the research investigators.

WHAT IF YOU DON'T WANT TO BE IN THE STUDY ANY LONGER?
If you enroll in this study, you may decide to stop participating at any time. Withdrawing from this study will not affect the benefits of your regular medical care.

CAN YOUR STUDY PARTICIPATION BE STOPPED WITHOUT YOUR CONSENT?
You may be taken off of study medicines before the end of the study if investigators or your doctor recommend this. You may be taken off the entire study without your consent if:

- Your study doctor decides that continuing in the study would harm you;
- Your lab results indicate that you are experiencing toxicity from study medications;

WHAT OTHER CHOICES DO YOU HAVE BESIDES THIS STUDY?
You may discuss other strategies for improving your health with your doctor.

WHAT ARE THE COSTS TO YOU?
The medications that are part of this study will be provided free-of-cost to you, and will be distributed during study visits. During the study, you, your insurance company, or some other third-party payer must pay for all other medicines, including HIV medicines not paid by the study and medicines needed to prevent or treat other illnesses. We will provide all clinical and professional services, lab work, and other tests that are part of this study and not part of your regular care at no cost to you.

HOW IS YOUR PRIVACY PROTECTED?
Any information that could be used to identify you will be treated in strict confidence to the extent allowed by law. Nevertheless, some uses and disclosures of your information are necessary to conduct the study. If you agree to be part of this study, you will also be allowing the uses and disclosures of your private health information as needed for the purposes of this study as described in this consent.

“Private health information” means information that identifies you and is collected:

- during this study;
- from your past and current medical records maintained by your regular health care providers, to the extent the information is relevant to this study or to your eligibility for this study; or
- from any payment records relating to items or services furnished to you during this study.

By signing this consent, you are agreeing that your private health information may be disclosed to and used by:

- the doctors and other health care providers involved in this study;
- their staff;
- the research center;
- members of this institution’s Human Subjects Research Committee/Institutional Review Board;
- Merck Pharmaceuticals;
- the sponsor of this study and its agents; and
- monitors from the United States Government and/or Food and Drug Administration (FDA).

The findings of this study may be used for scientific meetings, written reports, and publications, but no information that could be used to identify you will be disclosed for these purposes.

Once your private health information has been disclosed to a third party, federal privacy laws may no longer protect it from re-disclosure. However, anyone obtaining access to your private health information under this consent must agree to protect your information as required by this consent.

This consent to use your private health information as described above does not expire. However, if you later change your mind, you can revoke this consent by writing to [SITE INVESTIGATOR] saying that you no longer wish to allow your private health information to be used for this study. If you revoke your consent, you may no longer be able to participate in the study. Moreover, we cannot undo uses or disclosures of your private health information that have already taken place in reliance on your prior consent.

PLEASE NOTE:
In the event of a positive result for Hepatitis B or C, reporting of the results to the local state Department of Health may be required.

WHAT IF YOU ARE INJURED?
If you are injured as a result of being in this study, you will be given immediate treatment. The cost for treatment will be charged to you or your insurance company. There is no program through this institution to compensate participants who have research related injuries. You will not be giving up any of your legal rights by signing this consent.

WHAT IF YOU HAVE PROBLEMS OR QUESTIONS?
If you ever have questions or in the case of research-related injuries, you should contact:

[LIST STUDY INVESTIGATORS]

If you have questions about research subject's rights you can contact**:**

[SITE IRB CONTACT INCLUDING PHONE NUMBER]

RESEARCH STUDY REGISTRY
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at anytime.

SIGNATURE PAGE FOR CONSENT TO PARTICIPATE IN THE LIFE-HIV STUDY

If tissue samples are not fully used for this study, do you consent to allow further study of these specimens beyond this study: ☐**YES**☐**NO**

I have read this consent form, had the opportunity to ask questions and have received answers to any questions I have asked. I willingly give my consent to participate in this study, and authorize the use and disclosure of my health information as described in this form. By signing this consent form I do not give up any of my legal rights. Upon signing this form I will be given a signed copy of the form for my records.

If you have read the consent (or if you have had it explained to you) and understand the information, and you voluntarily agree to join this study, please sign your name below.

_____	_____	_____
Participant's name (typed or printed)	Participant's signature	Date

_____	_____
Person obtaining consent	Date

APPENDIX C: INFORMED CONSENT FORM (ICF) – WITHOUT BIOPSY

Protocol Name: **Losartan to reduce Inflammation and Fibrosis Endpoints in HIV**

Short Title: **LIFE-HIV**

Funding: **National Institute of Aging / National Institutes of Health**
Merck Pharmaceuticals (study drug)

Site: **[SITE]**

Investigators: **[SITE INVESTIGATOR(S)]**

CONSENT FOR PARTICIPATING IN AN NIA/NIH-FUNDED RESEARCH TRIAL

INTRODUCTION AND PURPOSE: WHY IS THIS STUDY BEING DONE?

You are invited to be in a research study that looks at the use of a medication that may improve the health of HIV positive people who are already on HIV medicines. The medication (losartan, or COZAAR) is approved by the Food and Drug Administration (FDA), but not for treatment of HIV infection; it is commonly used to treat high blood pressure. However, this medication may help address some of the damage that HIV causes in the body. One hundred HIV positive patients will be enrolled in this study, at 5 clinical sites in the U.S.

Damage caused by HIV infection results in problems with the immune system. These problems cannot be fully corrected, even with effective treatment using antiretroviral medications. One of these problems is inflammation. 'Inflammation' occurs when the body's immune system is responding to injury or infection. Inflammation can be helpful in the short term, but when it is persistent it can also cause more damage to the body over time. HIV also damages the immune system by causing scarring in tissues such as lymph nodes. This scarring limits recovery of the immune system after treatment with antiretroviral therapy is started.

Ongoing inflammation and damage to the immune system is thought to contribute to risk for heart disease and cancer among HIV positive persons. This study will determine if losartan, a commonly used medication for blood pressure, reduces inflammation and improves immune recovery (possibly by reducing scarring in lymph nodes).

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives you information about the clinical research study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign this consent form. You will be given a copy to keep.

Before you learn about the study, it is important that you know the following:
Your participation is entirely voluntary.

You may decide not to take part or to withdraw from the study at any time without losing the benefits of your routine medical care.

Eligibility: Who is being asked to be part of this research study?
You may be eligible for this study if you are over age 50, you have HIV infection, you are doing well with taking your antiretroviral medications, and you do not have contraindications to study procedures. Your HIV 'viral load' must be undetectable. If your doctor or the study investigators feel that it would not be safe to take the study medication then you will not be eligible to participate. Some factors that could make you ineligible for this study include low blood pressure, chronic inflammatory disease, and liver or kidney disease.

HOW LONG WILL YOU BE IN THE STUDY?
The study will then last approximately 12 months. After the screening visit, if you qualify, you will come in for a 'baseline' study visit, receive a blood draw after 2 weeks and then a follow-up visit at 1, 3, 6, 9, and 12 months.

HOW WILL THE STUDY WORK?
If you agree to participate in this study, you will be randomly assigned (like flipping a coin) to start either losartan or a placebo (inactive pill) for 12 months. Neither you, nor the study investigators, will know which study medication you will take. You will start out taking 50mg of losartan or matching placebo. If you are not having any problems after 2 weeks, your dose will be increased to 100mg of losartan or matching placebo for the duration of the study.

PROCEDURES: WHAT DO YOU HAVE TO DO IF YOU ARE IN THIS STUDY?

Screening visit:
You will be asked to come in for a screening visit, where your study investigators will review the study procedures and this consent form. If you agree to participate, you will have your blood drawn (approximately 2 tablespoons) and medical history reviewed (similar to a routine clinic visit) to determine if you are eligible to participate. If you meet study criteria, you will then return within 2 months to begin the study.

Baseline and follow-up (month 1, 3, 6, 9, 12) clinic study visits:
The baseline and follow-up clinical visit will last approximately 1-2 hours, and will consist of:

Medical History: You will be interviewed to review your medical history and assess your risk for developing heart disease. We will also access your medical chart to obtain the results of recent lab tests and medications you are taking.

Blood Draw: We will obtain a blood sample (up to 7-8 tablespoons) from a vein in your arm. The total blood draw volumes per visit will be less than 100 mL. <u>You must be fasting for at least 6 hours</u> prior to having your blood drawn at study visits. Blood samples will be used to measure markers in the blood related to inflammation and other markers that may contribute to heart disease and other complications of HIV infection. These samples may be stored for up to 20 years.

At the baseline visit, you will start taking losartan (or placebo). You will take 1 tablet (50 mg) every day, until a member of the study team tells you to stop or changes your dose. The nurses will give you the study medicine during your clinic visits. At follow-up visits you will be asked to bring in all of your study medication, and adherence and tolerability to the treatment will be assessed.

Blood draw at week 2:
Two weeks after you start taking study medication (at baseline), you will be asked to have your blood drawn to make sure you are not having significant toxicity from the study medication. You will also be asked several questions to determine if you are experiencing any side effects. If the study investigators determine that you are not having any toxicity or side effects, the dose of the study medication will be increased from 50mg to 100mg at (or immediately after) this visit.

Physical function assessments (baseline, month 6 and month 12):
We will also ask you to perform a short physical function test at three times during the study. The first test is grip strength. You will be asked to squeeze a hand held device for a few seconds that measures the force you can generate. The second test involves having you walk a short distance of four meters at your usual pace. This will be done twice. These physical functions tests will take 5 minutes.

STORED SAMPLES
During your participation in this study, blood will be collected by standard blood drawing techniques.

Samples will be used to evaluate the potential benefits of losartan. Additional samples will be stored up to 20 years for future research. These samples can help us learn more about HIV, AIDS, immune function, inflammation, fibrosis (scarring), or other related diseases. In general, the research tests we perform are not like routine medical tests and may not relate directly to your medical care. For this reason, we may not put future test results in your medical record or share these test results with your medical provider

Genetic Testing
Future research on stored samples might involve genetic testing. Genetic testing may tell researchers something about how health or illness is passed on to you by your parents or from you to your children. Some genetic information, such as the ability to make certain proteins in the body, has been associated with an increased risk of certain diseases like arthritis.

Any genetic information collected or discovered about you or your family will be confidential. Genetic information about you will not be revealed to others, including your relatives, without your permission. We will not release any information about you or your family to any insurance company or employer unless you sign a document allowing release of information.

HOW WILL YOU GET MEDICINES FOR THE STUDY?

Study medications will be provided to you by the study and will be distributed by study nurses during study visits. You will be provided with sufficient supply to last the duration of the study. You will be asked to return any unused study medication, including empty containers.

WHAT ARE THE RISKS AND/OR DISCOMFORTS OF THIS STUDY?

You will be monitored for side effects at each visit, and your lab tests will include an evaluation for signs of medication toxicity.

Risks of Study Procedures

You will have your blood drawn at each study visit. This is identical to having your blood drawn at a medical clinic, and can involve discomfort, light headedness and/or minor bruising. Physical examination of the groin may be uncomfortable and cause embarrassment. Discussion of past medical history or risk factors for HIV infection may be stressful and cause anxiety. You may decline to answer any questions that you do not feel comfortable answering.

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This medication should not be used during pregnancy. If a developing fetus is exposed to losartan, injury or even death may result. For this reason, if you are a woman of childbearing potential, you will be asked whether or not you are sexually active and if you are, what type of birth control you use. You must be willing to use a reliable form of birth control for the duration of the study period, such as a barrier method or spermicide. Condoms cannot be the only form of birth control you use. If you become pregnant during the study or think you may be pregnant, you should inform either the site investigator or a study nurse immediately. We will ask that you return any study medication. We will ask permission to contact you at the end of the pregnancy to check on the health of you and the baby.

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You may fall during the short 4-meter walk. If you feel unable to perform any of the walk test or grip strength test, you will not need to perform them. We will not ask you to perform any activities that put you at more than a minimal risk of falling or injuring yourself.

WHAT ABOUT PREGNANCY AND BREASTFEEDING?

If you are pregnant, breastfeeding or planning to become pregnant, you will not be eligible for this study. If you become pregnant during the study, you will be asked to stop study

medications and withdraw. Therefore, if it is possible that you could become pregnant, we ask that you use a reliable form of birth control for the duration of the study (listed above in 'Study Medication').

WHAT ARE THE BENEFITS OF THIS STUDY?
If you take part in this study, there are no anticipated benefits to your health. The research has the potential to benefit people with HIV infection, after the study is completed and the findings are analyzed.

COMPENSATION
You will be paid $XX for each study visit you attend after the screening visit. You may receive a total of $XXX for participating in the study. Study investigators may ask for your social security number as part of the monitoring process for this compensation.

WHAT IF THERE ARE NEW FINDINGS?
We will not be analyzing data during the study. However, if during the course of this research study, there are significant new findings discovered which might influence your willingness to continue, the researchers will inform you of those developments. You may request your own results after the study by contacting the research investigators.

WHAT IF YOU DON'T WANT TO BE IN THE STUDY ANY LONGER?
If you enroll in this study, you may decide to stop participating at any time. Withdrawing from this study will not affect the benefits of your regular medical care.

CAN YOUR STUDY PARTICIPATION BE STOPPED WITHOUT YOUR CONSENT?
You may be taken off of study medicines before the end of the study if investigators or your doctor recommend this. You may be taken off the entire study without your consent if:

- Your study doctor decides that continuing in the study would harm you;
- Your lab results indicate that you are experiencing toxicity from study medications;

WHAT OTHER CHOICES DO YOU HAVE BESIDES THIS STUDY?
You may discuss other strategies for improving your health with your doctor.

WHAT ARE THE COSTS TO YOU?
The medications that are part of this study will be provided free-of-cost to you, and will be distributed during study visits. During the study, you, your insurance company, or some other third-party payer must pay for all other medicines, including HIV medicines not paid by the study and medicines needed to prevent or treat other illnesses. We will provide all clinical and professional services, lab work, and other tests that are part of this study and not part of your regular care at no cost to you.

HOW IS YOUR PRIVACY PROTECTED?
Any information that could be used to identify you will be treated in strict confidence to the extent allowed by law. Nevertheless, some uses and disclosures of your information are necessary to conduct the study. If you agree to be part of this study, you will also be

allowing the uses and disclosures of your private health information as needed for the purposes of this study as described in this consent.

"Private health information" means information that identifies you and is collected:

- during this study;
- from your past and current medical records maintained by your regular health care providers, to the extent the information is relevant to this study or to your eligibility for this study; or
- from any payment records relating to items or services furnished to you during this study.

By signing this consent, you are agreeing that your private health information may be disclosed to and used by:

- the doctors and other health care providers involved in this study;
- their staff;
- the research center;
- members of the clinical site Human Subjects Institutional Review Board;
- Merck Pharmaceuticals;
- the sponsor of this study and its agents; and
- monitors from the United States Government and/or Food and Drug Administration (FDA).

The findings of this study may be used for scientific meetings, written reports, and publications, but no information that could be used to identify you will be disclosed for these purposes.

Once your private health information has been disclosed to a third party, federal privacy laws may no longer protect it from re-disclosure. However, anyone obtaining access to your private health information under this consent must agree to protect your information as required by this consent.

This consent to use your private health information as described above does not expire. However, if you later change your mind, you can revoke this consent by writing to [SITE INVESTIGATOR] saying that you no longer wish to allow your private health information to be used for this study. If you revoke your consent, you may no longer be able to participate in the study. Moreover, we cannot undo uses or disclosures of your private health information that have already taken place in reliance on your prior consent.

PLEASE NOTE:
In the event of a positive result for Hepatitis B or C, reporting of the results to the local state Department of Health may be required.

WHAT IF YOU ARE INJURED?
If you are injured as a result of being in this study, you will be given immediate treatment. The cost for treatment will be charged to you or your insurance company. There is no

program through this institution to compensate participants who have research related injuries. You will not be giving up any of your legal rights by signing this consent.

WHAT IF YOU HAVE PROBLEMS OR QUESTIONS?
If you ever have questions or in the case of research-related injuries, you should contact:

[LIST STUDY INVESTIGATORS]

If you have questions about research subject's rights you can contact**:**

[SITE IRB CONTACT INCLUDING PHONE NUMBER]

RESEARCH STUDY REGISTRY
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at anytime.

SIGNATURE PAGE FOR CONSENT TO PARTICIPATE IN THE LIFE-HIV STUDY

I have read this consent form, had the opportunity to ask questions and have received answers to any questions I have asked. I willingly give my consent to participate in this study, and authorize the use and disclosure of my health information as described in this form. By signing this consent form I do not give up any of my legal rights. Upon signing this form I will be given a signed copy of the form for my records.

If you have read the consent (or if you have had it explained to you) and understand the information, and you voluntarily agree to join this study, please sign your name below.

_____	_____	_____
Participant's name (typed or printed)	Participant's signature	Date

_____	_____
Person obtaining consent	Date