# Reversing Tissue Fibrosis to Improve Immune Reconstitution in HIV

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**Confidentiality Statement**

## 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.

## Signature Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

_____ _____

Timothy W. Schacker, MD, Professor of Medicine Date

## Signature Page 2

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

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## Table of Contents

## List of Abbreviations and Acronyms

| | |
|---|---|
| ACEi | Angiotensin converting enzyme inhibitor |
| ADL | Activities of daily living |
| AE | Adverse event |
| AIDS | Acquired Immunodeficiency Syndrome |
| ALT | Alanine aminotransferase |
| APC | Antigen presenting cell |
| ARB | Angiotensin-II receptor blocker |
| ART | Antiretroviral therapy |
| AST | Aspartate transaminase |
| cART | Combination antiretroviral therapy |
| CBC | Complete blood count |
| CD4 | Cluster of differentiation 4 |
| CFR | Code of Federal Regulations |
| CICP | C-terminal propeptide of type I collagen |
| CM | Central memory |
| CMP | Comprehensive metabolic panel |
| CMV | Cytomegalovirus |
| DNA | Deoxyribonucleic acid |
| DSMB | Data and Safety Monitoring Board |
| ELISA | Enzyme-linked immunosorbent assay |
| FDA | Food and Drug Administration |
| FRCn | Fibroblastic reticular cell network |
| GALT | Gut associated lymphoid tissue |
| GFR | Glomerular Filtration Rate |
| GM-CSF | Granulocyte macrophage colony stimulating factor |
| HA | Hyaluronic acid |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human Immunodeficiency Virus |
| HPV | Human Papillomavirus |
| IA | Immune activation |
| IHC | Immunohistochemisty |
| INFg | Interferon gamma |
| IPF | Idiopathic pulmonary fibrosis |
| IRB | Institutional Review Board |
| ISH | In situ hybridization |
| IL | Interleukin |
| IR | Immune reconstitution |
| LPS | Lipopolysaccharide |
| LT | Lymphoid tissue |
| MCRU | Masonic Clinical Research Unit |
| MMP | Matrix metalloprotease |
| MT | Microbial translocation |
| µL | Microlitre |
| mL | Milliliter |
| MRE | Magnetic Resonance Elastography |
| PB | Peripheral blood |
| PBMC | Peripheral blood mononuclear cell |
| PCR | Polymerase chain reaction |
| PI | Principal investigator |

| | |
|---|---|
| po | per os |
| QA | Quality assurance |
| QIA | Qualitative image analysis |
| RNA | Ribonucleic acid |
| SAE | Serious adverse event |
| sCD14 | Soluble CD14 |
| SIV | Simian immunodeficiency virus |
| TGF-β | Transforming growth factor beta |
| TUNEL | Terminal deoxynucleotidyl transferase dUTP nick end labeling |
| TNF | Tumor necrosis factor |
| TZ | T-cell zone |
| Treg | Regulatory T cell |
| UMMC | University of Minnesota Medical Center |
| VL | Viral load |

# Protocol Summary

| | |
|---|---|
| Full Title | Reversing Tissue Fibrosis to Improve Immune Reconstitution in HIV |
| Conducted By | National Institute of Allergy and Infectious Diseases (NIAID) |
| Principal Investigator | Timothy Schacker, MD<br>Professor of Medicine<br>University of Minnesota, Department of Medicine |
| Sample Size | 50 HIV infected subjects and 10 HIV uninfected control subjects. |
| Study Population | HIV infected: Inclusion: Healthy, HIV-1 positive men and women ≥18 years of age, with CD4 count 200-650cells/μl, on stable antiretroviral therapy (ART). Exclusion: Pregnancy, use of drugs that suppress or enhance immune function, current use of angiotensin converting enzyme inhibitor (ACEi) or angiotensin-2 receptor blocker (ARB).<br>HIV uninfected: Inclusion: Healthy, ≥18 years of age. Exclusion: Pregnancy, use of drugs that suppress or enhance immune function, current use ACEi or ARB. |
| Accrual Period | 14 months |
| Study Design | This is a single-center, double-blinded, placebo-controlled study of HIV infected, ART treated subjects randomized 1:1 to daily losartan or placebo for 30 months. All HIV infected subjects will undergo biopsies of inguinal lymph node (LN) and gut associated lymphatic tissue (GALT) at baseline, 12 and 30 months after study enrollment. Blood will be collected at least quarterly throughout the study and an intensive blood pharmacokinetic (PK) study will be conducted at month 1. All HIV infected subjects will be vaccinated with the quadrivalent human papilllomavirus (HPV) vaccine at months 23, 25 and 29.5. HIV uninfected control subjects will not receive study agents or HPV vaccination and will undergo blood draw and inguinal LN biopsy at one time point only. |
| Sub-study-MRI | 10 HIV+ subjects will be randomly selected to participate in the MRI sub-study and the 5 control subjects will be enrolled in the MRI sub-study. We are collecting preliminary data to determine if there is potential for a MRI to monitor levels of collagen in lymph nodes. We will image the node that will be removed in the losartan parent trial to have collagen levels measured. We think it possible that this non-invasive procedure might someday be used as a biomarker in studies of anti-fibrotic therapy in HIV infection. |
| Study Duration | HIV infected subjects will be followed for 30 months. HIV uninfected subjects will have up to four study visits. We expect to complete follow up of all subjects 44 months after the first subject is enrolled. |
| Study Agent/Intervention Description | Losartan 100 mg daily (starting at 50 mg and dose escalating to tolerance) vs. Placebo |
| | For the five additional controls: Stribild – 150 mg elvitegravir/150 mg cobicistat/200 mg emtricitabine/300 mg tenofivir disoproxil fumarate once daily and Prezista – 800 mg once daily |

| | |
|---|---|
| Primary Endpoint | Impact of losartan on lymphoid tissue (LT) fibrosis as determined by the amount of collagen deposition in LT and integrity of the fibroblastic reticular cell network (FRCn). |
| Secondary Endpoints | 1. Impact of losartan on immune reconstitution and T cell function.<br>2. Impact of losartan on immune activation.<br>3. Impact of losartan on HIV viral reservoir size.<br>4. Determination of potential drug-drug interactions between losartan and ART. |
| Exploratory Endpoints | 1. Assess the impact of losartan on frequency of dendritic cell and CD4 T-cell interactions with the FRCn using two-photon microscopy. Given that this is an exploratory endpoint, we will perform these assays in a subset of subjects (5 losartan treated, 2 placebo treated, and 10 HIV uninfected controls).<br>2. To develop methods to image collagen content of inguinal lymph nodes using MRI<br>3. To measure drug levels and pharmacokinetic (PK) profile of antiretrovirals in lymph tissues of HIV uninfected people. |
| | |

# 1. KEY ROLES

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# 2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

## 2.1 Background Information

The goal of combination antiviral therapy (cART) in HIV is to suppress viral replication and restore immune function. It is possible with modern cART to suppress plasma viremia to below detectable limits in most people; however immune reconstitution (IR) is often incomplete, and significant immunologic abnormalities persist, even after years of therapy. CD4 T cell populations in peripheral blood mononuclear cells (PBMC), lymph node (LN), and gut associated lymphoid tissue (GALT) do not reconstitute to normal levels [1-4], responses to vaccines remain abnormal [5-8], and life expectancy continues to be significantly decreased compared to age-matched controls [9]. The underlying mechanisms driving these persistent immune abnormalities are likely related to the fact that markers of immune activation (IA) do not normalize in treated patients [10-12]. Indeed, there is growing evidence to support a model of persistent IA during therapy driving disease progression, albeit at a much slower rate than in untreated patients.

Causes of persistent IA before and during therapy include ongoing virus production [13], co-infections such as cytomegalovirus (CMV) [14], and microbial translocation (MT) of bacterial products across a persistently damaged gut epithelium [12, 15, 16]. IA inevitably leads to pathologic changes in lymphoid tissues (LT) that significantly alter their function and limit immune reconstitution (IR). The primary pathology of persistent IA in LT is collagen deposition in the parafollicular T-cell zone (TZ) of secondary LN and GALT that gradually destroys TZ structure [17, 18]. Collagen deposition is mediated by transforming growth factor beta (TGF-ß) that is made by regulatory T cells (Treg) recruited into LT in an attempt to control virus production [19-21]. During chronic and treated infection, when virus production is either substantially or completely suppressed, co-infections and MT may play a larger role in TGF-ß production. Irrespective of the cause, TZ collagen accumulates over time and leads to depletion of naive and central memory (CM) CD4 T cells and prevents IR when cART is begun [17, 22]. Collagen destroys the fibroblastic reticular cell network (FRCn) which is a mesh of hollow fibers that form the skeletal anatomy of the TZ [23]. T cells require contact with the FRCn in order to migrate through the TZ and initiate immune responses and gain access to IL7 (made by the FRCn) required for survival [24-26]. Collagen formation in the TZ is a cause of naive and CM depletion and prevents their full reconstitution when cART is begun. Thus TZ collagen plays a critical role in HIV pathogenesis, and therapies to eliminate its presence may be an important adjunctive therapy to improve IR.

We have used the TGF-ß inhibitor pirfenidone in a non-human primate model of LT fibrosis induced by SIV infection. Pirfenidone is a drug licensed outside of the U.S. for the treatment of idiopathic pulmonary fibrosis (IPF) and it blocks TGF-ß at the level of phosphorylation of SMAD 2,3 in the TGF-ß signaling pathway [27-34]. We show that animals receiving pirfenidone plus cART had significantly less TZ collagen, a more normal FRCn and significantly greater numbers of CD4 T cells in PBMC and LN. Pirfenidone is not FDA approved and the manufacturer is unable to make it available for this study. However, an alternative drug exists that may be superior to pirfenidone. Losartan is an FDA-approved angiotensin receptor inhibitor (ARB) that inhibits TGF-ß at the level of phosphorylation of SMAD 2,3 [35-38] with animal and human studies to show it reverses existing fibrosis in lung, liver, and kidney [39-43]. It is widely used throughout the world in Marfan syndrome for its antifibrotic properties. Importantly, distinct from pirfenidone, losartan also decreases inhibitors of matrix metalloproteases that prevent tissue remodeling and increases proteases that induce tissue remodeling [39, 44, 45]. Further, it has anti-inflammatory properties that may be uniquely beneficial in the context of HIV infection. It inhibits LPS-induced inflammatory signaling, an important component of MT, and decreases levels of tumor necrosis factor (TNF), IL6, and soluble adhesion molecules [46, 47].

## 2.2 Study Hypothesis and Rationale

Our hypothesis is that treatment of HIV infected subjects with losartan, which has specific anti-inflammatory and anti-fibrotic actions will: 1) reverse existing fibrosis, 2) restore LN architecture, 3) increase the number and improve the function of peripheral and lymphatic CD4 T cells, 4) decrease levels of IA, 5) decrease HIV reservoirs, and 5) be safe and well tolerated. To address this hypothesis, we will enroll HIV infected subjects and randomize them 1:1 to losartan vs placebo, then serially sample blood and LT. In similar longitudinal tissue biopsy protocols our drop-out rate has ranged from 20-25%. Therefore, we plan to enroll 63 HIV infected subjects in order to have 50 complete the protocol. All HIV infected subjects randomized to losartan will initiate therapy at a dose of 50 mg per os (po) daily and increase the dose to 100 mg po daily if they have no side effects or laboratory abnormalities after 14 days. This is standard losartan dosing for the treatment of hypertension and stroke reduction. This dosing protocol was also used and was well tolerated by non-hypertensive subjects in a large clinical trial assessing the anti-fibrotic effect of losartan in patients with Marfan syndrome [48]. Given risk of teratogenicity with losartan, all female subjects of reproductive age who are enrolled in the protocol will be required to use a reliable and effective method of contraception for the duration of the study. Lymphatic tissue biopsies for primary endpoint analysis will be obtained at baseline and 12 and 30 months after initiation of losartan or placebo. We are following subjects for 30 months because we felt that a long period of follow up may be required to discern a significant difference in the treatment groups given the persistence of LT fibrosis in subjects treated with ART alone (unpublished data). HIV infected subjects will also be administered the human papillomavirus (HPV) quadrivalent vaccine (Gardasil) per the manufacturer's recommended dosing schedule to assess immune response (a secondary endpoint). Gardasil vaccine is FDA-approved for use in men and women ages 9 to 26 to prevent the complications related to infection with HPV types 6, 11, 16 and 18. Although not approved for adults >26 years of age, the vaccine has been well-studied in older populations and has been found to be safe and well-tolerated [49-51]. It is expected that many study subjects will have been exposed to 1 or more of the HPV serotypes included in the vaccine; however, based on epidemiologic data, it is not expected that subjects will have been exposed to all 4 serotypes [52-54]. Therefore, this vaccine can be used to assess both neo and recall immune responses. Vaccination will be initiated at the end of the study to maximize time on study drug prior to vaccination and to ensure that tissues are collected at time of maximal immune response.

Though there are no known or anticipated drug interactions between losartan and ARVs, this has not been extensively studied. Therefore an intensive pharmacokinetics experiment will be conducted on all HIV infected subjects at month 1 of the study (when losartan is at steady state). In addition, levels of ARVs and losartan in plasma and PBMCs will be performed quarterly over the 30-month study period and intracellular levels of losartan and ARVs will be measured in LT at month 30. These analyses will be performed in semi real-time to ensure that subjects are not put at risk from unexpected drug-drug interactions.

We will enroll 5 HIV uninfected subjects who will not be randomized to a treatment arm and will not be given HPV vaccination. They will undergo a blood draw and inguinal LN biopsy at one time point only. We will enroll an additional 5 HIV uninfected subject who will not be randomized to a treatment arm nor given HPV vaccine. These 5 subjects will be given Stribild and Prezista for two weeks. In previous studies with HIV-positive individuals, we have shown lymph node concentrations of all ARVs to be suboptimal. No one has yet measured ARV concentrations in lymph nodes of healthy volunteers; we feel this is an excellent opportunity to answer that question. These subjects will return to the medical center for an inguinal LN biopsy and colonoscopy and blood draws at baseline, hours 1, 2,3,4,6,8,12 and 24 at one time point only. The five control subjects who are taking Stribild and Prezista will be part of the MRI-sub-study (See Appendix I). This test will occur once during the two weeks while they are taking the antiretrovirals, prior to the biopsy.

In addition, these negative controls are important to the success of the two-photon microscopy experiment, which is used to measure motility of lymphocytes on the LN FRCn. The HIV negative control group will be

used to determine normal motility and samples obtained from HIV infected subjects on losartan and placebo will then be compared to these results. We have preliminary data from similar experiments conducted in mice to suggest that 10 control subjects will be adequate to establish normal motility.

## 2.3 MRI Sub-study

This sub-study will involve 10 regular subjects & the 5 new HIV-negative controls.

Subjects will be randomly selected to participate in the MRI sub-study. We are collecting preliminary data to determine if there is potential for a MRI to monitor levels of collagen in lymph nodes. We will image the node that will be removed in the losartan parent trial to have collagen levels measured. We think it possible that this non-invasive procedure might someday be used as a biomarker in studies of anti-fibrotic therapy in HIV infection. (See Appendix I for the MRI sub-study protocol).

## 2.4 Potential Risks and Benefits

### 2.4.1 Potential Risks

Potential risks of study procedures (venipuncture, inguinal LN biopsy, and colonoscopy with GALT biopsies) are detailed in Section 7.1 and in consent forms. According to the Losartan package insert, the most severe risks of losartan use include teratogenicity in the setting of pregnancy, hypersensitivity, symptomatic hypotension, worsening of renal function, and electrolyte abnormalities. The most common side effects of losartan include fatigue, weakness, diarrhea, chest pain, tachycardia and anemia. Subjects will be rigorously screened to ensure that they are at low risk for complications due to losartan and they will be monitored closely for side effects and signs of toxicity. The most common side effects of Gardasil include headache, fever, nausea, dizziness and injection site reactions. Severe reactions, including hypersensitivity, are rare. The most common side effects of Stribild are new onset or worsening of renal impairment, bone effects, fat distribution and immune reconstitution syndrome. The most common side effects of Stribild include nausea, diarrhea, abnormal dreams, headache, rash, fatigue, dizziness, insomnia, flatulence, somnolence, ocular icterus and jaundice. The most common side effects of Prezista are drug –induced hepatitis, skin reactions ranging from mild to severe, use with caution with a known sulfonamide allergy, new onset diabetes or hyperglycemia, redistribution/accumulation of body fat or immune reconstitution syndrome and subjects with hemophilia may develop increased bleeding events, The most common side effects of Prezista include diarrhea, nausea, rash, headache, abdominal pain and vomiting.

### 2.4.2 Potential Benefits

All subjects (excluding control subjects) will receive results of clinical laboratory testing and HPV vaccination at no cost over the course of this study. There are no other direct benefits of participation for the individual subjects. However, the proposed research has the potential to significantly benefit people infected with HIV.

# 3. STUDY OBJECTIVES

## 3.1 Primary Objective

We will assess the impact of losartan on LT fibrosis in HIV infected, ART treated adults by measuring the amount of collagen deposition in LT and the integrity of the FRCn using IHC.

## 3.2 Secondary Objectives

To determine the impact of losartan on immune reconstitution and T- cell function, immune activation, HIV viral reservoir size, and to determine any potential drug-drug interactions between losartan and ART.

## 3.3 Exploratory Objectives

1. Assess the impact of losartan on frequency of dendritic cell and CD4 T-cell interactions with the FRCn using two-photon microscopy.
2. To develop methods to image collagen content of inguinal lymph nodes using MRI
3. To measure drug levels and pharmacokinetic (PK) profile of antiretrovirals in lymph tissues of HIV uninfected people.

# 4. STUDY DESIGN

## 4.1 Description of the Study Design:

This is a randomized, double-blind, placebo-controlled trial of 50 HIV-1 infected individuals on stable ART randomized in a 1:1 ratio to losartan (50 mg orally daily titrated to 100 mg daily) vs placebo for 30 months. We plan to enroll a total of 63 HIV infected subjects to ensure that 50 complete the protocol. All HIV infected subjects will undergo biopsies of inguinal lymph node (LN) and gut associated lymphatic tissue (GALT) for primary endpoint analysis at baseline, 12 and 30 months after study enrollment. Blood will be collected at least quarterly throughout the study and an intensive blood pharmacokinetic (PK) study will be conducted at month 1. All HIV infected subjects will be vaccinated with the quadrivalent human papillomavirus (HPV) vaccine at months 23, 25 and 29.5. 5 HIV uninfected control subjects will also be enrolled. They will not receive study agents or HPV vaccination and will undergo blood draw and inguinal LN biopsy at one time point only. We anticipate completing subject enrollment over a 14 month period and plan to complete all subject follow up 44 months after the first subject is enrolled.

## 4.2 Study Endpoints:

### 4.2.1 Primary Endpoint

The primary endpoint is to determine the impact of losartan on LT fibrosis in HIV infected, ART treated adults. This will be determined by measuring the amount of collagen deposition in LT and the integrity of the FRCn using immunohistochemistry (IHC) and quantitative image analysis (QIA).

### 4.2.2 Secondary Endpoints

There are 4 secondary endpoints:

**1. We will assess the impact of losartan on immune reconstitution and T-cell function by measuring:**

⇒ Frequency of CD4+ T cells, TUNEL+CD3+CD8+ T cells and cells expressing TGF-beta and lymphotoxin-beta in LT using IHC.
⇒ Serum levels of IL-7 and TGF-beta using ELISA.
⇒ The immune response to HPV vaccination using flow cytometry to identify cells stimulated by specific HPV peptides.

**2. We will determine the impact of losartan on immune activation in HIV infected, treated individuals by determining:**

⇒ Frequency of activated T-cell populations (specifically CD3+CD4+CD38+, CD3+,CD8+CD38+, CD4+Ki67+ and CD8+Ki67+ T cells) in LT using immunofluorescence staining.
⇒ Percentage of activated T cells, macrophages and dendritic cells in PBMCs and LT using flow cytometry.
⇒ Intracellular levels of the inflammatory cytokines IL-17, IFNg, IL-2, TNF, IL-10, and GM-CSF in PBMCs and LT using cytokine staining.
⇒ Plasma levels of additional inflammatory markers including LPS, sCD14, I-FABP, IL-1b, IL-1RA, IL-6, TNF, amyloid A, CRP, and D-dimer using limulus assay (sCD14) and ELISA (all others).

**3. We will assess the potential for losartan to reduce the size of the viral reservoir by determining the frequency of HIV RNA+ and DNA+ cells in LN and GALT using both radiolabeled in situ hybridization (ISH) and RNAscope™ in situ technology.**

**4. To ensure that losartan is safe and well tolerated when used with ART, we will:**
⇒ Perform an intensive pharmacokinetics experiment in all HIV infected subjects at month 1 of the study (when losartan is at steady state).
⇒ Measure levels of ARTs and losartan in plasma and PBMCs quarterly over the 30-month study period.
⇒ Measure intracellular levels of losartan and ARTs in LT at month 30.

### 4.2.3 Exploratory Endpoints

1. Assess the impact of losartan on frequency of dendritic cell and CD4 T-cell interactions with the FRCn using two-photon microscopy.
2. To develop methods to image collagen content of inguinal lymph nodes using MRI
3. To measure drug levels and pharmacokinetic (PK) profile of antiretrovirals in lymph tissues of HIV uninfected people.

# 5. STUDY POPULATION

## 5.1 Selection of Study Population

83% of persons living with HIV/AIDS in the State of Minnesota are male, 50% are white, 32% black (with 10% being African born), 9% Hispanic/Latino, 4% American Indian, and 5% Asian or "Other" (Minnesota Department of Health 2010 data). By focusing on women and minority populations in our recruitment efforts, we anticipate that the demographic profile of our study participants will represent this distribution.

HIV infected subjects will be recruited using our well-established recruitment network in the Twin Cities that involves partnerships with local HIV clinics and community organizations such as the Minnesota AIDS Project. We will also design study specific advertisements and flyers for use in local publications and clinics. HIV uninfected subjects will be recruited through flyers placed around the University of Minnesota and advertisements in local magazines and newspapers.

As above, based on drop-out rates in similar studies we have conducted in the past, we plan to enroll a total of 63 HIV infected subjects to ensure that 50 complete the protocol.

## 5.2 Inclusion/Exclusion Criteria

**HIV infected participants:**

**a. Inclusion Criteria:**

Participants must meet all of the following inclusion criteria to participate in this study:

1. HIV-1 infected.

2. ≥ 18 years of age.
3. Baseline peripheral CD4+ T cell count 200-650 cells/$mm^3$ for at least two measures and no more than one measure outside the range over the 6 months prior to study enrollment.
4. ≥ 12 months of consistent use of antiretrovirals without disruption lasting ≥ 1 week in the period leading up to study enrollment.
5. HIV viral load (VL) < 50 copies/mL for at least two measures over the 6 months prior to study enrollment.
6. No contraindication to proposed study procedures.
7. Women of child-bearing potential must be willing to use a form of effective contraception for the duration of the study. Effective contraception includes hormonal injection, implant or oral medication, IUD, diaphragm, or cervical cap with spermicide. Condoms cannot be used as the sole form of contraception.

**b. Exclusion Criteria:**

Participants meeting any of the following exclusion criteria at baseline will be excluded from study participation:

1. Use of any immunomodulator within the 12 months prior to study enrollment**.** An immunomodulator for the purposes of this study is defined as a drug known to either diminish or augment a patient's immune system. Examples of these include, but are not limited to, systemic corticosteroids (use of topical steroids will be permitted), TNF-inhibitors, rituximab, cyclophosphamide, abatacept, cyclosporine, azathioprine, 6-mercaptopurine, methotrexate, sulfasalazine, cyclosporine, tacrolimus, sirolimus, and intravenous immune globulin. NOTE: It is considered permissible for a potential subject to have 1) taken a brief (≤ 2 week) course of oral corticosteroids for acute respiratory illness, 2) received a corticosteroid injection into a joint, and/or 3) inhaled corticosteroids for control of reactive airway disease.
2. Current use of an ARB or ACEi.
3. Current use of rifaximin, fluconazole or lithium given potential for drug interactions with losartan.
4. Prior reaction or intolerance to an ARB or ACEi.
5. Prior diagnosis of a chronic inflammatory disease with serologic or clinical evidence as diagnosed by a primary care physician or specialist. Examples of these include, but are not limited to, systemic lupus erythematosus, rheumatoid arthritis, scleroderma, Sjogren's syndrome, mixed connective tissue disease, psoriasis, polymyositis, dermatomyositis, vasculitis, sarcoidosis, Wegener's granulomatosis, giant cell arteritis, polyarteritis nodosa, gastrointestinal pemphigoid, eosinophilic colitis, Crohn's disease, ulcerative colitis, autoimmune hepatitis.
6. Prior diagnosis of a connective tissue disease with genetic, serologic or clinical evidence as diagnosed by a primary care physician or specialist. (Marfan's syndrome, Ehlers-Danlos syndrome).
7. Screening systolic blood pressure < 110.
8. Estimated Glomerular Filtration Rate (eGFR) of < 30ml/min/1.73 m2 within 60 days of study initiation or history of advanced renal disease.
9. AST and/or ALT > 3 times the upper limit of normal within 60 days of study enrollment.
10. Potassium > 5.0 within 60 days of study enrollment.
11. Pregnancy.
12. In women of childbearing age, unwillingness to use birth control for the duration of the study.
13. Breast feeding.
14. Prior vaccination with an HPV vaccine, including Cervarix (GlaxoSmithKline) or Gardasil (Merck).
15. History of hypersensitivity or severe allergic reactions to yeast.
16. At the principle investigator's discretion if not a suitable surgical candidate.
17. Currently undergoing treatment for hepatitis C or treatment completed within the last 3 months
18. History of ≥ 3 previous inguinal lymph node biopsies

**HIV-uninfected:**

**a. Inclusion Criteria**

Participants must meet all of the following inclusion criteria to participate in this study:

1. HIV uninfected.
2. ≥ 18 years of age.
3. No contraindication to proposed study procedures.

**b. Exclusion Criteria**

Participants meeting any of the following exclusion criteria at baseline will be excluded from study participation:

1. Use of any immunomodulator within the 12 months prior to study enrollment (as defined above).
2. Current use of an ARB or ACEi.
3. Prior diagnosis of a chronic inflammatory disease with serologic or clinical evidence (as defined above).
4. Prior diagnosis of a connective tissue disease with genetic, serologic or clinical evidence as diagnosed by a primary care physician or specialist. (Marfan's syndrome, Ehlers-Danlos syndrome).
5. AST and/or ALT > 1.5 times the upper limit of normal at screen
6. At the principal investigator's discretion if not a suitable candidate.
7. Sulfa allergy
8. Pregnancy.

## 5.3 Co-Enrollment Guidelines

Subjects are restricted from taking part in any other studies that involve tissue sampling or administration of pharmacologic agents while enrolled in this protocol.

# 6. STUDY AGENTS

## 6.1 Study Agent Acquisition

### 6.1.1 Losartan

#### 6.1.1.1 Formulation, Packaging, and Labeling

Losartan will be provided for this study by Merck and will be shipped directly to the Investigational Drug Services (IDS) pharmacy at the University of Minnesota. Matched placebo pills will also be provided by Merck. IDS pharmacy will maintain randomization codes and will provide appropriate labels for medications distributed to subjects.

#### 6.1.1.2 Preparation, Administration, Storage, and Dosage of Study Agent(s)/Intervention(s)

HIV infected subjects will be randomized to placebo or losartan. Starting dose of losartan will be 50 mg po daily. At week 2, dose will be increased to 100 mg po daily based on tolerability, blood pressure, and toxicity lab results. The IDS pharmacy will be responsible for preparing pill bottles, distributing medications to subjects, and storing medications. Research pharmacists and credentialed pharmacy technicians charged with dispensing research-related drugs will receive protocol-specific training and are compliant with State Board of Pharmacy requirements, FDA guidelines, and Good Clinical Practices.

#### 6.1.1.3 Study Agent Accountability Procedures

Losartan or placebo will be distributed to subjects at baseline visit, week 2 visit and approximately every 3 months thereafter. Pharmacy documents, including study drug accountability logs, study drug ordering/shipping logs, and regulatory documents are maintained by the pharmacy technician. All handling of study agents (e.g., drug receipt and storage, drug

accountability, etc.) will be done by a pharmacist. If subjects have study drug remaining at time of study completion or study withdrawal, designated study staff will collect the drug from the subject and return it to the UMN IDS where it will be properly disposed of.

### 6.1.2 Gardasil Vaccine

#### 6.1.2.1 Formulation, Packaging, and Labeling

Standard pre-filled syringes of Gardasil will be purchased commercially through Merck for use in this study and will be shipped directly to the IDS pharmacy.

#### 6.1.2.2 Preparation, Administration, Storage, and Dosage of Study Agent(s)/Intervention(s)

Gardasil will be stored in the IDS pharmacy and will be distributed by the pharmacy to designated study staff on the day of vaccine visits. Research pharmacists and credentialed pharmacy technicians charged with dispensing research-related drugs will receive protocol-specific training and are compliant with State Board of Pharmacy requirements, FDA guidelines, and Good Clinical Practices. Trained study staff will administer 0.5mL of Gardasil (which contains suspension of 120 mcg L1 protein from HPV types 6, 11,16, and 18) intramuscularly in the thigh of HIV infected subjects at months 23, 25 and 29.5.

#### 6.1.2.3 Study Agent Accountability Procedures

Pharmacy documents, including study drug accountability logs, study drug ordering/shipping logs, and regulatory documents are maintained by the pharmacy technician. All handling of study agents (e.g., drug receipt and storage, drug accountability, etc.) will be done by a pharmacist. IDS will properly dispose of any product that remains when the study is completed.

### 6.1.3 Stribild

#### 6.1.3.1 Formulation, Packaging, and Labeling

STRIBILD is a fixed-dose combination tablet containing elvitegravir, cobicistat, emtricitabine, and tenofovir DF for oral administration. Stribild will be provided for this study by the research study and will be shipped directly to the Investigational Drug Services (IDS) pharmacy at the University of Minnesota.

#### 6.1.3.2 Preparation, Administration, Storage, and Dosage of Study Agent(s)/Intervention(s)

HIV non-infected subjects will receive **150 mg elvitegravir/150 mg cobicistat/200 mg emtricitabine/300 mg tenofivir disoproxil fumarate to take by mouth once daily.** The IDS pharmacy will be responsible for preparing pill bottles, distributing medications to subjects, and storing medications. Research pharmacists and credentialed pharmacy technicians charged with dispensing research-related drugs will receive protocol-specific training and are compliant with State Board of Pharmacy requirements, FDA guidelines, and Good Clinical Practices

#### 6.1.3.3 Study Agent Accountability Procedures

Pharmacy documents, including study drug accountability logs, study drug ordering/shipping logs, and regulatory documents are maintained by the pharmacy technician. All handling of study agents (e.g., drug receipt and storage, drug accountability, etc.) will be done by a pharmacist. If subjects have study drug remaining at time of study completion or study withdrawal, designated study staff will collect the drug from the subject and return it to the UMN IDS where it will be properly disposed of.

### 6.1.4 Prezista

#### 6.1.4.1 Formulation, Packaging and Labeling

Prezista comes in tablets or oral suspension. Prezista will be provided for this study by the research study and will be shipped directly to the Investigational Drug Services (IDS) pharmacy at the University of Minnesota.

**6.1.4.2 Preparation, Administration, Storage and Dosage of Study Agent(s)/Intervention(s).**
HIV non-infected subjects will receive 800 mg of Prezista to take by mouth once daily. The IDS pharmacy will be responsible for preparing pill bottles, distributing medications to subjects, and storing medications. Research pharmacists and credentialed pharmacy technicians charged with dispensing research-related drugs will receive protocol-specific training and are compliant with State Board of Pharmacy requirements, FDA guidelines, and Good Clinical Practices.

**6.1.4.3 Study Agent Accountability Procedures**
Pharmacy documents, including study drug accountability logs, study drug ordering/shipping logs, and regulatory documents are maintained by the pharmacy technician. All handling of study agents (e.g., drug receipt and storage, drug accountability, etc.) will be done by a pharmacist. If subjects have study drug remaining at time of study completion or study withdrawal, designated study staff will collect the drug from the subject and return it to the UMN IDS where it will be properly disposed of.

## 6.2 Assessment of Participant Adherence with Study Product

Adherence to study drug (losartan or placebo) will be assessed by designated study staff at each study visit (these occur at least quarterly throughout the 30-month study). Subjects will be asked to bring their pill bottle to each of these visits so that remaining pills can be counted and used as a measure of adherence. Gardasil will be administered at appropriate dosing intervals by designated study staff, ensuring proper adherence.

## 6.3 Permitted Medications and Procedures

Medications not listed under prohibited medications and the manufacturers' package inserts are permitted during this study. If subjects develop a condition that requires a medical procedure while on study, we will determine impact of this on study participation on a case by case basis. Whenever a concomitant medication or study agent is initiated or a dose is changed, investigators must review the concomitant medications and study agents' most recent package inserts, and Investigator's Brochures to obtain the most current information on drug interactions, contraindications, and precautions.

## 6.4 Prohibited Medications

The following drugs are not permitted for HIV subjects while on study:

- Any immunomodulator (defined as a drug known to either diminish or augment a patient's immune system). Examples include, but are not limited to, systemic corticosteroids (use of topical steroids will be permitted), TNF-inhibitors, rituximab, cyclophosphamide, abatacept, cyclosporine, azathioprine, 6-mercaptopurine, methotrexate, sulfasalazine, cyclosporine, tacrolimus, sirolimus, and intravenous immune globulin.
- ACE inhibitors
- ARBs other than the study drug
- Rifampin
- Lithium
- Fluconazole

The following drugs are not permitted for uninfected control subjects while taking Stribild and Prezista:

- Any antiretroviral drugs

- Any nephrotoxic drugs
- Any drugs that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events

# 7. STUDY PROCEDURES/INTERVENTIONS

## 7.1 Description and Potential Risks of Clinical Procedures

### 7.1.1 Medical History

At screening and baseline visits, an extensive medical history will be obtained (including prior diagnoses, medications, surgeries, etc.). Interval medical histories will be obtained at each subsequent study visit. Only trained designated study staff or physicians will obtain the medical history. History will primarily be obtained by interviewing the subject. If the subject is agreeable and signs a release of information form, medical records may also be obtained and reviewed by the study clinical team. Obtaining a medical history may make subjects feel uncomfortable or embarrassed. There are no other potential risks.

### 7.1.2 Physical Exam

At screening, a physical exam will be performed which will include measurement of vital signs and evaluation of heart, lungs, abdomen, and neurologic and lymphatic systems. At all other time points indicated, a targeted physical examination including measurement of vital signs and evaluation of heart, lungs, abdomen, prior LN biopsy sites, and neurologic system will be performed. Only trained study staff and study physicians will perform exams. Performing a physical exam may make subjects feel uncomfortable or embarrassed. There are no other potential risks.

### 7.1.3 Medical Adherence Counseling

At each visit, designated study staff or physicians will counsel subjects about the importance of adherence to ART and study medications. Women of child-bearing potential will be counseled about the importance of adherence to contraceptives. There are no potential risks associated with this.

### 7.1.4 Venipuncture

Venipuncture will be performed at each visit and will be done only in the MCRU or Positive Care Center. Only trained research clinic staff or physicians will be allowed to perform venipuncture. The risks of venipuncture are transient pain, bleeding, lightheadedness, bruising, possible vasovagal reaction, and infection.

### 7.1.5 Gardasil Vaccination

Gardasil vaccine will be administered to HIV infected subjects at months 23, 25 and 29.5. Only study staff well trained in vaccine administration will be allowed to administer Gardasil. The vaccine will be administered intramuscularly in the thigh at the month 29.5 visit so that local response to the vaccine in the draining inguinal lymph node can be assessed. The most common side effects of this vaccination are headache, fever, nausea, dizziness and injection site reactions. Severe reactions, including hypersensitivity, are rare.

### 7.1.6 Inguinal Lymph Node Biopsy

Inguinal lymph node biopsies will be performed by Dr. Gregory Bielman or Dr. Jeffrey Chipman, members of the Department of Surgery at the University of Minnesota. HIV infected subjects will have

biopsies performed at baseline and months 12 and 30. HIV uninfected control subjects will have biopsies performed at one time point only. Procedures will be done in the MCRU under local anesthesia. The surgeon will first clean and sterilize the inguinal area, inject a local anesthetic and make a small (5 cm) incision over the lymph node. It will be removed and the incision closed. This technique has been successfully used in over 200 lymph node biopsies at the University of Minnesota in the past 5 years without any significant complications.

The risks of the lymph node biopsy are bleeding, infection, seroma, and scarring. Any participant who develops a seroma will have it drained with a syringe in the clinic. These complications are rare. All post-operative and post-biopsy care will be provided by designated study staff. If complications occur, the subject will be seen immediately by the appropriate study physician(s).

### 7.1.7 Colonoscopy with Ileal and Rectal Biopsies

Colonoscopies with ileal biopsies will be completed by Dr. Alex Khoruts, a member of the Division of Gastroenterology in the Department of Medicine at the University of Minnesota. HIV infected subjects will have biopsies performed at baseline and months 12 and 30. Procedures will be done in the UMMC Endoscopy Center under conscious sedation. Subjects will complete the required bowel regimen the night prior to their scheduled procedure. For the procedure, the participant will lie on their left side and the colonoscope will be inserted and guided through the colon. After 20 small tissue biopsies of the ileum and rectum are obtained, the colonoscope will be removed.

The risks of colonoscopy are abdominal cramping, bleeding, anxiety, and dizziness from the sedation. A very rare complication of colonoscopy is perforation of the intestine. All post-procedure and post-biopsy care will be given by designated study staff who are specially trained to do this.

### 7.1.8 MRI

MRI will be performed by Greg Metger, PhD, a faculty member of the Department of Radiology at the University of Minnesota. MRI sub-study participants will undergo MRI one to three weeks prior to the baseline, Month 12 or Month 30 visit. MRI will be performed at the Center for Magnetic Resonance Research (CMRR) without use of contrast dye. Sub-study participants will be screened and consented prior to undergoing MRI by trained CMRR staff. Subjects will be asked to avoid eating or drinking anything for four hours prior to the procedure. The subject will be positioned on their back on the scanner table, will enter the magnet tunnel feet first, and will have a surface coil consisting of a flexible plastic sheet placed around their body and secured with Velcro. A series of scans that in total will last between 60 – 180 minutes will be performed. These scans are intended to develop new methods for acquiring and analyzing MRI data to characterize structural and functional changes of the lymphatic tissue between the normal and disease state.

Risks of moving in/near the scanner and during the MR scanning include dizziness, warming, and muscle twitching. Some metallic objects are attracted to the magnet and cannot be brought into the room. Some participants may experience claustrophobia. The MR scanner makes loud noses. Some people report mild nausea, headache, a metallic taste in their mouth, or sensations of flashing lights that if present, subside shortly after leaving the magnet. There is a risk of unknown effects related to participation in MRI research. There are no benefits to participation. Incidental findings are not expected, but if found, a Radiologist associated with CMRR will review the MR images and will recommend the subject follow-up with their primary medical provider as needed.

See Appendix I for additional information about MRI

## 7.2 Plan to Minimize Risks to Subjects

All investigators and study staff have completed required training regarding human subject protections. Study staff will comply with all related regulations and laws, included, but not limited to 45 CFR 46 and 21 CFR 50, 56 (FDA version), and HIPAA Privacy Regulations. Study will be conducted in accordance with US and international standards for good clinical practices, applicable regulations, and institutional research policies and procedures.

Subjects will be rigorously screened against inclusion/exclusion criteria to ensure that their participation is safe. All staff members responsible for taking a medical history that could potentially make the participant uncomfortable have been specifically trained for how to do this in a sensitive manner. Only qualified study or clinic staff or physicians will draw blood to minimize risks. Pregnancy testing will be performed at screening, baseline, every 3 months, and anytime during follow up when the woman or investigator suspects pregnancy. In addition, study staff will stress the importance of adherence to contraceptives in order to minimize risks related to unintended fetal exposure. Subjects will be closely observed for systemic adverse events and toxicity related to the study drug on a routine basis through interviews, clinical assessment (including measuring vital signs and physical exam), and monitoring of renal function, liver function, and hematologic panel. All lymph node biopsies will be performed by either Dr. Gregory Beilman, Dr. Jeffrey Chipman, or Dr. Torfi Hoskuldsson who have performed >400 LN biopsies in the research setting without significant complications. All colonoscopies will be performed by Dr. Alexander Khoruts who has performed >300 colonoscopies and biopsies in related HIV studies in the past without any complications. Subjects will be monitored after biopsy procedures for at least 4 hours by study staff trained in managing postoperative patients. They will be monitored for at least 30 minutes after HPV vaccine administration to ensure that they tolerate the vaccine without difficulty.

Samples obtained for pharmacokinetic analysis will be immediately sent to Dr. Fletcher's laboratory and will be analyzed in semi real-time. Levels of losartan and all ARVs that an individual subject is taking will be monitored to ensure that subjects are not exposed to supratherapeutic levels of any drug, that losartan does not decrease levels of any ARVs, and that the active metabolite of losartan is within the expected range. Results of these analyses will be communicated to the PI as soon as they are available. If losartan is found to either increase or decrease levels of a particular ARV, the IRB will be notified and all enrolled subjects taking that ARV will be contacted and the finding explained. Depending on the type of drug-drug interaction, toxicity labs and/or HIV VL will be obtained within 3 days of obtaining the drug level results. If dose adjustments can be used to overcome these interactions, they will be made if the subject is willing. If dose adjustments are not possible, subjects taking the ARV will be withdrawn from the study and the protocol will be amended so that no additional subjects taking that ARV will be enrolled. If losartan metabolite levels are found to be lower than the expected range, the losartan dose will be increased. If losartan metabolite levels are higher than expected, toxicity labs will be obtained and the dose will be decreased. After any dose adjustments of losartan or ARVs, follow up drug levels will be monitored closely to ensure that target levels have been achieved.

## 7.3 Laboratory Evaluations and Specimen Collection

### 7.3.1 Clinical and Research Laboratory Evaluations

Comprehensive list of clinical laboratory evaluations are detailed in the Protocol Analyte List and a comprehensive list of non-FDA approved endpoint assays are detailed in the Non-FDA Approved End Point Test List. As a reference, both lists are included in the grant application appendix, located in the study files.

### 7.3.2 Biohazard Containment

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

### 7.3.3 Specimen Preparation, Handling and Shipping

Please refer to the Specimen Management Plan included in the appendix of this grant for further details pertaining to specimen preparation, handling and shipping.

#### *7.3.3.1 Blood*

All blood samples taken in the MCRU and Positive Care Clinic will be immediately transferred to the appropriate site. Research study staff will order clinical labs (e.g. CD4 T cell count, HIV VL, CMP, and CBC) and ensure that the appropriate blood collection tubes are sent to the appropriate clinical laboratory. Samples drawn for research purposes will be transported by a scientist in the Schacker lab from the MCRU to the laboratory for isolation of plasma, serum and PBMCs. Research samples obtained in the Positive Care Clinic will be transported to the Schacker lab by courier and immediately processed. Please refer to the Specimen Management Plan in the study files for details regarding the distribution of serum, plasma and PBMCs to specialized laboratories for analysis.

#### *7.3.3.2 Inguinal Lymph Node Biopsies*

Lymph nodes will be harvested in the MCRU and immediately sectioned at the bedside by a study physician. For HIV infected subjects, one portion will be placed into freshly made 4% paraformaldehyde (PFA) for 24 hours (and then transferred to 80% ethanol prior to paraffin embedding) and the other is placed into RPMI and immediately transported to the Schacker laboratory for preparation of cell suspension. Distribution of isolated cells is detailed in the Specimen Management Plan. For subjects enrolled in the two-photon microscopy study, a small section of the LN intended for histology will be immediately transferred fresh to the Fife laboratory. For HIV uninfected control subjects, half of the LN will be placed into fresh PFA and managed as above. The other half will be transferred fresh to the Fife laboratory.

#### *7.3.3.3 Colonoscopy with Ileal and Rectal Biopsies*

Approximately 20 snip biopsies of ileum and rectum will be obtained. Half of these samples will be placed in 4% PFA followed by 80% ethanol. They will then be paraffin embedded and used in IHC and ISH studies. The remaining samples will be immediately placed into RPMI and transferred to the Schacker lab for preparation of cell suspensions. Distribution of isolated cells is detailed in the Specimen Management Plan in the study files.

# 8. STUDY SCHEDULE

## 8.1 Schedule for HIV infected subjects (n=50)

### 8.1.1 Screening

At this visit, consents will be reviewed in detail and the subject will have the opportunity to ask any questions they may have. Subjects will be asked if they would like to participate in the additional two-

photon microscopy sub-study which entails additional blood draws at several scheduled study visits. Designated study staff or physician will interview the participant and a physical exam (which includes vital signs and exam of heart, lungs, abdomen, neurologic and lymphatic systems) will be performed. Blood will be obtained for CD4 T cell count, HIV VL, HIV antibody, liver function tests, electrolyte panel including creatinine, and complete hematology count (CBC - white blood cell count with differential, hemoglobin and platelets). Screening for hepatitis C will also be performed. Women of childbearing age will have a urine pregnancy test. Once lab results are available (within 3 days), they will reviewed by designated study staff to ensure that laboratory inclusion/exclusion criteria are met. If the subject has met all entry criteria, they will be contacted by the designated study staff. The study protocol will again be reviewed and if they are still interested in enrolling, they will be scheduled for the day 0 visit. Subjects who have not met the criteria will also be contacted by the designated study staff and told that they do not qualify to participate in the study. At this visit, an "enroll by date" will be designated that is no more than 60 days from the screen date to ensure screening labs are no more than 60 days old at the time of randomization. If more than 60 days passes between "Screen" and randomization, a comprehensive chemistry panel will be redrawn with the results known to be within study-required parameters prior to randomization.

### 8.1.2 Baseline Visit

Designated study staff will review medical history with the participant and perform a targeted physical exam (vitals, heart, lung, abdomen, neurologic system). The subject will not be randomized until lab results have been reviewed and found to be within limits designated in the protocol. The designated study staff or principal investigator will sign off on lab results and eligibility criteria on the day of randomization to ensure labs are no more than 60 days old and the subject still meets all eligibility criteria. Blood will then be drawn for CBC, comprehensive metabolic panel (CMP – includes electrolytes, creatinine, and liver function tests), CD4 and VL as well as plasma, serum and PBMCs for study endpoints. Subjects in the two-photon microscopy sub-study will also have an additional 90cc of blood drawn. Inguinal LN biopsy and colonoscopy with ileal and rectal biopsies will be performed (described in detail in section 7.1 above). They will remain in the MCRU for 4 hours after procedures for monitoring.

Following procedures, subjects will be randomized to receive either losartan or placebo. The drug will be dispensed to the participant to begin administration on that day. At this baseline visit, participants will start losartan or matching placebo at 50mg daily. Prior to the first dose, the subject will be counseled regarding dosing schedule, potential side effects, and the importance of adherence. A member of the study team will contact the subject by telephone at day 7 to check for tolerability.

### 8.1.3 Day 14 Visit

Subjects will be seen at day 14 for clinical assessment (interval history and targeted exam), assessment of drug adherence and to obtain toxicity labs (CBC and CMP). If the subject meets the following criteria: lack of dizziness, lightheadedness, or faintness upon rising, confusion, palpitations, and cough; GFR greater than or equal to 30 ml/min/1.73 m2 and potassium level less than or equal to 5.0, the dose of losartan/placebo will be increased to 100 mg po daily. The maximal tolerable dosage (up to 100 mg a day) will be continued for a total of 30 months.

At Day 14, side effects and blood chemistries (comprehensive chemistry panel) will be assessed. The dose of losartan/placebo will increase to 100mg daily at this Day 14 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

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 30-month study unless new side effects or adverse events necessitate re-evaluation (see below criteria for dose reduction or stopping study medication).

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 first 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.

### 8.1.4 Month 1 Visit

Subjects will be seen at month 1 for clinical assessment (interval history and targeted exam) and review of drug adherence. Blood will be drawn. They will then take their daily ART and study drug and will remain in the MCRU for additional blood draws at 1, 2, 3, 4, 6, 8, and 12 hours after drug administration in order to intensively study the pharmacokinetics of ARTs and losartan. They will then return to the MCRU the following morning for one additional blood draw at 24 hours after drug administration.

### 8.1.5 Months 3, 6, 9, 15, 18, 21 and 27 Visits

Subjects will be seen for clinical assessment (interval history and targeted exam), review of drug adherence, refill of study drug, and blood draw for CBC, CMP, CD4, VL, plasma, serum and PBMCs.

### 8.1.6 Month 12 Visit

Subjects will be seen at month 12 for clinical assessment (interval history and targeted exam), review of drug adherence, refill of study drug, and blood draw for CBC, CMP, CD4, VL, plasma, serum and PBMCs. Subjects in the two-photon microscopy sub-study will also have an additional 90cc of blood drawn. Subjects will then undergo repeat inguinal LN biopsy and colonoscopy with rectal and ileal biopsies. They will remain in the MCRU for 4 hours after procedures for clinical monitoring.

### 8.1.7 Month 23 Visit

Subjects will be seen for clinical assessment (interval history and targeted exam), review of drug adherence, refill of study drug, and blood draw for CBC, CMP, CD4, VL, plasma, serum and PBMCs. In addition, the first dose of Gardasil (human papillomavirus recombinant vaccine) will be administered. Subjects will remain in the MCRU for 30 minutes after administration for monitoring.

### 8.1.8 Month 25 visit

Subjects will return for clinical assessment (interval history and targeted exam), review of drug adherence, refill of study drug and blood draw for plasma, serum and PBMCs. The second dose of Gardasil will then be administered. Subjects will remain in the MCRU for 30 minutes after administration for monitoring.

### 8.1.9 Month 29.5 Visit

Subjects will be seen for clinical assessment (interval history and targeted exam), review of drug adherence and blood draw for CBC, CMP, CD4, VL, plasma, serum and PBMCs. In addition, the third and final dose of Gardasil will be administered. Subjects will remain in the MCRU for 30 minutes after administration for monitoring.

### 8.1.10 Month 30 Visit (Final Study Visit)

Subjects will be seen at the end of month 30 for the final scheduled study visit. Clinical assessment (interval history and targeted exam) and review of drug adherence will be performed and any remaining study drug will be collected by the designated study staff. Blood will be drawn for CMP, CBC, HIV VL, CD4 T cell count, serum, plasma, and PBMCs. Subjects in the two-photon microscopy sub-study will also have an additional 90cc of blood drawn. Subjects will undergo final biopsy procedures (ileal, rectal, and lymph node). They will remain in the MCRU for 4 hours after procedures for clinical monitoring. They will complete the off-study form prior to discharge from the MRCU. Subjects will be instructed to report any subsequent event(s) within 3 months of study completion that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

### 8.1.11 Early Termination Visit

Participants may withdraw voluntarily from the study at any time. If a subject opts to discontinue, attempts will be made to schedule and conduct an early termination visit for medical assessment and return of any unused study drug for proper disposal. If a subject withdraws after an adverse event (AE), they will be followed until resolution of the issue or until their condition is stable.

### 8.1.12 Pregnancy Visit

In the event that a study subject becomes pregnant, termination, reporting, and follow-up procedures outlined in Section 9.9 will be followed.

### 8.1.13 Unscheduled Visits

Additional visits will be scheduled as needed to follow up AEs, evaluate biopsy sites, address subject concerns, etc.

## 8.2 Schedule of Events for HIV Infected Subjects

**Table 1. Protocol summary for HIV infected subjects:**

| | Screen | Day 0 | Day 14 | Month 1 | Months 3, 6, 9, 15, 18, 21, 27 | Month 12 | Month 23 | Month 25 | Month 29.5 | Month 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| Medical history | X | X | | | | | | | | |
| Interval medical history | | | X | X | X | X | X | X | X | X |
| Physical Exam[1] | X | X | X | X | X | X | X | X | X | X |
| Adherence assessment and counseling | | X | X | X | X | X | X | X | X | X |
| Pregnancy test[2] | X | X | | X | X | X | | X | X | |
| HCV Ab | X | | | | | | | | | |
| CMP | X | X | X | X | X | X | X | | X | X |
| CBC w diff | X | X | X | X | X | X | X | | X | X |
| CD4 count | X | X | | X | X | X | X | | X | X |
| HIV viral load | X | X | | X | X | X | X | | X | X |
| Serum[3] | | X | | X | X | X | X | X | X | X |
| Plasma /PBMCs[4] | | X | | X | X | X | X | X | X | X |
| Additional PBMCs[5] | | X | | | | X | | | | X |
| Biopsies[6] | | X | | | | X | | | | X |
| HPV vaccine[7] | | | | | | | X | X | X | |
| PK assessment | | | | X[8] | X[9] | X[9] | | X[9] | | X[10] |

1. At screening, a full physical exam will be performed (described above). At subsequent visits, a targeted exam will be performed.
2. Women of childbearing age must have a negative pregnancy test prior to enrollment. Pregnancy testing will also occur roughly every 3 months during follow up and additionally if the subject or investigator suspects pregnancy.
3. Serum will be used to determine levels of IL-7 and TGF-beta.
4. Plasma will be used to determine levels of cytokines and markers of inflammation. PBMCs will be used in flow cytometry and intracellular cytokine staining.
5. Additional blood will be obtained from subjects enrolled in the two-photon microscopy sub-study.
6. Inguinal LN biopsy and colonoscopy with sampling of ileal and rectal tissues.
7. Human papillomavirus quadrivalent vaccine (Gardasil) will be administered at 3 time points per recommended vaccine schedule and immune response will be assessed at month 30.
8. Intensive 24-hour PK assessment will be performed at month 1 visit.
9. Plasma and intracellular PBMC concentration of losartan and ARVs will be determined approximately every quarter.
10. Plasma and intracellular PBMC concentration of losartan and ARVs PLUS concentrations in LN and GALT will be determined at month 30.

## 8.3 Schedule for Control - HIV Uninfected Subjects (n=5)

### 8.3.1 Screening

At this visit, consents will be reviewed in detail and the subject will have the opportunity to ask any questions they may have. The designated study staff or physician will interview the participant and a physical exam will be performed. Blood will be obtained for HIV antibody, liver function tests, electrolyte panel including creatinine, and CBC. Screening for hepatitis C will also be performed. Women of childbearing age will have a urine pregnancy test. Once lab results are available (within 3 days), they will reviewed by designated study staff to ensure that laboratory inclusion/exclusion criteria are met. If the subject has met all entry criteria, they will be contacted by the designated study staff. The study protocol will again be reviewed and if the subject is still interested in enrolling, they will be scheduled for the study visit. Subjects who have not met the criteria will also be contacted by the designated study staff and told that they do not qualify to participate in the study.

### 8.3.2 Study Visit

Designated study staff will review medical history with the participant and perform a targeted physical exam. Blood will then be drawn for CD4, plasma, serum and PBMCs. Inguinal LN biopsy will be performed. The subject will remain in the MCRU for 4 hours after procedures for monitoring. They will complete the off-study form prior to discharge from the MRCU. Subjects will be instructed to report any subsequent event(s) within 3 months of study completion that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

## 8.4 Schedule for Additional 5 Control HIV Uninfected Subjects (n=5)

Our first experiments with human tissue demonstrated that we need to change procedures for handling and imaging the tissue. The first few controls were not fully interpretable because of this. In addition the data we are generating suggests that increasing the number of controls by 5 subjects will significantly increase our power to detect changes in the FRCn attributable to the intervention.

### 8.4.1 Screening

At this visit, consents will be reviewed in detail and the subject will have the opportunity to ask any questions they may have. The designated study staff or physician will interview the participant and a physical exam will be performed. Blood will be obtained for HIV antibody, liver function tests, electrolyte panel including creatinine, and CBC. Screening for hepatitis C will also be performed. Women of childbearing age will have a urine pregnancy test. Once lab results are available (within 3 days), they will reviewed by designated study staff to ensure that laboratory inclusion/exclusion criteria are met. If the subject has met all entry criteria, they will be contacted by the designated study staff. The study protocol will again be reviewed and if the subject is still interested in enrolling, they will be scheduled for the study visit. Subjects who have not met the criteria will also be contacted by the designated study staff and told that they do not qualify to participate in the study

### 8.4.2 MRI Sub-Study

Within two weeks of the biopsy a MRI will be scheduled, see Appendix I for MRI sub-study protocol.

### 8.4.3 Study Visit(s)

If subject agrees to participate in the trial they will return to the MCRU to receive Stribild and Prezista. The subjects will take these medications for two weeks and return to be seen by the designated study staff. At this time a review of the medical history with the participant and a targeted physical exam will be performed. Blood will then be drawn for CD4, plasma, serum and PBMCs. Inguinal LN biopsy with colonoscopy with ileal and rectal biopsies will be performed. During this time blood draws will be taken at baseline and hours 1,2,3,4,6,8,12, and 24 post-drug administration. The subject will remain at the

MCRU for 12 hours for monitoring. They will then return to the MCRU the following morning for one additional blood draw at 24 hours after drug administration. They will complete the off-study form prior to discharge from the MRCU. Subjects will be instructed to report any subsequent event(s) within 3 months of study completion that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

## 8.5 Schedule of Events for Additional 5 Control HIV Uninfected Subjects

**Table 2. Protocol summary for HIV uninfected (control) subjects:**

| | Screen | Study Drug Pick-Up | MRI | Week 2 |
|---|---|---|---|---|
| Medical history | X | | | |
| Interval medical history | | | | X |
| Physical Exam[1] | X | | | X |
| Safety screening | | | X | |
| MRI | | | X | |
| Adherence counseling | | X | | |
| Pregnancy test[2] | X | | | |
| HIV & HCV Ab | X | | | |
| CMP | X | | | X |
| CBC w diff | X | | | X |
| CD4 count | X | | | X |
| Serum[3] | | | | X |
| Plasma /PBMCs[4] | | | | X |
| Biopsies[5] | | | | X |
| PK assessment[6] | | | | X |

1. At screening, a full physical exam will be performed. At the Week 2 visit, a targeted exam will be performed.
2. Women of childbearing age must have a negative pregnancy test prior to enrollment.
3. Serum will be used to determine levels of IL-7 and TGF-beta.
4. Plasma will be used to determine levels of cytokines and markers of inflammation. PBMCs will be used in flow cytometry and intracellular cytokine staining. Additional blood will be obtained for two-photon microscopy.
5. Inguinal LN biopsy and colonoscopy with sampling of ileal and rectal tissues.
6. Intensive 24-hour PK assessment

# 9. ASSESSMENT OF SAFETY

## 9.1 Definition of an Adverse Event (AE)

### 9.1.1 Basic Definition

An AE is any symptom, sign, illness, or experience that develops or worsens in severity during the course of the study but does not necessarily have a causal relationship with a study agent/intervention or procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study agent/intervention or procedures whether or not it is related to the study agent/intervention. Intercurrent

illnesses or injuries will be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal,
- is associated with a serious adverse event (SAE),
- is associated with clinical signs or symptoms,
- leads to additional treatment or to further diagnostic tests, or
- is considered by the Investigator to be of clinical significance.

### 9.1.2 Definition of a Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. An SAE is any AE that is:

- Fatal,
- life-threatening,
- requires or prolongs a hospital stay,
- results in persistent or significant disability or incapacity,
- a congenital anomaly or birth defect, or
- an important medical event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in inpatient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious. All AEs that do not meet any of the criteria for serious should be regarded as non-serious AEs.

### 9.1.3 Definition of a Suspected Unexpected Serious Adverse Reaction (SUSAR)

An AE that is:
• Serious (i.e., an SAE),
• Related (i.e., there is a reasonable possibility that the AE may be related to the study product), and
• Unexpected (i.e., an AE whose nature or severity [intensity] is not consistent with the applicable product information found in an investigator's brochure, a package insert or a summary of agent characteristics).

### 9.1.4 Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an AE if the frequency, intensity, or character of the condition worsens during the study period.

### 9.1.5 Post-study Adverse Event

All unresolved AEs will be followed by the Investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the Investigator will instruct each subject to report any subsequent event(s) within 3 months of study completion that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

### 9.1.6 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any 1 of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality,

- The abnormality suggests a disease and/or organ toxicity, or
- The abnormality is of a degree that requires active management; e.g., change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

### 9.1.7 Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization will be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.
Neither the condition, nor hospitalization, nor prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical Investigator.

### 9.1.8 Anticipated Adverse Events

AEs are not anticipated, but there are AEs associated with the use of both study agents and with the procedures being performed in the study. Possible AEs are described above in section 7.1 above and in consent forms. Plans to minimize the occurrence of these AEs are described in section 7.2 above. We do not expect any serious adverse events due to losartan, Stribild, Prezista, or Gardasil use or from any of the study procedures.

## 9.2 Unanticipated Problems

Unanticipated problems (UP), as defined by the OHRP include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

2. Related or possibly related to participation in the research (in this guidance document, “possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Upon becoming aware of any AE, the PI will assess whether the AE represents an unanticipated problem by applying the criteria described above. If the PI determines that the AE represents an unanticipated problem, the PI will report it promptly to the University of Minnesota IRB and DSMB within their reporting timelines. In addition, incidents, experiences and outcomes that occur during the conduct of this study that represent unanticipated problems but are not considered AEs may require reporting under the HHS regulations at 45

CFR 46.103(a) and 46.103(b)(5). Further details and examples of such scenarios are described in the OHRP policy (http://www.hhs.gov/ohrp/policy/advevntguid.html#Q1). Unanticipated problems involving risks to participants or others will be reported as detailed in the DAIDS Critical Event Policy (http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Pages/Safety.aspx).

## 9.3 Recording and Documentation of AEs

At each contact with the subject, designated study staff must seek information as to discomforts or adverse experiences by specific questioning and, as appropriate, by examination. At study visits, safety laboratory evaluations will also be performed to identify any laboratory AEs. All side effects will be reported to the PI and/or physician co-investigators on a daily basis and toxicity laboratory results will be reviewed in real time. Information about AEs to be recorded includes event description, time of onset, investigator assessment of severity, relationship to study agent(s)/Intervention(s), and time of resolution/stabilization of the event. All AEs occurring during the study period (from time of consent signing to 3 months after the last study visit) must be documented appropriately regardless of relationship to study products or procedures. Information on all AEs will be promptly recorded in the source document and also in the appropriate AE module of the case report form (CRF).

Any medical condition that is present at the time that the participant is screened should be considered as baseline and not recorded as an AE. However, if the condition deteriorates at any time during the study it should be recorded and reported as an AE. Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of the onset and duration of each episode. Changes in the assessment of relationship to the study agent(s)/interventions should also be clearly documented.

### 9.3.1 Scales Used to Grade Severity of Adverse Events

The grading system for severity of AEs is located in the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification August 2009), which can be found on the DAIDS RSC Web Site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

### 9.3.2 Scales Used to Attribute Adverse Events

The Principal Investigator will assess the relationship of all AEs to any drug or study procedure according to the following criteria:

1. Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study agent/intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study agent/intervention (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
2. Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the study agent/intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.
3. Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition or other concomitant events). Although an adverse drug event may rate only as "possible" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probable" or "certain" as appropriate.
4. Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to study agent/intervention administration makes a causal relationship improbable (e.g., the event did

not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition or other concomitant treatments).

5. Not Related: The AE is completely independent of study agent/intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative and definitive etiology documented by the clinician.

### 9.3.3 Analysis/Management

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Any measures taken or follow-up results are to be recorded in the study file. In addition, any clinically meaningful incidental findings from colonoscopies or lymph node biopsies will be promptly communicated to the study participant's primary care provider by fax, e-mail, letter, and/or telephone call. Documentation of appropriate follow up and reporting of all AEs will consist of written telephone contact reports, faxes, e-mail messages, and/or letters, which will be kept in the study files. SAEs that are still ongoing at the end of the study period must be followed to determine the final outcome. Study physicians will be responsible for evaluating subjects in the case of AEs. Study physicians and study staff are responsible for communicating to subjects information arising from the study (on harm or benefit, for example), or from other research on the same topic that could affect subjects' willingness to continue in the study.

## 9.4 Reporting Procedures

### 9.4.1 AEs to be Reported

The following AEs will be promptly reported within three reporting days to the DSMB and the DAIDS medical monitor:

- 
- all cancers, all autoimmune diseases, all fetal losses associated with an AE,
- all overdoses associated with an AE, and
- any definitely related, unexpected, and serious adverse events (SUSARS).

Other AEs or unanticipated problems will be reported to the IRB and DSMB within their reporting timelines (such as annual review or if prompt reporting is required, within the urgent reporting timeline requirements).

At the time of the initial report, the following information should be provided:

- Study identifier,
- Subject number,
- A description of the event,
- Date of onset,
- Current status,
- Whether study treatment was discontinued,
- The reason why the event is classified as serious, and
- Investigator assessment of the association between the event and study treatment.

Copies of each report and documentation of applicable IRB notification and receipt will be kept in the Clinical Investigator's binder. All reportable AEs will be followed until satisfactory resolution or until the PI or Sub-investigator deems the event to be chronic or the participant to be stable. If a previous SAE that was not initially deemed reportable under expedited reporting criteria is later found to fit the criteria

for reporting, the PI will submit the SAE in a written report as soon as possible. The full list of adverse events and complications will be reviewed by the IRB and DSMB on an annual basis.

**Table: Expedited Reporting to IRB, DSMB and DAIDS**

| Expedited Reporting to Whom | Criteria for Reporting | Timeframe | Submission Format |
|---|---|---|---|
| IRB | Unanticipated death of a locally enrolled subject(s);<br>New or increased risk;<br>Any adverse event that requires a change to the protocol or consent form;<br>Unanticipated problems;<br>Refer to the IRB website for complete details on required reporting criteria. | 5 working days | Local IRB's standard reporting form |
| DSMB | 1) SUSARS<br>2) all cancers, all autoimmune diseases, all fetal losses associated with an AE, and all overdoses associated with an AE<br>3) Unanticipated death of locally enrolled subject(s) | 3 reporting days (defined as M-F, including holidays) | Submit AE/SAE CRF, and IRB Report form, if available. |
| DAIDS | 1) SUSARS<br>2) all cancers, all autoimmune diseases, all fetal losses associated with an AE, and all overdoses associated with an AE<br>3) Grade 3 and 4 AEs that are attributed to study procedures (graded as per section 9.3.1 above) | 3 reporting days (defined as M-F, including holidays) | Submit SAE/AE CRF to DAIDS medical officer. |

## 9.4.2 Expedited Adverse Event (EAE) Reporting to DAIDS

***EAE Reporting to DAIDS***

The definitions of AEs requiring expedited reporting are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the DAIDS RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

### *EAE Reporting Requirements for the Study*

The events for which expedited reporting is required include Suspected, Unexpected Serious Adverse reactions (SUSARs). In addition to the EAE Reporting Category identified above, other AEs that will be reported in an expedited manner include all cancers, all autoimmune diseases, all fetal losses associated with an AE, all overdoses associated with an AE and all Grade 3 and 4 AEs that are attributed to study procedures (graded as per section 9.3.1 above).

The timeline for reporting of expedited events to the DAIDS medical officer is as defined in the DAIDS EAE manual.

The study agents for which expedited reporting is required are losartan, placebo for losartan, Gardasil, darunavir, and elvitegravir/cobicistat/emtricitabine/tenofovir.

### *Grading Severity of EAEs*

The grading table used for this study is the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification August 2009), which can be found on the DAIDS RSC Web Site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

### *Expedited AE Reporting Period*

The reporting period will be from the time the subject starts the study drug until the end of the study follow up for that participant. After the protocol-defined AE reporting period, only SUSARs as defined in Version 2.0 of the EAE Manual will be reported to the IRB and DSMB if the study staff become aware of the events on a passive basis (from publicly available information). A copy of these reports will also be sent to the DAIDS Medical Officer.

## 9.5 Reporting a Pregnancy

Pregnant women are not eligible to participate in the study. Women are counseled regarding prevention of pregnancy and encouraged to make every effort to avoid pregnancy during study participation. In addition to the screening and baseline visits, pregnancy testing will occur every 3 months and anytime during follow up when the woman or investigator suspects pregnancy. If a study participant becomes pregnant during study participation, no further doses of losartan or Gardasil will be given and no additional study procedures will be performed. Basic information about the pregnancy will be recorded on the “Pregnancy” CRF and permission to record survival data for her until the end of the pregnancy and of the baby at birth will be requested. If there are complications during the pregnancy, the complications are recorded as AEs in the usual way. The participant is asked to report outcome of the pregnancy. If there is a congenital anomaly in the infant, this is recorded as a serious adverse event (SAE) in the data forms for the mother (i.e., the study participant).

## 9.6 Toxicity Management

The grading system for drug toxicities is located in the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification August 2009), which can be found on the DAIDS RSC Web Site: http://rsc.tech-res.com/safetyandpharmacovigilance/. Participants experiencing side effects related to losartan, will be evaluated immediately by a study physician and may be referred to their primary HIV provider for evaluation and treatment if indicated. Subjects who develop drug-related toxicity of Grade 1 or 2 may continue study participation. If a participant experiences any drug-related toxicity >Grade 2 and it is judged to be due to losartan, losartan will be discontinued. If a participant experiences any drug-related toxicity >Grade 2 and it is judged to be due to Gardasil, further doses of Gardasil will not be administered.

All subjects who experience AEs will continue to be followed until resolution or stabilization of the issue.

## 9.7 Halting and Stopping Rules

### 9.7.1 Halting Rules for the Protocol

At the initial meeting, which will take place during the administrative start-up period of this project and prior to the enrollment of any subjects, the DSMB will develop the DSMB charter, which includes triggers set for stopping the study for safety concerns and for efficacy. Subsequent review of serious, unexpected and related adverse events by the Medical Monitor, DSMB, ethics review committee or IRB, the sponsor(s), and other regulatory authorities may also result in suspension of further trial interventions/administration of study agent at a site. The study sponsor(s) and other regulatory agencies retain the authority to suspend additional enrollment and study agent(s)/intervention(s) administration for the entire study as applicable.

### 9.7.2 Stopping Rules for an Individual Participant

Subjects may withdraw from the study (and all follow-up procedures) for any reason and at any time. Participants may also choose to discontinue study medication and continue all follow-up procedures. This is most likely in the event of a toxicity that, in the opinion of the investigators, is related to the study agents.

If a subject withdraws, attempts will be made to schedule and conduct an early termination visit as described in section 8.1.11. The reason and date of withdrawal will be documented on the subject's CRF. Date of withdrawal will be documented as the date of last study drug treatment, not the date that the decision to withdraw was made. Participants will be followed after withdrawal from the study for 3 months after discontinuation. All AEs during that period will be reported.

The PIs may withdraw participants to protect their safety or if participants are unable or unwilling to comply with visits, study procedures, or study medication. Participants will be removed from the study if they experience any drug-associated toxicity > grade 2 and it is judged to be due to losartan (e.g., a complication of the biopsy procedure may not necessitate removal from the study). Subjects will not be withdrawn if they experience toxicity > grade 2 that is deemed due to Gardasil but will not continue to be given Gardasil. Subjects will also be removed in the event of pregnancy. Healthy controls will be removed from the study if they experience rash, moderate GI symptoms and/or symptoms consistent with clinical hepatitis with no other medically reasonable cause. Other exit criteria include:

- completion of the study,
- withdrawal of consent,
- moving to an area that would prohibit the subject from returning for visits, and
- death.

Even though subjects may be withdrawn prematurely from the study, we will continue to collect survival data on such subjects throughout duration of the 30 months. If a subject withdraws consent to participate in the study, attempts will be made to obtain permission to record survival data up to the 30-month end point. Methods used for follow up will include at least 5 phone calls and 3 letters to the participant.

Any subject withdrawn from the study prior to Month 1 will be replaced by another enrolled participant to meet study objectives.

## 9.8 Unblinding Procedures

The Investigational Drug Service (IDS) Pharmacy is responsible for the administration of the blinded study agent as the intervention. This pharmacy holds the record of randomization for this study. The study participant, investigators, study coordinator, other study staff, statistician, and DSMB are blinded. The following conditions or events in which unblinding may occur are:

### 9.8.1 Emergency Unblinding

Please see Fairview IDS Pharmacy Emergency Blind SOP for detailed description of the unblinding process that will occur in the case of an emergency.

### 9.8.2 Individual Unblinding

For individual unblinding, the PI should assess the need for unblinding where a SAE has occurred and the treatment or allocation code is required in order to enable clinical treatments to be planned. In the event that this occurs, the IDS pharmacy will be contacted by a member of the study staff and given the following details: caller name and position, study site, site investigator, name of participant, study protocol number, and subject identification number. Upon unblinding, the study staff member will record the participant withdrawal and allocation in the person's clinical and trial notes along with appropriate clinical notations. The study staff member will log the unblinded reports and the PI will report unblind rates to the local IRBs, DSMB, OHRP, and DAIDS.

### 9.8.3 Unmasked Analysis

The DSMB will determine that an unmasked analysis is required. The decision will be documented in the committee meeting minutes. A DSBM designee will make a written request to the PI for study unblinding. The PI will request the IDS pharmacy to provide randomization schedules to the DSMB designee. The IDS pharmacy will check the allocation log for completions to date, sign and date the last entry with a note to indicate point of request, and provide the DSMB designee with the register of all study allocations. The lead statistician will merge the study data with the table of allocation codes as per the data transfer instructions within the data management plan and undertake unmasked analysis as determined by the DSMB.

### 9.8.4 Unblinding Upon Study Completion

The PI will determine that study recruitment is complete and unblinding is required for analysis. A study staff member will contact the IDS pharmacy for the schedules and a request for return all unblinded study agent. The IDS pharmacy will check the allocation log for completion, sign and dater the last entry, provide the study staff member with the schedule of all study allocations, and ensure all invoicing has been completed. The study staff member will enter all identification numbers and allocations into a file and send the file to the statistician as outlined in the Data Management Plan. The statistician will confirm receipt of the file containing the ID numbers and allocations, and merge the study data with the new file in preparation for analysis.

# 10. CLINICAL MONITORING STRUCTURE

## 10.1 Safety Monitoring Plan

The PI will oversee safety of study, including careful assessment and appropriate reporting of AEs, regardless of their clinical significance or their relationship to the study. Subjects will be routinely questioned about adverse events at all study visits. Study related monitoring, audits, inspections by the IRB, DSMB, regulatory bodies and University compliance and QA groups will be conducted. Information about subjects will be kept confidential and managed in accordance with HIPAA. Study data will be

recorded in study progress notes. Explanations will be mandated for any missing data. Study records will be retained according to federal regulations. Subjects will be instructed to seek necessary medical attention and to notify the investigator immediately if they experience any AEs.

### 10.1.1 Data and Safety Monitoring Board (DSMB)

A DSMB will be assembled by the University of Minnesota's Clinical and Translational Science Institute (CTSI). This DSMB will have at least 3 members, including a statistician, at least one member with previous DSMB experience, and at least one member with relevant clinical expertise and/or experience.

It is the role of the DSMB to protect trial subjects and to provide impartial advice and assistance to the PI regarding the conduct and continuation of the study, so as to protect the validity and credibility of the trial. The DSMB will have access to the clinical protocol, interim data, adverse event reports, clinical monitoring reports, annual and/or progress reports submitted to the IRB and/or regulatory agency, and correspondence between the PI and either the IRB or any Regulatory Agency of competent jurisdiction.

The relationship between the DSMB and the PI shall be an advisory role. The specific roles of this DSMB will be to:

- Assess data quality, including completeness.
- Monitor compliance with the protocol by participants and investigators.
- Monitor recruitment progress and losses to follow up.
- Monitor evidence for treatment differences in the main efficacy outcome measures.
- Monitor evidence for treatment harm (SAEs).
- Advise on protocol modifications suggested by investigators or sponsors.
- Develop and/or monitor clinical trial stopping rules.
- Recommend and/or decide whether trial termination is appropriate.

DSMB members shall disclose potential competing interests, both financial and intellectual. Members shall not use interim trial results to inform trading in pharmaceutical or device company shares or stock in competing companies.

The initial DSMB meeting will occur within the first two months of the study period, prior to enrollment of subjects. At this meeting the DSMB will discuss the protocol and the DSMB charter which includes triggers set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines will also address stopping the study for safety concerns and for efficacy based on plans specified in the protocol. At this meeting, the DSMB will also develop procedures for conducting business (e.g., voting rules, attendance, etc.).

The DSMB will then meet at least once annually to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed. The frequency of meetings may be increased if unanticipated adverse events (AEs) or safety issues are observed or enrollment rate exceeds expectations. A DSMB meeting may be requested by DSMB members, study sponsor, industrial collaborator, IRB, or study PI at any time to discuss safety concerns. Decisions to hold ad hoc meetings will be made by the DSMB Chair. Meetings may be held by conference calls or videoconferences or as face-to-face meetings. In the event a DSMB member cannot attend a meeting, he/she may receive a copy of the closed session DSMB report and either participate by conference call or provide written comments to the DSMB Chair for consideration at the meeting.

DSMB meetings will be held in two parts. First, an open section will be held in which demographic data and trial progress will be discussed. Quality assurance and compliance issues may be discussed in this

session, and this session is open to investigators, sponsors, funder, patient advocates, and others as appropriate. Open sessions will be followed by closed sections in which only the DSMB members and specifically invited guests will attend. In closed sessions, the outcomes data will be assessed and recommendations formulated. Closed session meetings may be conducted by teleconference at the discretion of the DSMB Chair.

Meeting notes will be prepared from both the open and closed sessions within 2 weeks of the meetings. The DSMB will not share confidential information, particularly trial interim data with anyone, including the PI. The recommendations of the DSMB will be communicated in writing to the Trial Sponsor, PI, IRB, and/or appropriate regulatory agency. The recommendations open to the DSMB will include the following:

- No action required; continue trial as planned.
- Early termination due to a clear benefit or harm in the interventional group.
- Early termination due to futility.
- Termination of recruitment and/or treatment within a subgroup or single arm of a multi-arm trial.
- Extending recruitment or follow up for all or individual arms of the protocol.

If immediate action is required, the DSMB Chair will personally notify the DAIDS program officer of any findings of a serious and immediate nature or recommendations to discontinue all or part of the trial. In addition to verbal communications, recommendations to discontinue or substantially modify the design or conduct of a study must be conveyed to DAIDS in writing by e-mail, fax, or courier on the day of the DSMB meeting. This written, confidential report may contain unmasked supporting data and include the DSMB member's rationale for his/her recommendations.

# 11. STATISTICAL CONSIDERATIONS

## 11.1 Overview and General Design Issues

From the perspective of statistical analysis, the aims of the proposed study are all quite similar: to determine if a randomized intervention has an impact on a longitudinally observed outcome. Despite this similarity, there are differences in the frequency with which some outcomes of interest will be observed and this will impact the details of how the analysis will be conducted. In particular, some variables will be measured quarterly (those that are assessed in peripheral blood, such as LPS, IL-6, TNF and ICAM-1) while those variables assessed via biopsy (such as the number of Ki67+ cells and the magnitude of the FDC pool) will only be available at baseline, and 12 and 30 months after initiating treatment with losartan. The approach that will be used to test for treatment effects in both scenarios will involve fitting mixed models with treatment group indicators, time effects and interactions between time and treatment group to allow for testing for differences in rates of change between the 2 groups. What will differ between the 2 scenarios will be the modeling strategy, as the data that is more frequently observed will permit more sophisticated modeling strategies. These strategies include allowing for nonlinear trajectories via cubic splines and more flexible covariance structures. These models will be fit via restricted maximum likelihood and tests for differences between the treatment groups will be conducted using Wald tests. Standard diagnostics will be employed to assess the adequacy of the fit and remedial measures (e.g. transforming the outcome variable) will be employed as appropriate. The most current version of the statistical software R and the lme package will be used to fit models and examine the data. With 25 subjects per group the study should have adequate power to find strong effects; however losartan has not previously been used in the proposed context.

Analysis of data from the MRI substudy will be descriptive in nature. We are seeking to identify MRI methods that will render an image of the inguinal lymph node that is about to be excised. We will compare

collagen signal by MRI with collagen content measured by trichome stain. Analysis of PK data will include a comparison to previous data collected from HIV infected persons using a similar protocol. We will determine if blood and tissue levels of drug are similar or if they are statistically greater, or smaller than those historical controls.

## 11.2 Sample Size Considerations

A previous study of collagen deposition in humans conducted in the Schacker lab found a standard deviation for collagen deposition of 0.81 (log scale). We can use this estimate to examine the power of a 2 sample *t*-test to find differences between 2 groups of 25 subjects as this power depends on the magnitude of the difference between the 2 groups.

| Difference in average of collagen | 0.5 | 0.6 | 0.7 | 0.8 |
|---|---|---|---|---|
| Power | 0.59 | 0.75 | 0.86 | 0.94 |

## 11.3 Enrollment/Stratification/Randomization/Blinding Procedures

HIV infected subjects will be randomized to receive losartan or placebo in a 1:1 manner using ordinary methods for generating random deviates. Study staff (other than those in the IDS pharmacy) and participants will be blinded to the random assignments of all HIV infected study participants. All study drug will be supplied in identical containers.

The two-photon microscopy sub-study of the HIV infected subjects requires 5 participants on losartan and 5 on placebo. Investigators are blinded to treatment assignment. To be able to ensure we have the 5 on losartan and 5 on placebo we will give the list of all subjects who agree to the sub-study to the investigation pharmacist who maintains the blind. They will identify the ten participants we should use for the sub-study.

Blinding will be maintained until all data are entered into the study database, all study endpoint data and other data included in the final analysis have been verified and the data are ready for final analysis. This will be explained to participants as part of the study. Unblinding will occur according to standard operating procedures as per section 9.13.

## 11.4 Maintenance of Treatment Randomization Codes

The investigational pharmacy at the University of Minnesota Medical Center will make randomization assignments and will maintain the treatment randomization codes. They will be responsible for informing the DSMB of these codes for any interim analysis that is planned.

## 11.5 Final Analysis Plan

As detailed in section 11.1, once all of the data is available, mixed effects models will be used to test for differences between treatment groups using standard methods for the analysis of longitudinal data. Conventional significance levels will be used and the fit of the models will be assessed using standard tools (e.g. residual plots).

# 12. QUALITY CONTROL AND QUALITY ASSURANCE

Please refer to detailed Site Quality Management Plan and Manual of Procedures in the grant application appendix, which is maintained in the study records, for quality control and assurance protocols.

# 13. ETHICS/PROTECTION OF HUMAN SUBJECTS

## 13.1 DAIDS Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol informed consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) *WILL NOT* be reviewed or approved by the DAIDS PRO, and sites will receive an Initial Registration Notification when the DAIDS PRO receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. Sites will not receive any additional notifications from the DAIDS PRO for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) *WILL NOT* be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

## 13.2 Institutional Review Board

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonization Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject. This protocol and associated informed consent documents must be reviewed and approved by the University of Minnesota Institutional Review Board prior to protocol implementation.

## 13.3 Informed Consent Process

All participants participating in this study will be required to sign an IRB approved informed consent form. Participants who enroll in any of the sub-studies will also sign an additional consent form specific to each applicable sub-study. Prior to signing the consent form(s), the participants will meet with designated study staff who has been specially trained in obtaining informed consent. The participant will review the consent form in detail with the designated study staff and have the chance to ask questions. The study coordinator will then ask the participant questions to ensure that they understand the information presented in the consent form. The rights and welfare of the participants will be protected by emphasizing to them that the

quality of their medical care will not be adversely affected if they decline to participate in this study. Participants will be given time to consider the risks and benefits of participating. A copy of the informed consent document will be given to the participants for their records. The participants may withdraw consent at any time throughout the course of the trial. Consent forms for HIV infected and HIV uninfected (control) participants are included in this application.

### 13.4 Participant Confidentiality

A Certificate of Confidentiality will be obtained and all information that identifies study subjects will be handled in accordance with regulatory bodies including HIPAA regulations and the University of Minnesota's IRB. Information will be made available to the PI and designated study staff who directly participate in the research visits. In addition, information may be reviewed by the NIH, the Office For Human Research Protections and the IRB. Prior to screening, participants in this study will sign an authorization to use or disclose protected health information for research purposes. All staff will have been trained in the use of protected health information. Subjects will be assigned to a subject ID number and a link between identifiers will be maintained in a log. This log will be kept in a locked cabinet in a secure office. Only the PI and designated study staff will have access to this link.

### 13.5 Study Discontinuation

After study completion or in the event that the study is discontinued, subjects will stop taking study agents (for the HIV group: losartan or placebo; for controls: Stribild and Prezista).

## 14. DATA HANDLING AND RECORD KEEPING

### 14.1 Data Management Responsibilities

Data collection is the responsibility of the clinical research staff under the supervision of the PI, Dr. Schacker. Study data will be recorded in study progress notes. Explanations will be mandated for any missing data. Study records will be retained according to federal regulations. For further detailed description of data management please refer to the Manual of Procedures and Data Management Plan maintained in the study files.

### 14.2 Source documents and Access to Source Data

In accordance with NIH policy we will make any source data accumulated as a result of this program available to others in a timely and reasonable fashion. We will make data available at the time it is accepted for publication. Written requests for access to data before publication can be made to the administrative core and, if reasonable, data will be forwarded to the individual(s) requesting access.

### 14.3 Quality Control and Quality Assurance

We have designed a multi-step process to ensure data quality and accuracy. Clinical data will be collected into a standardized CRF that includes collection of source documents from the clinical laboratory performing the assays. Designated study staff will enter this data in real-time into our database. Data generated from Dr. Schacker's laboratory, Dr. Fife's laboratory, Dr. Douek's laboratory, Dr. Fletcher's laboratory, and Dr. Estes' laboratory will be entered into the database by the research staff in the laboratory. In addition, a written report of the data with a full accounting of how the data was generated, how much sample was used, and any unusual circumstances surrounding the generation of data (need for repeated experiments, reason for rejecting data from a data set, specimen handling problems, etc.) will be sent on a monthly basis to designated study staff. They will place a copy of the written report into the study chart of each individual

participant with data in the report and will scan it into the database, which will contain links back to the subject and the laboratory. Designated study staff will conduct monthly audits of a randomly selected series of 10 subject study charts to compare source documentation with data entered locally and at remote laboratories. Discrepancies will be resolved immediately. If an error rate of data entry of more than 3% is discovered for an individual or a laboratory, we will conduct a full audit of data from that person or that site. We prefer to have data entered by the investigator performing the research (as opposed to a data entry person working off faxed records) because we have found that data is available for discussion more rapidly and this fosters better collaboration and a more interactive research environment.

## 15. PUBLICATION POLICY

Publication of the results of this trial will be governed by NIAID publication policies. Any presentation, abstract, or manuscript will be made available for review by the NIAID supporters prior to submission.

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# 17. APPENDIX I

MRI Sub-study

The purpose of the study is to develop new ways to acquire and analyze MRI data to characterize the structural and functional changes of the body between the normal and disease state. This information would be valuable if and when the developed methods are incorporated into future clinical studies to help guide and monitor therapeutic interventions. The methods being developed will be experimental and will only be used for research purposes. We think it possible that this non-invasive procedure might someday be used as a biomarker in studies of anti-fibrotic therapy in HIV infection.

We will explore several standard and novel contrasts and their parametric maps to assess lymph flow and collagen amounts in LT. MRI studies will be performed on a 3 Tesla and/or 7T imaging systems (Siemens Medical Solutions, Erlangen, Germany). To acquire data, surface or surface array coils will be used for signal reception.

Protocol:
1) Scout imaging to localize target anatomy. (1 minute)
2) Maps of B1+ by the 3D AFI technique (4 minutes)
3) T2 weighted imaging (turbo spin echo, 0.5x0.5x3.0 mm3 resolution in the three planes) (10 minutes)
4) T2 mapping with the methods by Liney et al. (5 minutes)
5) T1 mapping with DESPOT (72) (5 minutes)
6) Diffusion weighted imaging (DWI), using a standard single shot approach in three orthogonal directions with multiple diffusion encoding b-values (e.g. 0, 50, 400 and 800 s/mm2) allowing the calculation of ADC– how long? (5 minutes)
7) Magnetization transfer (MT) using GRE readout, with T1sat mapping (10 minutes)
8) T1ρ mapping, using GRE readout (not clear, the application mention them, but then does not give details) (5 minutes)
9) T2ρ mapping, using GRE readout (not clear, the application mentions them, but then does not give details) (5 minutes)
10) RAFFn mapping using GRE readout (10 minutes)

Inclusion Criteria: (See Section 5.2)
Same as parent losartan study.

Exclusion Criteria:

Exclusion criteria will be assessed during a safety screening interview and during final consent. A questionnaire will be provided regarding general health and exclusion/inclusion criteria. The investigator doing the interview will determine if any of the exclusion criteria apply. Subjects will be asked:
1) To describe any previous surgeries to help subjects and study staff open a dialogue as to any possible contraindications listed in the yes/no section below.
2) If they are claustrophobic and to rate the severity.
3) If they wear hearing aids and if they can be removed for the scan.
4) If they have a transdermal delivery system, and if yes where is it located and if it can be removed for the study.
5) If they wear colored contact lenses and, if yes, can they be removed for the study.
6) To provide a list of medications, dosages and time of last dose for medicines taken regularly.

7) To identify if they have any pre-existing medical conditions including Hypertension, Hypotension, Diabetes, Cardiovascular Disease and Fever.
8) To identify if they ever had an operation.
9) To indicate if they have ever been injured by a metallic foreign body which was never removed.
10) If they wear braces and/or if they have removable bridgework, false teetch or a permanent retainer.
11) If they have any tattoos, non-removable body piercings or hair extensions.
12) If they are currently using/wearing an IUD or diaphragm.
13) If they have any reason to believe they are pregnant. (If the subjects are unsure about their pregnancy status, test kits will be available to subjects).

In addition, a set of yes/no questions will be asked in the screening form for the participant to confirm or deny the presence of items that may be hazardous to their safety and/or some interfere with the MRI examination, including:

Yes No Cardiac pacemaker
Yes No Implanted cardiac defibrillator
Yes No Carotid artery vascular clamp
Yes No Intravascular stents, filters, or coils
Yes No Aortic clip
Yes No Internal pacing wires
Yes No Vascular access port and/or catheter
Yes No Swan-Ganz catheter
Yes No Shunt (spinal or intraventricular)
Yes No Aneurysm clip(s)
Yes No Neurostimulator
Yes No Electrodes (on body, head, or brain)
Yes No Heart valve prosthesis
Yes No Any type of prosthesis (eye, penile, etc.)
Yes No Artificial limb or joint replacement
Yes No Bone growth/fusion stimulator
Yes No Bone/joint pin, screw, nail, wire, plate
Yes No Metal rods in bones
Yes No Harrington rods (spine)
Yes No Metal or wire mesh implants
Yes No Wire sutures or surgical staples
Yes No Insulin pump or infusion device
Yes No Any metal fragments (i.e. metal shop)
Yes No Any implant held in place by a magnet
Yes No Cochlear, otologic, or ear implant

RISKS AND BENEFITS:

NOTE: Appendix F's for both 3T and 7T systems are on file at the IRB and will not need to be submitted with this addendum.

Other Potential Risks.

1) Magnetic Field in the MRI (subject environment)
2) Metallic objects carried into the MRI room
3) Other energies, such as radiofrequency pulses, or rapid changes in the field strength

4) Displacement of Implanted metallic devices
5) Acoustic noise

Any risks from high magnetic field strength that has been identified as a concern by the FDA's office of device evaluation, has been addressed by our researchers, who performed numerous animal experiments. The results indicate non-significant risks (either short term or long term) at these magnetic field strengths (3T/7T).

Data over the last 10+ years indicate that the 7 Tesla magnetic field does not pose a significant risk to human volunteers. Metallic objects that are entered into the magnetic field can accelerate into the magnet potentially causing damage to the magnet or persons in the magnet room.

In addition, implanted metallic objects can be displaced. The acoustic noise generated by the scanner without ear protection could possibly impose damage to a person's hearing.

Finally, radiofrequency pulses impart small amounts of energy into the subject, and needs to be monitored by both hardware and software monitor to prevent inadvertent application of significant energies which may result in heating.

Most people experience no ill effects from the strong magnetic field, but some people report dizziness, mild nausea, headache, a metallic taste in their mouth, or sensations of flashing lights. These symptoms, if present, subside shortly after leaving the magnet. No serious ill effects have been reported to date at any site operating at 7T. If any sensations experienced cause discomfort or pain, informing the researcher will terminate the ongoing study and the subject will be taken out of the magnetic field.

The risk of metallic objects being carried into the magnet room by subjects has been addressed by having volunteers remove all jewelry and valuables (keys, hair pins, coins, glasses, watches, etc. ) for secure storage in a locker provided to them, prior to entering the magnet room.

All risk of other energies involved in MRI imaging are controlled such that they are not a significant risk and are substantially equivalent to clinical MRI systems, such as the Philips and Siemens devices currently in use at the University of Minnesota Medical Center, Fairview. These factors are in compliance with FDA guidelines.

In addition, there are safety monitors in the MRI instrument that will prevent the machine from running if these energies exceed the FDA levels. Risks posed to persons with implanted devices are avoided by exclusion of such persons from the study. Any unknown risks posed to fetuses are avoided by exclusion of pregnant women. Acoustic noise is reduced to FDA acceptable levels by having the subjects wear ear plugs, providing padding around the subject ears, and by additional noise reduction foam place inside the magnet itself. The acoustic noise has been carefully measured using a special probe inside the magnet.

MRI scans have become a routine diagnostic tool widely used in medicine. Based on more than two decades of experience with this technique, appropriate guidelines exist for its use to minimize the risks of the study. Although there is no direct benefit to the research subjects, the parameters and research data garnered from the studies will lead to being able to make much improved clinical and diagnostic decisions. With minimal risk to the subjects, this research and the MRI instruments will allow us to obtain improved anatomic and functional throughout the human body. Twenty years of research at 4 tesla and 15 years at 7 Tesla has established this type of research as extremely safe, as well as extremely beneficial to the scientific and clinical communities.

There are no direct benefits to the individuals participating in this study as subjects. However, the new methods under development may provide novel biomarkers for monitoring disease progression and treatment response, aid in the development and evaluation of novel interventions, and/or provide unique tools to aid in the diagnosis and prognosis of disease.

INFORMED CONSENT PROCESS:

Upon arrival at the CMRR, the subject will be greeted by research staff, provided the consent and MRI safety screening forms and given a brief description of the study that will be conducted that day. Specific details of the study which will be reviewed including:

- the field strength of the study (3T or 7T)
- the region of the body that will be studied
- the RF coils that will be used
- any additional hardware such as ECG leads or respiratory monitoring equipment that will be necessary
- any particular tasks that they may be asked to perform such as breath holding during the MRI procedure.

After this introduction the subject will then be given an opportunity to read the consent form and ask questions. The researcher will provide the participant ample time to decide whether or not to participate. The researcher will also assess whether the participant understands the study's procedures and will stress the voluntary nature of the experiment. In addition, the subject will be reminded that this is purely a research study and that the data acquired cannot be used in place of standard imaging obtained through their physician.

Volunteers will be given the study specific consent form upon arriving at the CMRR at which time the investigator will detail the specific goals for the study that day and provide any study specific instructions.

The investigator will give the participant ample time to review the informed consent document. This may entail waiting with the subject while they review the material or leaving the room if they need more time to consider the study. When the participant has finished reading the document and signs (or chooses not to sign) it, they will notify the researcher. Participant questions will be answered prior to their consenting.

One of the investigators listed on the protocol will obtain informed consent. Usually it will be the individual who will be performing the MRI scan.
Gregory J. Metzger – Associate Professor – already trained and running similar studies
Silivia Mangia – Associate Professor – already trained and running similar studies
Shalom Michaeli – Associate Professor – already trained and running similar studies
Diane Hutter – Research Nurse – already trained and consenting on similar protocols

The subject will always provide the informed consent or the study will not be performed.

As described above, the investigator(s) will give the participant ample time to review the informed consent document. This may entail leaving the room if they need more time to consider the study reducing any pressure to sign it. Subjects are also instructed that they may terminate participation at any time without prejudice.