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Effect of IL-1β Inhibition on Inflammation and Cardiovascular Risk

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Signature Page

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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PROTOCOL-SPECIFIC GLOSSARY OF TERMS

ACTG	AIDS Clinical Trials Group
AE	adverse events
ALT	aspartate aminotransferase
ANC	absolute neutrophil count
ART	antiretroviral therapy
AST	alanine aminotransferase
AUC	area under the curve
BART	brachial artery reactivity testing (used to measure flow-mediated vasodilation)
°C	degrees Celsius
CAD	coronary artery disease
CAPS	cryopyrin-associated periodic syndrome
CDC	Centers for Disease Control and Prevention
CHR	Committee on Human Research
CK	creatinine kinase
CMV	cytomegalovirus
CRF	case report form
CV	cardiovascular
CVD	cardiovascular disease
DAIDS	Division of Acquired Immunodeficiency Syndrome
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EAE	expedited adverse event
EBV	Epstein-Barr virus
°F	degrees Fahrenheit
FDA	Federal Drug and Food Administration
FDG-PET/CT	Fluorodeoxyglucose-positron emission tomography/computed tomography
FMD	flow-mediated vasodilation (a marker of endothelial function, measured by BART)
GCP	Good Clinical Practices

HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high-density lipoproteins
HHS	Human Health Services
HIPAA	Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
hsCRP	high-sensitivity C-reactive protein
IATA	International Air Transport Association
ICH	International Conference on Harmonisation
IL	interleukin
IRB	Institutional Review Board
IRIS	immune reconstitution inflammatory syndrome
ITSG	Inflammation Transformative Science Group
LDL	low-density lipoprotein
LFT	liver function test
LS tandem MS	liquid chromatography/tandem mass spectrometry
MACE	Major Adverse Cardiac Event
MCV	mean corpuscular volume
MI	myocardial infarction
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PBMC	peripheral blood mononucleated cell
PCR	polymerase chain reaction
PHI	Personally-Identifiable Health Information

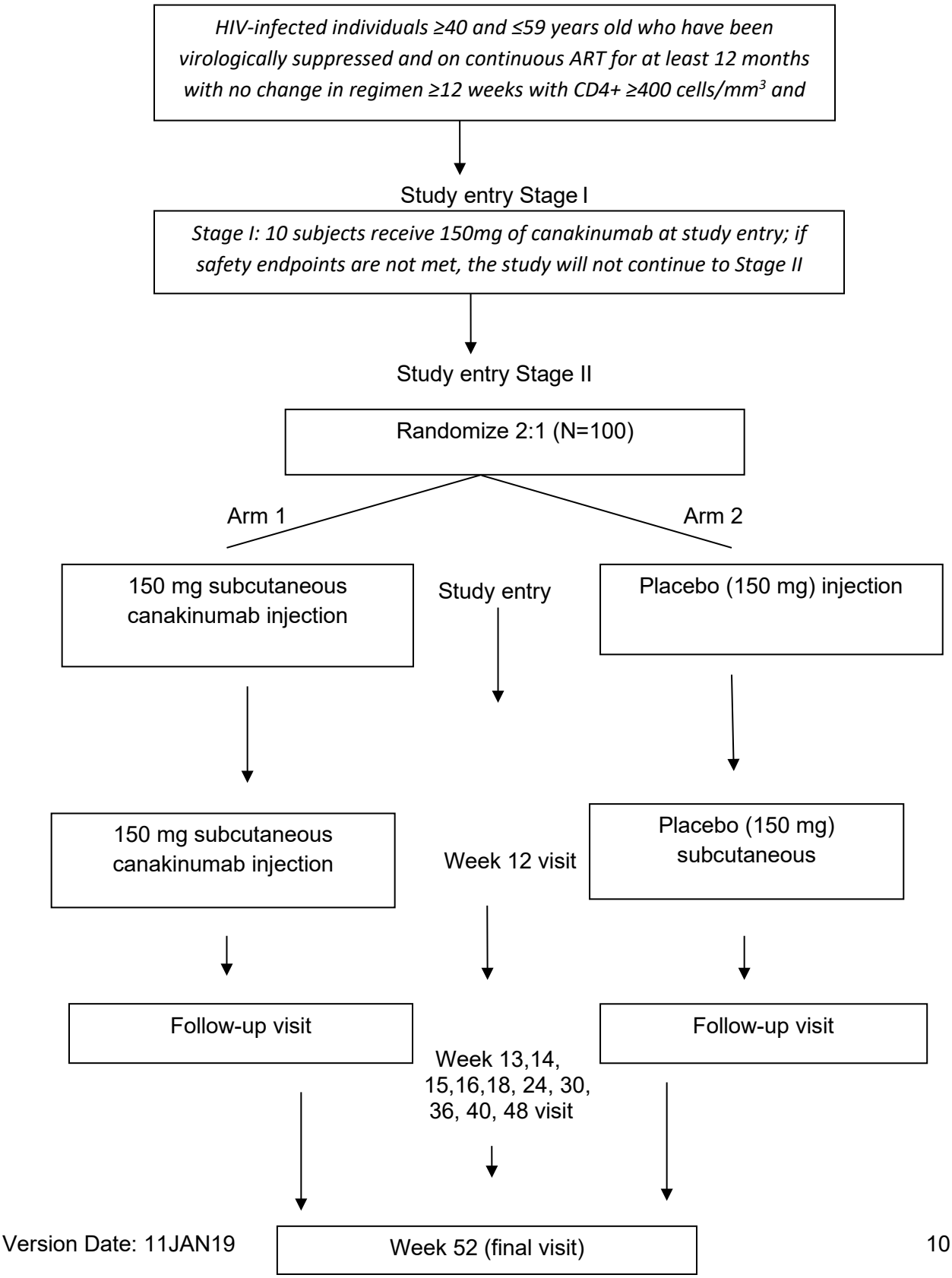
PHP	Positive Health Program
PI	Principle Investigator
PK	pharmacokinetic
PPD	purified protein derivative
RNA	ribonucleic acid
sCD	soluble CD
SCOPE	Study of the Consequences of the Protease Inhibitor Era
SD	standard deviation
SFGH	San Francisco General Hospital
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedures
TB	tuberculosis
TNF	tumor necrosis factor
UCSF	University of California San Francisco
ULN	upper limits of normal
WBC	white blood cell

SCHEMA

Effect of IL1β Inhibition on Inflammation and Cardiovascular Risk

DESIGN	This is a randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of canakinumab for the treatment for HIV-associated inflammation. This study will assess the safety and effects of canakinumab on endothelial function (assessed by flow-mediated vasodilation [FMD] of the brachial artery), vascular inflammation (assessed by FDG-PET/CT scanning), key inflammatory markers of cardiovascular disease (CVD) risk (high-sensitivity C-reactive protein [hsCRP]), interleukin-6 (IL-6), soluble CD163 (sCD163), D-dimer, T-cell and monocyte activation in the blood, and size of the HIV reservoir.
DURATION	52 weeks (24 weeks of drug treatment/placebo followed by observation for an additional 28 weeks)
SAMPLE SIZE	Stage I: 10 subjects, all who receive 150mg of canakinumab Stage II: 100 (randomized 2:1 to 150mg canakinumab arm or placebo arm)
POPULATION	HIV-infected men and women ≥40years old and ≤59 years old • who have been virologically suppressed on continuous antiretroviral therapy for at least 12 months with no change in regimen 4 weeks prior to entry; • who have a CD4+ T-cell count ≥400 cells/mm^3; AND • who have documented CVD or who are at increased CVD risk or have 1 CVD risk factor.
REGIMEN	Stage I: 10 subjects will be injected with 150 mg canakinumab. If the Stage I patients are clinically stable after 12 weeks of observation, study will then proceed to Stage II where 100 additional subjects will be enrolled and randomized 2:1 to 150mg of canakinumab vs. placebo All subjects in Stage II will receive a dose of canakinumab vs. placebo at study entry and second dose at Week 12 and followed until Week 52.

Figure 1: Study Schema



1.0 KEY ROLES

Our group is uniquely qualified to perform this study of canakinumab in HIV. Over the past 10 years, our group has established a highly effective cross-disciplinary team of investigators, each of whom has the requisite expertise to perform the type of research outlined in this proposal. **Dr. Hsue**, is among the few cardiologists devoting her efforts full time to understanding the epidemiology, pathogenesis and management of HIV-associated cardiovascular disease and the role of HIV-related inflammation in non-AIDS conditions. **Dr. Deeks,** is an expert on HIV pathogenesis and directs the SCOPE cohort, which will be used to support the canakinumab clinical trial. SCOPE is an outpatient, clinic based cohort of over 2000 HIV-infected individuals with detailed HIV characteristics and outstanding patient follow-up. He has overseen numerous clinical trials in the past, and in the past two years has enrolled over 300 patients into studies such as the one outlined in this proposal. **Dr. Ridker** is the PI of both the CANTOS study (testing canakinumab) and CIRT (testing methotrexate) and is an international expert on inflammation and atherosclerosis. **Dr. Ganz** is an international authority in endothelial function, inflammation, and biomarkers. He heads the Center of Excellence in Vascular Research at SFGH and will help oversee the endothelial function and cardiovascular biomarker studies. **Dr. Tawakol** (Massachusetts General Hospital, MGH) is a pioneer in vascular FDG-PET/CT and will oversee the quality control, performance, and interpretation for the FDG-PET/CT procedures**. Dr. Milush**, Director of Core Immunology Laboratory, will perform the microbial translocation assays and will oversee all of the immunologic studies proposed in Aim 2. **Dr. Rupert**, Functional Advisor at AIDS monitoring labs; Leidos Biomedical Research, will perform the assays for inflammation and coagulation and will help interpret the T cell activation and reservoir data. **Dr. Aweeka**, (director of the Drug Research Unit at SFGH and PI of the UCSF Pharmacology Laboratory for the ACTG) will perform the pharmacokinetic assays for the study. **Dr. Milush** will perform the assays for viral persistence. **Dr. Scherzer** (co-investigator) is an accomplished biostatistician with expertise in HIV studies. Drs. Deeks, Ganz, and Ridker are all co-investigators on the NHLBI study of HIV and methotrexate that was recently awarded to Dr. Hsue (PI).

2.0 INTRODUCTION

2.1 Rationale and Background

Need for novel strategies to reduce inflammation in the setting of HIV infection

By 2015, over half of HIV-infected individuals will be ≥ 50 years and CVD is becoming increasingly important in this unique patient population. Despite these unquestioned successes of therapy, antiretroviral treated-HIV infected adults have excess risk of morbidity and mortality[1, 2]. Compared to controls, HIV-infected individuals have an adjusted 50% increased risk of acute MI and this increased risk is present even among individuals with well-treated HIV[3]. Deaths due to CVD range from 6-15% in different HIV cohorts[4] and HIV-infected individuals have a higher mortality from MI compared to

controls[5] as well as higher rates of sudden cardiac death[6]. Although the contribution of HIV to atherosclerosis and CVD risk is becoming increasingly clear, the pathophysiological mechanism(s) underlying these observations are less well understood. The SMART study found that untreated HIV infection was associated with high levels of both interleukin-6 (IL-6) and D-dimers and that these biomarkers were strongly associated with all-cause mortality[7] and CVD events[8]. High sensitivity C-reactive protein (hsCRP), IL-6 and D-dimer levels remain high even among effectively treated HIV-infected individuals[9]. Treated and suppressed HIV-infected individuals are at risk for CVD and chronic inflammation in HIV is associated with increased carotid artery wall thickness as well as higher levels of arterial inflammation[10-12]. Chronic inflammation in the setting of ART is thought to underlie not only the higher rates of CVD in HIV, but also other non-AIDS conditions that are emerging in HIV-infected individuals such as kidney disease, bone disease, and neurologic complications[13]. Thus, therapeutic strategies to target inflammation in the setting of HIV infection may be beneficial in reducing not only CVD risk, but also other non-AIDS events resulting in improved mortality. The development of novel therapeutics which can decrease persistent inflammation and reduce cardiovascular risk depends on a precise understanding of the mechanism whereby HIV infection causes inflammation. Our group feels strongly that the most definitive way to define a host factor in the pathogenesis of human disease is to manipulate that factor experimentally with the aim of enhancing a potentially beneficial response or preventing a potentially harmful outcome. It is expected that a better understanding of these mechanisms may help identify other novel interventions to decrease the morbidity and mortality associated with HIV as well as non-AIDS conditions. These findings may also inform underlying mechanisms of atherosclerosis and treatment in the larger population as well.

Inflammation may cause CVD in uninfected adults

In the HIV-uninfected population, a strong body of evidence from over 20 cohort studies involving a wide range of patient populations demonstrates that markers of inflammation, particularly hsCRP, are strongly predictive of cardiovascular disease (CVD) events and mortality[14-18]. Targeting inflammation as a means of reducing CVD risk was partly addressed in the JUPITER trial, which showed that treatment with rosuvastatin among healthy individuals with normal LDL cholesterol and elevated hsCRP levels reduced myocardial infarction, stroke, and all-cause mortality by 44%[19]. This clinical benefit was proportional to the magnitude of hsCRP reduction, suggesting that rosuvastatin was acting partly through an anti-inflammatory mechanism[20]. However, in order to implicate inflammation in atherogenesis directly, an agent that targets inflammation without lipid-lowering and with an acceptable safety profile would be required. Such an agent would be particularly useful for patients at increased CVD risk on the basis of chronic inflammation rather than dyslipidemia; this may be the case for HIV-infected patients.

The interleukin-1 family, inflammation, and atherothrombosis

The interleukin-1 (IL-1) family which includes IL-1α, IL-1β and the IL-1 receptor antagonist plays a key role in mediating systemic inflammatory responses; namely imbalances in the IL-1β proteins which are proinflammatory and the IL-1Rα proteins which are anti-inflammatory are associated with clinical diseases. For example,

mutations in the NLRP3 inflammasome which increase the secretion of IL-1β and subsequently cause severe imbalances in IL-1β and IL-Rα result in autoinflammatory diseases such as Muckle-Wells syndrome, cryopyrin-associated periodic syndrome (CAPS), and neonatal-onset multisystem inflammatory disease[21] and treatment with canakinumab, a monoclonal antibody against IL-1β has successfully treated these conditions[22]. Moderate imbalances of the IL-1β system are associated with chronic inflammatory conditions including gout[23], type 2 diabetes[24], psoriasis[25] and inflammatory bowel disease[26]. Increased levels of IL-1β are present in atherosclerotic coronary arteries[27] and patients with acute coronary syndromes have higher levels of IL-1 receptor antagonist as compared to asymptomatic patients[28], interestingly among individuals with acute ST-segment elevation MI, increases in IL-1 receptor antagonist (which competitively blocks binding of IL-1β to the IL-1 receptor) precede elevations in troponin and CK, suggesting involvement in the pathogenesis of MI rather than being a consequence of myocardial necrosis[29]. Among individuals with rheumatoid arthritis who have a high prevalence of CVD attributable to chronic inflammation similar to HIV-infected individuals, treatment with anakinra, a IL-1 receptor antagonist reduced IL-6, hsCRP, and endothelin levels along with improving endothelial function as assessed by flow-mediated vasodilation of the brachial artery[30].

Canakinumab trial among individuals without HIV infection (CANTOS)

Published results and safety data from the CANTOS trial has showed that in over 10,000 individuals with prior MI and hsCRP level ≥2mg/L, canakinumab at a dose of 150 mg every 3 months led to a significantly lower rate of cardiovascular events as compared to placebo (HR 0.85, 95%CI 0.74 to 0.98, p=0.021)[31]. Notably, most of the benefit was observed in those with high levels of inflammation (hsCRP ≥ 2). The 150 mg dose met the pre-specified threshold for significance for the primary and secondary endpoints (HR vs placebo 0.83, 95% CI 0.73 to 0.95, P=0.005), although there was no significant difference in all-cause mortality (HR 0.94, 95%CI 0.83 to 1.06, p=0.31). Additionally, in the CANTOS study, IL-1β inhibition was not associated with increased risk of opportunistic infections, activation of TB or increased risk of cancers in the general population which suggests that canakinumab functions as an anti-inflammatory intervention as opposed to being immunosuppressive[32].

Those at greatest risk for infectious disease complications include older age and the presence of diabetes mellitus. Participants receiving canakinumab who achieved on-treatment IL-6 levels below the study median of 1.65ng/L had a 32% reduction in MACE, a 52% reduction in CV mortality, and a 48% reduction in all-cause mortality (p<0.0001 for all of these endpoints).

As HIV patients are excluded from CANTOS, the only way to determine safety in HIV is to perform a carefully done pilot study to confirm that the drug is safe in this patient population. We will work to ensure safety in our trial by only enrolling HIV-infected adults on long-term effective antiretroviral therapy with a CD4+ T-cell count ≥ 400 cells/mm^3 and by performing a two-stage milestone drive study as outlined below.

Safety

We constructed a two-stage, milestone driven study. In Stage I, we will treat a small cohort (n=10) of carefully monitored individuals. Pilot safety data will be reviewed by a Safety Monitoring Committee and NIH before moving on to randomized study (Stage II). This staged study design has been discussed extensively with NHLBI leadership, who support this approach. We have established protocols that were developed with experts in HIV, infectious disease, and oncology for carefully assessing the safety of canakinumab in the HIV-infected population. All subjects will be followed for possible adverse events (AEs) throughout their involvement in the study. Routine blood work will be performed on a regular basis. AEs will be graded according to the NIH/NIAID Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. The Principal Investigator will review these data daily, assess their degree of severity, and make a relationship assessment to study agent/intervention. Primary safety endpoints are detailed in the appendix and will be similar to endpoints used for our low-dose methotrexate study in HIV which have been carefully developed with the ACTG which include 1)For subjects with entry CD4+ T cell count <700 cells/mm^3, confirmed CD4+ decline >33% of baseline AND to <350 cells/mm^3. 2)For subjects with entry CD4+ T cell count ≥700 cells/mm^3, a confirmed CD4+ decline >50% of baseline, 3)Confirmed HIV-1 RNA level >200 copies/mL in the absence of an interruption in ART, 4)New or recurrent CDC category C AIDS-indicator condition, or 5)Evidence of HIV-associated infection including CMV end-organ disease, varicella zoster, EBV related clinical disease.

We will develop an independent Safety Monitoring Committee (SMC) prior to the initiation of the study. The final structure of this committee will be determined with input from NIH. We have recruited Dr. Judith Currier, Vice-Chair of the AIDS Clinical Trials Group (ACTG) from UCLA, Dr. Amir Lerman, a cardiologist with expertise in clinical trials and vascular function at the Mayo Graduate School of Medicine, Dr. Netanya Sandler Utay, a physician scientist with expertise in infectious diseases at University of Texas, Dr. Michael Lederman, a physician with expertise in infectious diseases at Case Western Reserve University, Dr. Vincent Marconi, director of infectious disease research at Atlanta VA Medical Center and Dr. Jason Baker, an infectious disease physician with research expertise in HIV and cardiovascular disease from the University of Minnesota (see letters of support). These individuals were selected based on their expertise in either the clinical management of HIV infection and/or their expertise with clinical trials. The committee will be chaired by Dr. Currier who has experience in the regulatory aspects of clinical trials.

The SMC will meet quarterly during the study and at approximately 2 to 4 weeks after the last person in Stage I has been dosed. The decision to move to Stage II will be made by the SMC. A study data coordinator will produce administrative reports after completion of each cohort describing study progress including the following: (1) accrual, (2) demographics, (3) study subject status, and (4) number and type of serious AEs. The SMC will review study progress, efficacy data, all interim and total AEs, and unanticipated problems involving risk to participants. Reviews will be communicated to the UCSF Committee on Human Research (CHR), study sponsor, and/or federal agencies, as appropriate. The SMC will have access to treatment assignment. The study will be discontinued if the SMC determines that it is in the best interest of the subjects.

We will work closely with the antiviral division of the NIH to assist in safety monitoring for the trial. Grade 1 or 2 AEs will not result in any change to our study plans. If there is evidence for a Grade 3 AE that is not caused by the study drug, the study will continue as planned. On the other hand, Grade 3 AEs thought to be possibly caused by the intervention and any Grade 4 AEs will result in a hold on any future enrollments until a decision to proceed or to stop the study is made by the SMC, the NIH, and Novartis.

Pharmacokinetic (PK) assessments:

There are no known drug-drug interactions with canakinumab. There are theoretical concerns that antibody-mediated reductions in inflammation may affect drug metabolizing enzymes such as CYP450. Elevated IL-6 has been associated with higher AUC of simvastatin and cyclosporine[33] and inhibition of IL-6 using tocilizumab has been associated with higher concentrations of simvastatin[34]. Given these theoretical concerns, with Dr. Aweeka's guidance, we plan to assess PK and carry out ART concentration measurements as troughs in N=60 subjects at 6 timepoints as shown in Table 1 which includes measurements prior to initiating and during treatment with canakinumab/placebo. For each trough sample collection, subjects will have PK trough levels drawn in the study clinic and will be instructed to hold their morning ART dose on the day of visit. Precise information on timing of the prior 3 doses will be collected from a patient diary as well as the precise time of sample collection. Venous PK sampling will be carried out prior to ART dosing. All samples will be processed for plasma and stored at -70ºC to await analysis. ART levels will be quantified using LC tandem MS instrumentation. Dr. Aweeka's laboratory has extensive experience in ART quantitation[35-41]. Methods are optimized and fully validated to permit quantification of all analytes at the sensitivity necessary for clinical PK evaluations. Inter- and intra-day coefficients of variation for all assays are consistently within 10%. PK trough estimates in 60 subjects will permit intrasubject comparison of exposure to the specific ART the subject is stabilized on prior to initiating study drug and following study drug administration. Subjects will serve as their own controls. In addition, PK exposure for the most commonly prescribed ART (e.g. atazanavir and efavirenz) will be compared between subjects receiving canakinumab to those subjects receiving placebo.

2.2 Summary

We will perform the first study to evaluate the effects of IL-1β inhibition on safety, measures of systemic and vascular inflammation and endothelial function (all indicators of cardiovascular risk) in treated and suppressed HIV infected individuals. In Stage I of our study, we will administer canakinumab as a single dose (150mg) by subcutaneous injection to 10 individuals on stable antiretroviral regimens with undetectable plasma HIV RNA levels using conventional assays. Based on safety data from the CANTOS study, we anticipate that this dose will be well tolerated and safe. In Stage II of our study, if the drug is well tolerated, we will continue enrolling patients for N=100 individuals and individuals will get two doses of 150mg (at baseline and 12 weeks). The pathogenesis-oriented approach will improve our understanding of the underlying mechanisms of the accelerated atherosclerosis observed in individuals with HIV infection, including the potential roles of inflammation and immune activation. By relating changes in inflammation and immune activation to changes in vascular function with IL-1β inhibition, we will establish specific mechanistic links between HIV infection and its

leading non-HIV cause of death, CVD. The results of this proof-of-concept trial could be potentially paradigm-shifting in this field; while the majority of studies to reduce HIV-related inflammation have focused solely on antiretroviral medication, the anti-inflammatory features of canakinumab can be applied to a variety of different disease manifestations in HIV. Theoretically, any intervention that decreases inflammation and T cell proliferation will result in a smaller reservoir. Thus IL-1β inhibition using canakinumab may have broad clinical implications for HIV-infected individuals favorably impacting HIV reservoirs and potentially leading to viral eradication and ultimately cure of HIV.

2.3 Hypothesis

Although antiretroviral therapy (ART) prolongs life, it does not fully restore health. For reasons that remain poorly defined, HIV-infected adults on otherwise effective therapy have a higher than expected risk of a number of “non-AIDS” conditions, including cardiovascular disease. Chronic inflammation predicts and possibly contributes to this risk. Although markers of T cell activation were dominant predictors in the era of untreated HIV disease, the most powerful and consistent predictors of morbidity in the modern ART era are those which reflect chronic activation of monocyte/macrophage systems. IL-6, sCD14, sCD163 and frequency of circulating monocytes with an activated phenotype have been found in multiple studies to be elevated in treated HIV disease and associated with increased risk of morbidity/mortality. Many of these pathways have also been associated with vascular dysfunction. These data collectively support a model in which a number of factors (e.g., microbial translocation, dysbiosis) activate monocyte/macrophages, leading eventually to vascular disease and end-organ tissue damage. There is ample precedent for this cardiovascular disease model in the general population, where resident inflammatory macrophages in vessels are central to the pathogenesis of atherosclerotic lesions and vascular dysfunction. We hypothesize that canakinumab administered at a dose of 150mg subcutaneously every 12 weeks will be safe and well tolerated in ART-treated HIV infected adults. We also hypothesize that IL-1β inhibition will reduce arterial inflammation, improve FMD, lower inflammatory markers, and reduce T-cell activation among treated and suppressed HIV-infected individuals. Finally, we predict that canakinumab-mediated inhibition of IL-1B signaling will reduce levels of CD4+ T cell activation, cell-associated RNA and cell-associated DNA.

2.4 Potential Risks and Benefits

Risks

The main risk is from the canakinumab intervention. The most common side effects of canakinumab used at doses for CAPS are rarely life-threatening but do warrant careful monitoring and management, especially in the setting of HIV infection. The risk of bacterial and viral infections may be higher during treatment with canakinumab, including fatal infections. All patients should contact their primary care doctor immediately if they develop symptoms of an infection. This risk is higher for the oldest old and those with comorbidities. For this reason, we have excluded those patients 60 years old or older. In clinical trials involving adults and children (ages 2-17 years), the most frequently reported adverse drug reactions were infections predominantly of the upper respiratory

tract. Commonly reported infections were nasopharyngitis, influenza, rhinitis, bronchitis, gastroenteritis, pneumonia, tonsillitis, sinusitis, urinary tract infection, and pharyngitis. There was one report of intra-abdominal abscess following appendectomy. TNF inhibitors have been associated with an increased risk of new tuberculosis or reactivation of latent tuberculosis and it is possible that IL-1 inhibitors may increase the risk of reactivation of TB. For this reason, we will screen and exclude any individuals with untreated latent or reactivated TB before study entry. To minimize risks, we will exclude individuals with active hepatitis prior to enrollment.

The impact of IL-1 therapy on development of malignancies is not known so we will exclude any individual with any history of malignancy from the study.

All participants must have adequate CD4+ T-cell counts and suppressed HIV prior to enrollment. CD4+ T cell counts and HIV-1 RNA titers will be measured frequently, as in the schedule of events. In clinical trials, injection site reactions were mild (10% of the clinical trial population) to moderate (less than 2% of the clinical trial population). No severe injection site reactions have been reported. Common toxicities associated with canakinumab also include, diarrhea, upper abdominal pain, headache, vertigo, and nausea. These will be queried by exam and interim medical history taking.

No hypersensitivity reactions have been reported with canakinumab therapy but we will exclude individuals if they have a known clinical hypersensitivity to the medication.

As canakinumab may affect the immune response to live vaccines and no data are available on the efficacy of live vaccines in individuals receiving canakinumab, adults would receive all recommended vaccines prior to initiation of therapy with canakinumab, and we will exclude any individual who has a requirement for live vaccines during the study.

Canakinumab has also been associated with a reduction in neutrophil count[22]. In a phase IIb study of 556 individuals, 10.7% of canakinumab treated patients and 4.6% of placebo patients had some neutropenia during the trial defined as an ANC < 1500/mm^3[2]. For this reason, we will exclude all individuals with ANC< 1500/mm and carefully monitor WBC and ANC during the study.

There may be temporary mild discomfort when the blood pressure cuff is inflated from the endothelial function studies. Some patients may feel weak or get a headache after taking the nitroglycerin medication which is also temporary. There is a risk of death if subjects are taking Viagra-like drugs and also take nitroglycerin at the same time; therefore subjects who have taken Viagra-like drugs within 48 hours of the study will not be administered nitroglycerin. Subjects will be monitored throughout the entire study and the exam will be terminated if severe symptoms develop. These have not been associated with any complications as patients are supervised and monitored during the studies. Trained technicians perform the procedures and Dr. Ganz and Dr. Hsue are available if any clinical issues arise. Incidental findings will be communicated to the study subject, and the study subject's physician and arrangements will be made to perform additional imaging or evaluation if clinically indicated. Phlebotomy may cause some discomfort, bleeding or bruising where the needle enters the skin, and rarely, fainting or infection may occur. No more than 480 ml (2 cups) of blood will be drawn over any two-

month period. This is within Red Cross Guidelines (less than 500 ml every two months). Risks of blood collection include anemia (low blood counts). Symptoms of anemia include tiredness, weakness and dizziness. Patients will be checked for anemia at each visit. If the investigator feels that an individual is at significant risk for anemia, the amount of blood collected will be reduced. If the study participant's hemoglobin falls below 9 g/dl or hematocrit falls below 27%, we will draw a 5 ml (1 teaspoon) of blood drawn to check the hemoglobin and hematocrit. Other than the blood required to check the hemoglobin and hematocrit, there will not have more blood drawn until the hemoglobin rises above 9 g/dl or the hematocrit rises above 27%.

The effects of canakinumab on an unborn baby are unknown. Female subjects of childbearing age and pregnant or nursing women will be excluded from the study. Male subjects will be counseled to use two forms of birth control during the study.

Other side effects that are not known at this time could occur during study treatment. All drugs have a potential risk of an allergic reaction, which if not treated promptly, could become life-threatening. During the course of the study, subjects will be told about any new information that may affect their decision to continue to participate.

Benefits

Although ART for patients with HIV prolongs life, it does not fully restore health. Several factors contribute to the excess CVD risk observed in patients with HIV, including chronic inflammation and immune activation. Thus, therapeutic strategies to target inflammation and immune activation in the setting of HIV infection may reduce CVD risk, leading to improved survival. We will perform the first study ever to evaluate the effects of an anti-inflammatory treatment strategy via IL-1B inhibition that does not affect traditional CVD risk factors and is not an HIV antiretroviral medication on endothelial function, inflammation, and immune activation in individuals with HIV infection. Treatment of inflammation, other than through use of ART or lipid-lowering medications is a novel approach to reducing CVD risk in patients with HIV, and could potentially change preventive cardiology treatment paradigms in these patients by adding an additional tool to the arsenal of CVD risk-reducing interventions used in patients with and without HIV infection.

While receiving the study drug, there is no anticipated benefit for patients participating in this exploratory study.

3.0 OBJECTIVES

3.1 Primary Objectives

1. To determine the safety, tolerability, and pharmacokinetics of IL1-β inhibition using canakinumab in effectively treated and suppressed HIV infected adults.
2. To demonstrate that IL-1β inhibition decreases systemic inflammatory markers, T cell activation, and improves vascular inflammation and endothelial dysfunction among treated and suppressed HIV-infected individuals.

3. To determine whether IL-1β inhibition decreases the size of the HIV reservoir in blood.

4.0 STUDY DESIGN

We will perform a double blinded study that will be performed in two stages. In Stage I, 10 individuals will receive a dose of 150mg canakinumab and then followed for 12 weeks as detailed in Table 1. If the drug is safely tolerated and none of the safety milestones have been reached at the end of followup period, we will proceed with Stage II of the study which entails the enrollment of a 100 additional study participants who will be treated with two doses of canakinumab (150mg subcutaneous baseline and 12 weeks) or placebo.

4.1 Inclusion Criteria

4.1.1 Currently on continuous ART for at least 12 months with no change in regimen in 4 weeks prior to study entry. This is defined as continuous active therapy for the 4-week period prior to study entry with no treatment interruption longer than 7 consecutive days and a total duration off treatment of no more than 14 days in the 90 days prior to study entry.

4.1.2 CD4+ T-cell count ≥400 cells/mm^3 obtained within 30 days prior to study entry by any US laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent.

4.1.3 HIV-1 RNA level below the limit of quantification using an FDA-approved assay for at least 52 weeks prior to study entry and confirmed within 60 days prior to study entry. The assay used for eligibility can be performed by any US laboratory that has a CLIA certification or its equivalent.
NOTE: Single determinations that are between the assay quantification limit and 200 copies/mL are allowed as long as the preceding and subsequent determinations are below the level of quantification.

4.1.4 The following laboratory values obtained within 60 days prior to study entry by any US laboratory that has a CLIA certification or its equivalent;

- ALT [serum glutamic pyruvic transaminase (SGPT)] <2 times upper limit of normal (ULN)
- Total bilirubin < 4 times ULN
- AST [serum glutamic oxaloacetic transaminase (SGOT)] <2 x ULN
- White blood cell (WBC) >3000/mm^3
- Hemoglobin >12.0 g/dL
- Platelets >150,000/mm^3
- ANC ≥ 1500/mm

4.1.5 Female subjects who are postmenopausal (i.e., of non-childbearing potential), defined as having either:

A) Appropriate medical documentation of prior hysterectomy and/or complete bilateral oophorectomy (i.e., surgical removal of the ovaries, resulting in “surgical menopause” and occurring at the age at which the procedure was performed),

OR

B) Permanent cessation of previously occurring menses as a result of ovarian failure with documentation of hormonal deficiency by a certified healthcare provider (i.e., “spontaneous menopause”). Hormonal deficiency should be properly documented in the case of suspected spontaneous menopause as follows:

1. If age >54 years and with the absence of normal menses for at least 24 consecutive months: serum follicle stimulating hormone (FSH) level elevated to within the post-menopausal range based on the laboratory reference range where the hormonal assay is performed;
2. If age ≤ 54 years and with the absence of normal menses for at least 24 consecutive months: Negative serum or urine (HCG) performed within 48 hours prior to study entry with concurrently elevated serum FSH level in the post-menopausal range, depressed estradiol (E2) level in the post-menopausal range, and absent serum progesterone level, based on the laboratory reference ranges where the hormonal assays are performed.

4.1.6 Moderate or high CVD risk or presence of one CVD risk factor defined as:

A) Documented CVD as assessed by meeting at least 1 of 3 criteria below:

1. Coronary artery disease (CAD): prior MI due to atherosclerosis, coronary artery bypass graft surgery, percutaneous coronary intervention, or angiographic CAD with luminal diameter stenosis of at least one coronary artery at least 50%.
2. Cerebrovascular disease: prior ischemic stroke of carotid origin, carotid endarterectomy or stenting, or angiographic carotid stenosis of at least 50%.
3. Peripheral arterial disease: prior lower extremity arterial surgical or percutaneous revascularization procedure, or angiographic lower extremity arterial stenosis of at least 50%.

OR

B) At increased risk for CVD by meeting the criteria below:

1. First degree relative who had heart attack, stroke, or documented CVD as defined in section 4.1.6.A that occurred when they were age:
   a. 55 years or younger for **male** relatives (father, uncle, or brother)
   b. 60 years or younger for **female** relatives (mother, aunt, or sister)

OR

C) One of the following CVD risk factors below:

1. Current smoking: subject report of smoking at least a half a pack of cigarettes a day, on average, in the past month.
2. Hypertension (HTN): two consecutive BP readings with either systolic >140 mmHg or diastolic > 90 mmHg; or currently on antihypertensive medications.
3. Dyslipidemia: defined as non-HDL-C >160mg/dL, or HDL-C ≤ 40mg/dL regardless of medication use; or currently on lipid lowering medications.
4. hsCRP ≥ 2mg/L obtained at screening.

4.1.7 Men and women age ≥40 years and ≤ 59 years

4.1.8 May be on stable lipid-lowering medication, and/or anti-hypertensive medication.

4.1.9 Ability and willingness of subject to provide informed consent

4.1.10 Appropriate documentation from medical records of prior receipt of pneumococcal vaccination (with both the 13 valent conjugant vaccine [PCV13] and 23 valent pneumococcal polysaccharide vaccine [PPV23]). Pneumococcal vaccination requirements will be determined on a case-by-case basis according to IDSA guidelines (http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/idsa-vaccinesimmunosupp2013).

- If necessary, the PCV13 vaccination can be provided and administered before study entry given that the subject meets all other inclusion/exclusion criteria and primary care providers are unable to provide immunization. Each subject's primary care provider will be notified.

4.1.11 Influenza and pneumococcal vaccination must be completed more than 14 days prior to study entry.

4.2 Exclusion Criteria

4.2.1 Men and Women age ≥60

4.2.2 Acute or serious illness requiring systemic treatment and/or hospitalization within 90 days prior to study entry.

NOTE: Treatment must have ended at least 60 days prior to study entry for eligibility.

4.2.3 Documentation of any CDC category C AIDS-indicator condition within 90 days prior to study entry.

4.2.4 Receipt of antibiotic therapy within 30 days prior to study entry.

4.2.5 Active or latent TB infection (defined as a positive PPD ≥5 mm positive interferon-gamma release assay, or positive T-spot test at any time in the past)

- Evidence of latent TB on a prior chest x-ray that has not been treated will be excluded
  - Subjects who have received latent TB treatment at least 6 months prior to study entry will be allowed
- Active TB disease requiring treatment will be excluded
  - Subjects who have received active TB treatment at least 48 weeks prior to study entry will be allowed

4.2.6 Women of child bearing age, pregnant or nursing women because the effects of canakinumab on fetal development are not known.

4.2.7 CABG surgery in the past 3 years.

4.2.8 New York Heart Association Class IV congestive heart failure.

4.2.9 Uncontrolled hypertension within the past 90 days prior to study entry.

4.2.10 A history of or current, active hepatitis B infection defined as positive hepatitis B surface antigen test or positive HBV DNA in subjects with isolated HBcAb positivity, defined as negative HBsAg, negative HBsAb, and positive HBcAb within 24 weeks prior to study entry.

NOTE: Subjects who are positive for hepatitis B surface antigen but who are HBV DNA negative are permitted in the study.

4.2.11 Chronic hepatitis C infection defined as a positive hepatitis C antibody and positive hepatitis C RNA at any time prior to study entry.

NOTE: Subjects who are positive for hepatitis C antibody but who are HCV RNA negative are permitted in the study. Participants who have been treated for hepatitis C must be HCV RNA negative for at least 24 weeks after completion of therapy to be eligible for the study.

4.2.12 Patients with diabetes mellitus.

4.2.13 Nephrotic syndrome or eGFR< 30ml/min/1.73m^2 or kidney transplant (regardless of renal function).

4.2.14 Any prior malignancies including Kaposi's Sarcoma (KS), Cervical Cancer, and Non Hodgkin Lymphoma (NHL) with the exception of basal and squamous cell carcinoma.

4.2.15 Use of immunomodulators (e.g., interleukins, interferons, cyclosporine), HIV vaccine, systemic cytotoxic chemotherapy, or investigational therapy within 30 days prior to study entry or during study.

4.2.16 Change in the ART regimen in the 4 weeks prior to study entry or intended modification of ART during the study.

NOTE: Some modifications of ART doses during the 4 weeks prior to study entry are permitted. In addition, the change in formulation (e.g., from standard formulation to fixed-dose combination) is allowed within 4 weeks prior to study entry. A within class single drug substitution (e.g., switch from nevirapine to efavirenz or from atazanavir to darunavir) is allowed within 4 weeks prior to study entry, with the exception of a switch from any other NRTI to abacavir. No other changes in ART in the 4 weeks prior to study entry are permitted.

4.2.17 Known allergy/sensitivity or any hypersensitivity to components of study drug(s) or their formulation.

4.2.18 Requirement for live active vaccination during the study and vaccination (e.g., influenza, pneumococcal polysaccharide) within 30 days prior to study entry and 30 days after study completion or requirement for inactive vaccination within 2 weeks prior to study entry.

4.2.19 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.20 Changes in lipid-lowering or antihypertensive medication within 90 days prior to study entry or expected need to modify these medications during the study.

NOTE: Lipid-lowering medication includes: statins, fibrates, niacin (dose ≥250 mg daily), and fish-oil/omega 3 fatty acids (dose >1000 mg of marine oils daily).

4.2.21 Planned surgery in the next 52 weeks because canakinumab may lead to changes in white blood cells may affect wound healing.

4.2.22 Patients who have taken PDE-5 inhibitors within 72 hours of the FMD test because nitroglycerin administered during the FMD can have fatal side effects with PDE-5 inhibitors.

4.2.23 Subjects with a history of multiple imaging studies associated with radiation exposure in order to reduce the overall burden of radiation.

4.2.24 Patients with neutropenia defined as an ANC <1500/mm.

4.2.25 Triglycerides > 400mg/dL because small increases in triglycerides are associated with canakinumab therapy.

4.2.26 Patients with a history of EBV-related lymphoproliferative disorders.

4.2.27 Known active or recurrent hepatic disorder (including cirrhosis, hepatitis B and hepatitis C (positive or indeterminate central laboratory results), or alanine aminotransferase/ aspartate aminotransferase (ALT/AST) levels > 2 times ULN or total bilirubin > 4 times ULN).

4.2.28 Additional exclusion criteria for the FDG-PET/CT imaging:

- Significant radiation exposure during the year prior to randomization. Significant exposure is defined as i) more than 2 PCI procedures, ii) more than 2 myocardial perfusion studies, or iii) more than 2 CT angiograms.
- Any history of radiation therapy.

4.3 Study Enrollment Procedures

4.3.1 Subject recruitment and enrollment process

As we have done in our prior studies, all HIV-infected subjects will either be recruited from the SCOPE study or co-enrolled in the SCOPE study. SCOPE is an ongoing prospective cohort of over 2000 HIV-infected adults and over 600 individuals meet the entry criteria for this study ensuring rapid recruitment. Advantages of using SCOPE to perform our proposed study include: (1) the ability to leverage existing funds to support the proposed research; (2) rapid recruitment of eligible subjects from a well-characterized cohort; and (3) reliance on an established infrastructure for patient retention, data collection, laboratory testing, and data management.

Quest Clinical Research will recruit from their cohort as well as screen referrals from sub-investigators.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject. The date of enrollment is the date the participant signs the informed consent form.

4.3.2 Randomization

Due to the objectives of the study, the identity of the test and control treatments will not be known to investigators, research staff, or patients. 100 patients will undergo double-blinded 2:1 randomization using a SAS-based computer-generated randomization scheme developed by the data management provider. Subjects will be assigned to receive canakinumab 150 mg or matching placebo for 6 months, while continuing their other antiretroviral drugs (Stage II). Those who enroll in Stage I of the study will receive an initial dose of 150mg. The dose

chosen for this study of 150 mg subcutaneous injection every 2 months is the FDA approved dose for CAPS.

The DAIDS-approved SFGH investigational pharmacy offers in house investigational drug services and dispensing of research drugs. The pharmacist and credentialed pharmacy technician charged with dispensing research-related drugs receive protocol-specific training and are compliant with State Board of Pharmacy requirements, FDA Guidelines, and Good Clinical Practices. Pharmacy documents, including study drug accountability logs, study drug ordering and shipping logs, and regulatory documents are maintained by the pharmacy technician. Medication will be dispensed at baseline for Stage I and at baseline and 12 weeks for Stage II.

5.0 STUDY TREATMENT

In Stage I, 10 subjects will receive 150mg of canakinumab. After 12 weeks, if no safety endpoints have been met and are clinically stable as defined in section 9.2 the study will proceed to Stage II. In Stage II, 100 additional patients will be randomized in a 2:1 ratio using a SAS-based computer-generated randomization scheme developed by the study data management provider to either Arm 1 or Arm 2 as shown in Figure 1 to receive 150mg canakinumab or placebo.

At the week 12 study visit, if subjects in Stage II remain clinically stable, patients will receive another subcutaneous injection of 150mg of canakinumab. The patients will receive a dose at entry and at 12 weeks.

5.1 Regimens, Administration, and Duration

5.1.1 Dose

Canakinumab is FDA approved for treatment of CAPS at 150mg SQ injection given every 2 months; for systemic juvenile idiopathic arthritis in patients as young as 2 years it can be given at a maximum dose of 300mg SQ every 4 weeks. No adjustments are needed for renal or liver impairment or in the elderly. In the CANTOS pilot study, doses given 5-150mg per month were associated with significant reductions in biomarkers. In Stage I, 10 individuals will be treated with 150mg injection once. This dose was chosen as it was well tolerated in the CANTOS pilot study and resulted in significant decreases in inflammatory markers.

If Stage I is safely tolerated, we will then proceed with Stage II with a 150mg subcutaneous injection at baseline and 12 weeks. This dose was chosen as it is the intermediate dose being used in the CANTOS study and after intensive discussion with Dr. Ridker and experts at Novartis.

5.1.2 Administration

Medication will be administered as a subcutaneous injection during the patient visit by a trained study nurse who will ensure complete compliance and

medication delivery once (at baseline) during Stage I of the study and twice (at baseline and 12 weeks) during Stage II of the study.

5.1.3 Duration

For stage I, subjects will be receiving study treatment at entry and followed for 12 weeks for observation. The total study duration for Stage 1 for each subject is 12 weeks. For Stage II, subjects will be on study treatment for 24 weeks and followed for 28 weeks of observation. The total study duration for Stage II for each subject is 52 weeks.

5.2 Study Product Formulation and Preparation

5.2.1 Canakinumab

Canakinumab is supplied as prefilled syringes (PFS) of canakinumab 150 mg in 1 mL in bulk. You will have to individually label these syringes for each patient but otherwise they are ready to use. The PFS has to be stored in refrigerator (2°.C - 8°C) and protected from light. Prior to use the PFS are taken out of refrigerator into room temperature 20 min prior to use so that the PFS reaches room temperature prior to administration.

5.2.2 Placebo

Placebo is supplied as a white powder for solution for subcutaneous injection. Reconstitution with 1mL preservative-free sterile water for injection in required prior to injection. The placebo will be stored in the refrigerator at 2°C -8°C (68°-77°F) and protected from light.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Product Supply

Canakinumab and matching placebo for canakinumab will be supplied through the study by Novartis. ART will not be supplied through the study.

5.3.2 Study Product Acquisition/Distribution

Novartis will ship the study drug and placebo to the SFGH investigational pharmacy after all required regulatory documentation has been received by the Sponsor and a contract has been executed.

5.3.3 Study Product Accountability

The DAIDS-approved SFGH investigational pharmacy offers in house investigational drug services and dispensing of research drugs. The pharmacist and credentialed pharmacy technician charged with dispensing research-related drugs receive protocol-specific training and are compliant with State Board of Pharmacy requirements, FDA Guidelines, and Good Clinical Practices.

Pharmacy documents, including study drug accountability logs, study drug ordering and shipping logs, and regulatory documents are maintained by the pharmacy technician. Medication will be dispensed on a 3 monthly basis.

5.4 Concomitant Medications

Subjects in both groups will continue to take their current anti-HIV medicines. There are no medications that are prohibited while the patients are on the study treatment. There may be a loss of therapeutic effects for medications that require individualized dose adjustments. Therefore, these concomitant medications will be continued during the trial if only medically necessary and with appropriate monitoring. All concomitant medication and concurrent therapies will be documented at screening, and at visits on weeks 1, 4, 8, 12, 24, 36, 40, 48, 52 and for any premature discontinuation.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events

6.1.1 Schedule of Events for Stage I (Table 1)[1]

Event	Screen	Pre-Entry	Entry	Wk 1	Wk 2	Wk 3	Wk 4	Wk 8	Wk 12
Medical History	x	x	x	x	x	x	x	x	x
Physical Exam	x		x	x			x		x
Concomitant Medications	x	x	x	x	x	x	x	x	x
Latent TB testing	x								
Hepatitis B and C	x								
Pregnancy testing	x		x						
Hemoglobin A_1C	x								
Canakinumab (150mg) Stage I			x[3]						
Safety Labs[2]	x		x	x	x	x	x	x	x
CD4 count; HIV-1 RNA	x	x	x	x	x	x	x	x	x
Lipid panel	x		x	x	x	x	x	x	x

[1]visit windows ± 4 days except weeks 1 and 2 (±3 days); [2]CBC, AST, ALT, bilirubin, creatinine, glucose; [3]Stage 1: ten subjects get 150mg canakinumab at entry with study visits as shown above.

6.1.2 Schedule of Events for Stage II (Table 1)[1]

Event	Screen	Pre-Entry	Entry	Wk 1	Wk 2	Wk 3	Wk 4	Wk 8	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 18	Wk 24	Wk 30[10]	Wk 36[4]	Wk 40[10]	Wk 48[10]	Wk 52[10]	Premature Discont.
Medical History	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical Exam	x		x	x			x		x					x		x		x	x	x	x
Concomitant Medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Latent TB testing	x																				
Hepatitis B and C	x																				
Pregnancy testing	x		x																		
Hemoglobin A_1C	x																				
Canakinumab/PBO Stage II[5]			x[5]						x[5]												
Safety Labs[2]	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CD4 count; HIV-1 RNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lipid panel	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				x
Phamacokinetics[6]			x				x		x					x	x		x				
Brachial Artery FMD			x[7]						x					x							x
FDG-PET/CT			x[8]											x[9]							

Inflammatory Markers[3]			x				x		x					x			x			x
Monocyte/T Cell/CMV			x				x		x					x			x			x
HIV Persistence			x				x		x					x						
Stored plasma/PBMCs		x	x				x		x					x	x		x			x

[1]visit windows ± 4 days except weeks 1 and 2 (±3 days); [2]CBC, AST, ALT, bilirubin, creatinine, glucose; [3]hsCRP, IL-6, fibrinogen, sCD163, D-dimers; [4]post-treatment visit for safety and to assess inflammatory marker rebound; [5]in Stage II, N=100 subjects will be randomized to receive 150mg canakinumab SQ baseline and 12 weeks vs. placebo; [6]PK studies will be done in Stage II of the study; CMV PCR will be done to assess for reactivation of latent CMV; [7]paired FMD (1 hour apart) and must occur within 30 days of entry injection; [8]FDG-PET/CT Scan and FMD visit and 30 days of entry injection; [9]FDG-PET/CT Scan must occur within ±1 week of FMD visit; if reimaging is deemed necessary at baseline or follow-up, second FDG-PET/CT scan must occur within 1 week; [10]Study visit added to the shchedule fo events

6.2 Timing of Evaluations

6.2.1 Screening Evaluations

Screening evaluations must occur prior to the subject's starting any study medications, treatments, or interventions.

Screening evaluations to determine eligibility must be completed within 120 days prior to study entry unless otherwise specified.

NOTE: The screening evaluations should be done in a fasting state (see section 6.3 for a definition of fasting), as indicated in section 6.1. If the potential subjects are not fasting at the screening visit, then they should come back to the clinic within 3 days of the originally scheduled screening visit for a fasting blood draw.

In addition to data being collected on subjects who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form.

6.2.2 Pre-Entry Visit

Prior to entry visit, subjects will come in for a blood draw to collect pre-intervention serum and PBMC.

6.2.3 Entry Evaluations

Subject must begin study treatment within 24 hours after randomization. Paired FMD will be performed within 30 days prior to delivery of drug and FDG-PET/CT will be scheduled for completion within 1 week of the paired FMD.

NOTES:

- A qualified FDG-PET interpreter will measure the image within a week of the scan and may find it necessary to repeat the scan within one week due to subpar image quality. Study drug dispensing will be delayed until after the repeat scan is completed.
- The second FMD assessment must be completed within 72 hours after the first FMD and must occur before the subject takes the first dose of study treatment.
- Paired FMD can be same day, at least 30 minutes apart.

<u>Depression Questionnaire (Optional) for Stage II Only</u>

- 3-5 minute survey that asks questions to gauge whether or not the participant is experiencing symptoms of depression in order to study the link between depression and heart disease in HIV positive and HIV negative patients.

Transcranial Doppler Imaging and Neurocognitive Sub study (Optional) for Stage II Only

- The transcranial Doppler imaging is a non-invasive ultrasound test to evaluate the blood vessels in the brain. The visit will require a 12 hours fast and will also include a walking speed assessment.
- The neurocognitive test consists of a series of paper and pencil tests to examine your thinking, memory, motor function, and coordination, as well as a questionnaire about your daily function

6.2.4 Post-Entry Evaluations

All post-entry evaluations occur in reference to the date on which the subject started study treatment.

Return Fasting and Brachial FMD Visit (as needed) for Stage II Only

If subjects are in a non-fasting state or have not abstained from exercise or smoking for at least 12 hours prior to the weeks 12 and 18 post-entry study visits, then they should return to the clinic within 3 days of the originally scheduled study visit for a fasting blood draw and brachial FMD assessment. In addition, the subject must be afebrile (oral T <38 °C or <100.4 °F) for at least 48 hours prior to the FMD assessments at weeks 12 and 18.

Return FDG-PET/CT visit (as needed) for Stage II Only

A qualified FDG-PET interpreter will measure the image within a week of the scan and may find it necessary to repeat the scan within one week due to subpar image quality. If the participant was already required to repeat the FDG-PET/CT at entry, the participant will not be asked to repeat the FDG-PET/CT at post-entry and vice versa.

Confirmation of Virologic Failure

For subjects with suspected virologic failure, a confirmatory plasma HIV-1 RNA test is to be performed within 30 days after the receipt of the results of the initial HIV-1 RNA sample indicating suspected virologic failure. In light of ART interruption (including identified periods of non-adherence), confirmatory HIV-1 RNA testing is not required; adherence counseling is recommended as needed. **If a participant has a second episode of virologic failure during the study after having achieved full suppression following the previous virologic failure, this should also be confirmed within 30 days.**

NOTE: Virologic failure is defined as confirmed HIV-1 RNA levels >200 copies/mL at any time during the study in the absence of an interruption of ART.

Modifications to ART medications only must be documented.

The study will trigger a DSMB review if ≥ 5% of subjects in Stage II have two confirmed episodes of virologic failure during the study.

Confirmation of CD4+ T Cell Count

For subjects with a suspected CD4 safety endpoint, a confirmatory CD4+ T-cell count is to be performed within 30 days after the receipt of the results of the initial CD4 result indicating suspected CD4 endpoint.

NOTE: The CD4 safety endpoint is defined as indicated below.

- For subjects with an entry CD4+ T cell count <700 cells/mm^3, a confirmed CD4 decline >33% of baseline and to an absolute value <350 cells/mm^3
- For subjects with an entry CD4+ T cell count ≥700 cells/mm^3, a confirmed CD4 decline >50% of baseline

Study Completion Evaluations

The week 52 evaluations will be completed as the subject's final study visit.

In addition to all the study visits mentioned above, participants will be contacted every week to ask about infectious symptoms and any other side effects by phone call.

6.2.5 Discontinuation Evaluations

Evaluations for Randomized Subjects Who Do Not Start Study Treatment

Subjects who do not start study treatment will be taken off study with no further evaluations required.

All case report forms (CRFs) must be completed and keyed for the period up to and including entry.

Premature Treatment Discontinuation Evaluations

Premature discontinuation of study treatment is defined as permanently discontinuing canakinumab prior to week 24.

Subjects who prematurely discontinue canakinumab will have the premature treatment discontinuation evaluations performed within 1 week and no later than the next scheduled study visit. Subjects will be encouraged to continue on study/off study treatment and receive all study evaluations per section 6.1 through completion of the study.

NOTE: Subjects who are on study/off study treatment do not need to have PK assessments or study treatment adherence assessments (subjects should continue to be evaluated for adherence to ART medications).

Premature Study Discontinuation Evaluations

Subjects who prematurely discontinue from the study prior to the final visit at week 52 will have the premature study discontinuation evaluations performed as per section 6.1 and be taken off study.

6.3 Instructions for Evaluations

All stated evaluations are to be recorded on the CRF and keyed into the database unless otherwise specified. This includes events that meet the International Conference on Harmonization (ICH) definitions for a serious adverse event.

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

Fasting Instructions

Fasting is defined as nothing to eat or drink except prescription medications and water for at least 12 hours prior to the procedure. If subjects are in a non-fasting state, they must come back to the clinic within 3 days of the originally scheduled study visit for a fasting blood draw and FMD assessments, as indicated in section 6.1. Patients will be fasting at every visit for both Stage I and Stage II.

6.3.1 Demographics

Demographic information (date of birth, gender, race) will be recorded at screening.

6.3.2 Medical History

In addition to reporting all diagnoses within the past 30 days, the following diagnoses should be reported regardless of when the diagnosis was made.

- AIDS-defining conditions
- Coronary heart disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes
- Tuberculosis
- Chronic hepatitis C
- Chronic hepatitis B

Any allergies to any medications and their formulations must also be documented.

6.3.3 Medication History and Study Treatment Modifications

Medication History

A medication history must be present, including start and stop dates (unless otherwise specified). The table below lists the medications that must be included in the history to be recorded on the CRFs.

Medication Category	Timeframe
Antiretroviral therapy	Complete history (start and stop dates only required for ART taken within past 2 years)
Immune-based therapy	Complete history
HIV-1-related vaccines	Complete history
Drugs for treatment or prevention of opportunistic infections	Complete history
Prescription and non-prescription drugs	All cardiac and diabetes medications taken currently and within past year All other medications within 6 months prior to entry
Dietary supplements	Within 6 months prior to entry

Study Treatment Modifications

All of the following criteria must be met at the week 1 and week 12 visits before Stage II of the study can be initiated.

- WBC count is >2500/uL and ANC ≥1500/mm
- Platelet count is >75,000/uL
- ALT/AST levels are <2 x ULN
- Hemoglobin >11.0 g/dL
- Platelets >75,000/mm^3
- No clinically important symptoms (defined as stomatitis, persistent fever, vomiting, clinical bleeding, or worsening shortness of breath).

Study participants who develop neutropenia (ANC < 1500/mm) after the initial canakinumab dose will not be re-dossed as these subjects are anticipated to be at increased risk of infection.

6.3.4 Clinical Assessments

Complete Physical Exam

At the screening visit, a complete physical examination is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; examination of the lower extremities for edema. The complete physical exam will also include signs and symptoms, height and weight, diagnoses, and vital signs (temperature, pulse and blood pressure).

Targeted Physical Exam

At entry and all post-entry visits, a targeted physical exam is to be driven by any previously identified or new signs or symptoms that the subject has experienced since the last visit. This exam also includes vital signs (temperature and blood pressure), height and weight, and diagnoses at all visits.

CV Risk Assessment

For screening, this should include evaluation of smoking within the past 30 days, treatment for high blood pressure, and subject self-reported history of prior diagnosis or treatment for CVD, MI, coronary artery bypass graft surgery, percutaneous coronary intervention [angioplasty or stent], stroke, transient ischemic attack, peripheral arterial disease, or diabetes mellitus [excluding gestational diabetes].

Smoking status will also be assessed at each post-entry FMD visit.

NOTE: Peripheral arterial disease should only be recorded if the subject reports a medical diagnosis of peripheral artery disease, a history of a revascularization procedure, or use of medications for peripheral arterial disease (i.e., cilostazol or pentoxifylline).

Signs and Symptoms

At entry, all grades of signs and symptoms that occurred within 30 days prior to entry must be recorded; at post-entry, only signs and symptoms Grade ε2 must be recorded. Record all signs and symptoms that led to a change in treatment, regardless of grade. Further evaluation will be required for those events that meet EAE or ICH reporting requirements.

Patients will be asked for any signs or symptoms of infection such as fever, chills, fatigue or rash. If present, the study participant will be asked to seek medical care immediately. The study team will notify the primary care physician, and arrange for expedited evaluation for infection and if indicated, provide antibiotics for immediate treatment.

Diagnoses

Record all of the following targeted diagnoses (as defined in Appendix 100, Diagnoses Appendix Criteria for Clinical and Other Events from the ACTG):

- CDC category C AIDS indicator condition
- oropharyngeal candidiasis (thrush)

- oral ulcers
- single episodes of bacterial pneumonia, herpes simplex ulcers, and cryptosporidiosis should be reported regardless of whether they meet the CDC category C AIDS indicator definitions.
- any serious clinical events
- any CVD, hematologic, infectious, and neoplastic diseases
- all hepatobiliary (i.e., viral hepatitis) and renal disorders (renal insufficiency)
- all mycobacterial infections, lymphoproliferative disorders, and pulmonary disorders, including all cases of immune reconstitution inflammatory syndrome (IRIS) and interstitial pneumonitis.

ART Medications

All modifications to ART medications including initial doses, subject-initiated and/or protocol-mandated interruptions (e.g., single dose interruption), modifications, permanent discontinuations, and last doses on study will be recorded.

6.3.5 Laboratory Evaluations

At screening and entry, all protocol-required laboratory values must be recorded. For post-entry assessments, fasting lipid, fasting glucose, hematology, liver function tests, and creatinine values regardless of grade and all other Grade ≥3 laboratory values will be recorded. All laboratory toxicities that led to a change in treatment, regardless of grade, must be recorded. Further evaluation will be required for those events that meet EAE or ICH reporting requirements.

hsCRP (real-time)

A real-time hsCRP is required only at screening for those subjects whose eligibility will be determined based on Category C of section 4.1.6. hsCRP results do not need to be recorded on a CRF.

Hematology

Blood will be obtained and analyzed for determination of hemoglobin, hematocrit, mean corpuscular volume (MCV), WBC, WBC differential (including neutrophils, lymphocytes, monocytes, basophils, eosinophils, other cell types present), and platelets.

Chemistries

Blood will be analyzed for determination of sodium, potassium, chloride, bicarbonate/CO2, BUN, and creatinine.

Liver Function Tests

Blood will be analyzed for determination of total bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase.

Lipid Panel (fasting, real-time)

Blood will be analyzed for determination of total cholesterol, high density lipoprotein cholesterol (HDL), calculated low-density lipoprotein cholesterol (LDL), and triglycerides.

NOTE: If the subject is not fasting, it is mandatory that the subject return to the clinic within 3 days of the originally scheduled study visit, after fasting for 12 hours.

Glucose (fasting)

Blood will be analyzed for glucose measurements..

NOTE: If the subject is not fasting, it is mandatory that the subject return to the clinic within 3 days of the originally scheduled study visit, after fasting for 12 hours.

TB Test

A PPD skin test, or interferon-gamma release assay must be performed prior to study entry to rule out latent TB unless there is documentation that the subject completed treatment for latent TB at least 6 months prior to entry.

NOTE: The TB test results do not need to be recorded on a CRF.

Hepatitis Serologies
Hepatitis Serologies will be performed at screening with results available prior to study entry.

For subjects with known hepatitis B immunity, prior documentation of positive HBsAb is acceptable. If documentation is not available, HBsAb, HBsAg, and HBcAb will be obtained at screening. Results must be available prior to study entry. Subjects who have positive HBcAb but negative HBsAg and HBsAb (isolated HBcAb positive status) must have HBV DNA polymerase chain reaction (PCR) performed and confirmed as negative for subject to be eligible.

Negative hepatitis B tests (i.e., negative HBsAb, HBsAg, and HBcAb) performed within 6 months prior to enrollment need not be repeated. If the last negative test result is more than 6 months prior to enrollment, then testing should be performed at screening. Results of the hepatitis B serology must be available prior to study entry so that subjects who have active hepatitis B can be excluded from participating in the study. Hepatitis B serology results will be recorded on the CRFs.

For subjects with known chronic HCV, prior documentation of positive

HCV antibody is acceptable. Negative HCV antibody obtained <6 months prior to enrollment is acceptable. If documentation of HCV status is not available or if the initial HCV test was negative but was obtained >6 months prior to enrollment,

then an HCV antibody test should be obtained at screening. Results must be available prior to study entry and recorded on the CRF.

6.3.7 Immunologic Studies for Stage II only

CD4+/CD8+

Absolute CD4+/CD8+ T-cell count and percentages within 120 days prior to entry must be obtained from a laboratory that possesses a CLIA certification or equivalent.

During the study, CD4+/CD8+ T-cell count and percentages must be performed by a laboratory that possesses a CLIA certification or equivalent and is certified for protocol testing by the DAIDS Immunology Quality Assurance (IQA) Program.

Stored Plasma and Serum for Inflammatory and Coagulation Markers

Plasma and serum for IL-6, hsCRP, D-dimer, sCD163, IP-10, sCD14, tissue factor, and microbial translocation such as lipopolysaccharide will be collected, processed, and stored for analyses.

Stored PBMC for Immunology Assays

PBMC for markers of HIV immune activation (CD38 and HLA-DR markers on peripheral CD4+ and CD8+ T-cells), immune senescence (CD28-/CD57+ T-cells), monocyte subpopulations (including adhesion/activation indices), and expression of CX3CR1 will be collected, processed, and stored for analysis.

6.3.8 Virologic Studies for Stage II only

Plasma HIV-1 RNA

Screening HIV-1 RNA must be performed within 120 days prior to study entry by a laboratory that possesses a CLIA certification or equivalent using any FDA-approved assay. Eligibility will be determined based on the screening value.

Stored Blood for Quantitative CMV Assay (Batched)

CMV DNA by PCR will be performed on frozen whole blood collected and batched for testing at a central laboratory that possesses a CLIA certification or equivalent and is certified for protocol testing by the DAIDS VQA Program.

6.3.9 Viral Reservoir Studies for Stage II only samples for the viral reservoir studies will be collected and stored from all subjects.

Stored PBMC for Cell-associated HIV-1 RNA and DNA

The cell-associated HIV-1 RNA and DNA will be performed on cryopreserved PBMC.

Stored Plasma for Single-copy HIV-1 RNA Testing

6.3.10 Other Studies for Stage II only

Stored Plasma for Future Unspecified Assays

Plasma will be collected, processed, and stored for analyses.

Stored PBMC for Future Unspecified Assays

PBMC will be collected, processed, and stored for analyses.

6.3.11 Brachial FMD for Stage II only

All FMD assessments should be performed at the same time of the day (i.e., in the morning within half an hour of a previous FMD). The subject must be afebrile (oral T <38 °C or <100.4 °F) for at least 48 hours, be fasting, and have abstained from exercise and smoking for at least 12 hours prior to the FMD assessments.

Paired FMD (At entry)

Two FMD assessments are requested on all subjects, but are not mandatory. During paired FMDs, first performance will be done without nitroglycerin and following performance will be done with nitroglycerin.

It is preferable to separate the paired FMDs by at least 24 hours, but if this is not possible, it is acceptable to separate the paired FMDs by at least 30 minutes.

The first FMD must occur within 30 days prior to study entry and must be confirmed as acceptable.

The second FMD assessment must be completed within 72 hours after the first FMD and must occur before the subject takes the first dose of study treatment.

Single FMD (At subsequent visits)

FMD assessments will also be performed at weeks 12 and 18 and at the premature discontinuation visit (if applicable). If the FMD cannot be performed within the specified window of the originally scheduled week 12 or week 18 visit, the subject must return to the clinic as soon as possible to complete the FMD assessment. The subject will remain on study for continued follow-up.

6.3.12 FDG PET/CT scan for Stage II only

The subject must be fasting and have eaten a protein only meal the night for at least 12 hours prior to the FDG PET/CT scan. The patient will be asked to avoid consuming alcohol and strenuous exercise for at least 24 hours prior to the scan. The scan will also be performed at entry and week 18. If the scan cannot be performed within the specified window of the originally scheduled entry or week 18 visit, the subject must return to the clinic as soon as possible to complete the FDG-PET/CT scan. A qualified FDG-PET/CT interpreter will measure the image within a week of the scan and may find it necessary to repeat the scan within one week due to subpar image quality at either entry or follow-up. Study drug dispensing will be delayed until after the repeat scan (if needed at entry) is completed.

The subject will remain on study for continued follow-up.

6.3.13 Adherence Assessments

Adherence to ART medications will be by self-report. Adherence to ART medications will be evaluated at entry. Post entry, adherence to study treatment and ART medications will be evaluated at all visits. Adherence to study treatment will be verified by nurses at the study site.

6.3.14 Medication Diary for Stage II only

At the entry study visit, a medication diary will be given to subjects who will have PK assessments done at entry and week 4, 12, 18, 24, and 36 study visits. Subjects will be instructed to record the doses of their ART medications on the 4 days preceding the PK study visit.

6.3.15 PK Assessments for Stage II only

PK assessments will be carried out at entry and week 4, 12, 18, 24, and 36 study visits.

6.3.16 Depression Questionnaire for Stage II only

Optional questionnaire administered at every study visit.

6.3.17 Transcranial Doppler Imaging and Neurocognitive Substudy for Stage II only

Optional substudy to occur at entry and week 18.

6.3.18 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form.

7.0 CLINICAL MANAGEMENT ISSUES

Criteria for subject management, dose interruptions, modifications, and discontinuation of treatment will be mandated only for toxicities attributable to canakinumab.

7.1 Toxicity

Clinical symptoms of any drug toxicities are required to be documented on the adverse event form and submitted within 10 days of the event, according to UCSF CHR protocol. The following guidelines come from the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events and should be used to grade severity.

Severity Grade	**Description**

Mild (1)	Symptoms causing no or minimal interference with usual social & functional activities
Moderate (2)	Symptoms causing greater than minimal interference with usual social & functional activities
Severe (3)	Symptoms causing inability to perform usual social & functional activities
Potentially Life-threatening (4)	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

7.1.1 Grade 1 or 2 Toxicity

Subjects who develop a Grade 1 or 2 AE or toxicity, except as stated in section 7.2, may continue study treatment. If a subject chooses to discontinue study treatment, complete the premature treatment discontinuation evaluations, and encourage the subject to complete any remaining study visits until the toxicity resolves.

7.1.2 Grade 3 Toxicity

If the site investigator has compelling evidence that the AE has NOT been caused by the study treatment, dosing may continue. Otherwise, subjects who develop a Grade 3 AE or toxicity should have study treatment withheld, except as stated in section 7.2. The subject should be reevaluated closely until the AE returns to Grade ≤2, at which time study treatment may be reintroduced at the discretion of the site investigator or according to standard practice. Subjects experiencing Grade 3 AEs requiring permanent discontinuation of study treatment should be followed closely for resolution of the AE to Grade ≤2 and the protocol core team must be notified.

7.1.3 Grade 4 Toxicity

Subjects who develop a Grade 4 symptomatic AE or toxicity will have study treatment discontinued, except as stated in section 7.2. If the site investigator has compelling evidence that the AE has not been caused by the study treatment, dosing may resume when the AE has resolved to Grade ≤2. Subjects experiencing Grade 4 AEs requiring permanent discontinuation of study treatment should be followed closely until resolution of the AE to Grade ≤2 and the protocol core team must be notified.

7.2 Management of Specific Toxicities

7.2.1 Hepatotoxicity

LFT monitoring will be performed. In the event that there is an increase in ALT or AST ≥2 x ULN, the canakinumab dose will be reduced downward and LFT

blood testing will be repeated every 2 weeks to determine if the values have returned to normal range.

7.2.2 Hypertriglyceridemia/Hyperlipidemia

If elevated triglycerides or lipid levels are from a non-fasting blood draw, repeat the draw after a 12 hour fast. Only levels done in a fasting state should be used to determine toxicity management. Subjects with asymptomatic Grade ≥ 3 triglyceride, total cholesterol, or LDL cholesterol elevations may continue study drugs, at the discretion of the site investigator.

7.3 Pregnancy

Pregnancy should be avoided if either partner is receiving canakinumab during the study and for a minimum of 3 months after therapy for male subjects. Male subjects will be required to agree to the use of two forms of contraceptive during the entire study duration. In this study, all individuals will be made fully aware of the risks while taking canakinumab. However, after study entry, male subjects whose partners become pregnant must notify site personnel within 24 hours after learning about or suspecting pregnancy. Male subjects may continue on study/on study treatment

Male subjects whose partners become pregnant will be considered on study until the outcome of the pregnancy and the 6 month infant assessment has been obtained and reported on the CRF.

Male subjects whose partners become pregnant within 12 weeks after the final study visit or last dose of study drugs should inform site personnel within 24 hours after learning about or suspecting pregnancy. Sites will collect data on pregnancy-related complications and pregnancy-related outcomes. A telephone contact or clinic visit will be conducted at 6 months post partner's delivery to collect data regarding the health of the infant.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Permanent Treatment Discontinuation

- Drug-related toxicity (see section 7.1 Toxicity).
- Reaching a primary safety endpoint (defined in section 9.2.1.1).
- Completion of treatment as defined in the protocol.
- Request by subject to terminate treatment.
- Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity section of the protocol.
- Not taking ART for 2 consecutive weeks.

8.2 Premature Study Discontinuation

The following is a list of possible reasons for study treatment discontinuation:

- Request by the subject to withdraw.

- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the IRB, Food and Drug Administration, Office for Human Research Protections (OHRP), NIAID, other government agencies as part of their duties, or investigator.

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This is a double-blind, 52 week study to evaluate if treating virologically suppressed HIV-infected individuals with canakinumab is safe and reduces CVD risk as measured by endothelial function and HIV-associated inflammation.

Due to the lack of safety data on canakinumab in patients with HIV, we will perform our study in two stages. In Stage I, 10 individuals will all receive 150mg canakinumab once and undergo followup for 12 weeks.

9.2 Endpoints

9.2.1 Primary Endpoints

9.2.1.1 Safety

Subjects who experienced any one of the following safety milestones during study follow-up (from entry to week 52) will be considered to have reached the primary safety endpoint:

- For subjects with an entry CD4+ T-cell count <700 cells/mm^3, a confirmed CD4 decline >33% of baseline and to an absolute value <350 cells/mm3
- For subjects with an entry CD4+ T-cell count ≥700 cells/mm^3, a confirmed CD4 decline >50% of baseline

- Confirmed HIV-1 RNA >200 copies/ml in the absence of interruption in HIV medication
- New or recurrent CDC category C AIDS indicator condition
- Evidence of HIV-associated infection including CMV end-organ disease, thrush, HSV reactivation, varicella zoster, EBV-related clinical disease

9.2.1.2 Endothelial function

Change from baseline to week 18 in brachial artery FMD (%) defined as the maximum FMD (%) calculated from RH 45, 60, 75, and RH 90 relative to resting artery diameter (baseline).

9.2.2 Secondary Endpoints

9.2.2.1 Secondary/supportive measures of endothelial function

- Change from baseline to week 12 in brachial artery FMD (%)
- Change from baseline to week 12 in brachial artery diameter (mm)
- Change from baseline to week 18 in brachial artery diameter (mm)
- Change from baseline to week 18 in brachial artery hyperemic flow velocity (time-integrated, cm)

9.2.2.2 Vascular inflammation as measured by FDG-PET/CT scan from baseline to week 18

9.2.2.3 Markers related to CVD risk, inflammation, and coagulation

Changes from baseline to week 24 in levels of hsCRP, IL-6, sCD163, and D-dimer.

9.2.2.4 Markers of monocyte subpopulations, adhesion and activation indices, and CX3CR1 expression

Change from baseline to week 24 in monocyte levels, adhesion and activation indices, and CX3CR1 expression.

9.2.3 Exploratory Endpoints

9.2.3.1 Exploratory soluble and cellular biomarkers

Change from baseline to week 24.

NOTE: The final choice of soluble and cellular biomarkers to be evaluated will be determined closer to the time of analysis based on the current state of knowledge and interest at that time. Biomarkers currently of interest include:

- Inflammation, coagulation, and microbial translocation: sCD14, IP-10, tissue factor, and lipopolysaccharide (LPS)
- Monocyte activation: Expression of CD14 and CD16, their activation marker (CD69) and homing molecule (CX3CR1) expression
- Immune activation: CD38+HLA-DR+ expression on peripheral CD4+ and CD8+

- Immune senescence: CD57+CD28+ expression on peripheral CD4+ and CD8+ cells

9.2.3.2 Reactivation of latent CMV and CMV replication

Qualitative (detectable and undetectable) and quantitative CMV DNA levels at baseline and over time to week 24.

NOTE: For qualitative outcome, baseline CMV level will be considered detectable if either the screening or entry samples have detectable virus.

9.2.3.3 HIV-1 persistence

HIV-1 RNA level at baseline and over time as quantified by Abbott assay with limit of quantification of 40 copies/mL categorized as no signal detected; signal <40 copies/mL; signal ≥40 copies/mL.

HIV-1 RNA level at baseline and over time as quantified by SCA. Quantitative and qualitative endpoint (detectable/undetectable) will be evaluated.

NOTE: For the qualitative outcome, week 18/24 HIV-1 RNA will be considered detectable if either the week 18 or week 24 samples have detectable virus.

9.2.3.4 Cellular HIV-1 expression

Changes in HIV-1 DNA: RNA ratios in CD4+ T-cells from baseline over time to the average of weeks 18 and 24.

9.3 Randomization and Stratification

Study participants will be randomized in a double-blinded fashion in a 2:1 ratio of canakinumab to placebo. A blocked randomization scheme will be generated using SAS Proc PLAN.

9.4 Sample Size and Accrual

Power calculations (Aim 1): All power calculations were performed using SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina, USA) for 80% power, with all calculations assuming a two-tailed $\alpha=0.05$.

Stage I: The primary focus of the initial pilot study is to verify the safety of canakinumab. Rates of AEs will be tabulated, with exact confidence intervals. With 10 participants, the ability of this pilot study to detect the occurrence of rare events is limited; however, common toxicities are likely to be detected. If none of the 10 subjects getting active treatment experience any AEs, then the upper confidence bound for the probability of having an AE would be 31% (using an exact binomial interval); hence, we would have strong evidence that the probability of having an AE is less than 50%.

The sample size was determined based on the primary endpoint for endothelial function, while at the same time ensuring that the study will be well powered to demonstrate the safety of the intervention.

9.4.1 Endothelial Function

Based on a superiority trial design with two-sided α=0.05, assuming a standard deviation (SD) of 3% and a 10% drop out rate, a sample size of 100 participants (67 active, 33 placebo) will ensure 80% power to detect a between-groups difference of 1.9%. An effect size of 1.5-2% change in FMD is considered clinically relevant and is detectable with brachial artery FMD studies of this size[42]. This magnitude of FMD improvement has been seen in studies of ART[43] and statins[44, 45] in patients with HIV-infection. In the Cardiovascular Health Study[46] a 1% FMD difference was associated with an ~14% adjusted CVD risk reduction.

9.4.2 Vascular Inflammation

We will also measure between-treatment arm differences in the change aortic TBR (the measure of arterial inflammation by PET/CT). The assumed SD for change in TBR is 0.18; this value is derived from Elkhwad et al[47] and takes into account the multi-center nature of PET/CT measurements in this study. That SD is chosen conservatively, since it is for change in TBR from the aorta and carotid arteries combined (which is a secondary endpoint in this application, and which is associated with a larger SD for change compared to single-vessel endpoints). Based on previous work in a comparable cohort by Subramanian et al[12], the anticipated average baseline value in our cohort will be approximately 2.23. Based on a superiority trial design with two-sided α=0.05, a total of 82 subjects entering and 66 subjects providing complete, evaluable data sets will ensure 93% power to detect a between-groups difference of 0.156 (which is 7% of a mean baseline TBR value of 2.23). The minimum meaningful expected change on therapy of 7% is based on the observation that low-dose and moderate dose statins are associated with ~8%[48] and ~19%[49] reduction in TBR, respectfully. Of note, the SD for change in TBR for the aorta, the primary endpoint in this application, is smaller (0.14)[50] and hence we expect to have larger residual power than calculated here.

9.4.3 Safety – Stage II:

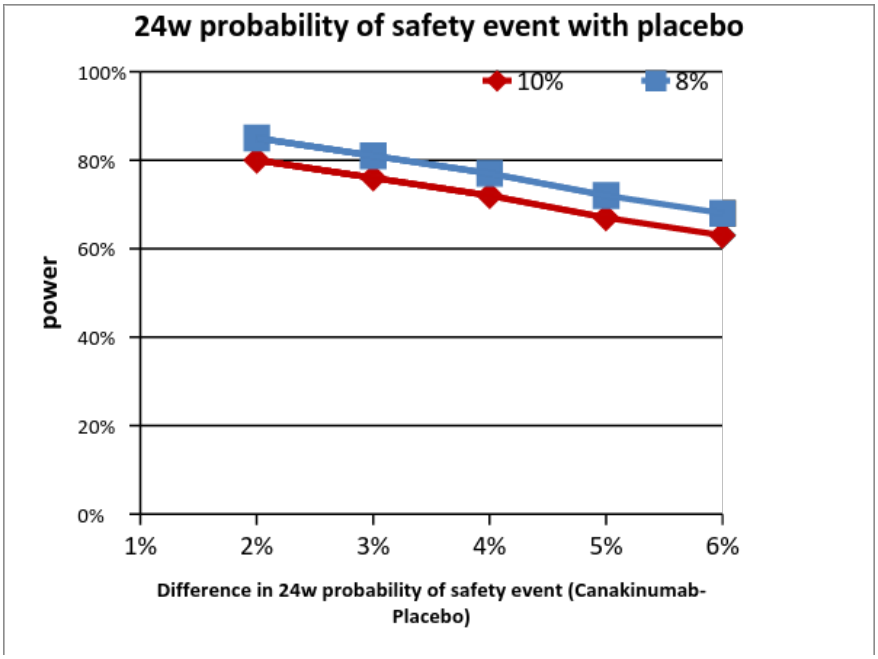
Safety of active treatment will be demonstrated if it can be shown that active treatment provides no more than an 18% point absolute higher rate of safety events than the background rate. That is, a non-inferiority hypothesis on the difference in the proportions of subjects with safety events between the groups utilizing a non- inferiority bound of 18%.

Assuming an underlying true probability of safety endpoints of 8% with placebo compared to 10% with treatment, accepting a one-sided 10% type 1 error rate, and assuming 10% dropout rate, a sample size of 30 subjects in placebo group and 60 subjects in treatment group will provide 80% power to

demonstrate that the difference in the probability of a safety endpoint between the two treatment strategies is less than 18%.

The power of the study to rule out a 18 percentage point (absolute) increase in the probability of a safety event over 24 weeks will be adversely impacted if the true event probability in the placebo group is higher than that assumed or if treatment results in more than an absolute 2 percentage point increase in the event probability (Figure 9.1).

Figure 9.1: Power to exclude a difference in the 24 week probability of safety events (canakinumab – placebo) of 18 percentage points (absolute) or greater with a sample size of N=30 in placebo and N=60 in canakinumab group.



9.4.4 Viral Reservoir Studies

A sample size of 30 subjects in placebo and 60 in canakinumab group will provide sufficient precision to estimate the difference in the proportion of subjects with undetectable viremia between the two groups with a 95% confidence interval with maximal width of 0.30 (+/-0.15).

9.5 Monitoring

Summaries of deaths and serious and targeted adverse events pooled across study regimens will be reviewed on a regular basis by the protocol core team. The core protocol team will also review study conduct in terms of pooled rates of accrual and study and treatment discontinuation as well as completeness of data and specimen collection for key study endpoints (pooled across study regimens).

The study will undergo quarterly reviews for safety by the Study Monitoring Committee (SMC) that includes Dr. Currier, Dr. Lerman, Dr. Marconi, Dr. Sandler, Dr. Lederman and Dr. Baker.

9.6 Analysis Plan

9.6.1 Safety

Analytic plan: We will compare baseline characteristics by treatment group (canakinumab vs. placebo) using a t-test for normally distributed variables and using the Mann-Whitney U test for continuous variables that are non-normally distributed, and Fisher's exact test for categorical variables. Analyses for aim 1 will involve comparisons of continuous measures at multiple visits following treatment initiation (through week 52). We will use linear mixed models to evaluate the association of treatment group with CD4 count and HIV RNA level, in separate models for each outcome. Because these measures are known to be right-skewed, each outcome will be log-transformed for analysis; results will be back-transformed to produce estimated percentage differences by treatment group. To account for the possibility of missing data due to loss to follow-up or study discontinuation, which could bias the treatment effect, we will adjust estimates using an inverse probability weighting approach[51]. We will model each participant's probability of having a non-missing outcome, with separate weights calculated at each visit. The inverse of this probability will then be used as a weight applied to persons with non-missing outcomes in the regression analyses.

9.6.2 Secondary/Exploratory Outcomes

Primary inference for all remaining continuous study outcomes will be based on a comparison of the change in the endpoints from baseline to week 24 between the two study groups using a stratified Wilcoxon rank sum test using an intent-to-treat approach including all randomized subjects. Subjects with missing week 24 outcome data will be imputed with worst rank. These results will be supported by baseline adjusted analyses using stratified analysis of covariance. For all analyses, missing outcome data assumed to be ignorable.

All analyses will be presented with longitudinal scatter plots of observed changes by study group and with two-sided 95% confidence intervals of the within group changes and differences in changes between the two study groups. The proportions of subjects with detectable HIV-1 RNA levels, and detectable CMV will be plotted over time and compared by study arms at week 18/24 using Fisher's exact test. Given sufficient numbers of individuals with detectable levels of HIV-1 RNA and CMV, the rate of change in virus levels over time will be estimated.

Given adequate sample sizes within each stratum, exploratory subgroup analyses by current use of statins within the past 8 weeks versus none will be performed for all endpoints.

9.7 Pharmacovigilance Plan

Given the small number of patients and short exposure time in our study, we do not anticipate many unexpected malignances in our study. An unblinded expert from the DSMB will monitor adverse events of malignancy throughout the entirety of the study. In addition to looking at malignancy adverse event numbers, the unblinded expert will also review narratives to detect any unusual malignancies. Our study will incorporate a cancer adjudication SOP that our group has used for prior NIH-sponsored studies. After the study is completed, we will call patients once a year for 2 years to evaluate for malignances.

10.0 DATA MANAGEMENT AND COLLECTION

10.1 Data Management

All data for our proposed study will be managed by the UCSF Data Coordinating Center, which is housed in the Department of Epidemiology and Biostatistics. All issues related to data management, specimen storage and data analysis for this study will be directed by Dr. Jeffrey Martin, who co-directs the SCOPE cohort with Dr. Deeks.

The UCSF Data Coordinating Center was created to develop and support data management systems for clinical researchers. The web-based systems currently in use encompass all aspects of a clinical research study's life cycle. They are utilized by small, medium and large-sized studies both within and outside UCSF. The standard research data system is a customized hybrid of off-the-shelf software that combines decentralized data submission, centralized and remote data editing, and a centralized database structure designed to collect, transfer, and store data for clinical research studies. The system integrates scannable forms with Internet capabilities to provide rapid and timely access to highly accurate data. In this system, data are collected and transmitted via the Internet to the Coordinating Center by remote clinical sites. After the data have been electronically received by the Coordinating Center, they are assessed for quality via a combination of automated and manual processes and then written to the study database, which has a powerful and flexible data dictionary for subsequent query generation. Every 24 hours, queries (data discrepancies) are generated to identify potential errors in the study data. These queries are accessible via a secure study web site so that clinic staff can immediately resolve them in a timely manner. When appropriate, sites can audit data in real-time via the web site, which automatically generates a full audit trail. Data from outside sources such as participating laboratories are integrated into the study database. The ultimate result is elimination of delays in identifying missing data, improved data quality, and faster time to data analysis. The standard system includes a password-protected study web site which can be accessed from anywhere in the world for administrative coordination and data operations. The typical study web site has several distinct components: administrative (meeting planner, document archives), data system support (user manuals, Q&A), forms tracking, querying and editing reports, and other types of reporting (recruitment, retention, editing). Finally, the standard system is compliant with all Federal

Government confidentiality guidelines. The 13 staff members of the Coordinating Center, with experience in programming, web design, server set-up and maintenance, form development, and training users how to use the systems, are available to assist with all aspects of system set-up and maintenance.

Work already performed in SCOPE in developing the forms, the relational database, the web-based reporting, and the creation of complex derived variables presents an enormous cost and time saving for the proposed study.

10.2 Data Collection Instruments

All study participants complete an interviewer-administered questionnaire that will include a validated form developed specifically for Dr. Hsue's cardiovascular studies. These forms collect data regarding antiretroviral medication usage and adherence, quality of life, recreational drug use, HIV transmission behaviors and traditional cardiac risk factors. Study forms are created using Teleform Elite software (Cardiff Corp., Vista, CA) and are transmitted via an internet scanner to the Data Coordinating Center, where they are read by optical character recognition and stored in a Microsoft SQL-server database. After human verification of key text fields, an automated error and consistency-checking algorithm is performed, and all errors resolved on-line in real time by the original interviewers. Data from outside sources such as participating laboratories are integrated into the study database. The ultimate result is elimination of delays in identifying missing data, improved data quality, and faster time to data analysis. The standard system is compliant with all Federal Government confidentiality guidelines.

Questionnaire development. All study participants will complete interviewer- and self-administered questionnaires developed for this study. These forms will collect data regarding antiretroviral medication usage and adherence, as well as quality of life, recreational drug use, and HIV transmission behaviors. Study forms will be created using Teleform Elite software (Cardiff Corp., Vista, CA) and eventually transmitted via an internet scanner form our clinical research sites to the UCSF Data Coordinating Center, which is located several miles away. Using an established system, these forms will be read by optical character recognition and stored in a Microsoft SQL-server database. After human verification of key text fields, an automated error and consistency-checking algorithm will be performed, and all errors reported the next day on the study's password-protected website where they are resolved on-line by the original interviewers.

Instruments for this study will be developed based upon existing questionnaires used in the SCOPE cohort. Data obtained from these forms will be will be housed using the same variable labels and coding in our existing SCOPE database, thus eliminating substantial start up time and expense on database development. Detailed questionnaires similar to that in SCOPE will be administered at baseline and at the primary outcome visits. We will also develop shorter forms to be given at the interim visits. These forms will contain a standard set of questions addressing the following domains:

Demographic characteristics. At baseline and annually thereafter, we will obtain information on race/ethnicity (baseline only), education, employment, income, and residence.

General health. Participants will be asked about the occurrence and degree of severity of a list of 20 symptoms developed by the ACTG. This index is widely used in ongoing studies throughout the world. It includes symptoms commonly related to antiretroviral therapy. Information will also be obtained regarding emergency room use and hospitalizations.

HIV-related history. Participant's self-reported last known negative HIV antibody test and first known positive test will be obtained. Self-reported CD4+ T-cell nadir will also be recorded, but this will be supplemented by objective review of the each participant's medical chart. A focused chart review will also obtain all plasma HIV RNA and CD4+ T-cell counts performed since entry into clinic.

AIDS-related diagnoses. Participants will be asked about the occurrence of any AIDS-defining diagnosis listed in the 1993 Revised CDC AIDS case definition. All affirmative responses will be confirmed by clinical chart review.

Use of opportunistic infection prophylaxis. Participants will be shown a list of drugs used for prophylaxis and will be asked about past 4 month and current use.

Antiretroviral medication use and adherence. At each visit, participants will be asked about their use of antiretroviral medications in the prior 4 months and be prompted with a list of names as well pictures. As is performed in SCOPE, participants taking antiretroviral medications will be asked about the number of missed dosages in the past 4 days (asking about each day separately) and the total number of dosages missed in the prior 30 days. In addition, the time of the last dose of each drug will be recorded for future pharmacokinetic work using stored samples.

11.0 CLINICAL SITE MONITORING AND ADVERSE EVENT REPORTING

11.1 Clinical Site Monitoring

Site monitors under contract to the NIH will visit our clinical research site to review the individual subject records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physician progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

11.2 Adverse Event Reporting

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

A Serious Adverse Event (SAE) is defined as any AE that results in any of the following outcomes: death, a life-threatening adverse experience, inpatient hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect. Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

All subjects will be followed for possible adverse events throughout their involvement in the study. At each interaction study personnel will elicit subject input as to discomforts or adverse experiences while taking the medications. Subjects will be seen and interviewed by a study nurse at screening, day 0, and weeks 1, 2, 4, 8, 12, 13, 14, 15, 16, 18, 24, 30, 36, 40, 48 and 52. At each visit, they will be assessed for any new symptoms and vital signs will be obtained. A complete blood count, complete metabolic panel, CD4+ T cell count, and plasma HIV-1 RNA level will be performed on most visits. Subjects will also be seen after study drug administration is completed on week 24 (12 weeks after the last dose).

Adverse events will be reported within 10 working days of the Investigator's awareness of the event, according to UCSF CHR Guidelines. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed. If a subject is withdrawn from treatment due to an adverse event, the subject will be followed by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

12.0 HUMAN SUBJECTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

All protocols receive approval from the Committee on Human Research, UCSF's institutional review board. The consent process is conducted in accordance with the division's SOP, which is based on GCP and HHS guidelines. All staff at PHP have completed the NIH computer based-training on the Protection of Human Research Subjects. Certificates for this training are on file. In addition, all staff have completed the Human Subject Protections online course developed by UCSF. In accordance with HIPAA regulations, subjects will be identified by a four-digit code (as is currently done in SCOPE).

Research staff will ensure that candidates understand all elements of the consent form by addressing questions posed during the consent procedure and by asking for verbal confirmation that the candidate has no additional questions and verbally understands the purpose of the study, study intervention, basic procedures, risks, and that participation is voluntary. Subjects will be given as much time as they need to read, understand and sign the approved informed consent document. The subject will receive a signed/dated copy of the consent form to keep along with the UCSF Subject Bill of Rights and the HIPPA form to keep.

12.2 Subject Confidentiality

All biologic specimens and clinical data obtained from this study will be linked to this code and not to personal identifying information (e.g., name, social security number, medical record number). A key which will link the four-digit code to the personal information will be maintained in a secure pass-word protected file maintained by Dr. Hsue.

HIPAA Compliance: All sites are committed to ensuring that appropriate measures are taken to protect the privacy and confidentiality of all Personally-Identifiable Health Information (PHI) for which it is responsible. This includes compliance with all regulations set forth by the HIPAA Privacy Rule, as well as with existing State and Federal laws pertaining to PHI. All entering study volunteers will be given a copy of the Notice of Privacy Practices. Good faith efforts will be made to obtain each individual's written acknowledgement of receipt of the Notice(s). The rights mandated by the HIPAA Privacy Rule concerning an individual's ability to access, amend, and disclose certain sections of his or her own PHI are fully respected, and every effort will be made to assist patients who choose to exercise these rights.

12.3 Subject Recruitment

Recruitment of eligible subjects will be greatly aided by an established prospective, clinic-based cohort study of HIV-infected adults (the "Study of the Consequences of the Protease Inhibitor Era," or SCOPE (S. Deeks, PI, H8211-17887)). To date, SCOPE has enrolled over 2000 subjects: over 600 are expected to meet criteria outlined in this proposed study. During the enrollment period all SCOPE patients who come in for regular cohort check-ups will be asked if they would like to learn more about the canakinumab trial. Subjects in the SCOPE study have already given consent allowing the SCOPE team to approach them for other studies.

Dr. Felicia Sterman, a HIV physician in the community will refer individuals to the study.

When a protocol opens to accrual, our group initiates a large-scale education effort to inform both providers and patients about study aims, requirements, and procedures. Drs. Hsue and Deeks maintain a large primary care clinic and are in the clinic nearly every day.

12.4 Subject Compensation

Subjects will receive reimbursements to cover costs related to the study. They will receive the following amounts for each procedure: $25 at the completion of each study visit that does not include imaging, $25 for each FMD and $100 for each FDG-PET/CT scan.

13.0 PUBLICATION OF RESEARCH FINDINGS

Research findings from this study will be published in a timely manner. The principal investigator will have full control over the content of such publications.

14.0 BIOHAZARD CONTAINMENT

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

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

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