AIDS 347

IL-6 Blockade in Treated HIV Infection

Funded by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institutes of Health

NIAID Funding Mechanism: U01-AI-105937-01
DAIDS Protocol Number: 11946

IND Holder and Sponsor: Benigno Rodriguez, MD
Study Phase: I/II
This protocol will be performed under the following IND#: **121325**

Protocol Co-Chairs: **Benigno Rodriguez, MD**
Michael M. Lederman, MD

Study Coordinator: **Jane Baum, RN**

FINAL Version 4.0
March 12th, 2015

CONTENTS

SITES PARTICIPATING IN THE STUDY

This is a single-site study that will open at the Case Western Reserve University/University Hospitals Case Medical Center Clinical Research Site only.

PROTOCOL TEAM ROSTER

Co-Chairs

Benigno Rodriguez, MD
2061 Cornell Road, Suite 401
Cleveland, OH 44106
Phone: (216) 844-2057
Fax: (216) 844-5523
E-Mail: rodriguez.benigno@clevelandactu.org

Michael M. Lederman, MD
2061 Cornell Road, Suite 401
Cleveland, OH 44106
Phone: (216) 844-8786
Fax: (216) 844-5523
E-Mail: MXL6@case.edu

Study Coordinator

Jane Baum, RN BSN
2061 Cornell Road, Suite 301C
Cleveland, OH 44106
Phone: (216) 844-2546
Fax: (216) 844-5523
E-Mail: baum.jane@clevelandactu.org

Data Manager

Preeti Tuscano
Case Western Reserve University
2061 Cornell Road, Suite 307
Cleveland, OH 44106
Phone: 216-844-3605
E-Mail: Tuscano.Preeti@clevelandactu.org

Research Nurses

Kristen Allen, RN BSN
2061 Cornell Road, Suite 301D
Cleveland, OH 44106
Phone: (216) 844-8136
Fax: (216) 844-5523
E-Mail: allen.kristen@clevelandactu.org

Patricia Walton, RN BSN
2061 Cornell Road, Suite 301B
Cleveland, OH 44106
Phone: (216) 844-3259
Fax: (216) 844-5523
E-Mail: walton.trisha@clevelandactu.org

Pharmacist

Michael Banchy, RPh
11100 Euclid Ave #122, Suite B-52
Cleveland, OH 44106
Phone: (216) 844-8018
Fax: (216) 844-1445
E-Mail: Michael.banchy@uhhospitals.org

Statistician

Curtis Tatsuoka, PhD
Departments of Neurology and Epidemiology and Biostatistics
Case Western Reserve University
2103 Cornell Road, Suite 6206
Cleveland, OH 44106
Phone: (216) 368-6724
Fax: (216) 368-4835
E- Mail: cmt66@case.edu

Laboratory Coordinator

Daniela Moisi
2109 Adelbert Road, Suite 1048B
Cleveland, OH 44106
Phone: (216) 368-4853
Fax: (216) 368-5415
E-Mail: Moisi.Daniela@clevelandactu.org

Regulatory Coordinator

Cheryl Smith
2061 Cornell Road, Suite 303
Cleveland, OH 44106
Phone: (216) 844-8052
Fax: (216) 844-5523
E-Mail: smith.cheryl@clevelandactu.org

DAIDS Medical Officer

Randall L. Tressler, MD
5601 Fishers Lane
Rm 9E47
Rockville, MD 20852
Phone: (917) 547-7279
E-Mail: randall.tressler@nih.gov

Unit Administrator

Mary Robertson
2061 Cornell Road, Suite 401
Cleveland, OH 44106
Phone: (216) 844-2342
Fax: (216) 844-5523
E-Mail: robertson.mary@clevelandactu.org

STUDY MANAGEMENT

All questions concerning this protocol should be sent to robertson.mary@clevelandactu.org. Ms. Robertson will direct the communications to the most appropriate team member. A response should generally be received within 24 hours (Monday-Friday).

Clinical Management

For questions concerning entry criteria, toxicity management, concomitant medications, and coenrollment, contact the protocol co-chairs. Send an e-mail message to rodriguez.benigno@clevelandactu.org AND MXL6@case.edu. One of the co-chairs will address clinical queries.

GLOSSARY OF ABBREVIATIONS

AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
Apo	Apolipoprotein
ART	Antiretroviral therapy
ARV	Antiretroviral
AST	Aspartate aminotransferase
AUC	Area under the curve
BART	Brachial arterial reactivity testing
BUN	Blood urea nitrogen
CBC	Complete blood count
CDC	(United States) Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFSE	Carboxyfluorescein succinimidyl ester
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	Maximum serum concentration
CRF	Case report form
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DAIDS	Division of AIDS
DCRU	Dahms Clinical Research Unit
DMARD	Disease-modifying antirheumatic drug
DMPA	Depot medroxyprogesterone acetate
DNA	Deoxyribonucleic acid
EAE	Expedited adverse event
ELISA	Enzyme-linked immunosorbent assay
FDA	(United States) Food and Drug Administration
FMD	Flow-mediated dilatation
GALT	Gut-associated lymphoid tissue
GCP	Good Clinical Practices
GFR	Glomerular filtration rate
GC-MS	Gas chromatography–mass spectrometry
HANC	HIV/AIDS Network Coordination
HBcAb	Hepatitis B core antibody
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HOMA-IR	Homeostatic model assessment of insulin resistance
IATA	International Air Transport Association

IFABP	Intestinal fatty acid binding protein
IGRA	Interferon-gamma release assay
IL	Interleukin
IND	Investigational new drug
IoR	Investigator of Record
IRB	Institutional review board
IUD	Intrauterine device
IV	Intravenous
LC-MS	Liquid chromatography–mass spectrometry
LDL	Low-density lipoprotein
Lp(a)	Lipoprotein(a)
LPS	Lipopolysaccharide
MOPS	Manual of operating procedures
mTOR	Mammalian target of rapamycin
NIAID	(United States) National Institute of Allergy and Infectious Diseases
NIH	(United States) National Institutes of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
OHRP	Office for Human Research Protections
OxLDL	Oxidized LDL
PBMC	Peripheral blood mononuclear cells
PD	Pharmacodynamic(s)
PD-1	Programmed cell death protein 1
PK	Pharmacokinetic(s)
PON-1	Paraoxonase/arylesterase 1
PRN	As needed
PRO	Protocol Registration Office
PT	Prothrombin time
PTID	Participant identification number
PTT	Partial thromboplastin time
RPR	Rapid plasma reagin
RA	Rheumatoid arthritis
RNA	Ribonucleic acid
SAE	Serious adverse event
sCD14	Soluble CD14
sCD163	Soluble CD163
sCD40L	Soluble CD40 ligand
SIU	Special Immunology Unit
SMC	Safety monitoring committee
SUSAR	Suspected and unexpected serious adverse reactions
sVCAM	Soluble vascular adhesion molecule
TCZ	Tocilizumab
TNF	Tumor necrosis factor
TNFr	Tumor necrosis factor receptor
UA	Urinalysis
UHCMC	University Hospitals Case Medical Center
ULN	Upper limit of normal
USPI	U. S. Prescribing Information

VLDL	Very low-density lipoprotein
VTI	Hyperemic velocity time integral

AIDS347: IL-6 Blockade in Treated HIV Infection

INVESTIGATOR SIGNATURE FORM

FINAL Version 4.0
12 March 2015

Funded by:
Division of AIDS, US National Institute of Allergy and Infectious Diseases
US National Institutes of Health

IND Holder and Sponsor:
Benigno Rodriguez, MD

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I will comply with all requirements regarding the obligations of investigators as outlined in the Statement of Investigator (Form FDA 1572), which I have also signed. I agree to maintain all study documentation for at least two years following the date of marketing approval for the study product for the indication in which it was studied. If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued, the US Food and Drug Administration is notified, and the site's final Financial Status Report is filed with the National Institutes of Health (NIH).

I have read and understand the information in the U.S. Prescribing Information (USPI) including the potential risks and side effects of the products under investigation, and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____
Name of Investigator of Record

_____ _____
Signature of Investigator of Record Date

SCHEMA

DESIGN — The study is a phase I/II, double-blind, placebo-controlled, randomized cross-over clinical trial of tocilizumab (TCZ) or placebo in HIV-infected subjects receiving antiretroviral therapy with suppressed viral replication and CD4+ T cell count ≥350 and ≤1,000 cells/mm^3)

DURATION — 48 weeks (40 weeks during cross-over treatment periods) total per subject.

SAMPLE SIZE — 30 subjects with complete data up to week 30. Up to 36 subjects may be enrolled in order to achieve an estimated final sample size of 30 participants with complete data up to week 30.

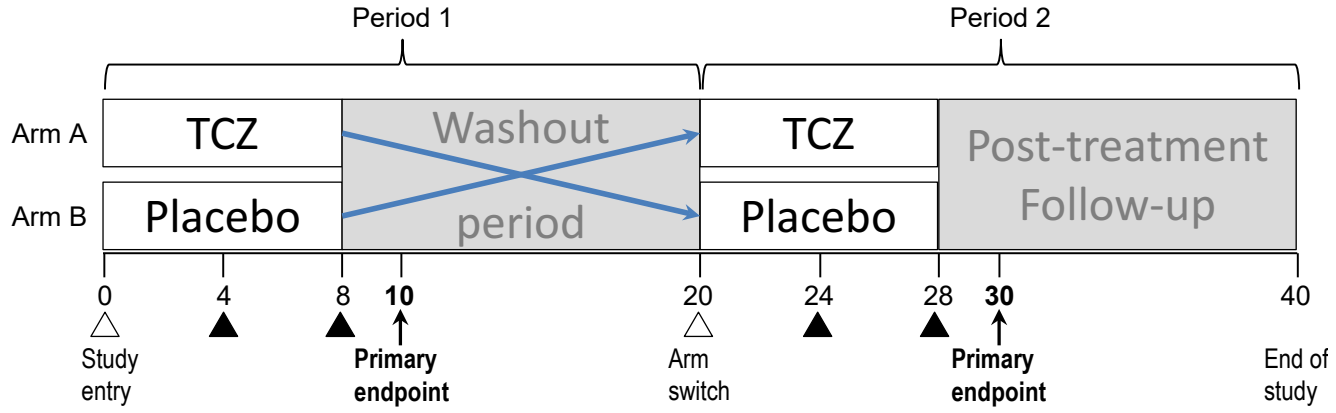
POPULATION — HIV-infected male and female subjects from 18 through 60 years of age receiving combination antiretroviral therapy (ART) without changes in the 24 weeks prior to enrollment (changes for reasons other than virologic failure allowed up to 8 weeks prior to enrollment), with suppressed plasma HIV RNA (<200 copies/mL, one blip up to <1,000 copies/mL permitted) for at least 96 weeks and a CD4+ T-cell count ≥350 and ≤1,000 cells/mm^3 at the time of study enrollment.

REGIMEN — Subjects will be randomized 1:1 to one of the following arms:

ARM A: TCZ, 4 mg/Kg by IV infusion over 60 minutes (not to exceed 400 mg) once at study entry, followed by TCZ, 8 mg/Kg by IV infusion over 60 minutes (not to exceed 800 mg) at weeks 4 and 8, and THEN placebo by IV infusion at weeks 20, 24, and 28.

ARM B: Placebo by IV infusion at study entry followed by placebo by IV infusion at weeks 4 and 8, and THEN TCZ, 4 mg/Kg (not to exceed 400 mg) by IV infusion over 60 minutes once at week 20, followed by TCZ, 8 mg/Kg (not to exceed 800 mg) by IV infusion over 60 minutes at weeks 24 and 28.

Figure 1. Study schema for the on-treatment period. Triangles indicate the dates of study agent administration. Empty symbols = 4 mg/Kg dose; solid symbols = 8 mg/Kg dose.



1.0 HYPOTHESES AND STUDY OBJECTIVES

1.1 Hypotheses

We hypothesize that TCZ, a humanized monoclonal antibody against the IL-6 receptor, at a dose of 4 mg/Kg once followed by two doses of 8 mg/Kg every four weeks beginning 4 weeks after the first dose, will be safe and well tolerated among HIV-infected subjects receiving suppressive antiretroviral therapy.

Additionally, we hypothesize that TCZ will reduce systemic inflammation, reduce abnormal central memory CD4 T cell cycling, and restore systemic and gut immune cell responsiveness to IL-7 through enhancement of CD127 expression.

We further hypothesize that TCZ will improve the cardiometabolic risk profile (as reflected by improved brachial artery endothelial function, decreased procoagulant indices, and/or diminished serum levels of inflammatory and atherogenic).

Finally, we hypothesize that TCZ will promote a transcriptomic and metabolomic profile that more closely resembles that seen in patients who have achieved robust CD4 T cell count reconstitution after suppressive antiretroviral therapy, with downregulation of the interferon and inflammatory pathways that we have previously described are commonly upregulated among patients with blunted CD4+ T cell count reconstitution.

1.2 Primary Objectives

1.2.1 To assess the safety and tolerability of tocilizumab, at a dose of 4 mg/Kg once followed by two monthly doses of 8 mg/Kg beginning 4 weeks after the first dose, among HIV-infected subjects fully suppressed on stable antiretroviral therapy with a plasma HIV RNA below 200 copies/mL and a CD4 T cell count ≥350 and ≤1,000 cells/mm^3.

1.2.2. To determine if the mean serum C-reactive protein (CRP, a marker of inflammation) level two weeks after the last dose of study agent (weeks 10 and 30) is significantly lower within subjects after TCZ than after placebo, in a mixed-effects model that includes CRP level at the beginning of each study period (study entry and week 20).

1.2.3 To determine if the proportion of central memory CD4 T cells expressing the nuclear antigen Ki67 (a reflection of cell cycling) two weeks after the last dose of study agent (weeks 10 and 30) is significantly lower within subjects after TCZ than after placebo, in a mixed-effects model that includes CD4 T cell cycling at the beginning of each study period (study entry and week 20).

1.3 Secondary Objectives

1.3.1 To determine if TCZ, at the doses described above, will enhance expression of the IL-7 receptor α chain CD127 on peripheral blood and gut CD4 T cells from

the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.2 To establish whether TCZ administration will reduce serum CRP and central memory cycling from the beginning of each study period (study entry and week 20) to any post-TCZ administration time point.

1.3.3 To determine if TCZ administration will enhance CD127 expression on peripheral CD4 T cells, as well as on rectal CD4 T cells among those subjects who choose to provide rectal biopsy specimens, from the beginning of each study period (study entry and week 20) to any post-TCZ administration time point.

1.3.4 To assess whether TCZ will increase bcl-2 expression and/or decrease annexin V binding by Ki67+ T cells from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.5 To assess whether TCZ will enhance ex vivo responsiveness to IL-7, as reflected by Stat 5 phosphorylation and α4β7 expression, and in vivo responsiveness to IL-7 as reflected by spontaneous expression of α4β7 by peripheral CD4 T cells from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.6 To establish whether TCZ will result in decreased CD4 T cell activation, as manifested by CD38/HLA-DR coexpression from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.7 To determine whether TCZ will reduce plasma levels of IL-7, and whether circulating IL-7 level changes correlate with CD127 expression on CD4 T cells at each timepoint and from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.8 To assess whether TCZ will increase CD4 T cell density in the gut associated lymphoid tissue (GALT), and GALT lymphocyte expression of IL-17, and IL-22 from baseline to 2 weeks after the last dose in each period of the trial (weeks 10 and 30).

1.3.9 To determine whether TCZ will replenish numbers and function of innate lymphoid cells (ILC) and cells expressing retinoid-related orphan receptor gamma from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.10 To establish whether TCZ will increase expression of mucosal barrier genes (occludins and claudins) by rectosigmoid epithelial cells from baseline to 2 weeks after the last dose in each period of the trial (weeks 10 and 30).

1.3.11 To assess whether TCZ will reduce the extent of mucosal breach and inflammatory infiltration of the distal colonic submucosa from study entry to 2 weeks after the last dose in each period of the trial (weeks 10 and 30).

1.3.12 To determine whether TCZ will decrease other plasma biomarkers of gut barrier integrity, microbial translocation, inflammation and coagulation, including lipopolysaccharide (LPS), soluble CD14 (sCD14), tumor necrosis factor receptors I and II (TNFrI and TNFrII), D-dimers, and intestinal fatty acid binding protein (IFABP), and increase levels of the plasma marker of intestinal barrier integrity zonulin from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.13 To study whether TCZ will lead to improved endothelial function, as reflected by the change in flow-mediated dilatation and hyperemic velocity time integral from baseline to 2 weeks after the last dose in each period of the trial (weeks 10 and 30), and whether changes in endothelial function indices will correlate with soluble markers of inflammation and microbial translocation at those time points.

1.3.14 To assess whether TCZ will reduce tissue factor expression on patrolling and inflammatory monocytes from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.15 To establish whether TCZ will reduce levels of selected cardiovascular risk markers, including soluble vascular adhesion molecule (sVCAM), soluble CD40 ligand (sCD40L), soluble CD163 (sCD163), P-selectin (CD62P), and E-selectin from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.16 To explore the effects of TCZ on systemic levels of inflammatory and atherogenic lipids and on lipoprotein profile, including HDL, LDL, and VLDL cholesterol, apolipoproteins A1 and B (ApoA1 and ApoB), pro-inflammatory HDL, paraoxonase/arylesterase 1 (PON-1), oxidized LDL, and lipoprotein(a) (Lp(a)) from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.17 To assess whether TCZ will improve indices of insulin resistance, as reflected by changes in homeostatic model assessment of insulin resistance (HOMA-IR) from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.18 To determine whether TCZ administration is associated with changes in systemic levels of the adipokines adiponectin, leptin, and resistin from the beginning of each study period (study entry and week 20) to 2 weeks after the last dose in each period of the trial (weeks 10 and 30, respectively).

1.3.19 To define the transcriptomic profile of naïve and central memory CD4 T cells and monocyte subsets associated with IL-6 blockade by TCZ, and to correlate it with the plasma metabolomic profile in HIV-infected subjects during antiretroviral-induced suppression of viral replication.

1.3.20 To describe the safety profile of short-term administration of TCZ in HIV-infected subjects receiving a stable antiretroviral regimen with suppressed viral replication and a CD4 T cell count ≥350 and ≤1,000 cells/mm^3.

1.3.21 To explore the effect of TCZ administration on trough levels of antiretrovirals, particularly protease inhibitors, in HIV-infected subjects receiving a stable antiretroviral regimen with suppressed viral replication and a CD4 T cell count ≥350 and ≤1,000 cells/mm^3.

2.0 INTRODUCTION

2.1 Background and Rationale for Blocking IL-6 as a Therapeutic Strategy in Immune Failure

2.1.1 Immune Failure and Immune Success with Antiretroviral Therapy

In the current treatment era, successful control of HIV replication typically promotes CD4 T cell restoration but a substantial minority of successfully treated subjects fails to increase CD4 T cell counts to levels that are within the normal range among HIV-uninfected persons. This phenomenon, which we have termed "immune failure", is linked to a greater risk of morbid complications (1-5), but its mechanisms are not well understood. We have established a cohort of immune failure subjects (the Cleveland Immune Failure –CLIF– cohort) who, despite at least 2 years of virologic control, failed to increase circulating CD4 T cell counts to at least 350 cells/mm^3 (6). In this group, there were profound decreases in all circulating CD4 T cell maturation subsets, as well as decreases in naïve CD8 T cells, implicating a failure of T cell homeostasis. Importantly, immune failure patients showed an elevated frequency of cycling CD4 central memory T cells but normal CD8 T cycling, despite persistent CD4 and CD8 T cell activation (CD38/HLA-DR coexpression) (6). Inflammatory (IL-6) and coagulation indices (D-dimers) were elevated as was monocyte activation, with increased levels of the soluble LPS receptor sCD14. While LPS levels tended to be higher in immune failures than in immune successes and healthy controls (21, 18 and 12 pg/mL respectively) these differences did not reach statistical significance (6). Thus, persons with immune failure on virologically suppressive antiretroviral therapy also have evidence of immune activation, inflammation and heightened coagulation.

2.1.2 Role of Immune Activation/Inflammation and Coagulation in HIV Disease.

There is increasing evidence that immune activation and inflammation are central to the pathogenesis of HIV-1-related disease. Suppressive antiretroviral therapies typically attenuate these phenomena, but persistent

activation/inflammation is often demonstrable in treated HIV-1 infection despite virologic control, especially among immune failures (6, 7). The drivers and determinants of activation and inflammation in this setting are not fully characterized. Yet, a growing body of data link activation/inflammation and coagulation to the morbidity of HIV-1 infection in the treatment era, as is also the case with other systemic inflammatory diseases.

In an analysis including both arms of the Strategic Management of Antiretroviral Therapy (SMART) trial, a large prospective, randomized trial comparing ART administration guided by CD4 count versus sustained virologic suppression(8), higher plasma levels of the cytokine interleukin-6, of C-reactive protein and D-dimers independently predicted morbidity and mortality (9).

2.1.3 Inflammatory Markers also Predict Mortality in Treated HIV-1 Infection.

In a recent study, higher plasma levels of inflammatory markers, markers of coagulation and of intestinal permeability were associated with mortality in a case-control study performed in two San Francisco cohorts (SOCA and SCOPE) (10). All participants had achieved virologic control with therapy and cases and controls were matched for CD4 T cell counts at treatment initiation. In these studies, being in the highest quartile of IL-6 levels was associated with an odds ratio for mortality of over 100-fold relative to subjects in the lowest quartile. Indices of gut mucosal barrier breach (high intestinal fatty acid binding protein and low zonulin) were also associated with mortality, while cellular markers of activation (coexpression of CD38 and HLA-DR), senescence (CD57, CD28-) or naïve cell numbers were not (10).

These results have been recently confirmed and expanded in a case control study of 450 HIV infected persons who initiated antiretroviral therapy and controlled viremia in the AIDS Clinical Trials Group ALLRT cohort (NWCS 329). In this study, before initiation of antiretroviral therapy, one year after treatment start and in the most recent sampling before the occurrence of a major morbid event (non-accidental death, MI, stroke, malignancy and major bacterial infection), inflammatory (IL-6, IP-10, sTFNr1, sTNFrII, sCD14), and coagulation (D-dimers) markers were significantly associated with increased odds ratios for these events. As in the study including SOCA and SCOPE participants cited above, IL-6 level was the most robust correlate of morbidity and mortality, and cellular activation markers did not predict outcome except for expression of programmed cell death protein 1 (PD-1) on CD4 T cells.

Thus, an inflammation profile, especially higher IL-6 levels, reproducibly predicts morbidity and mortality in treated HIV infection despite virologic control, and persons who maintain low CD4 T cell numbers are at greater risk for these events (1-5). Not surprisingly, these markers of inflammation and coagulation are strongly correlated with each other. While understanding cellular pathways of activation and inflammation may provide hints about the causal meaning of these associations (e.g., IL-6 can drive CRP(11) and TNF can drive IL-6 (12)), the numerous cell populations and tissues that express and respond to immune

signals *in vivo* may render these relationships even more complex than might be anticipated on the basis of pathway analyses in individual cell populations. Thus, an informative way to explore the relationships among these markers is to examine the response to an intervention that targets a plausible mediator and clinically correlated activity marker. In this study, we plan to explore the effects of IL-6, one of the two biomarkers consistently correlated with increased morbidity and mortality, by inhibiting IL-6 activity using TCZ. This will allow us to explore the effect the IL-6 pathway has on these inflammatory, activation and coagulation markers to determine the role of IL-6 in their regulation and interactions.

2.1.4 Interleukin-6 and its Potential Role in Immune Failure

IL-6 is a cytokine produced by multiple cell types, including T cells, B cells, monocyte/macrophages, and fibroblasts that exerts its biologic activity by binding to its cellular receptors CD126 and CD130. Soluble CD130 can bind IL-6 and inhibit its biologic activity, while IL-6 bound to soluble CD126 can interact with cellular CD130 inducing both JAK/STAT3 dependent and ERK 1 /2 MAP kinase activation. IL-6 induces acute phase reactant (e.g. CRP) expression in the liver and can act as an endogenous pyrogen. IL-6 has been implicated in demargination of circulating neutrophils and induction of adhesion molecules VCAM and ICAM-1 on endothelial cells (13, 14). First described as B cell stimulatory molecule, IL-6 promotes antibody production by promoting maturation of T follicular helper cells (15). IL-6 also is reported to increase mitogen-induced CD4 T cell proliferation, and this has been attributed to blockade of IL-2 production and Treg induction (16). In murine studies, IL-6 has been linked to induction of a TH2 response and inhibition of IL-12-induced Th1 responses (17). Together with TGFB, IL-6 may direct naïve T cells to acquire a TH17 phenotype (18). Thus, IL-6 exhibits both pro-inflammatory and anti-inflammatory features, and may be implicated in the maintenance of an accelerated, and perhaps maladaptive, state of T cell proliferation in response to a variety of stimuli.

While numerous mechanisms including virus-induced cell death likely contribute to CD4 T cell deficiency in untreated HIV infection, the determinants of persistent CD4 lymphopenia (immune failure) in treated HIV infection are incompletely understood but likely include both increased cellular death and a failure to sustain cellular production/maturation. It has been shown (19-21) that turnover of central memory CD4 T cells is an important feature of the immune deficiency in HIV infection and in the SIV model, the ultimate loss of these cells predicts mortality (22). While increased cycling of both CD4 and CD8 memory cells is readily demonstrable in untreated HIV infection (20, 23), immune failures with controlled HIV replication have both sustained high plasma levels of IL-6 and increased cycling and turnover of central memory CD4 T cells, while CD8 T cell cycling is normal (6). We have found that central memory CD4 T cell cycling and death can be induced *in vitro* by exposure to microbial TLR ligands (24). This appears to be mediated largely by the induction of IL-6, as the cycling of memory CD4 T cells induced by LPS is inhibited in the presence of the IL-6 receptor inhibitor TCZ and IL-6 alone is sufficient to induce cycling of central memory CD4 T cells. Meanwhile, CD8 T cells are unaffected, mirroring a phenotype

characteristic of immune failure in the presence of virologic control (6). *We therefore propose that elevated systemic levels of IL-6 are important drivers of central memory CD4 T cell cycling and turnover in immune failure.*

2.1.5 IL-6 May Block Cellular Restoration in Immune Failure by Decreasing Expression of the IL-7 Receptor α Chain (CD127).

Failure of CD4 T cell restoration in HIV infection has been linked to impaired thymic production (25, 26) and to increased lymphoid tissue fibrosis (27, 28). More recent work has suggested that collagen deposition in the fibroblastic reticular cell network in these nodes may block T cell access to the homeostatic cytokine IL-7 (29). IL-7 drives T cell proliferation and supports T cell survival by inducing expression of anti-apoptotic bcl-2 (30, 31). In HIV infection, diminished T cell expression of CD127, the IL-7 receptor α chain, may also limit the effectiveness of whatever IL-7 levels are available (32, 33). And while diminished CD127 expression has been linked to both immune failure and to immune activation in HIV infection (34, 35), the mechanisms whereby this might take place are not at all understood (36, 37).

In vitro, IL-6 exposure decreases the expression of CD127 on both CD4 and CD8 T cells, and that decrease in CD127 expression induced by LPS exposure is reversed by TCZ(38). We therefore hypothesize that sustained systemic exposure to IL-6 decreases T cell expression of CD127 *in vivo* and contributes to HIV associated immune failure.

2.1.6 HIV Replication does not Appear to Be a Major Determinant of IL-6 Expression and IL-6 Levels in Plasma Remain Elevated despite ARV-induced Control of HIV Replication.

Although several studies have demonstrated weak but significant relationships between HIV and IL-6 levels in plasma of untreated patients (39, 40), this relationship disappears within the first weeks of ARV therapy (41) and IL-6 levels in plasma remain elevated in effectively treated HIV infection (6, 41). *In vitro*, neither productive infection with HIV nor exposure to HIV could induce IL-6 expression by PBMC, macrophages, lymph node histocultures or colonic mucosal explants. Moreover, lymph node histocultures prepared from HIV infected subjects actually expressed less IL-6 spontaneously than did lymph node histocultures prepared from control subjects (41). Unlike exposure to HIV, exposure of lymphoid tissue to microbial products such as LPS and flagellin results in robust IL-6 induction.

2.1.7 The Gut May Be a Major Source of the Elevated IL-6 Levels in Treated HIV Infection.

Substantially elevated levels of IL-6 mRNA levels have been found in colonic biopsies of HIV-infected subjects when compared to levels among uninfected controls and were unrelated to the magnitude of plasma viremia (42, 43). We therefore hypothesize that the gut mucosa is a major source of the elevated

systemic levels of IL-6 that characterize treated and untreated HIV infection. In this model, HIV infection and elimination of gut mucosal CD4 T cells during the first few weeks of infection (44) result in diminished expression of the T helper cytokines IL-22 and IL-17, which are necessary to maintain an intact gut epithelium (45). The resultant breakdown in the gut epithelial tight junction permits greater transition of luminal microbial products into the mucosal lamina propria that then induce greater monocyte expression of pro-inflammatory cytokines, including upregulation of IL-6 expression. Exposure to increased IL-6 levels decreases CD127 expression on other mucosal T cells and ILC resulting in their death and/or failure to express IL-17 and IL-22, with attendant further breach of the intestinal barrier. A relationship between systemic translocation of microbial products and systemic IL-6 levels is also supported by our observation in a previous study (6) of a significant correlation between plasma IL-6 levels and plasma levels of bacterial lipopolysaccharide ($r = 0.287$; $P = 0.009$)(41). Yet, these relationships do not establish causality. The observations below suggest that such a causal relationship is plausible.

2.1.8 Increased Gut Mucosal Expression of IL-6 in HIV Infection May Be both Cause and Consequence of Microbial Translocation.

There is increasing evidence that gut mucosal defenses are dependent upon the interplay among mucosal defense cells, the mucosal epithelium, which comprises a key barrier to intraluminal retention of microbial elements, and the expression of mucosal defense cytokines such as IL-22, IL-23 and IL-17 (45). Mucosal defense cells include T cells, dendritic cells of several distinct lineages, natural killer cells, B lymphocytes, and a group of innate lymphoid cells (ILC) that lack lineage markers but are important sources of IL-22. These cells can be induced to express IL-22 by exposure to IL-23 (46) and IL-22 expressed by mucosal defense cells is a key inducer of the T helper cytokine IL-17, which is an important regulator of gut mucosal integrity (47). ILC are identifiable in human and mouse gut mucosae. In murine systems, their depletion permits systemic dissemination of a gram negative bacterium, Alcaligenes sp, that is at least partially corrected after systemic administration of IL-22 (46). Thus, ILC and their IL-22 product appear to play an important role in compartmentalization and containment of a gut luminal microbe.

In rhesus macaques, colonic mucosal CD4 and CD8 T cells express both IL-22 and IL-17 and in SIV infection, the frequencies of IL-22 producing and IL-17 producing T cells are diminished dramatically (48). In SIV infected rhesus, extensive LPS infiltration can be demonstrated by immunostaining (49), and this has substantial consequences for gut integrity as in SIV infected rhesus, the frequencies of IL-22 producing CD4 and CD8 T cells in colonic tissues and mesenteric lymph nodes correlate indirectly with an index of intestinal barrier breach (the ratio of claudin positive to claudin negative sites) (48). In acute SIV and HIV infection, depletion of CD4+ IL-22 producing cells is attributable to viral infection and cell death, yet this does not explain the CD8+ T cell defects or the frequent failure of gut CD4 T cell restoration after antiretroviral therapy (50) and the persistence of systemic microbial translocation despite virologic control (51).

2.1.9 Expression of the IL-7 Receptor Alpha Chain (CD127) May Be Necessary to Maintain the Frequency and Survival of Gut T Cells and ILC that Sustain Mucosal Integrity and Barrier Function.

IL-7 promotes T cell expansion and survival (52) and, when administered exogenously to HIV-infected persons with immune failure, induces dramatic expansion of circulating CD4 and CD8+ T cells (30, 31) without induction of a T regulatory phenotype such as is seen after systemic IL-2 administration (53). IL-7 may also play a critical role in maintaining gut mucosal integrity as both *in vitro* and *in vivo* IL-7 upregulates T cell expression of the α4β7 integrin, which promotes lymphocyte homing to gut mucosal sites (54, 55). By inducing expression of bcl2, IL-7 also promotes T cell survival and resistance to pro-apoptotic signals. This effect may not be restricted to an effect on T cells as ILC also express high levels of CD127 (46) and these cells are thought to be critically dependent upon IL-7 for survival (45). We hypothesize that IL-7 plays an important role in gut mucosal barrier integrity by both inducing expression of the gut homing receptor α4β7 and promoting survival of the gut mucosal T cells and ICL that are key sources of IL-22. Preliminary results from our group suggest that this could be the case as systemic IL-7 administration both increases CD4 T cell restoration in the gut and decreases systemic levels of sCD14 and D-dimers, known predictors of morbidity and mortality in treated HIV infection (55). We hypothesize that microbe-induced expression of IL-6 renders gut mucosal T cells and ILC less responsive to IL-7 dependent survival signals that perpetuates breach in the gut epithelial barrier, and anticipate that blockade of IL-6 action by TCZ will reverse this cycle, increasing mucosal T cell and ILC CD127 expression, promoting their survival, increasing IL-22 and IL-17 expression, and restoring gut barrier function with resulting attenuation of microbial translocation and further inflammation.

2.1.10 Cardiovascular Risk in Treated HIV Infection, the Role of Inflammation and IL-6

In the current HIV treatment era, a major cause of morbidity and mortality is cardiovascular disease (CVD) (56)**.** There is mounting evidence that CVD risk is increased in HIV infection despite virologic suppression and while “traditional” risk factors for cardiovascular events are important drivers of this risk, it is likely that the systemic inflammation that characterizes even treated HIV infection contributes to this risk as it does in a number of inflammatory rheumatologic conditions (57). A number of observations suggest that IL-6 may play a causal role in increased CVD risk. High plasma levels of IL-6 are associated with subsequent risk of myocardial infarction in the general population, and a systematic review of 17 large population-based prospective studies of IL-6 found a strong association between long-term average IL-6 levels and clinical coronary outcomes (MI or coronary death) (58). As shown in our preliminary data above, increased IL-6 levels predict overall morbidity and mortality, including cardiovascular morbidity and mortality in HIV infection. There is genetic evidence to support a role for IL-6 in coronary artery disease risk as a single nucleotide polymorphism in the IL-6 receptor gene that results in a non-synonymous receptor variant (Asp358Ala) has recently been linked to decreased CAD risk

and a clinical profile compatible with IL-6 receptor blockade in the general population (59, 60). Moreover, Mendelian analysis strongly suggested that the association is causal, rather than correlative (60). Finally, there is evidence that IL-6 blockade results in improved endothelial function, as reflected by flow-mediated dilatation after 6 months of treatment with TCZ in RA patients (61).

2.1.11 Other Proposed Interventions to Manage Inflammation in HIV Infection

Whereas a highly targeted approach such as the one proposed in this study is valuable in providing a narrowly focused intervention that allows for a greater understanding of the role of a critical inflammatory mediator in HIV pathogenesis, more broadly acting agents have also been proposed. Thus, the non-absorbable polymer sevelamer (ClinicalTrials.gov Identifier NCT01543958) and the non-absorbable antibiotic rifaximin (ClinicalTrials.gov Identifier NCT01866826) have been studied as options to block absorption and production of intestinal microbial products, respectively. The toll-like receptor (TLR) signaling inhibitor chloroquine has been shown to reduce cellular immune activation modestly in chronic HIV infection (62), and the antimetabolite methotrexate is now being studied as an anti-inflammatory in HIV-infected persons as well (ClinicalTrials.gov Identifier NCT00000834). Rosuvastatin has been shown to decrease markers of monocyte activation and the vascular inflammation marker Lp-PLA2 in treated HIV-infected subjects (63). Other proposed applications of statins in HIV infection take the opposite approach, and instead of using these agents as broadly anti-inflammatory interventions, they are being used as modulators of risk with the specific goal of reducing the occurrence of comorbid endpoints such as cardiovascular disease (ClinicalTrials.gov Identifier NCT00965185) and neurocognitive impairment (ClinicalTrials.gov Identifier NCT01263938). The angiotensin receptor inhibitor telmisartan has been shown to reduce visceral adiposity in HIV infection(64), but its effects on inflammation appear inconclusive and might include paradoxical increases in monocyte activation (65).

Thus, the intervention proposed in this study complements other proposed approaches, targets one of inflammatory biomarkers most consistently correlated with morbidity and mortality in HIV infected individual, modulates inflammation and immune activation in HIV infection, and is distinct from other planned strategies. In addition, it isolates a single step in the inflammatory cascade, thereby representing a unique opportunity to understand the contribution of IL-6 to the global state of heightened inflammation in treated HIV infection.

2.2 Rationale

2.2.1 Rationale for the Choice of Study Agent

As outlined above, IL-6 may play a role not only as a reflection of systemic inflammation and immune activation in treated HIV infection, but as a true mediator of pathogenesis, particularly in the setting of incomplete immune reconstitution. Blocking the biological activity of IL-6 in vivo would potentially have the double appeal of helping elucidate the precise etiologic role of IL-6 in

the pathogenesis cascade in treated HIV infection and exploring whether TCZ can reduce markers of inflammation, coagulation and immune activation that have been associated with increased risk of morbidity and mortality.

Tocilizumab (TCZ) is a humanized monoclonal antibody that binds and neutralizes both soluble and membrane bound IL-6 receptors and is a potent anti-inflammatory. It has been approved by the FDA for the treatment of:

- Adult patients with moderately to severely active rheumatoid arthritis (RA) who have had an inadequate response to one or more disease-modifying anti-rheumatic drugs (DMARD)
- Patients 2 years of age and older with active polyarticular juvenile idiopathic arthritis (PJIA)
- Patients 2 years of age and older with active systemic juvenile idiopathic arthritis (SJIA).

In Japan, TCZ has also been approved for treatment of Castleman's disease.

The onset of action and clinical benefits of TCZ in RA are rapid, with dramatic reductions of inflammatory markers observed by day 14 after starting therapy (66). Clinical signs and symptoms also may respond early and continue to improve over the first 12-24 weeks. CRP levels fall dramatically and rapidly after TCZ administration, as do other inflammatory markers. In a small pilot study of patients with rheumatoid arthritis, TCZ administration was associated with improvements in endothelial function, as assessed by flow-mediated dilatation (61). Thus, TCZ appears to be a potent blocker of IL-6 activity with easily demonstrable systemic anti-inflammatory activity. While the macrolide rapamycin has been shown to inhibit lymphocyte proliferation in response to IL-2, IL-4, and IL-6, it does so by binding to and inhibiting the downstream effects of the protein kinase mammalian target of rapamycin (mTOR), a regulator of protein translation essential to the control of the cell cycle; it likely has little effect on events proximal to IL-6. Moreover, the lack of specificity of rapamycin makes it difficult to attribute any observed effects to IL-6 inhibition alone.

2.2.2 Safety of TCZ

An overview of known adverse events associated with TCZ administration is provided below. Details of the TCZ risk mitigation strategy are provided in section 12.6.1.

Risks associated with the use of TCZ include infections, some leading to hospitalization or death, hypersensitivity reactions including anaphylaxis, gastrointestinal (GI) perforations, infusion reactions, laboratory abnormalities (neutropenia, thrombocytopenia, AST and ALT elevations, elevations of lipids) and malignancies. In the five Phase III clinical trials the most common AEs (>5% of patients) treated with 8 mg/kg TCZ monotherapy through 6 months of therapy were upper respiratory tract infection, nasopharyngitis, headache, hypertension and increased ALT. Viral reactivation has been reported with immunosuppressive

biologic therapies and cases of herpes zoster exacerbation were observed in clinical studies with TCZ.

2.2.2.1 Serious Infections

Serious infections leading to hospitalization or death due to bacterial, mycobacterial, invasive fungal, viral, protozoal or other opportunistic pathogens, have occurred in patients receiving immunosuppressive agents including TCZ for RA. In the 24-week, controlled clinical studies, the rate of serious infections in the TCZ monotherapy group was 3.6/100 patient-years compared to 1.5 per 100 patient-years in the methotrexate group. The rate of serious infections in the 4 mg per kg and 8 mg per kg TCZ plus DMARD group was 4.4 and 5.3 events per 100 patient-years, respectively, compared to 3.9 events per 100 patient-years in the placebo plus DMARD group. The most common serious infections included pneumonia, urinary tract infection, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis. Cases of opportunistic infections have been reported. Patients presented with disseminated rather than localized disease, and were often taking concomitant immunosuppressants such as methotrexate or corticosteroids which in addition to rheumatoid arthritis may have predisposed them to infections.

To mitigate the risk of serious infections, patients with a recent history or current active infections will be excluded, and patients with concomitant conditions or indications for medications that can contribute to further immunosuppression will also be excluded. Details of the risk mitigation strategy for serious infections can be found in section 12.6.1.1.

2.2.2.2 Hypersensitivity Reactions

Hypersensitivity reactions, including anaphylaxis and death, have been reported in association with infusion of TCZ. Clinically significant hypersensitivity reactions, including anaphylaxis associated with TCZ and requiring treatment discontinuation were reported in 0.1% (3/2644) in the 24-week, controlled trials and in 0.2% (8/4009) in the all-exposure population.

Appropriate medical treatment will be available for immediate use in the event of a serious hypersensitivity reaction, and all the doses will be given on an inpatient basis. Details of the risk mitigation strategy for hypersensitivity reactions can be found in section 12.6.1.2.

2.2.2.3 Gastrointestinal Perforations

Gastrointestinal perforations, mostly colonic, have been reported in patients receiving TCZ. During the 24-week, controlled clinical trials, the overall rate of gastrointestinal perforation was 0.26 events/100 patient-years with TCZ therapy. In the all-exposure population, the overall rate of

gastrointestinal perforation remained consistent with rates in the controlled periods of the studies. Reports of gastrointestinal perforation were primarily reported as complications of diverticulitis including generalized purulent peritonitis, lower gastrointestinal perforation, fistula and abscess. Most patients who developed gastrointestinal perforations were taking concomitant nonsteroidal anti-inflammatory medications (NSAIDs), corticosteroids, or methotrexate. The relative contribution of these concomitant medications versus TCZ to the development of gastrointestinal perforations is not known.

To mitigate the risk of GI perforations, patients at risk for these complications will be excluded, concomitant immunosuppressive agents will be prohibited, and abdominal symptoms will be promptly evaluated to identify possible cases of perforation as early as possible. Details of the risk mitigation strategy for gastrointestinal perforation can be found in section 12.6.1.3.

2.2.2.4 Infusion Reactions

Adverse events associated with the infusion (occurring during or within 24 hours of the start of infusion) were reported in 8% and 7% of patients in the 4 mg/kg and 8 mg/kg TCZ plus DMARD groups, respectively, compared to 5% of patients in the placebo plus DMARD group in the 24 week, controlled clinical studies. The most frequently reported event during the infusion was hypertension (1% for both the 4 and 8 mg/kg doses), while the most frequently reported events occurring within 24 hours of finishing an infusion were headache (1% for both doses) and skin reactions (1% for both doses), including rash, pruritus and urticaria. There was no difference in infusion reactions between the 4 and 8 mg/kg doses, and they were not treatment limiting.

To reduce the risk of severe infusion reactions, subjects will be closely monitored during and after infusion (for which they will be admitted as inpatients to the Dahms Clinical Research Unit) with frequent vital sign monitoring, and immediate availability of qualified medical treatment and resuscitation medications and equipment. Details of the risk mitigation strategy for infusion reactions can be found in section 12.6.1.4.

2.2.2.5 Laboratory Abnormalities

2.2.2.5.1 Neutropenia

In the 24 week, controlled clinical studies, neutropenia, defined as decreases in absolute neutrophil counts (ANC) to below 1,000 cells/mm^3, occurred in 1.8% and 3.4% of patients in the 4 mg per kg and 8 mg per kg TCZ plus DMARD group, respectively, compared to 0.1% of patients in the placebo plus DMARD group. Approximately half of the instances of ANC

below 1,000 cells/mm^3 occurred within 8 weeks of starting therapy. Decreases in neutrophil counts to below 500 cells/mm^3 occurred in 0.4% and 0.3% of patients in the 4 mg/kg and 8 mg/kg TCZ plus DMARD group, respectively, compared to 0.1% of patients in the placebo plus DMARD group. There was no clear relationship between decreases in neutrophils to below 1,000/mm^3 and the occurrence of serious infections. In the all-exposure population, the pattern and incidence of decreases in neutrophil counts remained consistent with what was seen in the 24-week controlled clinical studies.

To reduce the risk of neutropenia, subjects with an ANC below 2,000 cells/mm^3 will be excluded, and study treatment will be temporarily or permanently discontinued if the ANC falls during the study to between 500 and 1,000 cells/mm^3 or below 500 cells/mm^3, respectively, as recommended in the USPI. Details of the risk mitigation strategy for neutropenia can be found in section 12.6.1.5.1.

2.2.2.5.2 Thrombocytopenia

In the 24 week, controlled clinical studies, decreases in platelet counts below 100,000/mm^3 occurred in 1.3% and 1.7% of patients on 4 mg/kg and 8 mg/kg TCZ plus DMARD, respectively, compared to 0.5% of patients on placebo plus DMARD, without associated bleeding events. In the all-exposure population, the pattern and incidence of decreases in platelet counts remained consistent with what was seen in the 24 week controlled clinical studies.

To reduce the risk of thrombocytopenia, subjects with a platelet count below 100,000/mm^3 will be excluded, and study treatment will be temporarily or permanently discontinued if the platelet count falls during the study to between 50,000 and 100,000/mm^3 or below 50,000/mm^3, respectively, as recommended in the USPI. Details of the risk mitigation strategy for neutropenia can be found in section 12.6.1.5.2.

2.2.2.5.3 Liver Enzyme Elevations

Treatment with TCZ was associated with a higher incidence of transaminase elevations than placebo. Liver enzyme abnormalities in the 24-week controlled period of registrational studies are summarized in table 1. In patients experiencing liver enzyme elevation, modification of treatment regimen, such as reduction in the dose of concomitant DMARD, interruption of TCZ, or reduction in TCZ dose, resulted in decrease or normalization of liver enzymes. These elevations were not

associated with clinically relevant increases in direct bilirubin, nor were they associated with clinical evidence of hepatitis or hepatic insufficiency. In the all-exposure population, the elevations in ALT and AST remained consistent with what was seen in the 24 week, controlled clinical trials.

Table 1. Incidence of liver enzyme abnormalities in the 24 week controlled period of studies

	TCZ 8 mg/Kg MONOTHERAPY N = 288 (%)	Methotrexate N = 284 (%)	TCZ 4 mg/Kg + DMARDs N = 774 (%)	TCZ 8 mg/Kg + DMARDs N = 1582 (%)	Placebo + DMARDs N = 1170 (%)
AST (U/L)					
> ULN to 3x ULN	22	26	34	41	17
> 3x ULN to 5x ULN	0.3	2	1	2	0.3
> 5x ULN	0.7	0.4	0.1	0.2	< 0.1
ALT (U/L)					
> ULN to 3x ULN	36	33	45	48	23
> 3x ULN to 5x ULN	1	4	5	5	1
> 5x ULN	0.7	1	1.3	1.5	0.3

To reduce the risk of liver enzyme abnormalities, subjects with underlying serious liver disease will be excluded, as will subjects with an AST or ALT $\geq$1.5 times the upper limit of normal (ULN). The dose of study treatment will be reduced for AST or ALT values persistently greater than 1.4 xULN but below 3xULN, and study treatment will be temporarily or permanently discontinued if AST or ALT values are persistently 3xULN to 5xULN or above 5xULN, as recommended in the USPI. Details of the risk mitigation strategy for liver enzyme abnormalities can be found in section 12.6.1.5.3.

2.2.2.5.4 Lipid Elevations

Total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides were first assessed at 6 weeks following initiation of TCZ in the controlled 24 week clinical trials. Increases were observed at this time point and remained stable thereafter. Increases in triglycerides to levels above 500 mg per deciliter were rarely observed. Mean changes in other lipid parameters from baseline to week 24 in the 8 mg/kg TCZ monotherapy arm are summarized below:

- Mean LDL cholesterol increased by 25 mg per dL
- Mean HDL cholesterol increased by 4 mg per dL
- Mean LDL/HDL cholesterol ratio increased by 0.26
- ApoB/ApoA1 ratios were essentially unchanged.

In the all-exposure population, the elevations in lipid parameters remained consistent with what was seen in the 24 week,

controlled clinical trials. Elevated lipids responded to lipid lowering agents.

There was no increase in myocardial infarctions associated with TCZ use in the integrated TCZ safety data base (control group 0.49/100 PY, 0.18/100PY TCZ 4 mg/kg, 0.17/100PY TCZ 8 mg/kg) (67), and although lipids increased with TCZ therapy, CRP levels and insulin resistance were dramatically reduced and arterial flow mediated dilatation was increased (68). The net effect of TCZ on CVD risk is unknown, but is unlikely to be affected by 8 weeks of therapy. No specific risk mitigation steps have been implemented for lipid perturbations seen with TCZ.

2.2.2.6 Malignancies

Malignancies were diagnosed in 15 patients receiving TCZ, compared to 8 malignancies in patients in the control groups during the 24-week, controlled period of the registrational studies. Exposure-adjusted incidence was similar in the TCZ groups (1.32 events/100 patient-years) and in the placebo plus DMARD group (1.37/100 patient-years). In the all-exposure population, the rate of malignancies remained consistent with the rate observed in the 24 week, controlled period. The impact of TCZ on malignancies is unknown.

To minimize the risk or emergent or recurrent malignancies, subjects with a history of or active cancer will be excluded. Details of the risk mitigation strategy for malignancies can be found in section 12.6.1.6.

2.2.2.7 Viral Reactivation

Herpes zoster exacerbations, or shingles, have been reported in patients receiving TCZ(69). About one in ten adults who have experienced wild type varicella-zoster viral infection will have shingles when the virus reemerges during a period of stress. The seroprevalence of varicella-zoster virus antibodies among adults ages 20-49 in the United States, which presumably represents a group of individuals infected with varicella zoster prior to the approval of the varicella live virus vaccine in 1995, exceeds 95%(70), suggesting that excluding individuals with a history of chicken pox or a positive antibody titer would make the study infeasible. Consistent with the USPI, a history of or a positive antibody titer to herpes zoster will therefore not be exclusionary for this study.

Reactivation of hepatitis B has occurred in patients previously exposed to hepatitis B virus (HBV) receiving DMARDs, most commonly tumor necrosis factor (TNF) inhibitors. The risk of HBV reactivation after administration of TCZ is unknown, but may be lower than that associated with TNF inhibitors(71), as the most recent post-marketing report from Japan in 2013 showed no cases of HBV reactivation among 7,901 RA

patients treated with TCZ despite the high background prevalence of HBV infection in that country (Genentech, data on file). More specifically with regards to patients with serological evidence of previous HBV exposure, a recent study reported outcomes among 8 RA patients with positive hepatitis B core antibody (HbcAb), but negative HbsAg and HBV DNA below the limit of quantitation at the beginning of treatment with TCZ. None of these patients received prophylactic antivirals, and none experienced HBV reactivation after 18 months of follow-up(72). In another study that included 17 RA patients who were either HbcAb- or HbsAb-positive and had HBV DNA below the limit of quantitation at the start of TCZ treatment, two patients had a detectable HBV during a median of 18 months of follow-up, but HBV levels never increased beyond the quantitation limit of the assay (2.1 $\log_{10}$ copies/mL), were not associated with liver enzyme elevation or clinical symptoms, and reverted to undetectable spontaneously without antiviral treatment(73). In a third study including 157 HbsAg-negative, HbcAb- or HbsAb-positive RA patients who received treatment with any of six biological DMARDs, including TCZ, 13 patients developed a detectable HBV DNA during 18 months of follow-up, one of them while receiving TCZ(74). HBV DNA positivity in the patient receiving TCZ resolved spontaneously within a month, and none of the 13 patients who developed reactivation had concomitant liver enzyme abnormalities, although ten of them received treatment with entecavir(74). TCZ has also been used safely in two patients with RA and one patient with adult-onset Still's disease and preexisting chronic active hepatitis B(75-77). Importantly, almost all reported cases of HBV reactivation during DMARD treatment have involved patients receiving other immunosuppressive agents together with DMARDs.

Expert opinion(78) and guidelines from the American Association for the Study of Liver Diseases(79), the American Society of Clinical Oncology(80), the European Association for the Study of the Liver(81), the US National Institutes of Health(82), and the US Centers for Disease Control and Prevention(83), all coincide in recommending, with various degrees of certainty, screening using a combination of HbsAg, HbcAb, and HBV DNA at the beginning of various immunsuppressive therapies. The role of active antiviral prophylaxis, on the other hand, is unclear except in the setting of profoundly immunosupressive chemotherapy and is likely to be most beneficial among patients with evidence of ongoing HBV replication (e.g., positive HbsAg, HBV DNA, or HbeAg). In this study, subjects with positive HbsAg, HBV DNA, or HbeAg will be excluded, and those with evidence of resolved HBV infection (i.e., positive HbcAb) will be required to be receiving an HIV antiretroviral regimen that includes at least one agent with anti-HBV activity, such as lamivudine, emtricitabine, or tenofovir. Details of other features of the protocol that might mitigate the risk of viral reactivation can be found in section 12.6.1.7.

2.2.2.8 Cytochrome P450 Inhibition and Decreased Plasma Levels of Antiretrovirals

Inhibition of IL-6 signaling in RA patients treated with TCZ may restore CYP450 activities to higher levels than those in the absence of TCZ, leading to increased metabolism of drugs that are CYP450 substrates. In vitro studies showed that TCZ has the potential to affect expression of multiple CYP enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Its effects on CYP2C8 or transporters are unknown. In vivo studies with omeprazole, metabolized by CYP2C19 and CYP3A4, and simvastatin, metabolized by CYP3A4, showed up to a 28% and 57% decrease in exposure one week following a single dose of TCZ, respectively.

The effects of TCZ on individual ARV levels are unknown, but based on the data, they can be assumed to represent a restoration to exposure levels that are comparable to those in patients with lower levels of inflammation, e.g., those with immune reconstitution success. Thus, even if TCZ administration results in relatively diminished exposure to antiretrovirals metabolized by CYP3A4, it is not expected to reach levels where therapeutic efficacy would be compromised.

Nonetheless, to mitigate the risk of reduced levels of antiretroviral medications, subjects receiving non-boosted protease inhibitors will be excluded, and trough levels of protease inhibitors and non-nucleoside reverse transcriptase inhibitors will be monitored throughout the study. Details of the risk mitigation strategy for reduced levels of antiretrovirals can be found in section 12.6.1.8.

2.2.2.9 Demyelinating Disorders

The impact of TCZ on demyelinating disorders is not known, but multiple sclerosis and chronic inflammatory demyelinating polyneuropathy have been reported rarely in clinical studies.

To minimize the risk of emerging demyelinating disorders, subjects with a history of such conditions will be excluded, and all subjects will be monitored closely at each visit for signs or symptoms indicative of a demyelinating disease. Details of the risk minimization strategy for demyelinating disorders can be found in section 12.6.1.9.

2.2.2.10 Immunogenicity

In the 24-week, controlled clinical studies, a total of 2,876 patients have been tested for anti-TCZ antibodies. Forty-six patients (2%) developed positive anti-TCZ antibodies, of whom 5 (0.17%) had an associated, medically significant, hypersensitivity reaction leading to withdrawal.

Thirty patients (1%) developed neutralizing antibodies. The data reflect the percentage of patients whose test results were positive for antibodies to TCZ in specific assays. No specific risk mitigation interventions are planned to mitigate the risk of TCZ immunogenicity.

2.2.2.11 Safety of TCZ in HIV-infected Individuals

Limited data are available about the safety and tolerability of TCZ in HIV-infected patients. TCZ is approved for treatment of multicentric Castleman's disease in Japan, where it has been used successfully to treat both HHV-8-positive and HHV-8-negative cases (84), but few cases with HIV infection have been reported. At least one HIV-infected patient has been treated in Europe[a], and it is likely that a number of other patients with HIV infection have been treated in Japan, although the experience has not been published in the peer-reviewed literature.

A National Cancer Institute (NCI)-sponsored open-label, pilot clinical trial of TCZ for treatment of Castleman's disease in HIV-infected and HIV-uninfected patients is now underway in the US (ClinicalTrials.gov identifier: NCT01441063). As of April 18, 2013, 3 HIV-positive subjects had been enrolled[b]. All subjects were well controlled on ritonavir-boosted protease inhibitor-containing regimens, and their CD4+ T cell counts at entry were 203, 323, and 421 cells/mm^3. All patients received 8 mg/Kg of TCZ every 2 weeks for 8 doses total. All three patients have completed the course of study medication, although one of the patients did not receive all the scheduled doses because progression of the Castleman's disease was documented during follow-up (in total, 17 doses were administered among 3 subjects). None of the patients had dose-limiting toxicities. One patient experienced a mild infusion reaction consisting of grade 2 pruritus, which resolved with symptomatic treatment, and tolerated subsequent doses with the use of a low-dose antihistamine. There were several transient episodes of mild cytopenias, which were attributed by the investigators to the concomitant use of zidovudine and valganciclovir. Subjects maintained control of HIV replication during exposure to TCZ. There have been no infectious complications.

2.2.3 Rationale for Rectosigmoid Biopsies

The gastrointestinal tract and in particular the gut-associated lymphoid tissue have long been recognized as a major site of HIV replication beginning at the earliest stages of HIV infection, regardless of the route of infection. More recently, the gastrointestinal tract has also emerged as one of the most active sites of HIV pathogenesis, both as a rich source of HIV-susceptible cellular targets, and as the potential source, through impaired integrity of the barrier

a Personal communication 1 May 2013, Mark Bower, Department of Oncology, Chelsea and Westminster Hospital, London, UK

b Personal communication, 18 April 2013, Thomas Uldrick National Cancer Institute, Bethesda, MD

function of the intestinal epithelium, for entry of microbial-derived products into the systemic circulation, which in turn likely contribute to the heightened state of cell cycling and immune activation that is characteristic of progressive HIV infection.

IL-6 mRNA expression levels in colonic biopsy specimens are elevated in HIV infection (42), and we propose that IL-6 might, through both direct and indirect mechanisms, contribute to enhanced microbial translocation from the intestinal lumen and to disruption of the mucosal barrier function integrity even after suppression of systemic HIV replication by antiretroviral therapy (see sections 2.1.7 through 2.1.9). Therefore, this study will provide an invaluable opportunity to test these hypotheses and to improve our understanding of the role of IL-6 in the causal cascade leading to impaired CD4 T cell reconstitution in treated HIV infection. While only a subset of participants is expected to participate in the rectosigmoid biopsy collection part of this study, the insights gained even from a small number of subjects before and after the administration of TCZ will greatly enhance the contribution of this study to the field of HIV immunopathogenesis.

2.2.3.1 Risks of Rectosigmoid Biopsy

Risks associated with lower gastrointestinal endoscopy include colitis from chemicals for endoscope sterilization, bowel perforation, bleeding, diverticulitis, and infection. The frequency of serious complications after flexible sigmoidoscopy is extremely low. In two large studies (85, 86) including a combined 144,832 clinically indicated procedures, the incidence of serious complications ranged from 0.06 to 0.8%. Obtaining biopsies may be associated with an increased risk of complications. The best available data on the risk of multiple biopsies comes from studies of dysplasia surveillance among patients with long-standing inflammatory bowel disease, in whom large numbers of “blind” biopsies are obtained throughout the colon for early detection of malignant transformation. In two such studies (87, 88) including a combined 3,011 procedures and a median of eight (88) and 17 biopsies (87), respectively, there was only one serious complication, for an incidence of approximately 0.33%.

More relevant to the present protocol, in a study of subjects undergoing endoscopic procedures exclusively for research purposes (89), including 64 sigmoidoscopies with a mean of 25 biopsies obtained from the rectosigmoid, there were no major complications. Thirteen subjects experienced minor symptoms (self-limited bleeding and pain), which were not related to the number of biopsies. Thus, the risk of serious complications from the proposed study procedures, even with up to 20 biopsy specimens, is expected to be very low.

To minimize the risks of flexible sigmoidoscopy with biopsies, subjects with conditions or exposure to medications that might increase the risk of complications from these procedures will be excluded, and procedures will be performed only by trained endoscopists using the accepted clinical

protocols of the University Hospitals Case Medical Center Digestive Health Institute. Details on the risk mitigation strategy for the flexible sigmoidoscopy procedure can be found in section 12.6.3.

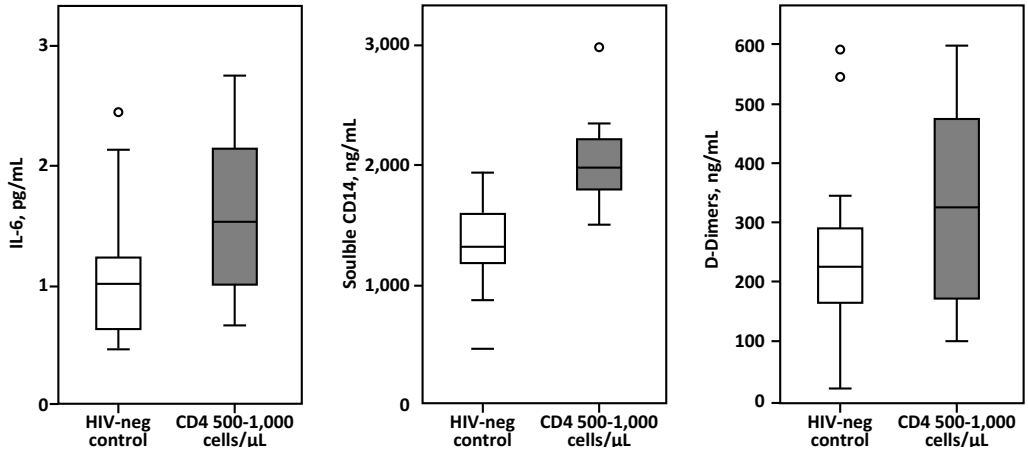
2.2.4 Rationale for Study Population

As outlined above, we have an extensive body of preliminary data in HIV-infected individuals to suggest that heightened immune activation is not only a constant feature of immune failure on successful antiretroviral therapy, but also a strong predictor of adverse outcomes in this clinical scenario. Moreover, we have shown that IL-6 likely plays a role in both impairing CD4 T cell reconstitution and in mediating some of the forms of increased morbidity and mortality observed in these patients, including cardiovascular disease. Therefore, HIV-infected individuals who are suppressed on ART, but who continue to have elevated IL-6 levels may be most likely to benefit from an intervention aimed at interrupting a critical step in the inflammatory process. The long duration of virologic suppression required for enrollment will ensure that subjects are receiving a well-tolerated and effective antiretroviral regimen and that they have reached steady-state levels of immune activation and inflammation secondary to effective ART. Requiring a CD4 cell count ≥350 cells/mm^3 will reduce the risk of opportunistic complications.

The determination of a threshold to segregate patients who experience “immunologic failure” and those who experience “immunologic success” is a source of considerable controversy. In our previous study (6), we used a threshold of 350 cells/mm^3 to define immunologic failure, based on the lower limit of normal CD4 T cell count at our institution and on the threshold for initiation of antiretroviral treatment at the time of that work.

More recently, however, we have examined subjects in our previously published experience who have less pronounced blunting of CD4 T cell reconstitution, in the range that will be included in this study. In that segment of the population, those with CD4 T cell counts between 500 and 1,000 cells/μL, we found that levels of soluble plasma markers of inflammation and coagulation, including IL-6, the monocyte activation marker soluble CD14, and the intravascular coagulation marker D-dimer remain elevated to levels that are distinguishable from those seen among HIV-uninfected healthy controls (figure 2). Therefore, we feel confident that even after excluding patients with extremely blunted CD4 T cell reconstitution (below 350 cells/μL), who might have an elevated residual risk of opportunistic complications, we will still be able to target a population with persistent abnormal inflammatory changes.

Figure 2. Plasma levels of IL-6, sCD14, and D-dimers among HIV-infected patients with suppressed plasma viremia for at least 2 years and CD4+ T cell counts between 500 and 1,000 cells/µL, compared to healthy, HIV-uninfected controls. One extreme outlying value is not shown.



2.2.5 Rationale for Inclusion of Women of Reproductive Potential

Given that globally more than 50% of HIV-infected individuals are women, and that both men and women are at increased risk of morbidity and mortality associated with chronic immune activation and inflammation seen in HIV-infected individuals who are virologically suppressed on ART, it is important to include women in the interventional clinical trials. Further, inclusion of women of reproductive potential might be important, if it can be done safely, to assess the potential impact of sex hormones on the intervention.

TCZ has been approved by the FDA for the treatment of RA in adult men and women with an inadequate response to other approved therapies and for the treatment of active SJIA and/ active PJIA in pediatric patients $\geq$ 2 years of age. It is a Pregnancy Category C compound, indicating that there are no adequate and well-controlled studies in pregnant women. In pregnant cynomolgus monkeys treated during organogenesis, at doses IV at up to 6.25 times the levels used in this study, there was no evidence of teratogenic or dysmophogenic effect. However, there was an increased incidence of abortion/embryo-fetal death. In mice, an analogue of TCZ, dosed at 50 mg/kg IV three times per week, from implantation through 21 days after delivery, showed no evidence for any functional impairment of the development and behavior, learning ability, immune competence and fertility of the offspring. Based on these data, the USPI states that TCZ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is also not known whether tocilizumab is excreted in human milk or absorbed systemically after ingestion. Therefore, because there is no proven benefit of TCZ to treat chronic inflammation and immune activation in HIV+ individuals suppressed on ART, pregnant and breastfeeding women will be excluded from this study.

For women of reproductive potential, multiple safeguards to minimize the risk of unintended exposure to TCZ during pregnancy have been incorporated into the

study, including required negative pregnancy tests before each dose of study treatment, requirement of two valid methods of contraception verified before each study treatment dose, and careful monitoring of FDA updates regarding safety of the study agent in this population. Details about the risk mitigation strategy for women of reproductive potential can be found in section 12.6.1.10.

2.2.6 Rationale for a Crossover Design

A crossover design increases efficiency and reduces sample size requirements by reducing inter-subject variability, since each subject is his/her own control.

The condition treated here, immune activation and inflammation, fulfills generally accepted criteria for a crossover design, as it is a chronic state that cannot be expected to be permanently eliminated by the therapeutic intervention. Because of the gain in efficiency, we will be able to conduct the trial while exposing as small a group of subjects as possible to the study agent in this exploratory, pathogenesis-driven trial.

The intervention, TCZ administration, has a rapid onset of action and a limited duration after discontinuation of treatment, as shown by the return of erythrocyte sedimentation rate and C-reactive protein to baseline values within 4 to 8 weeks after the last infusion of TCZ to RA patients (90). Thus, we have built in a prolonged washout period of 12 weeks that should provide sufficient protection against a carryover effect. The terminal half-life of TCZ is 151 ± 59 hours, indicating that the proposed washout period is adequate to ensure minimal residual concentrations of TCZ. We will monitor serum TCZ levels by ELISA to confirm that this is the case. The information collected will also be of interest to understand potential differences in pharmacokinetics and pharmacodynamics of TCZ in HIV-infected persons receiving antiretroviral therapy.

2.2.7 Rationale for Duration of Study

We sought to identify the minimum exposure necessary to ensure that a biological effect could be observed while minimizing exposure risks and sample size. In RA trials (91), a plateau in clinical benefit appears to be reached around month 3 of treatment, and PK modeling suggests that a steady-state is reached after approximately 8 weeks of treatment with the 8 mg/Kg dose (92). Thus, we will administer the full 8 mg/Kg dose for 2 months, the minimum interval we expect to offer a good probability of observing the peak biological activity. According to the USPI, we will initiate treatment with the lower dose of 4 mg/Kg.

3.0 STUDY DESIGN

This is a phase I/II, double-blind, crossover trial of TCZ, 4 mg/Kg IV followed 4 weeks later by TCZ, 8 mg/Kg IV every 4 weeks for 2 doses, or matching placebo, in male and female HIV-infected subjects between 18 and 60 years of age receiving combination antiretroviral therapy without changes due to virologic failure (i.e., confirmed detectable HIV RNA) in the 24 weeks prior to enrollment and with a suppressed plasma HIV RNA

(<200 copies/mL) for at least 96 weeks and a CD4 T-cell count ≥350 and ≤1,000 cells/mm^3 at the time of study enrollment. Subjects will be assigned randomly to one of the following sequences: TCZ as described above followed by a 12 week washout period and then matching placebo administered in an identical schedule, or vice versa.

All subjects will be offered the option to undergo rectal mucosal biopsy via flexible sigmoidoscopy at baseline, week 10, and week 30, but participation in the rectal tissue collection procedures will not be necessary in order to be enrolled in the study. Flexible sigmoidoscopies will be performed on the dates of regularly scheduled visits that all study participants, regardless of involvement in the rectal specimen collection part, will be asked to complete. Subjects who choose to participate in the rectal specimen collection part of the study will have to meet all the same inclusion and exclusion criteria as those who choose not to participate, with the difference that a few additional precautions and prohibited medications and conditions apply to subjects in the rectal sampling section of the study (section 4.2.17).

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 Men and women age 18-60 years.

4.1.2 Ability and willingness to communicate in English or Spanish

4.1.3 Ability and willingness of subject to provide informed consent.

4.1.4 Ability and willingness to provide adequate locator information.

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

4.1.6 Receiving a stable antiretroviral regimen consisting of 3 or more drugs belonging to two or more classes, one of which must be a protease inhibitor, an integrase inhibitor, or a non-nucleoside reverse transcriptase inhibitor, without any changes due to virologic failure in the past 24 weeks, and with no plans to change antiretroviral regimen in the 40 weeks following enrollment. If the regimen includes a protease inhibitor, ritonavir or an approved boosting agent known to increase trough levels of the protease inhibitor by at least 50% must also be a part of the regimen.

NOTE A: Changes to the antiretroviral regimen 8 weeks or more prior to enrollment are allowed if the plasma HIV RNA was <50 copies/mL at the time of the change, the reason for the change was not suspicion or documentation of virologic failure, and all other criteria for virologic control, as outlined in criterion 4.1.9 below, are met.

NOTE B: For the purposes of this protocol, "a change due to virologic failure" is defined as any of the following events or combinations thereof happening in a patient who is receiving uninterrupted combination antiretroviral therapy including at least three agents belonging to at least two classes: a) plasma HIV RNA is >200 copies/mL on two or more consecutive occasions; b) the treating physician determines that any given plasma HIV RNA value is indicative or suggestive of virologic failure, and recommends that the patient's regimen be modified as a result; c) a genotypic or phenotypic antiretroviral resistance test identifies the presence of resistance to any component of a patient's regimen that was not present before, and a physician recommends that the patient's regimen be modified as a result.

4.1.7 **For subjects who have a positive HBcAb only:**

- An antiretroviral regimen containing one or more of the following medications: lamivudine, emtricitabine, or tenofovir.

4.1.8 Screening CD4+ T-cell count ≥350 cells/mm^3 but ≤1,000 cells/mm^3 performed in a laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent.

4.1.9 HIV-1 RNA <200 copies/mL at every time a plasma HIV RNA has been obtained, but no fewer than twice, in the 96 weeks prior to enrollment.

NOTE: One plasma HIV RNA above 200 copies/mL but lower than 1,000 copies in the 96 weeks prior to enrollment is permissible if flanked by two measurements that are both below 200 copies. The HIV-1 RNA at screening must be <50 copies/mL.

4.1.10 The following laboratory values obtained within 60 days prior to entry:

- Hgb ≥10 g/dL
- Platelet count >100,000/mm^3
- Absolute neutrophil count (ANC) ≥2,000 cells/mm^3
- Aspartate aminotransferase (AST) [serum glutamic oxaloacetic transaminase (SGOT)] <1.5x ULN
- Alanine aminotransferase (ALT) [serum glutamic pyruvic transaminase (SGPT)] <1.5x ULN
- **For subjects who consent to undergo rectal tissue sampling only:** International normalized ratio (INR) < 1.7
- Total bilirubin <3.0 mg/dL, EXCEPT for subjects receiving atazanavir
- For subjects receiving atazanavir: Direct bilirubin ≤1.0 mg/dL
- Calculated GFR ≥60 mL/min/1.73m^2

4.1.11 Female subjects of reproductive potential must have a negative serum or urine pregnancy test at study entry and must affirm that they do not intend to become

pregnant for the duration of the study.

NOTE: For the purposes of this protocol, a female subject of reproductive potential is defined as one who has reached menarche, has not been post-menopausal for at least 24 consecutive months (i.e., has had menses within the preceding 24 months), and has not undergone surgical sterilization (e.g., hysterectomy, tubal ligation, or bilateral oophorectomy).

4.1.12 Female subjects of reproductive potential participating in sexual activity that could lead to pregnancy must agree to use at least one of the following forms of birth control for at least 30 days prior to study entry through 30 days after the final study visit:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide

PLUS at least one of the following:

- An FDA-approved copper intrauterine device (IUD), e.g. ParaGard®, or an FDA-approved hormone-releasing IUD, e.g. Mirena®
- An FDA-approved transdermal hormonal contraceptive, e.g. Ortho-Evra®
- An FDA-approved injectable hormonal contraceptive, e.g. Depo-Provera®
- An FDA-approved hormonal contraceptive in vaginal ring form, e.g. NuvaRing®
- An FDA-approved implantable hormonal contraceptive, e.g. Implanon® or Jadelle®

NOTE A: Oral hormonal contraceptives, either combined or progestin-only, are not acceptable as a second contraceptive method.

NOTE B: Women of reproductive potential who are using a hormonal contraceptive not included above may consider switching their contraceptive to one of the approved forms, but will be required to do so in consultation with their primary healthcare providers. Study investigators will not prescribe or recommend a specific form of contraception to study participants. The study will not cover the costs of contraceptives.

4.1.13 Female subjects who are not of reproductive potential [i.e., women who have been post-menopausal for at least 24 consecutive months or women who have undergone surgical sterilization (e.g., hysterectomy, tubal ligation, or bilateral oophorectomy)] are eligible without requiring the use of a contraceptive. Acceptable documentation of sterilization, other contraception methods, menopause and reproductive potential is patient-reported history at any time prior to screening.

4.1.14 Subjects must be determined by the investigators to have at least one fully active regimen available to them other than the regimen they are receiving at study

entry. The determination of whether or not an alternative regimen is available must take into account both the subject's antiretroviral history and any previously obtained resistance testing. A valid alternative regimen must meet the recommendations for an active regimen outlined in the current DHHS guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents (93).

4.2 Exclusion Criteria

4.2.1 History of one of the following opportunistic infections at any time in the past, as demonstrated by either a patient-reported or physician-documented diagnosis AND the initiation of specific treatment if applicable:

- Tuberculosis, pulmonary or extrapulmonary
- Non-tuberculous mycobacterial infection, disseminated or extrapulmonary
- *Pneumocystis jirovecii* pneumonia
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis or isosporiasis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or lymph nodes) and including retinitis with loss of vision
- Histoplasmosis, disseminated or extrapulmonary
- Kaposi's sarcoma
- Lymphoma, Burkitt's or immunoblastic, regardless of anatomical location
- Lymphoma, primary of brain
- Progressive multifocal leukoencephalopathy
- Toxoplasmosis of brain

4.2.2 Latent tuberculosis infection, defined as a positive or borderline FDA-approved interferon-gamma release assay (IGRA).

4.2.3 Pregnant or breastfeeding.

4.2.4 Active local or systemic infection (defined as requiring systemic antibiotics or other medical or surgical treatment) at the time of enrollment.

4.2.5 Use of systemic cancer chemotherapy or radiation therapy, immunosuppressive or immunomodulatory therapy (e.g., interferons, tumor necrosis factor antagonists, interleukins) or any investigational therapy within 90 days prior to study entry or continued indication for such medications. A list of prohibited study medications is provided in appendix 1 of the Manual of Operations (MOPS).

NOTE A: Use of inhaled or nasal steroids, the equivalent of 10 mg of prednisone or less per day or a less than 2-week course of steroids are not exclusionary.

NOTE B: A single course of 1% hydrocortisone cream or equivalent applied up to 3 times a day to <10 square inches area for <2 weeks is permitted. The investigators will determine if other forms of limited, short-term topical steroid use are expected to interfere significantly with the study objectives and are therefore exclusionary.

4.2.6 Receipt of a live attenuated vaccine within 30 days prior to study entry or expected need for such vaccines at any time during and for 30 days after the study.

4.2.7 Known allergy/sensitivity or any hypersensitivity to components of the study drug or its formulation.

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

4.2.9 Serious illness requiring systemic treatment and/or hospitalization within 45 days prior to study entry.

4.2.11 Known cirrhosis or severe liver disease (e.g., ascites, encephalopathy, history of variceal bleeding).

4.2.12 Active hepatitis B (positive HBsAg, HBV DNA, or HBeAg) or hepatitis C (positive HCV antibody and positive HCV RNA in plasma) documented at the screening visit.

NOTE A: For purposes of documenting hepatitis B status, an HBsAg or HBV DNA obtained in the 6 months prior to and up to the date of the screening visit is acceptable EXCEPT for subjects with a positive HBcAb at screening (see Note B). A negative HBeAg during the same period in the absence of an HBsAg or HBV DNA is not sufficient evidence of HBV-negative status. If a clinically obtained test is not available at screening, an HBsAg will be drawn together with other evaluations at this visit.

NOTE B: For subjects with a positive HBcAb at screening, an HBV DNA below the limit of quantification at any time from screening to study entry is required.

NOTE C: For purposes of documenting HCV status, a negative HCV antibody obtained in the 6 months prior to and up to the date of the screening visit is sufficient. For any subject with a positive HCV antibody, an HCV RNA measurement is required.

4.2.13 History of diverticulitis, intestinal perforation, distal intestinal obstruction, or lower gastrointestinal bleeding.

4.2.14 An antiretroviral regimen containing maraviroc at study entry.

4.215 History of a demyelinating disorder such as multiple sclerosis or chronic demyelinating polyneuropathy at any time in the past.

4.2.16 A trough level of any protease inhibitor (other than a boosting dose of ritonavir) or non-nucleoside reverse transcriptase inhibitor included in the subject's antiretroviral regimen at screening that is below the lower limit of the steady-state trough level range observed in published studies, according to Table 2, below.

Table 2: Cutoffs of selected antiretroviral trough levels

Drug	Lower limit of range (levels below this at screening are exclusionary)
Efavirenz(94)	1 µg/mL
Nevirapine(94)	3 µg/mL
Etravirine(95)	75 ng/mL
Rilpivirine(96)	4 ng/mL
Atazanavir/r(94)	0.15 µg/mL
Darunavir/r(96)	1036 ng/mL
Lopinavir/r(94)	1 µg/mL
Amprenavir(94)	0.4 µg/mL
Fosamprenavir/r(94)	0.4 µg/mL
Indinavir/r(94)	0.1 µg/mL
Saquinavir SGC/r(94)	0.1 µg/mL
Nelfinavir(94)	0.8 µg/mL

4.2.17 Active malignant disease, other than a resected basal cell carcinoma of the skin.

4.2.18 **For subjects who consent to undergo rectal tissue sampling only:**

- History of a bleeding diathesis of any kind, or contraindication for lower GI endoscopy
- Major GI tract surgery within 45 days prior to study entry. Minor procedures involving only the anal canal such as condyloma ablations, hemorrhoidectomy, and anoscopy are permitted if cleared by the responsible surgeon.
- Continued need for, or use during the 7 days prior to the scheduled flex-sig procedure, of the following medications:
    - Aspirin, systemic antiplatelet agents, or more than 4 doses of NSAIDs
    - Warfarin, heparin, thrombin inhibitors, factor Xa inhibitors, and systemic thromobolytics and fibrinolytics. A list of prohibited medications for the flex-sig procedure can be found in the appendix of the MOPS. A bridge course of short-acting low-molecular weight heparin within the 7-day pre-procedure window in patients receiving a chronic systemic anticoagulant who can be safely maintained off anticoagulation for several days is permitted if cleared by the subject's primary healthcare provider and the UHCMC Digestive Health Institute.
- Abnormalities of the colorectal mucosa, or significant colorectal symptoms,

which in the opinion of the clinician represent a contraindication to biopsy (including but not limited to presence of any unresolved injury, infectious or inflammatory condition of the local mucosa)

4.3 Study Enrollment Procedures

4.3.1 Study Initiation

Prior to implementation of this protocol, and any subsequent full version amendments, the protocol and the protocol consent form(s) must be approved by the University Hospitals Case Medical Center IRB and the FDA. Upon receiving final approval, the UHCMC CRS will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

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

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

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

4.3.2 Recruitment

Participants will be recruited from a variety of sources, using the following key strategies:

- Referrals from clinicians in the Special Immunology Unit
- Direct outreach to patients who are participating in an existing protocol that allows for collection of clinical data for research purposes who have provided advance informed consent to be contacted for future research studies

- Participant referrals from existing or past study participants
- Passive self-referral by subjects after receiving IRB-approved publicity materials published through print and electronic media
- Referrals from other area HIV practices

Study staff will meet as needed to discuss current recruitment status, targets, and strategies. Staff also will follow-up with all persons who express an interest in the study to ensure that screening appointments are scheduled and carried out in a timely manner.

To reduce the number of subjects simultaneously exposed to study agent during the early stages of the study, the first ten subjects who enroll into the study will be scheduled to have their entry visit at least 3 days apart from each other, so that no more than two new subjects will initiate study treatment in a given week during this period. The decision to remove this gating restriction after the first ten subjects will be made by the principal investigators in consultation with the SMC and the medical officer.

4.3.3 Subject Registration

Subjects who provide informed consent will be registered to the study according to standard UHCMC procedures.

4.3.4 Retention

Once participants enroll in this study, the study site will make every effort to retain them for the duration of follow-up in order to minimize possible bias associated with loss-to-follow-up. The study staff is responsible for developing and implementing local standard operating procedures to target this goal. Components of such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process, and re-emphasis at each study visit
- Thorough explanation of the importance of completing all study visits to the overall success of the study
- Use of appropriate and timely visit reminder mechanisms (via email and/or telephone)
- Immediate and multifaceted follow-up on missed visits

4.4 Coenrollment Guidelines

Coenrollment in any other studies will be decided on a case-by-case basis by the protocol chairs.

5.0 STUDY TREATMENT

Study treatment is defined as tocilizumab (ACTEMRA®) and placebo (Sodium Chloride Injection, USP 0.9%).

5.1 Regimens, Administration, and Duration

On study day 0 (study entry), subjects will receive 4 mg/Kg of TCZ intravenously or matching placebo, infused over 60 minutes, with a total dose not to exceed 400 mg. The final volume of both the placebo and active drug bags will be 100 cc. If the initial dose is tolerated and the subject does not experience a grade 3 or greater clinical or laboratory adverse event that is thought to be related to study treatment (see section 7.1 for AE management), he/she will receive two more doses of 8 mg/Kg of TCZ each, not to exceed 800 mg, or matching placebo at weeks 4 and 8.

The subjects will be followed on study through week 20, when they will receive the opposite arm of the regimen they received during the first period (i.e., if they were assigned to placebo during the first period, they will receive TCZ during the second period, and vice versa). The first dose of the second period will generally be administered at week 20, but could be given later if the week 20 dose is held for any reason.

The weight used for dose calculation will be obtained on the date of each of the study agent administration visits.

All doses of study agent will be administered as an inpatient in the Dahms Clinical Research Unit (DCRU), and subjects will be observed as inpatients for at least 30 minutes after study drug administration. This change is consistent with recommendations for the use of study drug for clinical purposes(97), and with the USPI(69). Subjects will be followed on study through week 40 from the enrollment visit.

5.2 Study Product Formulation and Preparation

Tocilizumab, solution for infusion, reconstituted as per package insert recommendations or matching placebo, will be supplied in sterile vials by the investigational pharmacy and delivered in a light-opaque pouch ready for administration. Preparation steps are as described below:

- Step 1. For participants at or above 30 kg, dilute to 100 mL in 0.9% Sodium Chloride for intravenous infusion using aseptic technique. Withdraw a volume of 0.9% Sodium Chloride Injection, USP, equal to the volume of the TCZ solution required for the participant's dose from the infusion bag or bottle.
- Step 2. Slowly add TCZ from each vial into the infusion bag or bottle. To mix the solution, gently invert the bag to avoid foaming.
- Step 3. Allow the fully diluted TCZ solution to reach room temperature prior to infusion.

For placebo, commercially available 100 mL bags of Sodium Chloride injection, USP

0.9% will be used. The investigational pharmacy will shroud bags in a light-opaque pouch, and label the bags on the outside with the date, patient's PTID, visit number, and sequence number, leaving the tubing out of the pouch for access.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

Tocilizumab (ACTEMRA®) and matching placebo will be obtained commercially.

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products. The procedures to be followed are provided in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks in the section Study Product Management Responsibilities.

5.3.3 Storage

TCZ must be refrigerated at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect the vials from light by storage in the original package until time of use.

Once TCZ solution is prepared for infusion it may be stored at 2° to 8°C (36° to 46°F) or room temperature for up to 24 hours and should be protected from light. TCZ solutions do not contain preservatives; therefore, unused product remaining in the vials should not be used. Storage conditions for protocol-provided study products will include segregation, security, temperature monitoring, and sanitation. Study products will be stored in a limited access area that is locked when not in use. The study products will be accessible only to authorized personnel.

5.3.4 Administration

TCZ or placebo will be administered by intravenous infusion. Both the subjects and study personnel involved with the administration of study agent or the adjudication of adverse events will be blinded to study assignment.

The solution will be inspected visually for particulate matter and discoloration at the time of reconstitution and prior to administration. If particulates and discolorations are noted, the product will not be used. Fully diluted TCZ solutions are compatible with polypropylene, polyethylene and polyvinyl chloride infusion bags and polypropylene, polyethylene and glass infusion bottles. The infusion will be administered over 60 minutes using an infusion set and pump. The solution will not be administered as an intravenous push or bolus. No other drugs will be infused concomitantly.

5.3.5 Dispensing

Study products will be dispensed from the pharmacy to study staff for an enrolled participant only upon receipt of a written prescription from an authorized prescriber.

5.3.6 Retrieval of Unused Product

Any dispensed but unused product will be handled according to the appropriate site and pharmacy procedures and destroyed once it is confirmed that it will not be used by the intended recipient. A record of the destruction of the product will be maintained by the investigational pharmacy.

5.4 Concomitant Medications

Whenever a concomitant medication is initiated or a dose changed, investigators must review the concomitant medications' and study agents' most recent package inserts, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

5.4.1 Prohibited Medications

The medications that are excluded prior to study entry as stated in the exclusion criteria are also prohibited while on study. A list of prohibited medications can be found in Appendix 1 of the MOPS.

6.0 CLINICAL AND LABORATORY EVALUATIONS
6.1 Schedule of Events

Event	Timepoint and window															
	Visit 1: Screening	**Visit 2: Baseline[1]**	**Visit 3: Study entry**	**Visit 4: D3**	**Visit 5: W4**	**Visit 6: W8**	**Visit 7: W10**	**Visit 8: W20 (arm shift)**	**Visit 9: W20 + 3d**	**Visit 10: W24**	**Visit 11: W28**	**Visit 12: W30**	**Visit 13: W40 (EOS)**	**Premature treatment/study discontinuation**	**Pharmacologic interaction confirmation**	**Virologic failure confirmation**
	-60 to -10 d	-10 to 0 d	0 d	3 d 2 to 4 d	28 d 24 to 32 d	56 d 52 to 60 d	70 d 66 to 74 d	140 d 133 to 147 d	143 d W20 + 2d to W20 + 4d	168 d 164 to 172 d	196 d 192 to 200 d	210 d 206 to 214 d	280 d 273 to 287 d		Within 5 weeks of a trough PI/NNTTI level below threshold	Within 2 weeks of an HIV-1 RNA >200 copies/mL
Study procedure review; informed consent signature	X															
Documentation of HIV	X															
Randomization			X													
Medical/Medication History	X	X	X													
Clinical assessments	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Complete physical exam	X		X				X	X				X		X		
Targeted physical exam		X		X	X	X			X	X	X		X			
Weight			X		X	X	X	X		X	X	X	X			
Height			X													
Verification of contraceptive methods	X	X	X	X	X	X	X	X	X	X	X	X	X			
Tests processed by the clinical lab																
CD4+/CD8+ T cell counts	X		X	X	X	X	X	X	X	X	X	X	X	X		
Plasma HIV-1 RNA	X		X	X	X	X	X	X	X	X	X	X	X	X		X[2]
CBC + diff and platelet count	X		X	X	X	X	X	X	X	X	X	X	X	X		
Chemistry panel	X		X	X	X	X	X	X	X	X	X	X	X	X		
HBsAg	X[3]															
HBcAb (with reflex HBV DNA if positive)	X[3]															
HBV DNA			X[4]		X[4]	X[4]	X[4]	X[4]		X[4]	X[4]	X[4]	X[4]			
HCV Ab	X[3]															
HCV RNA	X[5]															
Interferon-gamma release assay	X[6]						X[6]									
PT/PTT	X[7]					X[7]					X[7]					
For women: rapid pregnancy test	X[8]		X[8]		X[8]	X[8]	X[8]	X[8]		X[8]	X[8]	X[8]				
Tests processed by the research labs																
Pharmacologic monitoring (ARV and TCZ levels)	X[9]		X[9]		X	X	X	X		X	X	X		X	X[10]	
Immunologic monitoring			X	X	X	X	X	X	X	X	X	X	X	X		
Transcriptomic monitoring			X				X	X				X	X	X		
Metabolic monitoring			X				X	X				X	X	X		
Study agent administration[11]			X[12]		X	X		X[12]		X	X					
Cardiovascular monitoring (BART testing)		X					X	X				X				
Sigmoidoscopy and biopsies		X[13]					X[13]					X[13]				

[1] Any of the baseline evaluations can be done at any time in the 10 days prior up to and including the day of entry
[2] Only when the previous HIV-1 RNA is >200 copies/mL
[3] Only for subjects who do not have one available within 6 months prior to screening
[4] Only for subjects who have a positiveHBcAb at screening
[5] Only for subjects who do not have one available within 6 months prior to screening AND have a positive HCV Ab
[6] And whenever tuberculosis exposure or disease is suspected
[7] Only for subjects who will undergo rectosigmoidoscopy and biopsies
[8] And whenever pregnancy is suspected
[9] Antiretroviral levels only
[10] Only when the trough level of a protease inhibitor or an NNRTI at the previous visit was below the lower limit of the population range (refer to section 4.2.16)
[11] Frequent vital sign monitoring will be a part of study agent administration visits
[12] First dose in each cycle will be 4 mg/Kg (up to 400 mg). Subsequent doses will be 8 mg/Kg (up to 800 mg). Dose may be reduced according to lab results.
[13] Only a subset of participants will undergo tissue sampling at each timepoint.

Subjects must be fasting (defined as nothing by mouth except medications and water for at least 8 hours) for all study visits after the screening visit. Subjects must refrain from strenuous exercise and smoking 4 hours prior to BART. Otherwise BART will be rescheduled within the visit window.

6.2 Timing of Evaluations

6.2.1 Overview of Study Visits

In addition to any interim visits that may occur in response to clinical events during the study, the following visits will take place for study participants:

Visit 1:	Screening Visit
Visit 2:	Baseline Visit
Visit 3:	Day 0 Study Entry - Dosing Visit
Visit 4:	Day 3 F/U
Visit 5:	Week 4 - Dosing Visit
Visit 6:	Week 8 - Dosing Visit
Visit 7:	Week 10 – Primary Endpoint Visit
Visit 8:	Week 20- Crossover Dosing Visit
Visit 9:	Week 20 + 3 Days F/U
Visit 10:	Week 24 - Dosing Visit
Visit 11:	Week 28 - Dosing Visit
Visit 12:	Week 30 - Primary Endpoint Visit
Visit 13:	Week 40 End of Study Visit

Participants will have a 12-week washout period before crossover to the next arm.

Prescreening via a phone call will take place with potential participants, and those interested in the study can request that the informed consent documents be mailed out to them prior to the Screening Visit.

6.2.2 Visit 1: Screening Evaluations (Day -60 to -10)

Both screening and baseline evaluations must occur prior to the subject's starting any study medications, treatments or interventions. Written informed consent will be obtained before any procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined. Participants not meeting the eligibility criteria may be rescreened at a later date, if appropriate (e.g., to allow washout of a discontinued prohibited medication).

Screening evaluations to determine eligibility must be completed within 60 days, but no less than 10 days prior to study entry unless otherwise specified.

NOTE: Subjects do not need to be fasting for the screening evaluations.

Table 3: Visit 1 (Screening)

Component	Procedure/Analysis
Administrative	• Obtain written informed consent • Assign participant ID (PTID) • Assess eligibility • Provide reimbursement for study visit • Confirm documentation of HIV status • Obtain locator information
Clinical	• Record medical history • Record medication history • Verify method(s) of contraception • Perform complete physical exam
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Hepatitis testing (if no results available within prior 6 months), including: - Hepatitis B surface antigen (HBsAg) - Hepatitis B core antibody (HBcAb, with reflex HBV DNA if positive) - HCV Ab - HCV RNA (only if HCV Ab+) o Interferon-gamma release assay (IGRA) o PT/PTT (for subjects participating in rectosigmoid specimen collection only) o Qualitative hCG for women of childbearing potential, if not performed on urine • Collect blood specimens and send to the research laboratory for: o Pharmacologic studies (ARV levels only)
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood

6.2.3 Visit 2: Baseline Evaluations (Day -10 to 0)

Baseline evaluations must be completed within the 10 days prior to entry evaluations unless otherwise specified. If needed for ease of scheduling, baseline evaluations may be conducted over more than one day, as long as all evaluations are completed within the visit's window. Baseline evaluations may be conducted on the same day as study entry.

NOTE A: Subjects will be asked to fast for the baseline evaluations. Fasting is defined as nothing by mouth except medications and water for at least 8 hours prior to the visit, but this visit will not be rescheduled if the subject is not fasting. Instead, this deviation will be recorded in the CRF.

NOTE B: All laboratory, cardiovascular, and gastrointestinal sampling procedures at the baseline visit must be completed before the first dose of study agent.

NOTE C: Subjects will be asked to refrain from smoking and from strenuous exercise for at least 4 hours prior to the baseline visit. BART testing will be rescheduled if the subject smoked or exercised vigorously in the 4 hours prior to BART.

Table 4: Visit 2 (Baseline)

Component	Procedure/Analysis
Administrative	• Confirm informed consent and eligibility • Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Review/update medical history • Review/update medication history • Verify method(s) of contraception • Perform targeted physical exam if indicated
Cardiovascular studies	• Perform brachial arterial reactivity testing (BART)
Rectosigmoid specimens (If subject has consented to rectosigmoid biopsies)	• Confirm that subject followed instructions for preparation, including enemas • Collect rectosigmoid biopsies (~20) via flexible sigmoidoscopy

6.2.4 Visit 3: Study Entry (Day 0)

All entry evaluations must occur prior to the administration of the first dose of study agent. Randomization will occur at the beginning of this visit. Randomization procedures are described in the MOPS. Baseline evaluations may be completed on the same day as study entry.

NOTE: Subjects will be asked to fast for the entry (day 0) and all post-entry study visits, defined as nothing by mouth except medications and water for at least 8 hours prior to the visits, but these visits will not be rescheduled if the subject is not fasting. Instead, this deviation will be recorded in the CRF.

THIS SPACE INTENTIONALLY LEFT BLANK

Table 5: Visit 3 (Study entry)

Component	Procedure/Analysis
Administrative	• Confirm and complete randomization • Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Pre-dose Procedures	
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Review/update medical and medication history • Perform complete physical exam, including height and weight
Blood specimens (~120 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic studies (ARV levels only) o Immunologic studies o Transcriptomic studies o Metabolic studies
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Study product dose	• Administer 4 mg/kg dose of TCZ or placebo via intravenous infusion over 60 minutes

6.2.5 Visit 4: Day 3 (+/-1 day)

Table 6: Visit 4 (Day 3)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 • Collect blood specimens and send to the research laboratory for: o Immunologic studies

6.2.6 Visit 5: Week 4 (+/-4 days)

Table 7: Visit 5 (Week 4)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Pre-dose Procedures	
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated, obtain weight
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic studies o Immunologic studies
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Study product dose	• Administer 8 mg/kg dose of TCZ or placebo via intravenous infusion over 60 minutes

THIS SPACE INTENTIONALLY LEFT BLANK

6.2.7 Visit 6: Week 8 (+/-4 days)

Table 8: Visit 6 (Week 8)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Pre-dose Procedures	
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated, obtain weight
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o PT/PTT(for subjects participating in rectosigmoid specimen collection only) o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic monitoring o Immunologic monitoring
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Study product dose	• Administer 8 mg/kg dose of TCZ or placebo via intravenous infusion over 60 minutes

6.2.8 Visit 7: Week 10 (+/-4 days)

NOTE: Subjects will be asked to refrain from smoking and from strenuous exercise for at least 4 hours prior to the week 10 visit. BART testing will be rescheduled if the subject smoked or exercised vigorously in the 4 hours prior to BART.

If needed for ease of scheduling, week 10 evaluations may be conducted over more than one day, as long as all evaluations are completed within the visit's window.

THIS SPACE INTENTIONALLY LEFT BLANK

Table 9: Visit 7 (Week 10)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform complete physical exam
Blood specimens (~120 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Interferon-gamma release assay (IGRA) o Qualitative hCG for women of reproductive potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic monitoring o Immunologic monitoring o Transcriptomic monitoring o Metabolic monitoring
Cardiovascular studies	• Perform brachial arterial reactivity testing (BART)
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Rectosigmoid specimens (If subject has consented to rectosigmoid biopsies)	• Confirm that subject followed instructions for preparation, including enemas • Collect rectosigmoid biopsies (~20) via flexible sigmoidoscopy

6.2.9 Visit 8: Week 20 Crossover (+/- 7 days)

NOTE: Subjects will be asked to refrain from smoking and from strenuous exercise for at least 4 hours prior to the week 20 visit. BART testing will be rescheduled if the subject smoked or exercised vigorously in the 4 hours prior to BART.

If needed for ease of scheduling, week 20 evaluations may be conducted over more than one day, as long as all evaluations are completed within the visit's window.

Table 10: Visit 8 (Week 20, crossover)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Pre-dose Procedures	
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform complete physical exam, obtain weight
Blood specimens (~120 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic studies o Immunologic studies o Transcriptomic studies o Metabolic studies
Cardiovascular studies	• Perform brachial arterial reactivity testing (BART)
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Study product dose	• Administer 4 mg/kg dose of TCZ or placebo via IV infusion over 60 minutes

THIS SPACE INTENTIONALLY LEFT BLANK

6.2.10 Visit 9: Week 20 and 3 Days (+/- 1 day)

Table 11: Visit 9 (Week 20 + 3 days)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 • Collect blood specimens and send to the research laboratory for: o Immunologic studies

6.2.11 Visit 10: Week 24 (+/-4 days)

Table 12: Visit 10 (Week 24)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Pre-dose Procedures	
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated; obtain weight
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic studies o Immunologic studies
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Study product dose	• Administer 8 mg/kg dose of TCZ or placebo via intravenous infusion over 60 minutes

6.2.12 Visit 11: Week 28 (+/-4 days)

Table 13: Visit 11 (Week 28)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Pre-dose Procedures	
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated; obtain weight
Blood specimens (~60 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o PT/PTT(for subjects participating in rectosigmoid specimen collection only) o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic monitoring o Immunologic monitoring
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Study product dose	• Administer 8 mg/kg dose of TCZ or placebo via intravenous infusion over 60 minutes

6.2.13 Visit 12: Week 30 (+/-4 days)

NOTE: Subjects will be asked to refrain from smoking and from strenuous exercise for at least 4 hours prior to the week 30 visit. BART testing will be rescheduled if the subject smoked or exercised vigorously in the 4 hours prior to BART.

If needed for ease of scheduling, week 30 evaluations may be conducted over more than one day, as long as all evaluations are completed within the visit's window.

THIS SPACE INTENTIONALLY LEFT BLANK

Table14: Visit 12 (Week30)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform complete physical exam; obtain weight
Blood specimens (~120 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o Qualitative hCG for women of childbearing potential, if not performed on urine o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Pharmacologic monitoring o Immunologic monitoring o Transcriptomic monitoring o Metabolic monitoring
Urine specimens	• Collect urine sample for qualitative hCG for women of childbearing potential, if not performed on blood
Cardiovascular studies	• Perform brachial arterial reactivity testing (BART)
Rectosigmoid specimens (If subject has consented to rectosigmoid biopsies)	• Confirm that subject followed instructions for preparation, including enemas • Collect rectosigmoid biopsies (~20) via flexible sigmoidoscopy

6.2.14 Visit 13 (End of Study): Week 40 (+/- 7 days)

Table 15: Visit 13 (Week 40, end of study)

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Verify method(s) of contraception • Record any AEs and concomitant medications • Perform targeted physical exam if indicated; obtain weight
Blood specimens (~120 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 o HBV DNA (for HBcAb-positive subjects only) • Collect blood specimens and send to the research laboratory for: o Immunologic studies o Transcriptomic studies o Metabolic studies

6.2.15 Interim Contacts and Visits

Interim visits may be performed at any time during the study. All interim contacts and visits will be documented in the source documentation and on applicable case report forms.

Some interim visits may occur for administrative reasons. For example, the participant may have questions for study staff. Other interim contacts and visits may occur in response to AEs experienced by study participants. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care.

When a trough level of a protease inhibitor or non-nucleoside reverse transcriptase inhibitor that is part of the subject's regimen is found to be below the pre-specified threshold (refer to section 4.2.16), a pharmacologic interaction confirmation visit will occur and a repeat antiretroviral level measurement will be obtained, unless the confirmation visit coincides with another scheduled visit. When a plasma HIV RNA level is above 200 copies/mL in a subject who reports continued adherence to his/her antiretroviral regimen, a virologic failure confirmation visit will occur and only a specimen for repeat plasma HIV RNA measurement will be obtained, unless the confirmation visit coincides with another scheduled visit.

Table 16: Interim contacts and visits

Component	Procedure/Analysis
Administrative	• Review/update locator information • Provide test results, if applicable
	• Record any AEs and concomitant medications • Perform targeted physical exam if indicated
Blood Specimens (~20 mL)	• If pharmacologic interaction confirmation, collect blood specimens and send to the research laboratory for: o Pharmacologic studies • If virologic failure confirmation, collect blood specimens and send to the clinical laboratory for: o Plasma HIV-1 RNA
Additional specimens	Collect appropriate specimens and perform testing as clinically indicated

6.2.16 Participants Who Become Pregnant

Study staff will capture pregnancies on study documents. Women who become pregnant will be considered to be on-study until the outcome of the pregnancy is known. Women who become pregnant will be encouraged to enroll in the Actemra® pregnancy registry by calling 1-877-311-8972. Protocol-specified procedures will continue except:

- Product administration
- Verification of contraception
- Qualitative hCG
- Flexible sigmoidoscopy and biopsies
- BART

6.2.17 Discontinuation Evaluations

If the participant withdraws or is withdrawn from the study after receiving study product, a premature treatment/study discontinuation visit will be conducted if possible.

6.2.17.1 Final Contact

After the end of study visit or early termination visit, a final contact may be required to provide laboratory test results or follow-up regarding an AE. In addition, for participants who become pregnant during study participation, an additional contact may be required to ascertain the participant's pregnancy outcome. All final contacts will be documented in participant study records.

6.2.17.2 Evaluations for Registered Subjects Who Do Not Start Study Treatment

A subject who does not start study treatment will be taken off study with no further evaluations required, and replaced by the next subject who attends the baseline visit. Demographic data, clinical diagnosis data, and screening laboratory results will be retained on all subjects who are not enrolled for any reason after the screening visit and who have consented to have basic demographic information stored for this purpose.

6.2.17.3 Premature Study Treatment Discontinuation Evaluations

Premature discontinuation of study treatment is defined as permanently discontinuing all study treatment prior to week 30.

Subjects who prematurely discontinue study treatment will have the premature treatment/study discontinuation evaluations performed within 2 weeks and no later than the next scheduled study visit. Subjects who prematurely discontinue study treatment will be encouraged to continue on study/off study treatment and receive all study evaluations per section 6.1 through week 40.

6.2.17.4 Premature Study Discontinuation Evaluations

Subjects who discontinue prematurely from the study prior to the final visit will have the premature treatment/study discontinuation

evaluations performed as per section 6.1 as soon as possible after the study team learns about study discontinuation.

Table 17: Premature treatment/study discontinuation visit

Component	Procedure/Analysis
Administrative	• Confirm/update locator information • Provide available test results • Provide reimbursement for study visit
Clinical	• Record any AEs and concomitant medications • Perform complete physical exam
Blood specimens (~120 mL)	• Collect blood specimens and send to the clinical laboratory for: o CD4+/CD8+ T cell counts o Plasma HIV-1 RNA o CBC with differential and platelets o Chemistry panel as defined in 6.3.5.1 • Collect blood specimens and send to the research laboratory for: o Pharmacologic studies o Immunologic studies o Transcriptomic studies o Metabolic studies

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Results of all approved laboratory tests performed as part of the study will be provided to the subjects' physicians, and any resulting clinical action will be taken by the subject and their physician without interference from the study personnel.

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

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- May jeopardize the subject or may require intervention to prevent one of the events listed above, even if not immediately life-threatening or resulting in death or hospitalization.

To grade diagnoses, signs and symptoms, and laboratory results, the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, available at http://rsc.tech-res.com/safetyandpharmacovigilance, will be used.

6.3.1 Documentation of HIV

Refer to section 4.1.5 regarding assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the CRF.

6.3.2 Medical History

The medical history must include all diagnoses that were either established within 45 days prior to or ongoing at the time of enrollment. Any allergies to any study medications and their formulations must be documented. All elements of the medical history will be recorded on the CRF.

6.3.3 Medication History

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

Table 18. Medications to be recorded

Medication Category	Timeframe
Antiretroviral medications	Complete history
Immune-based therapies	Within 90 days prior to study entry
Drugs for treatment or prevention of opportunistic infections	Within 96 weeks prior to study entry
Vaccines	Within 30 days prior to study entry
All other prescription and non-prescription medications	Within 90 days prior to entry

6.3.4 Clinical Assessments

6.3.4.1 Complete Physical Exam

At the screening, study entry, week 10, week 20, and week 30 visits, a complete physical examination will be performed. A complete physical exam is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the heart and lungs; abdominal exam; and examination of the lower extremities for edema. The complete physical exam will also include vital signs (temperature, heart rate, respiration rate, and blood pressure).

6.3.4.2 Targeted Physical Exam

At all other visits, a targeted physical exam will be performed. A targeted physical exam is directed to the site or organ system involved in any previously identified or new signs or symptoms that the subject has experienced since the last visit. This exam also includes vital signs (temperature, heart rate, respiration rate, and blood pressure).

6.3.4.3 Additional Vital Sign Monitoring at Study Agent Administration Visits

In addition to vital signs recorded as part of either targeted or complete physical exams, vital signs (temperature, heart rate, respiration rate, and blood pressure) will be measured and recorded every 15 minutes for 60 minutes after beginning infusion of the study agent and 30 minutes after completion of infusion at the entry and week 20 visits. Vital signs will also be measured and recorded every 30 minutes for 90 minutes from the beginning of study agent infusion at the week 4, 8, 24, and 28 visits.

6.3.4.4 Height

Height without shoes in centimeters will be recorded on the CRF at entry.

6.3.4.5 Weight

Weight without shoes in kilograms will be recorded on the CRF at each TCZ administration visit and at the end of study visit.

6.3.4.6 Signs and Symptoms

At entry, all signs and symptoms, regardless of grade, that occurred within 45 days before entry will be recorded in the source documents. Post-entry, only grade ≥ 2 signs and symptoms, or those that led to a change in treatment, regardless of grade, will be recorded on the CRF.

6.3.4.7 Diagnoses

After study entry, all new diagnoses established while on study will be recorded on the CRF, and relationship to study agent will be assessed.

6.3.4.8 Concomitant Medications

Concomitant prescription or over-the counter medications initiated or discontinued since the last visit will be recorded on the source documents.

6.3.4.9 Study Treatment Modifications

All study drug modifications, including initial doses or protocol-mandated modifications at each visit will be recorded. Any permanent discontinuation of treatment will be recorded.

6.3.4.10 Antiretroviral Medications

All modifications to antiretroviral medications post-entry will be recorded on the CRF. If the modification includes discontinuation of antiretrovirals, the rules for treatment discontinuation (section 8) must be followed.

6.3.5 Laboratory Evaluations

At entry and at week 20, record hemoglobin, platelet count, ANC, WBC, hematocrit, serum creatinine, AST, ALT, serum glucose, total cholesterol, LDL cholesterol, and triglyceride values, regardless of grade, on the CRFs. After entry and at times other than week 20, only laboratory values grade 2 or higher must be recorded on the CRFs.

6.3.5.1 Clinical Laboratory Tests

All clinical labs as indicated in section 6.1 and listed below will be processed in the CLIA-certified clinical laboratory of University Hospitals Case Medical Center. Specimens for all other laboratory tests for pharmacologic, transcriptomic, immunologic, and metabolic/metabolomics studies will be received, aliquoted, and distributed by the Lederman-Sieg laboratory. Research laboratory studies are described in the next several sections. Clinical laboratories include:

- CBC with differential and platelet count. This test will be submitted and results obtained in real time.
- Metabolic panel, including at least glucose, BUN, creatinine, sodium, potassium, chloride, bicarbonate, total and direct bilirubin, AST, ALT, alkaline phosphatase, total cholesterol, triglycerides, and LDL cholesterol. This test will be submitted and results obtained in real time.
- For subjects participating in the rectosigmoid specimen collection, prothrombin time (PT), international normalized ratio, and partial thromboplastin time (PTT). This test will be submitted and results obtained in real time.
- HIV-1 RNA testing will be done using an FDA-approved method with a lower limit of detection ≤50 copies/mL. This test will be submitted in real time, but the turnaround time is approximately 3 days.
- HBsAg, HBcAb with reflex HBV DNA, HCV Ab, and HCV RNA will be performed using FDA-approved kits as needed to determine eligibility (refer to section 4.2.12). These tests will be submitted in real time. Results will be obtained, however, prior to study entry.
- The QuantiFERON®-TB Gold in-tube test will be used for tuberculosis screening and processed in the clinical laboratory of University Hospitals of Cleveland, according to the manufacturer's instructions. Whole blood will be processed within 16 hours of drawing. Subjects with a positive result will be referred for clinical follow-up, and will be

not eligible to enroll, regardless of previous treatment history. Refer to the MOPS for details about timing of specimen collection, tube type, blood volume required and processing. This test will be submitted in real time. Results will be obtained prior to study entry and prior to the week 20 dose administration.

Additionally, for women of reproductive potential, an FDA-approved rapid pregnancy test (either blood or urine) will be performed at study entry, at each dosing visit, and whenever pregnancy is suspected. At each dosing visit, administration of study agent will begin only after a negative pregnancy test result has been obtained.

6.3.5.2 Pharmacologic Studies

6.3.5.2.1 Tocilizumab levels

Tocilizumab (TCZ) levels in plasma will be quantified by ELISA at each post-entry study visit, except the day 3 and week 20+ 3 days visits, in the Lederman-Sieg laboratory. Before testing samples derived from the study, the assay will be fully validated in the Lederman-Sieg lab. This testing will be done in batch. Refer to the MOPS for details about timing of specimen collection, tube type, blood volume required and processing.

6.3.5.2.2 Antiretroviral Level Monitoring

Trough levels of protease inhibitors and non-nucleoside reverse transcriptase inhibitors will be sent in real time to the laboratory of Edmund Capparelli at the University of California San Diego Antiviral Research Center for processing. Every effort will be made to coordinate dosing times to obtain specimens at a time point as close to the end of the dosing interval as possible. Dates and times of the previous three doses of all antiretrovirals will be recorded on the CRF. A specimen for antiretroviral levels will not be collected if a subject has been off antiretrovirals for 72 hours or longer prior to the visit. Refer to the MOPS for details about timing of specimen collection, tube type, blood volume required and processing.

Specimens for pharmacologic monitoring will be submitted as they are collected, but turnaround time is expected to be 2-3 weeks, not including shipping times. Results will be recorded on the CRF upon receipt and reviewed by investigators by the following workday. If trough levels of a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor are found to be lower than the inferior limit of the range observed in previous population studies for that agent (refer to section 4.2.16 for cutoffs), a new level will be drawn within 5 weeks of the initial

value below the cutoff. This repeat draw can occur at the time of the subsequent scheduled visit if within the 5-week window from the initial lower-than-expected value.

6.3.5.3 Transcriptomic Studies

Gene expression profiles on whole blood, sorted naïve and central memory CD4+ T cells, and monocyte subsets, will be constructed using the Illumina platform from specimens obtained at the study visits indicated in section 6.1. Additionally, mucosal snips and single-cell suspensions prepared by collagenase digestion from rectosigmoid biopsies from subject participating in the flex-sig substudy will be preserved in RNAlater for RNAseq analysis. All gene expression profiling will be performed in the laboratory of Dr. Rafick-Pierre Séklay at the Vaccine & Gene Therapy Institute (VGTI) of Florida in Port St. Lucie, FL. Refer to the MOPS for details about timing of specimen collection, tube type, blood volume required and processing.

PBMCs will be isolated and frozen according to the HANC Cross-Network Consensus Cryopreservation SOP and aliquoted at 5 to$10x10^6$ cells per vial in one ml. Specimens for transcriptomic studies will be shipped and processed in batch. Refer to the MOPS for details about timing of specimen collection, tube type, blood volume required and processing.

6.3.5.4 Metabolic Studies

6.3.5.4.1 Lipid fractions and other markers of cardiovascular risk

Conventional lipid fractions, ApoA1, ApoB, Lp(a), OxLDL, piHDL, PON1, the adipokines adiponectin and leptin, and insulin resistance as reflected by HOMA-IR and resistin levels will be measured in serum or plasma. All the metabolic studies will be performed in batch at the end of the study, except for conventional lipid fractions done in the clinical laboratory as part of the comprehensive metabolic panel (Chem23), as described in 6.3.5.1. Refer to the MOPS for details about timing of specimen collection, tube type, blood volume required and processing.

6.3.5.4.2 Metabolomic profile

Levels of acylcarnitine, amino acid and free carnitine metabolites in plasma and metabolites modulated by IL-6 blockade will be analyzed in collaboration with Metabolon, Inc. (Durham, NC) using liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS). All metabolomics assays will be performed in batch and specimens will be collected at the study visits specified in

section 6.1. Samples will be prepared in Dr. Ghannoum's laboratory using an automated MicroLab STAR® system (Hamilton). Refer to the MOPS for details about specimen collection timing, tube type, required volume, and processing.

6.3.5.5 Immunologic Studies

CD4/CD8 T-cell counts will be done in real-time at screening, study entry and all post-entry study visits in a CLIA-certified or equivalent laboratory.

CRP measurements will be performed in batch in the Lederman-Sieg laboratory using a commercial ELISA kit. Specimens for CRP testing will be collected at each visit, beginning with the study entry visit.

PBMCs will be isolated and frozen according to the HANC Cross-Network Consensus Cryopreservation SOP and aliquoted at 5 to$10x10^6$ cells per vial in one ml. Refer to the MOPS for details of tube type, blood volume required and processing. PBMCs will be used for the following batched assays, all of which will be performed using standard flow cytometry techniques in the Lederman-Sieg laboratory:

- CD4 and CD8 T cell maturation subset frequencies
- Expression of CD127, bcl-2, α4β7, and Ki-67 on each CD4 and CD8 subset
- *Ex vivo* responsiveness of circulating T cells to IL-7 will be measured by Stat 5 phosphorylation and induction of α4β7 expression
- Expression of the activation markers CD38 and HLA-DR on CD4 and CD8 T cells
- Frequency of CD4 T cells expressing high levels of the coinhibitory molecule PD-1

PBMCs will be also be used for the following real-time assays, all of which will be performed using standard flow cytometry techniques in the Lederman-Sieg laboratory:

- Survival and ex vivo completion of cell division by cycling cells by CFSE dye dilution within each T cell maturation subset
- Monocyte subsets and tissue factor expression by flow cytometry

On plasma, the following elements will be measured in batch from specimens obtained at the visit times outlined in section 6.1:

- Bacterial LPS and its soluble receptor sCD14
- Other inflammatory/coagulation markers (IL-6, IP-10, TNFrI, TNFrII, D-dimers)
- Zonulin (reflective of gut barrier integrity) and intestinal fatty acid binding protein (IFABP) as a marker of gut barrier breach (10)

6.3.5.6 Cardiovascular Studies (BART testing)

Assessment of endothelial function will be done by flow-mediated dilatation (FMD), a measure of endothelium-dependent vasoreactivity obtained by brachial artery reactivity testing (BART). Hyperemic velocity time integral (VTI), also obtained during BART, is a complementary measure of *microvascular* health that may be more predictive of cardiovascular events than FMD. Both indices will be measured in the UHCMC Harrington Heart and Vascular Institute. BART will be performed at baseline and at the time of arm shift at week 20, as well as 2 weeks after the last dose in each of the study periods (weeks 10 and 30). Refer to the MOPS for further instructions.

6.3.5.7 Sigmoidoscopy and Biopsies

All subjects will be offered the opportunity to participate in the rectosigmoid specimen collection program, but consent to provide rectosigmoid biopsy specimens will not be a requirement to be on the main study. It is expected, based on previous experience at the UHCMC CRS that approximately 50% of the subjects will consent to flexible sigmoidoscopy and biopsies. Since 2011, the investigators have maintained an IRB-approved protocol to obtain rectosigmoid biopsies via flexible sigmoidoscopy from HIV-infected patients and normal volunteers at the UHCMC site. Since then, approximately 45 subjects have been approached for participation, of whom 25 (56%) consented. Thus, we do not anticipate difficulties recruiting subjects to participate in the rectosigmoid specimen collection part of the study.

There are no published data on the effects of tocilizumab on GALT in HIV-infected subjects. Therefore, while approximately 50% of the entire study population is expected to agree to participate in the gut sampling section of the study, even a smaller number of observations will provide valuable information, if the actual proportion of participants willing to undergo rectosigmoid biopsies is lower than anticipated.

Procedures will be performed in the endoscopy suite of University Hospitals Case Medical Center at the visits specified in section 6. Subjects will receive instructions for preparation before the procedure as per established practice at the UHCMC Digestive Health Institute.

Briefly, the procedure will include the following steps:

- Subjects will be asked to fast after midnight on the evening before the procedure.
- Subjects will be asked to self-administer two enemas 2 hours prior to the procedure
- Visual and digital rectal exam: The examiner will conduct a visual examination of the anus and surrounding area and note any abnormality. The examiner will then insert a lubricated gloved finger into the anal canal and sweep around the internal anal circumference.

- Flexible sigmoidoscopy and biopsy: A flexible sigmoidoscope will be inserted to approximately 15 cm from the anal verge, in order to reach beyond the dentate line and minimize discomfort for the subjects, noting any lesions or abnormalities. Approximately 20 biopsies will be taken using disposable large-cup biopsy forceps.

All procedures will be performed by a trained gastroenterologist and endoscopist in the endoscopy suite at the UHCMC Digestive Health Institute. Further instructions are provided in the MOPS.

6.4 Specimen Collection, Handling, and Processing

6.4.1 General Specimen Management Guidance

The study site will adhere to the standards of good clinical laboratory practice, DAIDS Laboratory Requirements and site standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens at the local laboratories, as described in (http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/gclp.pdf)

Specimen collection, testing, and storage at the site laboratories will be documented per standard site practice. In cases where laboratory results are not available due to administrative or laboratory error, specimens intended for use in the screening as well as ongoing safety assessments process may be re-drawn.

6.4.2 Storage of Specimens for Future Use

Study staff will store all specimens collected in this study on site at least through the end of the study. Specimens will not be labeled with any personal identifiers. Storage of all study samples will follow local standard operating procedure to ensure the anonymity and confidentiality of the trial research participants. Specimens remaining at the end of the study will be transferred to a designated bio-repository with appropriate participants' permission and after all protocol-required and quality assurance testing has been completed. If such permission is not obtained, those participant's samples will be destroyed.

Informed consent for storage of specimens for future studies will be obtained through an existing IRB-approved protocol that governs the existing bio-repository at UHCMC. This protocol exists under the oversight of the Case Western Reserve University Center for AIDS Research (CFAR), and is one of the resources of the CFAR Clinical Services Core, which is directed by Dr. Rodriguez. The Clinical Services Core maintains a fully HIPAA-complaint repository management system, and the protocol provides for separate consents to use stored specimens for: a) use of deidentified data for research purposes; b) non-genetic studies; c) genetic studies; and d) authorization to contact participants for future studies. Participants can consent to any, all, or none of the four activities. The repository management system permits tracking and updating

in real time, for each record in the database and for each specimen, whether a valid consent of any of the four types described above exists in the system.

7.0 CLINICAL MANAGEMENT ISSUES

If at any time, a decision is made to discontinue study agent in all participants, the IND Sponsor (Dr. Benigno Rodriguez), after consultation with the DAIDS MO, and the protocol team will inform the US Food and Drug Administration (FDA), the IRB, and the site investigators.

The grading system for drug toxicities is located in the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, available at http://rsc.tech-res.com/safetyandpharmacovigilance.

7.1 Management of Specific Adverse Events

7.1.1 Serious Infections

All subjects will be monitored for signs or symptoms of a serious or local infection at each visit. All subjects who develop a serious infection while on study will be referred for immediate medical care to a qualified healthcare professional, and will be required to discontinue study treatment, as specified below. Instructions for evaluations in subjects who discontinue study treatment are provided in section 6.2.17.3.

- Participants who develop any infection listed in section 8.1 will discontinue study treatment permanently.
- Participants who experience an infection that is considered life-threatening or that requires inpatient treatment, will discontinue study treatment permanently.
- Subjects who experience other serious infections not listed in 8.1 and that do not require inpatient treatment and are not considered life-threatening will have study treatment temporarily stopped until the infection is controlled. These subjects may restart study drug once the infection is controlled.

Other infections, including local infections, will also require referral to qualified medical treatment, and all of them will also require, at a minimum, temporary discontinuation of study treatment until the infection is adequately controlled. The study co-chairs, in consultation with the Medical Officer, will determine if an infection not listed in section 8.1 warrants permanent treatment discontinuation even after the infection has been adequately controlled.

7.1.2 Acute Hypersensitivity Reactions

To minimize the risk of a severe, immediate-type hypersensitivity reaction, all doses of study agent will be administered in the Dahms Clinical Research Unit, which is located within the Rainbow Babies & Children's Hospitals and has full

inpatient management capabilities, including the ability to provide Advanced Cardiac Life Support and to transfer a patient to an intensive care bed without leaving the building complex. Full resuscitation equipment is available within the DCRU, and personnel trained in the management of anaphylaxis are available 24 hours a day.

Development of a grade ≥3 hypersensitivity reaction OR a grade 2 hypersensitivity reaction that includes manifestations of angioedema will require permanent treatment discontinuation.

7.1.3 Gastrointestinal Perforation

All subjects will be monitored for signs or symptoms of gastrointestinal perforation at each visit. Subjects who present with new onset of abdominal symptoms will be immediately referred for immediate evaluation by a qualified healthcare professional to rule out gastrointestinal perforation. Subjects who develop a gastrointestinal perforation during study will be required to discontinue study treatment permanently, and will be followed until resolution of the AE.

7.1.4 Infusion Reactions

To minimize the risk of severe infusion reactions, all doses of study agent will be administered in the Dahms Clinical Research Unit, which is located within the Rainbow Babies & Children's Hospitals and has full inpatients management capabilities, including the ability to provide Advanced Cardiac Life Support and to transfer a patient to an intensive care bed without leaving the building complex. Full resuscitation equipment is available within the DCRU, and personnel trained in the management of infusion reactions are available 24 hours a day.

Development of a grade ≥3 infusion reaction will require permanent treatment discontinuation.

7.1.5 Laboratory Abnormalities

7.1.5.1 Neutropenia

A CBC with differential will be obtained at each study visit from entry to end of study. Subjects who develop neutropenia will be referred for treatment by a qualified healthcare professional. Subjects with an ANC <2,000 cells/mm^3 at screening will be excluded.

Subjects who develop an ANC ≤1,000 but ≥500 cells/mm^3 will be required to discontinue study treatment temporarily for the visit at which the low ANC was recorded, and the pharmacy will be notified to provide a 4 mg/Kg dose of the assigned study agent, regardless of scheduled dose, until the ANC has risen to above 1,000 cells/mm^3. Unblinding will not be required.

Subjects who develop an ANC <500 cells/mm^3 will be required to discontinue study treatment permanently.

7.1.5.2 Thrombocytopenia

A platelet count will be obtained at each study visit from enrollment to end of study. Subjects who develop thrombocytopenia will be referred for treatment by a qualified healthcare professional. Subjects with a platelet count below 100,000 cells/mm^3 at screening will be excluded.

Subjects who develop a platelet count ≤100, 000 but ≥50,000 cells/mm^3 will be required to temporarily discontinue study treatment for the visit at which the low platelet count was recorded, and the pharmacy will be notified to provide a 4 mg/Kg dose of the assigned study agent, regardless of scheduled dose. The 4 mg/Kg dose will be continued until the platelet count has risen to above 100,000 cells/mm^3. Unblinding will not be required.

Subjects who develop a platelet count <50,000 cells/mm^3 will be required to discontinue study treatment permanently.

7.1.5.3 AST and ALT Abnormalities

AST and ALT will be monitored at each visit from study entry to the end of study. Subjects with AST or ALT over 1.5 times the upper limit of normal or who have evidence of hepatitis B or hepatitis C at screening will be excluded.

7.1.5.3.1 AST or ALT >1.4 to 3 Times the Upper Limit of Normal

Subjects who develop an AST or ALT in this range while on study may continue study treatment, but the pharmacy will be notified to provide a dose of 4 mg/Kg of the assigned study agent, regardless of the scheduled dose. Unblinding will not be required. The dose will not be increased to 8 mg/Kg until both the AST and ALT have returned to below the upper limit of normal.

7.1.5.3.2 AST or ALT >3 to 5 Times the Upper Limit of Normal

Subjects who develop an AST or ALT in this range will temporarily discontinue study treatment, until both the AST and ALT return to below 3 times the upper limit of normal, at which time guidelines outlined in section 7.1.5.3.1 will apply. Unblinding will not be required.

7.1.5.3.3 Liver Enzymes >5 Times the Upper Limit of Normal

Subjects who develop an AST or ALT in this range will be required to discontinue study treatment permanently.

7.1.5.4 Lipid Abnormalities

Subjects who experience incident lipid abnormalities will be referred for adequate medical treatment by a qualified healthcare professional, but in light of the short duration of the study, isolated lipid abnormalities will not require study treatment modification or discontinuation. In general, treatment for dyslipidemias by providers in the John T. Carey Special Immunology Unit of UHCMC follows the ATP III guidelines. Consultation with experts in cardiovascular risk is available on-site in the Special Immunology Unit by co-investigator Dr. Chris Longenecker.

7.1.6 Malignant Conditions

All subjects will be monitored for signs or symptoms of a malignant condition at each visit. Subjects who develop a malignancy other than a basal cell skin carcinoma requiring no more than local excision during study will be required to discontinue study treatment permanently.

7.1.7 Viral Reactivation

Subjects who develop pain, a skin rash, or other clinical manifestation deemed consistent with an episode of herpes zoster, will be immediately referred for evaluation by a qualified clinician, and will be required to discontinue study treatment until any pharmacologic treatment for varicella-zoster has been completed and the clinical manifestations have resolved in the judgment of the treating physician. Subjects who develop recurrence of signs or symptoms or herpes zoster reactivation, and those who develop involvement of more than two dermatomes or have evidence of visceral involvement while on study will be require to discontinue study treatment permanently.

Subjects who develop a detectable HBV DNA at any time during the study will be required to discontinue study treatment permanently, and will be referred for specialized medical care a soon as the study team becomes aware of a positive HBV DNA result.

7.1.8 Cytochrome P450 Inhibition and Decreased Plasma Levels of Antiretrovirals

If a trough level of an antiretroviral agent that is part of a subject's regimen is reported to be below the cutoff levels listed in table 2 (section 4.2.16), a confirmation visit will be scheduled as soon as the result is received. The confirmation visit must occur within 5 weeks of the initial specimen collection date (due to an expected turnaround time of 2-3 weeks for the antiretroviral level testing), and can coincide with the next scheduled study visit. Additionally, the

treating physician will be informed of the result, as well as the confirmatory test result when available.

The results of the plasma HIV RNA must also be reviewed each time an antiretroviral level test is below the cutoff. If the concomitant plasma HIV RNA level is below the limit of detection of the assay, subjects will be allowed to continue study treatment until the confirmation test result is obtained.

If the plasma HIV RNA corresponding to the initial low antiretroviral level result is above the limit of detection of the assay, the subject will be required to discontinue study treatment temporarily until the confirmatory result is available, and a confirmatory virologic failure blood draw will be scheduled. If the confirmation antiretroviral level result is also below the cutoff level, the subject will be required to discontinue study treatment permanently.

7.1.9 Demyelinating Conditions

All subjects will be monitored for signs or symptoms of a demyelinating condition at each visit. Subjects who develop a demyelinating condition such as multiple sclerosis or chronic inflammatory demyelinating polyneuropathy during study will be required to discontinue study treatment permanently.

7.2 Management of Other Adverse Events

7.2.1 Grade 1 or 2 AE

Subjects who develop a Grade 1 or 2 AE other than those discussed in Section 7.1 may continue study treatment.

7.2.2 Grade 3 AE

If the investigators have compelling evidence that the AE has NOT been caused by the study treatment, dosing may continue. Otherwise, subjects who develop a Grade 3 AE will have study treatment withheld, except as stated in section 7.1. The subject will be reevaluated closely until the AE returns to Grade ≤2, at which time study treatment may be reintroduced at the discretion of the investigators or according to standard practice.

If the same Grade 3 AE recurs during the study, study treatment must be permanently discontinued unless an alternative etiology for the AE that is not related to study treatment has been identified.

Subjects experiencing Grade 3 AEs requiring permanent discontinuation of study treatment will be followed closely for resolution of the AE to Grade ≤ 2 and the protocol core team will be notified.

7.2.3 Grade 4 AE

Subjects who develop a Grade 4 symptomatic AE will have study treatment discontinued. If the investigators have compelling evidence that the AE has NOT been caused by the study treatment, after consultation with the safety monitoring committee (SMC, refer to section 9.4) and the DAIDS MO, dosing may resume when the AE has resolved. Subjects experiencing Grade 4 AEs requiring permanent discontinuation of study treatment will be followed closely until resolution of the AE to Grade ≤ 2 and the protocol core team will be notified.

7.3 Resumption of Study Treatment Administration when the Crossover Dose Is Held

If the first dose of the second period (week 20) is held for any of the reasons described above, the first dose given at a subsequent visit will always be 4 mg/Kg.

7.4 Pregnancy

All women of reproductive potential will be asked to affirm that they currently use and will continue to use throughout the study two different methods of contraception as specified in section 4.1.11. At each visit, continued use of acceptable contraceptive methods will be ascertained and recorded in the source documents. Additionally, on each of the study dosing visits, women of childbearing potential will be required to undergo rapid pregnancy testing. Both the ascertainment of continued use of acceptable contraceptive methods and verification of a negative pregnancy test will be done before administering the study medication. Women who have interrupted one or both contraceptive methods will discontinue study treatment until they have resumed use of acceptable contraceptive methods.

Female subjects who become pregnant while on study treatment despite the above measures must immediately discontinue study treatment and consult with their primary healthcare providers. In consultation with the subject, her healthcare provider, and the SMC, a decision may be made to unblind the participant if she requests access to unblinding information to assist in her decision making process about the pregnancy. Subjects will be encouraged to remain in the study to be followed on study/off study treatment until study completion and will be followed by telephone contact thereafter to determine the pregnancy outcome. Pregnant women will also be invited to enroll in the pregnancy registry by calling 1-877-311-8972. Pregnancy-related outcomes (health of the infant) and any pregnancy-related complications (e.g., fetal loss/abnormalities) must be reported on the CRF. Subjects should have the premature treatment discontinuation evaluations completed within 2 weeks after stopping study treatment.

NOTE: Pregnant subjects will be considered on study until the outcome of the pregnancy has been obtained and reported on the CRF.

7.5 Reporting of Adverse Reactions to the Responsible IRBs

The investigators will report adverse reactions to the responsible IRB in accordance with respective IRB policies and procedures.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting, the investigator will report it as soon as possible in accordance with respective IRB policies and procedures.

7.6 Social Harms Reporting

Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities.

Social harms that are judged by the study chairs to be serious or unexpected will be reported to responsible site IRB at least annually or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Permanent Treatment Discontinuation

- Interruption of antiretroviral medications for >4 consecutive weeks
- Change of antiretroviral medications to a regimen that does not meet the criteria for eligible antiretroviral treatment in section 4.1.6 for >4 consecutive weeks.
- Loss of virologic control, defined as a plasma HIV RNA >200 copies/mL on two consecutive occasions at least two weeks apart. A single plasma HIV RNA measurement >200 copies/mL is permissible if the subsequent measurement is <200 copies/mL.
- Occurrence of a grade ≥3 hypersensitivity reaction or infusion reaction OR a grade 2 hypersensitivity reaction that includes angioedema of any grade
- Drug-related AE meeting the criteria for discontinuation in sections 7.1 or 7.2.
- Occurrence of any of the following opportunistic complications:

    - Tuberculosis, pulmonary or extrapulmonary
    - Non-tuberculous mycobacterial infection, disseminated or extrapulmonary
    - *Pneumocystis jirovecii* pneumonia
    - Coccidioidomycosis, disseminated or extrapulmonary
    - Cryptococcosis, extrapulmonary
    - Cryptosporidiosis or isosporiasis, chronic intestinal (greater than 1 month's duration)
    - Cytomegalovirus disease (other than liver, spleen, or lymph nodes) and including retinitis with loss of vision

- Histoplasmosis, disseminated or extrapulmonary
- Kaposi's sarcoma
- Lymphoma, Burkitt's or immunoblastic, regardless of anatomical location
- Lymphoma, primary of brain
- Progressive multifocal leukoencephalopathy
- Toxoplasmosis of brain

- Occurrence of herpes zoster reactivation involving more than one dermatome or visceral involvement including but not limited to viral pneumonia, encephalitis and hepatitis.
- Recurrence of herpes zoster reactivation while on study, regardless of anatomic location.
- Development of a detectable HBV DNA at any time during the study.
- Occurrence of a serious infection that is considered life-threatening or requires inpatient treatment.
- Occurrence of an incident cancer other than a localized basal cell carcinoma of the skin that requires local excision only
- Occurrence of a suspected or confirmed demyelinating condition.
- Occurrence of an AST or ALT level above 5 times the upper limit of normal.
- Occurrence of an ANC below 500 cells/mm^3.
- Occurrence of a platelet count below 50,000 cells/mm^3.
- Requirement for prohibited concomitant medications (see appendix 1 of the MOPS).
- A confirmed trough level of a protease inhibitor or non-nucleoside reverse transcriptase inhibitor included in the subject's antiretroviral regimen that is lower than the lower limit of the steady-state trough level range observed in published studies. Refer to section 4.2.16 for cutoff values.
  NOTE: For the purposes of this treatment discontinuation criterion, "confirmed" refers to two consecutive trough levels of the antiretroviral agent no more than 5 weeks apart that are both below the expected level, as defined in section 4.2.16.
- Pregnancy or breastfeeding.
- Completion of treatment as defined in the protocol.
- Request by subject to terminate treatment.
- Reasons believed to be clinically significant by the investigators, even if not addressed in the adverse event management section of the protocol.

8.2 Premature Study Discontinuation

- Failure by the subject to attend 3 consecutive visits or 2 medication visits during the study defined study windows.
- 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 institutional review board (IRB), Office for Human Research Protections (OHRP), FDA, NIAID or investigator.

8.3 Temporary Treatment Discontinuation

- Subjects who meet a temporary treatment discontinuation criterion (sections 7.1 and 7.2) will be required to discontinue study treatment temporarily.
- If a subject has not received any component of his/her antiretroviral treatment for ≥72 hours prior to a scheduled treatment administration visit, study treatment will be held for that visit, and this treatment modification will be recorded on the CRF. Study treatment administration can resume once the subject has received continuous antiretroviral treatment (without interruption ≥72 hours) for at least 28 days.
- Interruption of contraceptive methods in a female participant of reproductive potential such that at any time, the participant is using fewer than two methods as detailed in section 4.1.11. Study treatment administration can resume once the participant has been receiving an acceptable contraceptive regimen for at least 30 days.

8.4 Criteria for Unblinding

The SMC, in consultation with the statistician, will determine whether unblinding of a subject's arm assignment is warranted, and which personnel must receive the unblinding information. Regardless of determination, the decision will be reported to both the DAIDS MO and the FDA, in addition to any additional reporting requirements related to the event precipitating the unblinding. Unblinding will be considered in the following cases:

- Pregnancy
- Occurrence of a life-threatening adverse event that is thought to be probably or definitely related to study treatment
- Occurrence of a number of AEs that meets criteria for study discontinuation, where ascertainment of arm assignment is needed to establish relationship to TCZ (see section 12.3).
- Emergence of a concomitant medical condition that requires knowledge of the study agent administered for optimal medical management
- Upon instructions from the FDA, DAIDS, OHRP, or IRB

9.0 STATISTICAL CONSIDERATIONS

9.1 Design and Primary Endpoints

This is a randomized, double-blind crossover (AB/BA) study. The data analysis will evaluate the effect of TCZ on safety, inflammation, cellular cycling, and various secondary endpoints. The analysis of all secondary endpoints will be exploratory only. The primary endpoints are described below.

9.1.1 Primary Endpoint 1 (Safety)

The primary safety endpoint is the frequency of grade ≥2 clinical or laboratory adverse events related to study treatment during each period.

9.1.2 Primary Endpoint 2 (Inflammation)

The primary inflammation endpoint is serum C-reactive protein level at 10 weeks and 30 weeks.

9.1.3 Primary Endpoint 3 (Cellular Cycling)

The primary cellular cycling endpoint is the proportion of central memory T cells expressing the nuclear antigen Ki67 at 10 weeks and 30 weeks.

9.2 Statistical Analysis

All data analyses will be performed in the intent to treat population and will analyze subjects using the sequence to which they were randomly assigned. Additionally, a planned secondary analysis will be conducted that will include only subjects who completed study treatment and were followed up to week 30 at a minimum. Because it is desired to obtain a sufficient level of precision for both the primary (intention-to-treat) and the secondary (complete observation) analyses, the sample size and power calculations are based on the expected number of subjects who will have complete follow-up data (thirty subjects out of up to 36 enrolled subjects).

9.2.1. Baseline Comparability

To examine subject differences between the two sequences, variables measured prior to treatment initiation in the first period as well as the within-subject treatment effect on each primary and secondary endpoint (defined by the differences in endpoints between week 10 and week 30) will be summarized by sequence using standard summary statistics and subject-profile plots as necessary. Similarly, to examine differences between the two periods, within-subject differences in each endpoint between study entry and week 10 (or week 20 and week 30) will be summarized by group (tocilizumab or placebo) and period. The approach is the same for cardiovascular values and for rectal tissue studies, EXCEPT for those endpoints, all changes will be measured relative to the baseline visit (visit 2).

9.2.2 Safety Analysis

Safety will be assessed in all participants who are randomized and begin study treatment. The number and percentage of participants experiencing at least one grade ≥2 clinical or laboratory adverse event related to study treatment during each period (0 to 10 weeks for Period 1 or 20 to 30 weeks for Period 2) will be tabulated separately for each treatment arm. Based on the Clopper-Pearson (98) method, an exact 95% confidence interval of the probability of a safety event will be estimated separately for each arm.

9.2.3 Primary Analysis of Two Co-Primary Endpoints

All co-primary endpoints will be assessed in all participants who are randomized

and begin study treatment. To test the null hypothesis that the mean of each co-primary endpoint is the same in both arms, a separate linear mixed model will be used to estimate the mean (and 95% CI) of the difference in each endpoint between treatment arms, adjusting for period. To increase the precision of the estimated treatment effect, the within-period baseline measurements of each endpoint will be included as responses in the linear mixed model using the method described in Kenward and Roger (99). If all subjects complete all visits, such an analysis will provide estimates of the treatment effect that are identical to an analysis using within-period baseline measurements as covariates.

9.2.4 Secondary Safety Analyses

To test the null hypothesis that the incidence of safety events is the same during treatment with TCZ as during treatment with placebo (versus the alternative hypothesis that the incidence of safety events is not the same during TCZ treatment as during placebo treatment), an estimate, 95% CI, and p-value of the relative risk of a safety event (TCZ: placebo) will also be calculated using a generalized linear mixed model that is estimated via Gaussian quadrature. The model will be based on a binomial distribution with a log link function and will incorporate fixed effects for period and treatment as well as a random effect representing subjects within sequence.

Secondary analyses using the number of grade ≥2 clinical or laboratory adverse events related to study treatment during each period (0 to 10 weeks for Period 1 or 20 to 30 weeks for Period 2) will also be performed. To test the null hypothesis that the rate of such events is the same during TCZ as during placebo treatment (versus the alternative hypothesis that the rate of these events is not the same during TCZ as during placebo treatment), the relative rate of a safety event (TCZ: placebo) will be estimated using a generalized linear mixed model based on a Poisson distribution and an offset variable to account for varying follow-up times within each period (which may arise due to study discontinuation). Fixed and random effects in the model will remain the same as previously described.

9.2.5 Secondary Analyses of Two Co-Primary Endpoints

The primary analysis of the two co-primary (CRP and T cell cycling) endpoints will specifically focus on the changes from study entry to week 10 and from week 20 to week 30. However, some endpoints are measured at other time points as well (see Table 6.1) and so to estimate the treatment effect at these time points, a secondary analysis will be done by fitting the model defined in Kenward and Roger (99) to include these additional measurements.

9.2.6 Adjustment for Baseline Covariates Measured prior to Randomization

In the case of a cross-over study with complete data, adjustment for baseline covariates measured prior to randomization has no effect on estimates of the treatment effect (or their precision) because the treatment effect is based on a

within-subject difference. However, this property does not hold if any endpoint data on a subject is missing. Because we expect some participants will drop-out of the study before the 30 week timepoint, secondary analyses will be conducted that incorporate fixed effects for CD4 T cell count, type of ART, and time since HIV diagnosis into the mixed models previously described.

9.2.7 Carry-over Effects

An important consideration in any cross-over design is the existence of a carry-over effect. In the past, a formal test for a carry-over effect was done using a test of the interaction between treatment arm and period and if one was detected, an analysis of Period 1 data was performed. However, as discussed by Senn (100), this type of sequential strategy is not very useful because many cross-over designs are underpowered to detect such interactions and even if one is detected, tests based on Period 1 data are also underpowered. With this in mind, no formal test for a carry-over effect will be conducted. Instead, a 12 week washout period (over 10 times the half-life of tocilizumab) has been incorporated into the study design to account for a possible carryover effect.

9.2.8 Multiplicity

There are 3 co-primary endpoints in this study: safety, CRP, and T cell cycling. The primary analysis for the safety endpoint is based on an estimate of precision, rather than a formal hypothesis test, because the rate of adverse events is expected to be low and the sample size is too small to detect any clinically meaningful differences between the two arms. Consequently, adjustment for multiplicity will only be done for the two co-primary endpoints, CRP and T cell cycling using a Bonferroni-Holm's method. This method will ensure that the familywise error rate (e.g., the probability of at least one Type I error arising from the two tests) does not exceed 0.05.

Note that all secondary objectives are exploratory and so no adjustment will be made to account for inflation of the Type I error rate arising from hypothesis tests of the secondary endpoints. Any results from these endpoints will be considered hypothesis-generating and will require further confirmation and replication to be considered established.

9.2.9 Missing Data

While simpler statistical methods could have been chosen to test our null hypotheses (e.g., a matched pairs t-test to compare the mean of continuous endpoints between arms; a McNemar's test to compare the proportion of safety events between arms), these methods remove subjects with any missing endpoint data from the entire analysis and assume that the mechanism for missing data is completely at random (e.g., subjects with complete data are a random sample of subjects from the study population). Because this assumption does not typically hold, a maximum likelihood approach based on generalized linear mixed models was selected. This approach incorporates all subjects

having an endpoint measured on at least one time point and produces unbiased parameter estimates when response data is missing at random (e.g., the missing data mechanism depends only on observed, not unobserved responses).

9.3 Sample Size Justification

9.3.1 Sample Size Considerations for Safety

Published TCZ safety studies have not used the DAIDS AE grading system, and therefore specific estimates of the expected frequency of grade ≥2 AEs are not available. Nonetheless, in the integrated database, the frequency of AEs requiring study discontinuation was 6.9/100 PY in the placebo group and 10.2 in the TCZ 8 mg/Kg group (67). The follow-up time in each period is 10 weeks and so assuming similar frequencies of adverse events occur, we expect to observe zero or one event in each arm among 30 subjects.

A single summary outcome of this type (yes/no) can be assumed to follow a Bernoulli distribution. Consequently, Table 19 shows the probability of zero, one or more, two or more, and three or more subjects experiencing a qualifying AE in each arm in a sample of 30 subjects with complete follow-up data through week 30. Selected true underlying probabilities of at least one grade ≥2 clinical or laboratory adverse event related to study treatment vary between 0.01 and 0.10. Table 20 shows the exact 95% confidence intervals for the probability of a qualifying event in each arm when 0 to 5 events are observed among 30 subjects with complete follow-up data through week 30.

Table 19. Probability of events for selected true probabilities of qualifying AEs

True rate	Zero subjects	One or more subjects	Two or more subjects	Three or more subjects
0.01	0.74	0.26	0.036	0.0033
0.02	0.55	0.45	0.12	0.022
0.03	0.4	0.6	0.23	0.06
0.04	0.29	0.71	0.34	0.12
0.05	0.21	0.79	0.45	0.19
0.06	0.16	0.84	0.54	0.27
0.07	0.11	0.89	0.63	0.35
0.08	0.082	0.92	0.7	0.43
0.09	0.059	0.94	0.77	0.51
0.1	0.042	0.96	0.82	0.59

9.3.2 Sample Size Considerations for Inflammation and Cellular Cycling

In the 8 mg/kg arm of a tocilizumab registrational trial, CRP decreased from 2.8±3.37 to approximately 0.5 mg/dL mg/dL at week 12 (101). Although there are no published data on the effect of TCZ on T cell cycling, in our CLIF study, 5.18±2.54% of central memory CD4 T cells were in cycle among immune failure subjects compared to 2.91±1.03 among immune success subjects(6).

Table 20. Exact 95% CIs for probability of a qualifying AE

Observed number of events	Estimated probability	Lower bound	Upper bound
0	0	0	0.12
1	0.033	0	0.17
2	0.067	0.0082	0.22
3	0.1	0.021	0.27
4	0.13	0.038	0.31
5	0.17	0.056	0.35

While this value is not related to the administration of TCZ, we will use this difference as a plausible observable difference because both immune failure and immune success subjects in the CLIF study were virologically controlled subjects, similar to those who will be included in the present study who were not receiving an immunomodulatory agent.

Under these assumptions, power calculations were performed under a variety of scenarios. In particular, we assumed that the within-subject mean difference between TCZ and placebo arms in CRP and T cell cycling would vary between 2 and 3 units, the standard deviation in CRP in either arm would vary between 3 and 4, the standard deviation in T cell cycling in either arm would vary between 1 and 3, the correlation between the first and second period measurements for either endpoint would vary between 0.2 and 0.5, and no carryover effect. Under these assumptions, we then conducted 5000 simulations to estimate empirical power. For each simulation, data for 30 subjects was simulated, a paired t-test was applied to each endpoint, and the p-values were adjusted using Holm's method to ensure that the familywise-error rate arising from the two tests was maintained at 0.05. We then calculated the empirical power for: 1) Detecting the specified mean difference in CRP between the 2 arms; 2) Detecting the specified mean difference in T cell cycling between the 2 arms; 3) Detecting the specified mean difference in CRP or T cell cycling between the 2 arms (any pair power); and 4) Detecting the specified mean difference in both CRP and T cell cycling between the 2 arms (all pair power). The results are provided in Table 21 and show that, assuming a sample size of 30 subjects with complete follow-up through week 30, there is ample power to detect the hypothesized mean difference in T cell cycling between TCZ and placebo arms in the vast majority of scenarios and adequate power to detect the hypothesized mean difference in T cell cycling between TCZ and placebo arms in most scenarios.

9.3.3 Accrual and Loss to Follow-up

Given the intensive nature of the study, losses to follow-up and premature treatment or study discontinuations are possible. An additional subject will be recruited for every subject who is lost to follow-up or discontinues study or study treatment before week 30, until up to 36 subjects total have been enrolled. Based on previous experience at the site, we estimate that this will yield approximately 30 subjects with complete follow-up through week 30.

Table 21. Empirical power to detect mean differences in CRP and T cell cycling

Mean difference in CRP	Mean difference in T cell cycling	SD in CRP	SD in T cell cycling	Correlation between first and second period measurements	Power to detect mean difference in CRP	Power to detect mean difference in T cell cycling	Any pair power	All pair power
2	2	3	1	0.2	0.79	1	1	0.79
				0.5	0.94	1	1	0.94
			3	0.2	0.77	0.77	0.91	0.63
				0.5	0.93	0.93	0.99	0.87
		4	1	0.2	0.55	1	1	0.55
				0.5	0.75	1	1	0.75
			3	0.2	0.53	0.74	0.83	0.44
				0.5	0.74	0.93	0.96	0.7
	3	3	1	0.2	0.8	1	1	0.8
				0.5	0.94	1	1	0.94
			3	0.2	0.8	0.98	0.99	0.79
				0.5	0.95	1	1	0.95
		4	1	0.2	0.55	1	1	0.55
				0.5	0.75	1	1	0.75
			3	0.2	0.54	0.98	0.98	0.54
				0.5	0.76	1	1	0.76
3	2	3	1	0.2	0.99	1	1	0.99
				0.5	1	1	1	1
			3	0.2	0.98	0.79	0.99	0.78
				0.5	1	0.94	1	0.94
		4	1	0.2	0.88	1	1	0.88
				0.5	0.98	1	1	0.98
			3	0.2	0.86	0.78	0.94	0.7
				0.5	0.97	0.94	1	0.92
	3	3	1	0.2	0.99	1	1	0.99
				0.5	1	1	1	1
			3	0.2	0.99	0.99	1	0.98
				0.5	1	1	1	1
		4	1	0.2	0.88	1	1	0.88
				0.5	0.98	1	1	0.98
			3	0.2	0.88	0.98	0.99	0.87
				0.5	0.98	1	1	0.98

9.4 Monitoring

The study data manager and statistician will prepare a monthly report of accrual, early study treatment and study discontinuations (and related reasons), baseline characteristics, Grade ≥2 signs and symptoms, Grade ≥2 laboratory abnormalities and reported serious adverse events (SAEs). Tables in the report will be based on the entire cohort of subjects that are randomized and will not be stratified by either sequence or

treatment arm. This report will be reviewed by the research team and DAIDS Medical Officer on a monthly basis.

The study data manager and statistician will also prepare a similar report on a quarterly basis for review by the DAIDS Medical Officer or designee. In this case, tables in the report will be stratified by treatment arm using a label of A and B to maintain blinding.

Approximately 3 months after enrollment of the first subject and every 3 months thereafter, interim reviews of the study will occur. A Safety Monitoring Committee (SMC) will review reports similar to those described for the DAIDS Medical Officer but the reports will also include a summary of longitudinal changes in CD4 T-cell count and HIV-1 RNA levels as well as the availability of stored samples for planned assays.

In addition to the regularly scheduled reviews, the SMC will perform expedited reviews of the safety data, unblinded to treatment assignment, whenever any of the following happens:

- Three or more participants have experienced a grade 3 AE that is deemed possibly, probably, or definitely related to study treatment
- Two or more participants have experienced a grade 4 AE that is deemed possibly, probably, or definitely related to study treatment
- Any death occurs on study that is deemed possibly, probably, or definitely related to study treatment

Whenever any of the events above occurs, enrollment into the study will be paused until the SMC review has taken place and a determination has been made that enrollment can resume.

The SMC will be composed of an investigator affiliated with Case Western Reserve University in a department other than the Division of Infectious Diseases and HIV Medicine, an investigator not affiliated with Case Western Reserve University, a biostatistician not connected to the trial, and a representative of the community or bioethicist. In addition to reviewing the monthly reports and all SAEs and EAEs, the SMC will meet every 3 months on a regular schedule, and every time a reason is identified by the DAIDS Medical Officer, study chairs, or study statisticians in consultation with the team.

The DAIDS Medical Officer and DAIDS Program Officer will receive the following safety-related documents sent to the FDA at the time they are reported to the FDA or earlier: all SAE reports, study drug-related AE reports expected or not, and a quarterly listing of all AEs. The DAIDS Medical Officer and DAIDS Program Officer will also receive copies of any correspondence received from the FDA.

10.0 PHARMACOLOGY PLAN

As discussed above, trough levels of antiretrovirals will be measured at the following visits: screening, study entry, week 4, week 8, week 10, week 20, week 24, week 28, and week 30, as well as premature study discontinuation or when needed for

confirmation of a possible pharmacologic interaction. Levels of TCZ will be measured in batch at the end of the follow-up period. Refer to section 4.2.16 for cutoff values for trough NNRTI and protease inhibitor levels. Confirmed trough levels below the cutoff values (found to be persistently below the threshold within 5 weeks of the initial measurement) will require study treatment discontinuation (refer to section 8.1).

11.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

11.1 Records to Be Kept

CRFs will be provided for each subject. Subjects will not be identified by name on any CRFs. Subjects will be identified by the patient identification number (PID). Source documents and data will be maintained in accordance with Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/sourcedocpolicy.pdf).

Study personnel will maintain and securely store complete, accurate, and current study records throughout the study. Per US regulations, for each of the investigational products tested, the investigators will retain all study records on site for at least two years after the investigation is discontinued and the US FDA is notified.

Study records will be maintained on site for the entire period of study implementation. No study records will be moved to an off-site location or destroyed prior to receiving approval from both DAIDS.

11.2 Study Coordination

Dr. Benigno Rodriguez holds the IND for this study. DAIDS will be informed of all communications with the FDA. Copies of all correspondence from the FDA, and all safety reports will be forwarded to DAIDS, for cross-referencing with other INDs for the study products.

Study implementation will follow the most recent DAIDS Medical Officer and FDA approved protocol. All amendments will be signed off by the DAIDS Medical Officer and FDA prior to implementation, unless the change is required to assure patient safety. Study implementation will also be guided by a Manual of Operation Procedures that provides further instructions and operational guidance on conducting study visits; data and forms processing; specimen collection, processing, and shipping; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations.

Close coordination among protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, retention, follow-up, and AE incidence will be monitored closely by the team.

11.3 Clinical Site Monitoring and Record Availability

Monitoring of the study will be done in compliance with the Requirements for On-Site Monitoring of DAIDS Funded and/or Sponsored Clinical Trials, GCP, and FDA regulations 21 CFR Part 312: http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/onsitemonitor_reqs.pdf

Monitoring visits may include any or all of the following activities:

- Assess compliance with the study protocol, Good Clinical Practices (GCP) guidelines, and applicable regulatory requirements, including US CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312
- Review informed consent forms, procedures, and documentation
- Perform source document verification to ensure the accuracy and completeness of study data
- Verify proper collection and storage of biological specimens
- Verify proper storage, dispensing, and accountability for investigational study products
- Assess implementation and documentation of internal site quality management procedures
- Assess site staff training needs

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of the DAIDS and the US regulatory authorities. A site visit log will be maintained at the study sites to document all visits. The outcomes of the monitoring visits and the subsequent reports of resolutions of any identified problems will be provided to the Sponsor of the IND application, Dr. Benigno Rodriguez.

The site investigator will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the NIAID, the OHRP, or the FDA or designee for confirmation of the study data.

11.4 Adverse Event Reporting

11.4.1 Definitions

11.4.1.1 Adverse event (AE)

An AE is any untoward medical occurrence in a participant who is being administered a study agent/intervention, but does not necessarily have a causal relationship with the study agent/intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom,

or disease temporally associated with the use of the study agent/intervention, whether or not related to the study agent/intervention.

11.4.1.2 Adverse Reaction

An *adverse reaction* is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

11.4.1.3 Suspected

A *suspected* adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, “reasonable possibility” indicates that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

11.4.1.4 Unexpected

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the package insert, or is not listed with the same characteristics or severity observed in the trial, or is not consistent with the risk information described elsewhere in the current protocol.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the package insert as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation.

11.4.1.5 Serious

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigators or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are not life-threatening, and do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

11.4.1.6 Life-Threatening

An adverse event or suspected adverse reaction is considered *life-threatening* if, in the view of either the investigators or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it hypothetically occurred in a more severe form, might have caused death.

11.4.2 Expedited Adverse Event Reporting to the DAIDS Medical Officer

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the investigators or his/her designee must notify the SMC Chair (or qualified alternate) and the DAIDS Medical Officer no later than 3 reporting days after the investigators become aware of the event. For the purposes of expedited reporting to the SMC and the DAIDS Medical Officer, the definition of a “reporting day” in Version 2.0 of the DAIDS EAE Manual will be used.

11.4.3 Reporting to the University Hospitals of Cleveland Case Medical Center (UHCMC) Institutional Review Board (IRB)

In compliance with the UHCMC IRB policy titled “Event Reporting – Unanticipated Problems, Adverse Events, and Protocol Deviations”, the Principal

Investigators will report all non-fatal events meeting the UHCMC IRB definition of *unanticipated problem involving risk to participants or others* within 14 calendar days of awareness of the event. All fatal events will be reported to the IRB within 7 calendar days of awareness of the event.

11.4.4 Expedited Reporting to the Food and Drug Administration and the DAIDS Medical Officer

This study is being conducted under an IND held by Dr. Benigno Rodriguez. Dr. Rodriguez and Dr. Lederman, study co-chairs, will be responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21CFR 312.32).

The Investigators will report in an IND safety report any suspected adverse reaction (see section 11.4.1.3) that is both serious (see section 11.4.1.5) and unexpected (see section 11.4.1.4). If the adverse event does not meet all three of the definitions, it will not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

11.4.5 Reporting Requirements for this Study

The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study. The study agent for which expedited reporting is required is tocilizumab (ACTEMRA®)/placebo. In addition to the EAE reporting category identified above, other AEs that will be reported in an expedited manner are:

- All incident cancers
- All opportunistic infections listed in section 8.1
- All recurrent serious infections
- All demyelinating conditions
- All occurrences of an AST or ALT level above 5xULN
- All occurrences of an ANC below 500 cells/mm^3
- All occurrences of a platelet count below 50,000/mm^3
- All gastrointestinal perforations

11.4.6 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, available at http://rsc.tech-res.com/safetyandpharmacovigilance will be used for grading severity of events in this study.

11.4.7 Relationship to Study Agent

The Principal Investigators will determine if there is a reasonable possibility that TCZ/placebo caused or contributed to the AE. The relationship assessment, based on clinical judgment, will rely on the following:

- A temporal relationship between the event and administration of TCZ/placebo,
- A plausible biologic mechanism for TCZ/placebo to cause the AE
- Another possible etiology for the AE,
- Previous report of similar AEs associated with TCZ/placebo
- Recurrence of the AE after re-challenge or resolution after de-challenge.

The terms used to assess the relationship of an event to TCZ/placebo are:

- Definitely related – The AE is **clearly related** to TCZ/placebo
- Probably related – The AE is **likely related** to TCZ/placebo
- Possibly related – The AE **may be related** to TCZ/placebo
- Probably not related – The AE is **not likely related** to TCZ/placebo
- Not related – The AE is **clearly NOT related** to TCZ/placebo

When an AE is assessed as “not related” to TCZ/placebo, an alternative etiology, diagnosis or explanation for the SAE will be provided. If new information becomes available, the relationship to assessment of any AE will be reviewed and updated, as required.

11.4.8 Expedited AE Reporting Period

The protocol-defined EAE Reporting Period for this protocol is the entire study duration for an individual subject (from study enrollment until study completion or discontinuation of the subject from study participation for any reason). After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

11.4.9 Reporting Procedures

All subjects will be followed for possible adverse events throughout their involvement in the study, including issues with study conduct. At each study visit, research staff will elicit subject input as to discomforts or adverse experiences.

All grade ≥2 AEs will be reported to the Principal Investigators on a daily basis. A complete blood count, complete metabolic panel as defined in 6.3.5.1, CD4 T cell count, and plasma HIV-1 RNA level will be performed on most visits. The Principal Investigator will obtain these safety data in "real-time" (i.e., within 1-10 days of a study visit) when laboratory values become available. The Principal Investigators will review these data within that timeframe, assess degree of severity, and assess the relationship to study agent/intervention.

The following adverse events will be reported: adverse events graded 3 and above, and any possibly, probably, or definitely related, unexpected, and serious adverse events. These AEs will be reported to the Safety Monitoring Committee (SMC), study sponsor, FDA, and DAIDS Medical Officer. Reporting to the UHCMC IRB will follow current UCHCMC IRB policy (see section 11.4.3).

Details of all serious, life-threatening, or fatal adverse events will be sent to the SMC and DAIDS Medical Officer no later than 3 reporting days after the investigators become aware of the event. For the purposes of expedited reporting to the SMC and the DAIDS Medical Officer, the definition of a "reporting day" in Version 2.0 of the DAIDS EAE Manual will be used. Reporting to the UHCMC IRB will follow current UCHCMC IRB policy (see section 11.4.3). Specifically, the following will be reported in writing: (1) all serious adverse events associated with the study procedures; and (2) any serious incidents or problems involving the conduct of the study or patient participation, including problems with the recruitment or consent processes.

12.0 HUMAN SUBJECTS

12.1 IRB Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB of University Hospitals Case Medical Center. A signed consent form will be obtained from the subjects. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record.

12.2 Subject Confidentiality

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

12.3 Study Discontinuation

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

The SMC will monitor the occurrence of all grade ≥2 signs, symptoms, diagnoses, or laboratory abnormalities.

The SMC will review accrual, toxicity summaries, off-treatment and off-study rates, and reasons broken down by study arms. Meetings of the SMC are described in section 9.4. The SMC will be asked to consider the following regarding safety events: the grade of the event, the time to resolution (if any), and the site clinician's judgment of the relationship to treatment. The first interim safety review by the SMC is planned 3 months after the first subject enrolls and approximately every 3 months thereafter, but expedited reviews will occur whenever the threshold for temporary enrollment discontinuation is reached. Criteria for temporary enrollment discontinuation and expedited SMC review are:

- Three or more participants have experienced a grade 3 AE that is deemed possibly, probably, or definitely related to study treatment
- Two or more participants have experienced a grade 4 AE that is deemed possibly, probably, or definitely related to study treatment
- Any death occurs on study that is deemed possibly, probably, or definitely related to study treatment

The SMC will review the events whenever any of the criteria above is met, and recommend, based on the results of the review, whether the study can proceed as planned, proceed with modifications, or be discontinued.

12.4 Human Subjects Involvement, Characteristics, and Design

Up to 36 participants will be enrolled in the clinical trial. Potential participants will be recruited primarily from the Special Immunology Unit, the HIV clinic of University Hospitals Case Medical Center, although recruitment from other area hospitals through direct outreach and targeted advertisement is also possible. Characteristics of the study population, inclusion/exclusion criteria, and planned study procedures are described in the clinical protocol. No vulnerable populations will be included in these studies.

12.5. Sources of Materials

Laboratory and clinical staff have received Good Clinical Practices (GCP) and Good Clinical Laboratory Practices (GCLP) training per their role in the study. Clinical monitoring and screening laboratories will be run in CLIA certified labs at University Hospitals Case Medical Center. All specimens will be stored with coded identifiers only in restricted-access laboratories. The CWRU/UHC CFAR Patient Care and Research Database personnel and the study data manager will store, manage and retrieve all study-related data, which will be stored on firewalled servers at UHCMC, in full compliance with relevant HIPPAA requirements. All study records will be stored and accessed using only a participant identifier (PID), and locator data linking identifiable information to the PID will be kept in a separate database in a different location to ensure the confidentiality of these data. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, the

FDA, the NIAID, the OHRP or the subject's designees. Information about the subject's participation will not be shared with persons who are not directly involved with the research subjects or as identified in the HIPAA consent.

12.6 Potential Risks and Risk Mitigation Strategies

12.6.1 Risks of Tocilizumab

12.6.1.1 Risk Mitigation Strategy for Serious Infections

To mitigate the risk of serious infections, individuals with any of the following conditions will be excluded from enrolling in this study:

- A screening CD4 T-cell count <350 cells/mm^3
- A history of AIDS-defining opportunistic infections, as listed in section 4.2.16
- An absolute neutrophil count <2000 cells/mm^3
- A history of TB, latent TB, or a positive screening test for TB
- An active local or systemic infection
- Use of immunosuppressive or immune modulating therapy
- Use of any investigational agent
- Co-infected with HBV or HCV
- History of having received a live attenuated vaccine within 30 days of study entry or an indication for a live attenuated vaccine

In addition to the exclusion criteria above, exposure to TCZ will be minimized by administering only three doses, the first of which will be at the lower 4 mg/Kg level, and the last of which will be given at day 56. In previous studies of TCZ for treatment of RA, the earliest opportunistic infection occurred at day 115 after initiation of treatment with TCZ. In the 6-month safety database, most serious non-opportunistic infections occurred after the fifth dose of TCZ. Therefore, by reducing the duration of exposure, it is expected that the overall risk of serious infections will be reduced as well.

If a serious infection develops, study drug will be held until the infection is adequately controlled. Study drug administration will be interrupted if the ANC is <1,000 cells/mm^3. Screening for tuberculosis will be done at screening and at week 10, and a negative result will be required before the first dose of study agent in each of the two periods of the trial (week 0 and week 20 visits).

12.6.1.2 Risk Mitigation Strategy for Hypersensitivity Reactions

To mitigate the risk of hypersensitivity reactions including anaphylaxis:

- Patients with a known allergy/sensitivity or any hypersensitivity to

components of the study drug or its formulation will be excluded.
- All doses of study agent will be administered on an inpatient basis in the Dahms Clinical Research Unit, where subjects will be monitored for a minimum of 30 minutes following the dose of study drug by personnel with life support training and access to adequate life support equipment and resuscitation medication.
- A severe allergic reaction related to study treatment will be grounds for study treatment discontinuation.

12.6.1.3 Risk Mitigation Strategy for Gastrointestinal Perforations

To mitigate the risk of gastrointestinal perforations, subjects at increased risk for gastrointestinal perforation, such as those with a history of diverticulitis, intestinal perforation, distal intestinal obstruction, or lower gastrointestinal bleeding, will be excluded. In the integrated safety review from clinical trials (67), the average age of patients who developed gastrointestinal perforations was 58.5 years. Therefore, subjects over 60 years of age will be excluded from this study. In addition, use of other immune suppressive agents will be prohibited. All subjects will be monitored for signs or symptoms of gastrointestinal perforation at each visit. Subjects who present with new onset of abdominal symptoms will be immediately referred for evaluation by a qualified healthcare professional to rule out gastrointestinal perforation. Subjects who develop a gastrointestinal perforation during study will be required to discontinue study treatment permanently.

12.6.1.4 Risk Mitigation Strategy for Infusion Reactions

To reduce the risk of severe infusion reactions, subjects will be closely monitored during and after infusion as inpatients with frequent vital sign monitoring, and immediate availability of qualified medical treatment and resuscitation medications and equipment. A qualified healthcare professional will be present in the room for each infusion.

12.6.1.5 Risk Mitigation Strategy for Laboratory Abnormalities

12.6.1.5.1 Neutropenia

The risk mitigation plan for neutropenia is consistent with the USPI and includes:

- Patients with an ANC <2000 cells/mm^3 will not be permitted to enroll in the study;
- ANC will be measured at each visit
- Should the ANC decrease to ≥500 but ≤1,000 cells/mm^3, the TCZ dose will be interrupted until the ANC is >1,000 cells/mm^3 and then TCZ will be

restarted at 4 mg/kg. If the ANC remains above 1,000 cells/mm^3, the dose will be increased again to 8 mg/Kg with close clinical and laboratory monitoring.
- Should the ANC decrease to <500 cells/mm^3,TCZ will be discontinued.

12.6.1.5.2 Thrombocytopenia

The risk mitigation plan for thrombocytopenia is consistent with the USPI and includes:

- Patients with a platelet count <100,000/mm^3 will not be enrolled in the study;
- Platelet counts will be measured at each visit;
- Should the platelet count decrease to ≥50,0000 but ≤100,000/mm^3 the TCZ dose will be interrupted until the platelet count is >100,000/mm^3 and then TCZ will be restarted at 4 mg/kg. If the platelet count remains above 100,000 /mm^3, the dose will be increased again to 8 mg/Kg with close clinical and laboratory monitoring.
- Should the platelet count decrease to <50,000/mm^3 TCZ will be discontinued.

12.6.1.5.3 AST and ALT Abnormalities

The risk mitigation plan for AST or ALT abnormalities is + consistent with the USPI and includes:

- Patients with an AST or ALT $\geq$ 1.5x ULN will be excluded from the study.
- Patients with cirrhosis, sever liver disease or active hepatitis B or C infection will be excluded from the study.
- AST and ALT will be evaluated at each study visit.
- For AST or ALT values persistently > 1.4x to <3x ULN, the TCZ dose will be reduced to 4 mg/kg or TCZ will be interrupted until the AST and ALT have normalized.
- For AST or ALT values confirmed 3x to 5x ULN, the TCZ dose will be interrupted until the values are 1x to <3x ULN and the above guidelines will be used.
- For AST or ALT values > 5x ULN TCZ dosing will be permanently discontinued.

12.6.1.5.4 Lipid abnormalities

Total and LDL cholesterol and serum triglycerides will be monitored in real time at each study visit beginning with

study entry, as well as at screening, as part of the Chem23 panel. Subjects who experience incident lipid abnormalities will be referred for adequate medical treatment by a qualified healthcare professional, but in light of the short duration of the study, isolated lipid abnormalities will not require study treatment modification or discontinuation. In general, treatment for dyslipidemias by providers in the John T. Carey Special Immunology Unit of UHCMC follows the ATP III guidelines. Consultation with experts in cardiovascular risk is available on-site in the Special Immunology Unit by co-investigator Dr. Chris Longenecker.

12.6.1.6 Risk Mitigation Strategy for Malignancies

To minimize the risk or emergent or recurrent malignancies, subjects with a history of or active cancer will be excluded, and a review of interval diagnoses or medical events and a full or targeted physical exam will be performed at each visit. Additionally, concomitant immunosuppressive medications will be prohibited during the study.

12.6.1.7 Risk Mitigation Strategy for Viral Reactivation

Consistent with the USPI, a history of or a positive antibody titer to herpes zoster will not be exclusionary for this study. Nonetheless, the prohibition of corticosteroid or immune suppressants in this study, and the short course of TCZ dosing, 8 weeks, may to some degree mitigate the risk of herpes zoster exacerbation in this study.

To reduce the risk of hepatitis B or hepatitis C reactivation, subjects with evidence of active hepatitis B (HbsAg, HbeAg, or HBV DNA positivity) or C (HCV antibody and HCV RNA positivity) will be excluded, and those who are HbcAb-positive at screening will be required to receive an antiretroviral regimen including at least one agent with HBV activity and will have HBV DNA monitoring throughout the study. Subjects who develop a detectable HBV DNA while on study will require permanent study discontinuation. Liver enzymes will be monitored throughout the study on all subjects, regardless of serological status.

12.6.1.8 Risk Mitigation Strategy for Cytochrome P450 Inhibition and Decreased Plasma Levels of Antiretrovirals

To minimize the risk of reaching levels of antiretrovirals that might compromise antiviral activity, subjects receiving non-boosted protease inhibitors will be excluded from the study, as will subjects whose trough levels of these agents are low at screening. Trough levels of protease

inhibitors and non-nucleoside reverse transcriptase inhibitors will be measured at each study agent administration visit, as well as at weeks 10 and 30, two weeks after the last dose of study agent in each of the periods of the trial using validated LC/MS/MS methods. If there is evidence of incomplete virologic suppression in a subject whose trough levels of antiretroviral are low or low antiretroviral levels are documented in two consecutive visits, study treatment will be permanently discontinued.

12.6.1.9 Risk Mitigation Strategy for Demyelinating Disorders

To minimize the risk of emerging demyelinating disorders, subjects with a history of these conditions will be excluded from the study. At each visit, a complete review of symptoms and new diagnoses will be conducted, and subjects who describe any neurologic symptoms suggestive of a demyelinating disorder will be immediately referred for evaluation by a qualified specialist. Subjects who develop a demyelinating disorder, or a neurologic condition that is deemed to be consistent with a demyelinating disorder, will be required to discontinue study treatment permanently.

12.6.1.10 Risk Mitigation Strategy for Women of Reproductive Potential

The following measures will be taken to minimize the risks of fetal TCZ exposure in women of childbearing potential:

- A negative pregnancy test will be required at enrollment and at each study visit where drug is administered, prior to administration of study drug.
- Women must agree to not to participate in a conception event
- Two forms of birth control are required - oral hormonal contraception will not be considered adequate because of potential drug-drug interactions.
- Use of contraception will be verified at each visit before administration of study drug.
- A sexually active woman who does not report using two protocol defined acceptable methods of contraception will not receive study medication at that visit.
- Should a woman become pregnant while participating in the study, study drug will be discontinued; the woman will be enrolled in the ACTIMERA (TCZ) Pregnancy Registry and followed for the outcome of the pregnancy.

In addition, the investigators will follow FDA updates to the USPI and the literature for reports from the TCZ pregnancy registry, and reassess the inclusion of women of child bearing potential if appropriate.

12.6.2 Enemas (for the subset of participants agreeing to rectal biopsies)

The main risk from having an enema is temporary discomfort. To receive an enema, a hollow soft plastic tube about the thickness of a pencil is used to put approximately 120 mL of enema fluid into the rectum to stimulate a bowel movement with stool evacuation and flush it out. This may cause a “bloated” or “cramping” feeling. Some air may be pumped into the rectum as well, causing flatulence. The tube is small, but it might cause some anal or rectal discomfort if the subject has any hemorrhoids or other painful conditions. To minimize the risk of symptoms derived from enema administration, all subjects will receive guidance on safe administration techniques, and will be instructed to follow the package directions closely.

12.6.3 Flexible Sigmoidoscopy and Biopsies

Risks associated with lower gastrointestinal endoscopy include colitis from chemicals for endoscope sterilization, bowel perforation, bleeding, diverticulitis, and infection. Rectosigmoid biopsies in this study will be obtained through flexible sigmoidoscopy rather than full colonoscopy, as colonoscopy has been shown to be associated with a still low, but significantly greater risk of complications than rectosigmoidoscopy (85). To minimize these risks, subjects with a history of a bleeding disorder, recent major anorectal surgical procedures, or significant anatomic lesions or active conditions involving the distal large bowel will be excluded. Additionally, agents containing aspirin and other antiplatelet agents will be prohibited within 7 days of the flexible sigmoidoscopy procedure, and subjects who require anticoagulant agents whose pharmacologic action cannot be interrupted rapidly will be excluded. Further, all procedures will be performed by trained, experienced endoscopists using state-of-the-art equipment, and a safety follow-up contact will be included to help detect potential complications.

12.6.4 Intravenous Infusion

Intravenous infusion can be associated with infection, superficial or deep thrombophlebitis, hematomas, air embolism, and tissue damage. The study agent will be administered through a peripheral IV line, so the risk of serious complications is exceedingly small. Sterile supplies and technique will be used throughout the study, and all personnel administering the study drug have extensive experience with infusion of biological products and intravenous medications. If peripheral vascular access cannot be secured for a given participant, no attempt will be made to obtain central access in order to avoid the additional risk that this entails, and the participant will be removed from the study.

12.6.5 Blood Sample Collection

The most common risks of blood sample collection are pain at the puncture site, bruising, and a feeling of lightheadedness. To minimize these risks, blood draws will be performed by trained personnel, and will be performed in a secure

environment with access to first aid equipment, bandages, and trained healthcare professionals.

12.7 Adequacy of Protection against Risks

12.7.1 Recruitment and Informed Consent

Before the initiation of any study procedures, subjects will be informed about the study and asked to sign an IRB approved informed consent/HIPAA document. Subjects will be consented in a private exam room. Subjects will be given time to read the consent, ask questions and consider the risks and/or benefits to participation in this research study prior to obtaining their signature. All subjects enrolled in the study will be given a copy of their signed and dated informed consent document. This consenting process will be done by trained research staff at the Case Clinical Trials Unit or at the DCRU.

12.7.2 Protections against Specific Study-related Risks

An extensive risk mitigation strategy has been implemented to address risks derived specifically from the study treatments and procedures. Details of the risk minimization strategy for each risk category are provided above in section 12. 6. All study-related activities will be conducted in private areas to protect patient confidentiality.

12.8 Potential Benefits of the Proposed Research to Human Subjects and Others

Participants in this trial are not expected to derive a direct benefit from their participation. If this trial provides proof of concept for the notion that IL-6 may play a central role in the development of inflammation-related complications in treated HIV infection, the results could offer the starting point for the implementation of a therapeutic strategy to halt this inflammatory process and that could result in a tangible clinical benefit to persons living with HIV/AIDS, including those directly involved in the study.

12.9 Importance of the Knowledge to Be Gained

Immune activation and inflammation are critical pathogenesis determinants in HIV infection in the era of effective antiretroviral therapy. Identifying the critical pathways that perpetuate this process and that lead to end-organ complications is a central goal of current HIV research. A large body of data now exists indicating that uncontrolled inflammation is one of the most significant contributors to the excess mortality observed even among HIV-infected persons with controlled plasma HIV RNA. Therefore, if a pivotal mediator can be found that is shown to play a meaningful causal role in this process, as we propose IL-6 does, therapeutic manipulation of that mediator might yield reductions in disease burden and even survival benefits in HIV infection. Therefore, the potential general benefit of the proposed studies is considerable.

12.10 Data and Safety Monitoring Plan

The two PIs, Drs. Rodriguez and Lederman, both experienced HIV clinical trialists, will review all adverse events, determine their relationship to study procedures, and generate reports to the IRB and FDA as needed. The data manager will prepare quarterly blinded data reports, and inconsistencies in data elements will be resolved by review of primary sources, interview with research personnel, and general consensus among study team members. The protocol statistician will be responsible for preparing the overall data reports, and for alerting the team to trends in adverse event frequency or data discrepancies. External data monitoring will be conducted by the DAIDS contractor PPD, which monitors most other network studies carried out in the Case/UHCMC Clinical Trials Unit. Trial safety will be monitored by an SMC (see section 9.4).

12.11 ClinicalTrials.gov Requirements

When regulatory approval is secured, this study will be registered with ClinicalTrials.gov. Results information (including adverse events) will be reported no later than 1 year after the primary completion date per Food and Drug Administration Amendments Act (FDAAA) requirements (http://grants.nih.gov/ClinicalTrials_fdaaa/steps.htm).

12.12 Inclusion of Women and Minorities

Both men and women will be included in the trial. Minorities, including Spanish-speaking persons, will be eligible to enroll in the trial, and a Spanish language consent will be available for them.

12.13 Inclusion of Children

Children ages 18 to 21 will be included in this study.

13.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be decided by consensus of the study team.

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, will be transported using packaging mandated by CFR 42 Part 72, as per the International Air Transport Association (IATA) Dangerous Goods Regulations.

15.0 REFERENCES

1. Baker JV, Peng G, Rapkin J, Abrams DI, Silverberg MJ, MacArthur RD, Cavert WP, Henry WK, Neaton JD. CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection. AIDS. 2008;22(7):841-8.
2. Lewden C, Chene G, Morlat P, Raffi F, Dupon M, Dellamonica P, Pellegrin JL, Katlama C, Dabis F, Leport C, Agence Nationale de Recherches sur le Sida et les Hepatites Virales COA-CSG, Agence Nationale de Recherches sur le Sida et les Hepatites Virales COASG. HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. J Acquir Immune Defic Syndr. 2007;46(1):72-7.
3. Baker JV, Peng G, Rapkin J, Krason D, Reilly C, Cavert WP, Abrams DI, MacArthur RD, Henry K, Neaton JD. Poor initial CD4+ recovery with antiretroviral therapy prolongs immune depletion and increases risk for AIDS and non-AIDS diseases. J Acquir Immune Defic Syndr. 2008;48(5):541-6.
4. Lapadula G, Cozzi-Lepri A, Gori A, Antinori A, Marchetti G, Chiodera A, Narciso P, Parruti G, Galli M, d'Arminio Monforte A, Icona Foundation Study. Risk of AIDS and non-AIDS-related Events among Patients with Conserved CD4+ T Cell Counts at Baseline but Blunted Immune Response Despite Virological Suppression after ART. 19th Conference on Retroviruses and Opportunistic Infections, Abstract # 641; Seattle, WA2012.
5. van Lelyveld SF, Gras L, Kesselring A, Zhang S, De Wolf F, Wensing AM, Hoepelman AI. Long-term complications in patients with poor immunological recovery despite virological successful HAART in Dutch ATHENA cohort. AIDS. 2012;26(4):465-74.
6. Lederman MM, Calabrese L, Funderburg NT, Clagett B, Medvik K, Bonilla H, Gripshover B, Salata RA, Taege A, Lisgaris M, McComsey GA, Kirchner E, Baum J, Shive C, Asaad R, Kalayjian RC, Sieg SF, Rodriguez B. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. J Infect Dis. 2011;204(8):1217-26. PMCID: 3218674.
7. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, Hsue P, Emu B, Krone M, Lampiris H, Douek D, Martin JN, Deeks SG. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis. 2008;197(1):126-33.
8. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, Cohen CJ, Cohn D, Cooper D, Darbyshire J, Emery S, Fatkenheuer G, Gazzard B, Grund B, Hoy J, Klingman K, Losso M, Markowitz N, Neuhaus J, Phillips A, Rappoport C. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006;355(22):2283-96.
9. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, Ledergerber B, Lundgren J, Neuhaus J, Nixon D, Paton NI, Neaton JD. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008;5(10):e203. PMCID: 2570418.
10. Hunt PW, Rodriguez B, Shive C, Clagett B, Funderburg N, Epling L, Van Natta M, Medvik K, Huang Y, Deeks SG, Martin JN, Meinert C, Lederman MM. Gut Epithelial Barrier Dysfunction, Inflammation and Coagulation Predict Higher Mortality during Treated HIV/AIDS. CROI; Seattle, Washington2012.
11. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci (Lond). 2012;122(4):143-59.
12. Sha BE, Valdez H, Gelman RS, Landay AL, Agosti J, Mitsuyasu R, Pollard RB, Mildvan D, Namkung A, Ogata-Arakaki DM, Fox L, Estep S, Erice A, Kilgo P, Walker RE, Bancroft L, Lederman MM. Effect of etanercept (Enbrel) on interleukin 6, tumor necrosis factor alpha, and markers of immune activation in HIV-infected subjects receiving interleukin 2. AIDS Res Hum Retroviruses. 2002;18(9):661-5.

13. Modur V, Li Y, Zimmerman GA, Prescott SM, McIntyre TM. Retrograde inflammatory signaling from neutrophils to endothelial cells by soluble interleukin-6 receptor alpha. J Clin Invest. 1997;100(11):2752-6. PMCID: 508479.
14. Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, Faggioni R, Luini W, van Hinsbergh V, Sozzani S, Bussolino F, Poli V, Ciliberto G, Mantovani A. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity. 1997;6(3):315-25.
15. Cucak H, Yrlid U, Reizis B, Kalinke U, Johansson-Lindbom B. Type I interferon signaling in dendritic cells stimulates the development of lymph-node-resident T follicular helper cells. Immunity. 2009;31(3):491-501.
16. Yoshida H, Hashizume M, Suzuki M, Mihara M. Anti-IL-6 receptor antibody suppressed T cell activation by inhibiting IL-2 production and inducing regulatory T cells. Eur J Pharmacol. 2010;634(1-3):178-83.
17. Diehl S, Rincon M. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol. 2002;39(9):531-6.
18. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441(7090):235-8.
19. Patki AH, Zielske SP, Sieg SF, Lederman MM. Preferential S phase entry and apoptosis of CD4(+) T lymphocytes of HIV- 1-infected patients after in vitro cultivation. Clin Immunol. 2000;97(3):241-7.
20. Sieg SF, Rodriguez B, Asaad R, Jiang W, Bazdar DA, Lederman MM. Peripheral S-phase T cells in HIV disease have a central memory phenotype and rarely have evidence of recent T cell receptor engagement. J Infect Dis. 2005;192(1):62-70.
21. Picker LJ, Hagen SI, Lum R, Reed-Inderbitzin EF, Daly LM, Sylwester AW, Walker JM, Siess DC, Piatak M, Jr., Wang C, Allison DB, Maino VC, Lifson JD, Kodama T, Axthelm MK. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. J Exp Med. 2004;200(10):1299-314.
22. Okoye A, Meier-Schellersheim M, Brenchley JM, Hagen SI, Walker JM, Rohankhedkar M, Lum R, Edgar JB, Planer SL, Legasse A, Sylwester AW, Piatak M, Jr., Lifson JD, Maino VC, Sodora DL, Douek DC, Axthelm MK, Grossman Z, Picker LJ. Progressive CD4+ central memory T cell decline results in CD4+ effector memory insufficiency and overt disease in chronic SIV infection. J Exp Med. 2007;204(9):2171-85.
23. Lempicki RA, Kovacs JA, Baseler MW, Adelsberger JW, Dewar RL, Natarajan V, Bosche MC, Metcalf JA, Stevens RA, Lambert LA, Alvord WG, Polis MA, Davey RT, Dimitrov DS, Lane HC. Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of CD4+ and CD8+ T cell turnover in HIV-infected patients. Proc Natl Acad Sci U S A. 2000;97(25):13778-83.
24. Funderburg N, Luciano AA, Jiang W, Rodriguez B, Sieg SF, Lederman MM. Toll-like receptor ligands induce human T cell activation and death, a model for HIV pathogenesis. PLoS ONE. 2008;3(4):e1915.
25. Dion ML, Bordi R, Zeidan J, Asaad R, Boulassel MR, Routy JP, Lederman MM, Sekaly RP, Cheynier R. Slow disease progression and robust therapy-mediated CD4+ T-cell recovery are associated with efficient thymopoiesis during HIV-1 infection. Blood. 2007;109(7):2912-20.
26. Teixeira L, Valdez H, McCune JM, Koup RA, Badley AD, Hellerstein MK, Napolitano LA, Douek DC, Mbisa G, Deeks S, Harris JM, Barbour JD, Gross BH, Francis IR, Halvorsen R, Asaad R, Lederman MM. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. AIDS. 2001;15(14):1749-56.
27. Schacker TW, Nguyen PL, Beilman GJ, Wolinsky S, Larson M, Reilly C, Haase AT. Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis. J Clin Invest. 2002;110(8):1133-9.

28. Schacker TW, Reilly C, Beilman GJ, Taylor J, Skarda D, Krason D, Larson M, Haase AT. Amount of lymphatic tissue fibrosis in HIV infection predicts magnitude of HAART-associated change in peripheral CD4 cell count. AIDS. 2005;19(18):2169-71.
29. Zeng M, Smith AJ, Wietgrefe SW, Southern PJ, Schacker TW, Reilly CS, Estes JD, Burton GF, Silvestri G, Lifson JD, Carlis JV, Haase AT. Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. J Clin Invest. 2011;121(3):998-1008. PMCID: 3049394.
30. Levy Y, Sereti I, Tambussi G, Routy JP, Lelievre JD, Delfraissy JF, Molina JM, Fischl M, Goujard C, Rodriguez B, Rouzioux C, Avettand-Fenoel V, Croughs T, Beq S, Morre M, Poulin JF, Sekaly RP, Thiebaut R, Lederman MM. Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo-controlled, multicenter study. Clin Infect Dis. 2012;55(2):291-300. PMCID: 3381639.
31. Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, Battaglia CA, Landay AL, Pahwa S, Fischl MA, Asmuth DM, Tenorio AR, Altman JD, Fox L, Moir S, Malaspina A, Morre M, Buffet R, Silvestri G, Lederman MM. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. Blood. 2009;113(25):6304-14. PMCID: 2710926.
32. Vranjkovic A, Crawley AM, Patey A, Angel JB. IL-7-dependent STAT-5 activation and CD8+ T cell proliferation are impaired in HIV infection. J Leukoc Biol. 2011;89(4):499-506.
33. MacPherson PA, Fex C, Sanchez-Dardon J, Hawley-Foss N, Angel JB. Interleukin-7 receptor expression on CD8(+) T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. J Acquir Immune Defic Syndr. 2001;28(5):454-7.
34. Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Jacod S, Delfraissy JF, Theze J. Regulatory dysfunction of the interleukin-7 receptor in CD4 and CD8 lymphocytes from HIV-infected patients--effects of antiretroviral therapy. J Acquir Immune Defic Syndr. 2006;42(3):277-85.
35. Marziali M, De Santis W, Carello R, Leti W, Esposito A, Isgro A, Fimiani C, Sirianni MC, Mezzaroma I, Aiuti F. T-cell homeostasis alteration in HIV-1 infected subjects with low CD4 T-cell count despite undetectable virus load during HAART. AIDS. 2006;20(16):2033-41.
36. Crawley AM, Angel JB. The influence of HIV on CD127 expression and its potential implications for IL-7 therapy. Semin Immunol. 2012;24(3):231-40.
37. Kiazyk SA, Fowke KR. Loss of CD127 expression links immune activation and CD4(+) T cell loss in HIV infection. Trends Microbiol. 2008;16(12):567-73.
38. Shive CL, Mudd JC, Funderburg NT, Sieg SF, Kyi B, Bazdar DA, Mangioni D, Gori A, Jacobson JM, Brooks AD, Hardacre J, Ammori J, Estes JD, Schacker TW, Rodriguez B, Lederman MM. Inflammatory Cytokines Drive CD4 T cell Cycling and Impaired Responsiveness to Interleukin-7: Implications for Immune Failure in HIV Disease. J Infect Dis. 2014.
39. Dezube BJ, Lederman MM, Chapman B, Georges DL, Dogon AL, Mudido P, Reis-Lishing J, Cheng SL, Silberman SL, Crumpacker CS. The effect of tenidap on cytokines, acute-phase proteins, and virus load in human immunodeficiency virus (HIV)-infected patients: correlation between plasma HIV-1 RNA and proinflammatory cytokine levels. J Infect Dis. 1997;176(3):807-10.
40. Eastburn A, Scherzer R, Zolopa AR, Benson C, Tracy R, Do T, Bacchetti P, Shlipak M, Grunfeld C, Tien PC. Association of low level viremia with inflammation and mortality in HIV-infected adults. PLoS ONE. 2011;6(11):e26320. PMCID: 3206804.
41. Shive CL, Biancotto A, Funderburg NT, Pilch-Cooper HA, Valdez H, Margolis L, Sieg SF, McComsey GA, Rodriguez B, Lederman MM. HIV-1 is not a major driver of increased plasma IL-6 levels in chronic HIV-1 disease. J Acquir Immune Defic Syndr. 2012.
42. McGowan I, Elliott J, Fuerst M, Taing P, Boscardin J, Poles M, Anton P. Increased HIV-1 mucosal replication is associated with generalized mucosal cytokine activation. J Acquir Immune Defic Syndr. 2004;37(2):1228-36.

43. Schulbin H, Bode H, Stocker H, Schmidt W, Zippel T, Loddenkemper C, Engelmann E, Epple HJ, Arasteh K, Zeitz M, Ullrich R. Cytokine expression in the colonic mucosa of human immunodeficiency virus-infected individuals before and during 9 months of antiretroviral therapy. Antimicrob Agents Chemother. 2008;52(9):3377-84. PMCID: 2533489.
44. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med. 2004;200(6):749-59. PMCID: 2211962.
45. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. Annu Rev Immunol. 2012;30:647-75.
46. Sonnenberg GF, Monticelli LA, Alenghat T, Fung TC, Hutnick NA, Kunisawa J, Shibata N, Grunberg S, Sinha R, Zahm AM, Tardif MR, Sathaliyawala T, Kubota M, Farber DL, Collman RG, Shaked A, Fouser LA, Weiner DB, Tessier PA, Friedman JR, Kiyono H, Bushman FD, Chang KM, Artis D. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. Science. 2012;336(6086):1321-5.
47. Dubin PJ, Kolls JK. Th17 cytokines and mucosal immunity. Immunol Rev. 2008;226:160-71.
48. Klatt NR, Estes JD, Sun X, Ortiz AM, Barber JS, Harris LD, Cervasi B, Yokomizo LK, Pan L, Vinton CL, Tabb B, Canary LA, Dang Q, Hirsch VM, Alter G, Belkaid Y, Lifson JD, Silvestri G, Milner JD, Paiardini M, Haddad EK, Brenchley JM. Loss of mucosal CD103+ DCs and IL-17+ and IL-22+ lymphocytes is associated with mucosal damage in SIV infection. Mucosal Immunol. 2012.
49. Estes JD, Harris LD, Klatt NR, Tabb B, Pittaluga S, Paiardini M, Barclay GR, Smedley J, Pung R, Oliveira KM, Hirsch VM, Silvestri G, Douek DC, Miller CJ, Haase AT, Lifson J, Brenchley JM. Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. PLoS Pathog. 2010;6(8):e1001052. PMCID: 2924359.
50. Dandekar S, George MD, Baumler AJ. Th17 cells, HIV and the gut mucosal barrier. Curr Opin HIV AIDS. 2010;5(2):173-8.
51. Mavigner M, Cazabat M, Dubois M, L'Faqihi FE, Requena M, Pasquier C, Klopp P, Amar J, Alric L, Barange K, Vinel JP, Marchou B, Massip P, Izopet J, Delobel P. Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest. 2012;122(1):62-9. PMCID: 3248296.
52. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol. 2011;11(5):330-42.
53. Sereti I, Imamichi H, Natarajan V, Imamichi T, Ramchandani MS, Badralmaa Y, Berg SC, Metcalf JA, Hahn BK, Shen JM, Powers A, Davey RT, Kovacs JA, Shevach EM, Lane HC. In vivo expansion of CD4CD45RO-CD25 T cells expressing foxP3 in IL-2-treated HIV-infected patients. J Clin Invest. 2005;115(7):1839-47. PMCID: 1142113.
54. Cimbro R, Vassena L, Arthos J, Cicala C, Kehrl JH, Park C, Sereti I, Lederman MM, Fauci AS, Lusso P. IL-7 induces expression and activation of integrin alpha4beta7 promoting naive T-cell homing to the intestinal mucosa. Blood. 2012;120(13):2610-9. PMCID: 3460683.
55. Sereti I, Estes JD, Thompson WL, Morcock DR, Fischl MA, Croughs T, Beq S, Lafaye de Micheaux S, Yao MD, Ober A, Wilson EM, Natarajan V, Imamichi H, Boulassel MR, Lederman MM, Routy JP. Decreases in colonic and systemic inflammation in chronic HIV infection after IL-7 administration. PLoS Pathog. 2014;10(1):e1003890. PMCID: 3907377.
56. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007;92(7):2506-12. PMCID: 2763385.

57. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685-95.
58. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, Wensley F, Higgins JP, Lennon L, Eiriksdottir G, Rumley A, Whincup PH, Lowe GD, Gudnason V. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med. 2008;5(4):e78. PMCID: 2288623.
59. Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet. 2012;379(9822):1214-24. PMCID: 3316968.
60. Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, Gao P, Saleheen D, Rendon A, Nelson CP, Braund PS, Hall AS, Chasman DI, Tybjaerg-Hansen A, Chambers JC, Benjamin EJ, Franks PW, Clarke R, Wilde AA, Trip MD, Steri M, Witteman JC, Qi L, van der Schoot CE, de Faire U, Erdmann J, Stringham HM, Koenig W, Rader DJ, Melzer D, Reich D, Psaty BM, Kleber ME, Panagiotakos DB, Willeit J, Wennberg P, Woodward M, Adamovic S, Rimm EB, Meade TW, Gillum RF, Shaffer JA, Hofman A, Onat A, Sundstrom J, Wassertheil-Smoller S, Mellstrom D, Gallacher J, Cushman M, Tracy RP, Kauhanen J, Karlsson M, Salonen JT, Wilhelmsen L, Amouyel P, Cantin B, Best LG, Ben-Shlomo Y, Manson JE, Davey-Smith G, de Bakker PI, O'Donnell CJ, Wilson JF, Wilson AG, Assimes TL, Jansson JO, Ohlsson C, Tivesten A, Ljunggren O, Reilly MP, Hamsten A, Ingelsson E, Cambien F, Hung J, Thomas GN, Boehnke M, Schunkert H, Asselbergs FW, Kastelein JJ, Gudnason V, Salomaa V, Harris TB, Kooner JS, Allin KH, Nordestgaard BG, Hopewell JC, Goodall AH, Ridker PM, Holm H, Watkins H, Ouwehand WH, Samani NJ, Kaptoge S, Di Angelantonio E, Harari O, Danesh J. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet. 2012;379(9822):1205-13. PMCID: 3316940.
61. Protogerou AD, Zampeli E, Fragiadaki K, Stamatelopoulos K, Papamichael C, Sfikakis PP. A pilot study of endothelial dysfunction and aortic stiffness after interleukin-6 receptor inhibition in rheumatoid arthritis. Atherosclerosis. 2011;219(2):734-6.
62. Murray SM, Down CM, Boulware DR, Stauffer WM, Cavert WP, Schacker TW, Brenchley JM, Douek DC. Reduction of immune activation with chloroquine therapy during chronic HIV infection. J Virol. 2010;84(22):12082-6. PMCID: 2977889.
63. Ross Eckard A, Jiang Y, Debanne SM, Funderburg NT. Effect of 24 Weeks of Statin Therapy on Systemic and Vascular Inflammation in HIV-Infected Subjects Receiving Antiretroviral Therapy. J Infect Dis. 2014;209.
64. Lake JE, Tseng CH, Currier JS. A pilot study of telmisartan for visceral adiposity in HIV infection: the metabolic abnormalities, telmisartan, and HIV infection (MATH) trial. PLoS ONE. 2013;8(3):e58135. PMCID: 3597631.
65. Lake JE, Currier JS, Tseng CH, Timmer JK, Douek DC, Sandler NG. Effects of Telmisartan on Markers of Monocyte Activation in HIV-Infected Patients on Antiretroviral Therapy. 19th Conference on Retroviruses and Opportnistic Infections, Abstract #O-227; Seattle, WA2012.
66. Yazici Y, Curtis JR, Ince A, Baraf H, Malamet RL, Teng LL, Kavanaugh A. Efficacy of tocilizumab in patients with moderate to severe active rheumatoid arthritis and a previous inadequate response to disease-modifying antirheumatic drugs: the ROSE study. Ann Rheum Dis. 2012;71(2):198-205.
67. Schiff MH, Kremer JM, Jahreis A, Vernon E, Isaacs JD, van Vollenhoven RF. Integrated safety in tocilizumab clinical trials. Arthritis Res Ther. 2011;13(5):R141. PMCID: 3308069.
68. Schultz O, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M, Krone W, Laudes M. Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. PLoS ONE. 2010;5(12):e14328. PMCID: 3001451.
69. Actemra (R) package insert, accessed 11 February 2015. 2015.

70. Reynolds MA, Kruszon-Moran D, Jumaan A, Schmid DS, McQuillan GM. Varicella seroprevalence in the U.S.: data from the National Health and Nutrition Examination Survey, 1999-2004. Public Health Rep. 2010;125(6):860-9. PMCID: 2966667.
71. Vassilopoulos D, Calabrese LH. Management of rheumatic disease with comorbid HBV or HCV infection. Nat Rev Rheumatol. 2012;8(6):348-57.
72. De Nard F, Todoerti M, Grosso V, Rossi S, Caporalli R, Montecucco C. Safety of the Newer Biologocal DMARDSs, Tocilizumab and Abatacept, in Rheumatoid Arthritis (RA) Patients with a History of HBV Infection: A Real Life Experience 2014 EULAR Annual European Congress of Rheumatology, Abstract # 0314; Paris, France2014.
73. Nakamura J, Nagashima T, Nagatani K, Yoshio T, Iwamoto M, Minota S. Reactivation of hepatitis B virus in rheumatoid arthritis patients treated with biological disease-modifying antirheumatic drugs. Int J Rheum Dis. 2014.
74. Urata Y, Uesato R, Tanaka D. Prevalence of Reactivation of Hepatitis B Virus DNA Replication in Rheumatoid Arthritis Patients. In: Seligmann H, editor. DNA Replication - Current Advances: InTech; 2011.
75. Kishida D, Okuda Y, Onishi M, Takebayashi M, Matoba K, Jouyama K, Yamada A, Sawada N, Mokuda S, Takasugi K. Successful tocilizumab treatment in a patient with adult-onset Still's disease complicated by chronic active hepatitis B and amyloid A amyloidosis. Mod Rheumatol. 2011;21(2):215-8.
76. Nagashima T, Minota S. Long-term tocilizumab therapy in a patient with rheumatoid arthritis and chronic hepatitis B. Rheumatology (Oxford). 2008;47(12):1838-40.
77. Tsuboi H, Tsujii A, Nampei A, Yoshihara H, Kawano K, Takeuchi E, Shi K. A patient with rheumatoid arthritis treated with tocilizumab together with lamivudine prophylaxis after remission of infliximab-reactivated hepatitis B. Mod Rheumatol. 2011;21(6):701-5.
78. Winthrop KL, Calabrese LH. Let the fog be lifted: screening for hepatitis B virus before biological therapy. Ann Rheum Dis. 2011;70(10):1701-3.
79. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50(3):661-2.
80. Artz AS, Somerfield MR, Feld JJ, Giusti AF, Kramer BS, Sabichi AL, Zon RT, Wong SL. American Society of Clinical Oncology provisional clinical opinion: chronic hepatitis B virus infection screening in patients receiving cytotoxic chemotherapy for treatment of malignant diseases. J Clin Oncol. 2010;28(19):3199-202.
81. European Association For The Study Of The L. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 2012;57(1):167-85.
82. Hoofnagle JH. Reactivation of hepatitis B. Hepatology. 2009;49(5 Suppl):S156-65.
83. Weinbaum CM, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW, Centers for Disease C, Prevention. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. MMWR Recomm Rep. 2008;57(RR-8):1-20.
84. Nishimoto N, Kanakura Y, Aozasa K, Johkoh T, Nakamura M, Nakano S, Nakano N, Ikeda Y, Sasaki T, Nishioka K, Hara M, Taguchi H, Kimura Y, Kato Y, Asaoku H, Kumagai S, Kodama F, Nakahara H, Hagihara K, Yoshizaki K, Kishimoto T. Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. Blood. 2005;106(8):2627-32.
85. Gatto NM, Frucht H, Sundararajan V, Jacobson JS, Grann VR, Neugut AI. Risk of perforation after colonoscopy and sigmoidoscopy: a population-based study. J Natl Cancer Inst. 2003;95(3):230-6.
86. Levin TR, Conell C, Shapiro JA, Chazan SG, Nadel MR, Selby JV. Complications of screening flexible sigmoidoscopy. Gastroenterology. 2002;123(6):1786-92.
87. Koobatian GJ, Choi PM. Safety of surveillance colonoscopy in long-standing ulcerative colitis. Am J Gastroenterol. 1994;89(9):1472-5.

88. Rutter MD, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, Williams CB, Price AB, Talbot IC, Forbes A. Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. Gastroenterology. 2006;130(4):1030-8.
89. Yao MD, von Rosenvinge EC, Groden C, Mannon PJ. Multiple endoscopic biopsies in research subjects: safety results from a National Institutes of Health series. Gastrointest Endosc. 2009;69(4):906-10.
90. Maini RN, Taylor PC, Szechinski J, Pavelka K, Broll J, Balint G, Emery P, Raemen F, Petersen J, Smolen J, Thomson D, Kishimoto T, Group CS. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. Arthritis Rheum. 2006;54(9):2817-29.
91. Smolen JS, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E, Woodworth T, Alten R. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. Lancet. 2008;371(9617):987-97.
92. Frey N, Grange S, Woodworth T. Population pharmacokinetic analysis of tocilizumab in patients with rheumatoid arthritis. J Clin Pharmacol. 2010;50(7):754-66.
93. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Available at http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed 30 January 2015. Washington, D.C.2014.
94. C.J.L. LP, Back DJ, Boucher CAB, Blaschke T, Boucher CAB, Fletcher CV, Flexner C, Gerber J, Kashuba ADM, Schapiro J, Burger DM. Updated guidelines to perform therapeutic drug monitoring for antiretroviral agents. Rev Antiviral Ther. 2006;3:3-14.
95. Scholler-Gyure M, Kakuda TN, Raoof A, De Smedt G, Hoetelmans RM. Clinical pharmacokinetics and pharmacodynamics of etravirine. Clin Pharmacokinet. 2009;48(9):561-74.
96. Kakuda T, Sekar V, Vis P, Coate B, Ryan R, Anderson D, De La Rosa G, Mrus J. Pharmacokinetics and Pharmacodynamics of Darunavir and Etravirine in HIV-1-Infected, Treatment-Experienced Patients in the Gender, Race, and Clinical Experience (GRACE) Trial. AIDS Res Treat. 2012;2012:186987. PMCID: 3318205.
97. American College of Rheumatology. ACTEMRA (tocilizumab). 2014 [updated 2014; cited 11 Feb 2015]; Available from: https://www.rheumatology.org/practice/clinical/drugsafety/actemra.asp.
98. Clopper CH, E.S. P. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika. 1934;26:404-13.
99. Kenward MG, Roger JH. The use of baseline covariates in crossover studies. Biostatistics. 2010;11(1):1-17.
100. Senn SJ. Cross-over trials, carry-over effects and the art of self-delusion. Stat Med. 1988;7(10):1099-101.
101. Emery P, Keystone E, Tony HP, Cantagrel A, van Vollenhoven R, Sanchez A, Alecock E, Lee J, Kremer J. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. Ann Rheum Dis. 2008;67(11):1516-23.