TITLE

A DOUBLE BLIND RANDOMIZED PLACEBO CONTROLLED STUDY EXAMINING THE EFFECTS OF A NON-ABSORBABLE (RIFAXIMIN) ANTIBIOTIC ON THE CHRONIC IMMUNE ACTIVATION OBSERVED IN HIV-INFECTED SUBJECTS

NIAID Protocol Number:

13-I-0062

Sponsored by:

National Institute of Allergy and Infectious Diseases (NIAID)

Principal Investigator:

Frank Maldarelli MD, PhD

Version Number: 8.0

Study Staff Roster

Principal Investigator:

NIAID- Frank Maldarelli MD, PhD

HIV Drug Resistance Program
National Cancer Institute
National Institutes of Health
Building 10, Room 5A06
Phone: (301) 435-8019
Email: fmalli@mail.nih.gov

WRNMMC- Anuradha Ganesan MD, MPH

Research Physician, Infectious Disease Clinical Research Program, USUHS
Assistant Professor of Medicine, USUHS
Staff Physician
Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD-20889
Phone: (301) 295-2972
Email: anuradha.ganesan.civ@health.mil

University of Pittsburgh, Deborah McMahon, MD

Professor of Medicine
Clinical Director, HIV/AIDS Program
Keystone Building, Suite 510
3520 Fifth Avenue
Pittsburgh, PA 15213
Phone: (412) 383-1675
Email: mcmahond@pitt.edu

Research Contact/Study Coordinator(s):

Jennifer M. Bell, RN, BS, CCRC
Protocol Nurse Coordinator II
Clinical Monitoring Research Program
Frederick National Laboratory for Cancer Research Leidos Biomedical Research, Inc.
Support to: Host Virus Interaction Branch (NIAID/LIR)
Building 10, 9N214.10
Phone: (301) 402-7032
Email: jennifer.bell@nih.gov

Heather Hairston MPH
Clinical Research Coordinator
Division of Infectious Diseases Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD-20889
Phone: (301) 295-5542
Email: heather.s.hairston.ctr@health.mil

Carol Oriss RN
Division of Infectious Diseases
University of Pittsburgh
3520 Fifth Avenue, Suite 510
Pittsburgh, PA 15213
Phone: 412-383-1434
Email: orissca@upmc.edu

Associate Investigator(s):

CDR Patrick Young, MD

Staff Physician
Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD-20889
Phone: (301) 295-4600
Email: patrick.young2@med.navy.mil

LCDR Ruben Acosta, MD

Staff Physician
Walter Reed National Military Medical Center
8901 Wisconsin Avenue,
Bethesda, MD-20889
Phone: (301) 295-4600
Email: ruben.acosta@med.navy.mil

Dr. Dean Follmann, PhD

Biostatistics Research Branch, NIAID
6700B Rockledge Drive MSC 7609
Bethesda, MD 20892
Phone: (301) 402-4518
Email: dfollmann@niaid.nih.gov

Dr. Jing Qin, PhD

Biostatistics Research Branch, NIAID
6700B Rockledge Drive MSC 7609
Bethesda, MD 20892
Email: jingqin@niaid.nih.gov

Sheila Kumar, MD MS

Digestive Diseases Branch, NIDDK
10 Center Drive
Bldg 10, Room 9C101
Bethesda, MD 20892
Phone: (301) 496-9730
Email: sheila.kumar@nih.gov

Collaborators:

Dr. Netanya Sandler Utay, MD

University of Texas Medical Branch
301 University Blvd, Marvin Graves Building, Room 4.202
Galveston, Texas 77555-0435
Email: neutay@UTMB.EDU

Dr. Daniel Douek, MD, MRCP, PhD

Vaccine Research Center
Bldg 40, Room 3504
Phone: (301) 594-8484
Email: Daniel.douek@nih.gov

Dr. Jacob Estes, PhD

Head, Tissue Analysis Core
Leidos Biomedical Research, Inc./
NCI-Frederick
Building 535, Suite 413B
Frederick, MD 21702
(301) 846-7641
Email: estesj@mail.nih.gov

Dr. Galit Alter, PhD

Assistant Professor in Medicine
Harvard University
Ragon Institute
149 13th Street, Room 6618b

Charlestown, MA, 02114
Phone: (301) 724-0546
Email: galter@partners.org

Dr. D. Scott Merrell, PhD

Associate Professor
Department of Microbiology and Immunology
Uniformed Services University
4301 Jones Bridge Road
Bethesda, MD 20814
Phone: (301) 295-1584
dmerrell@usuhs.mil

WRNMMC Medical Monitor:

Dr. Roseanne Ressner, MD
Staff Physician
Infectious Disease Division, WRNMMC
8901 Wisconsin Avenue,
Bethesda, MD-20889
Phone: 301-295-6400
roseanne.a.ressner.mil@health.mil

TABLE OF CONTENTS

List of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event/Adverse Experience
ART	antiretroviral therapy
CAD	coronary artery disease
CFR	Code of Federal Regulations
CIB	Clinical Investigator's Brochure
CIOMS	Council for International Organizations of Medical Sciences
CLIA	Clinical Laboratory Improvement Amendment of 1988
COI	Conflict of Interest
CRADA	Cooperative Research and Development Agreement
CRF	Case Report Form
CRO	Contract Research Organization
CRIMSON	Clinical Research Information Management System of the NIAID
DCR	Division of Clinical Research
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GALT	gut-associated lymphoid tissue
GCP	Good Clinical Practice
HE	hepatic encephalopathy
HIPAA	Health Insurance Portability and Accountability Act
IA	immune activation
IB	Investigator's Brochure
IBD	inflammatory bowel disease
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LPS	lipopolysaccharide
N	Number (typically refers to participants)
NCI	National Cancer Institute
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control

SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
USUHS	Uniformed Services University of the Health Sciences
WHO	World Health Organization

Protocol Summary

Title:	A Double Blind Randomized Placebo-Controlled Study Examining the Effects of a Non-Absorbable Antibiotic (Rifaximin) on the Chronic Immune Activation Observed in HIV-Infected Subjects
Short Title:	The Effect of Rifaximin on Immune Activation in HIV-Infected Subjects
Clinical Phase:	IIb
Conducted by:	National Institute of Allergy and Infectious Diseases
Principal Investigators:	NIH: Dr. Frank Maldarelli NNMC: Dr. Anuradha Ganesan University of Pittsburgh: Dr. Deborah McMahon
Sample Size:	44 subjects completing both dosing phases
Accrual Ceiling:	N = 200 subjects. It is anticipated that about 1 in 4 patients with an undetectable viral load on standard testing will have a detectable viral load on the single copy assay. Hence, to recruit 44 patients we anticipate screening approximately 200 patients
Study Population:	Suppressed HIV-infected adult subjects currently on antiretroviral therapy (ART)
Accrual date:	Accrual will be completed 2 years from enrollment of the first subject
Study Design:	Utilizing a randomized, double-blind, placebo-controlled study, with a case cross-over study design, we will assess the effects of rifaximin on markers of lipopolysaccharide (LPS) bioreactivity, cellular markers of activation, and microbial translocation.
Study Duration:	We anticipate that accrual and follow up will be completed over a 3 year period. Each participant will be in the study for 18 weeks
Study Agent:	Rifaximin

Intervention Description: Oral rifaximin at a dose of 550 mg twice daily will be administered for 4 weeks to HIV-infected subjects who are virologically suppressed on ART

Primary Objective: The primary objective of this study is to compare changes in soluble CD14 (sCD14) levels (a marker of monocyte activation and LPS bioreactivity) during the rifaximin phase of the study and compare it with the changes in sCD14 levels during the placebo phase of the study in HIV-infected aviremic subjects

Secondary Objectives:

1. To compare changes in HIV-1-RNA levels (measured using either the single copy assay or the HIV RT PCR assay) between the placebo and the rifaximin phases of the study.
2. To compare changes in T-cell activation (measured as changes in the proportion of CD4 or CD8 positive T cells that express either HLA-DR and/or CD38) during the rifaximin phase of the study and compare it with the changes in cellular markers of activation during the placebo phase of the study in both groups of subjects.
3. To compare changes in soluble markers of inflammation between the placebo and rifaximin phases of the study.
4. To investigate the safety profile of rifaximin in aviremic HIV-infected subjects.

Exploratory Objectives:

1. To compare changes in gut mucosal biopsies between the placebo and the rifaximin phases of the study. Immunohistochemistry and routine hematoxylin & Eosin stains will be used to examine gut mucosal biopsies. Gut mucosal biopsies will be examined for T-cell subtypes and the presence of HIV-DNA
2. To compare changes in the fecal microbiome during the rifaximin and placebo phases of the study.
3. To investigate other cellular markers of inflammation and activation, other than those listed in the secondary objective.

Précis

The introduction of antiretroviral therapy (ART) has resulted in dramatic reductions in AIDS-related morbidity and mortality [1]. Therapy is not curative, however, and the nature of HIV replication during therapy remains unclear. Understanding mechanisms involved in HIV persistence will be useful in identifying effective strategies for HIV eradication. Immune activation (IA) plays a central role in the pathogenesis of HIV-infection, and may play a critical role in HIV persistence during therapy. In comparison with the levels detected in HIV uninfected subjects, both cellular markers of activation and biomarkers of inflammation are elevated in HIV-infected individuals [2]. Levels of inflammatory cytokines and cellular markers of activation independently correlate with disease progression in HIV-infected subjects [2-3]. Chronic, persistent IA is associated with the observed CD4 depletion in untreated subjects and among ART- treated and virologically suppressed subjects and may contribute to the failure to reconstitute CD4 counts[4]. IA also plays a role in the pathogenesis of non-AIDS related complications such as chronic kidney and coronary artery disease (CAD)[5].

Although chronic persistent IA may play a role in HIV persistence [6-7], the source of immune activation itself is unknown. Low level viremia may represent a virologic stimulus for IA. Viremia persists at low levels during therapy, but it is not known whether HIV infection is maintained by ongoing cycles of replication in sanctuary sites, production from long-lived cells with integrated proviruses, or both. Using sensitive assays for HIV-1 viremia, we and others have detected the presence of persistent HIV viremia in the majority of subjects throughout prolonged antiretroviral therapy [8-9]. Drug intensification studies suggest little contribution of active replication to levels of persistent viremia, suggesting that factors other than complete cycles of HIV replication may contribute to HIV-1 persistence [10-11]. Activation of HIV-1 from long-lived cells in reservoir sites is another potential source of viremia, but the nature of such reservoirs is not yet well understood.

The mechanism of immune activation in HIV infection remains to be clarified and is likely multifactorial. Additional potential mechanisms of persistence include a central role for the gastrointestinal tract. The gastrointestinal epithelium and gut-associated lymphoid tissue (GALT) are thought to represent important barriers to microbial translocation, but HIV infection results in substantial destruction of both barriers. The reservoir of bacteria in the gastrointestinal tract is substantial, and small amounts of bacterial products are reported to translocate across the gastrointestinal tract into the bloodstream; microbial translocation across this defective GALT is an important driver of the observed immune activation in HIV infection [12-15]. The precise effects of ART on gut microbial translocation remain uncertain; some studies suggest that ART incompletely reverses the effects of microbial translocation, others have failed to demonstrate any effect, yet other studies have demonstrated complete reversal with ART [12, 16-18].

In this study, we will examine the potential role of bacterial translocation on IA by studying the effects of the antibiotic rifaximin on markers of microbial translocation, immune activation, and HIV viremia in the gut reservoir in ART treated aviremic subjects. Rifaximin is an orally administered antibiotic with potent qualitative and quantitative effects on gut bacterial flora. Rifaximin is not systemically absorbed, and drug effects appear to be confined to the

gastrointestinal tract. Rifaximin has been studied as maintenance therapy in both inflammatory bowel disease (IBD) and hepatic encephalopathy (HE), disease states in which endogenous gut flora play an important role in the pathogenesis [19-20]. It is anticipated that the use of rifaximin will result in an alteration and reduction in gut bacterial flora. We hypothesize that the reductions in gut bacterial flora will result in a corresponding reduction in bacterial translocation and reductions in biologically active LPS levels leading to reductions in immune activation, and HIV.

In this protocol, the role of gut microbial translocation in the pathogenesis of HIV infection will be examined by performing a randomized, double-blind, placebo-controlled study of rifaximin with a case cross-over design in virologically-suppressed HIV-infected persons receiving ART.

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

Role of chronic persistent immune activation in the pathogenesis of HIV infection
The widespread use of ART has resulted in significant reduction in AIDS-related morbidity and mortality; however, the consequences of chronic inflammation and persistent immune activation have emerged as an important concern in HIV-infected subjects [1-2]. The term immune activation (IA) encompasses a wide range of events observed in HIV-infection these include increases in the levels of activated T cells and proinflammatory cytokines. When compared to the levels detected in HIV-negative subjects, levels of both cellular markers of activation (i.e. activated T cells) and biomarkers of inflammation are elevated in HIV-infected persons [4, 21]. In general, the presence of higher levels of activated T cells has been associated with a poor prognosis [22-23]. The seminal work by Giorgi et al. examined the associations of T lymphocyte activation in subjects with advanced immunosuppression [22, 24]. However, recent studies have examined subjects identified early in HIV infection with relatively preserved CD4 counts, and the previously observed associations with IA and survival hold true even in this population [23]. In general, levels of activated T cells correlate inversely with gains in CD4+ T cells observed on ART [25]. The postulated effects of activation are pervasive; persistent IA is even thought to contribute to the CD4 declines observed among HIV-infected elite controllers [26]. In general, use of suppressive ART is associated with reductions in activated T cells [4]. However, despite suppressive ART, levels of activated T cells in HIV-infected persons remain elevated when compared with uninfected controls [4, 27] and the results of an unpublished study suggest that even prolonged suppressive ART (8 or more years) does not completely reverse the activated state observed in this disease (F. Cossarini and F. Maldarelli, unpublished observations).

Role of microbial translocation as a driver of immune activation
IA mechanisms are diverse and poorly understood, but recently, generalized IA by microbial products that have translocated from the intestinal lumen into the systemic circulation has been elegantly investigated [12]. The intestinal epithelium and the intestinal microflora play an important role in the generation of the mucosal immune response. The colonic epithelium is cloaked by large numbers of commensal microorganisms; these commensal organisms are in a state of equilibrium with their host rarely inciting a significant inflammatory response. However, when this equilibrium is disturbed it can result in disease. For example, the pathogenesis of inflammatory bowel disease (IBD) has been characterized by some as an abnormal inflammatory response to intestinal pathogens in a susceptible host. IBD is characterized by abnormal epithelial integrity; loss of epithelial integrity predisposes to microbial translocation with resultant stimulation of the innate and adaptive arms of the immune system resulting in chronic inflammation[28].

The Role of the Gastrointestinal (GI) tract in the pathogenesis of HIV infection
Gut-associated lymphoid tissue (GALT) has a central role in immune responses, and recent studies have demonstrated particular relevance to HIV infection [13-14, 29-30].

Clinical effect of HIV infection on the GI tract
A number of studies demonstrated early and profound involvement of the gut after HIV infection. Clinically, acute seroconversion syndrome includes a characteristic diarrheal syndrome [31-32]. Untreated, the course of HIV infection has long been recognized as a syndrome

characterized by continued enteropathy, including recurrent diarrhea and malabsorption syndromes[33]; although a number of specific opportunistic pathogens may explain many clinical presentations, persistent GI dysfunction is a frequent disturbance during the course of HIV infection independent of detectable pathogens[34-35]. Endoscopic evaluation during chronic infection typically reveal upper and lower GI disease, including crypt hyperplasia with villous atrophy and without evidence of other pathology and regardless of disease state [34, 36-38].

Effects of HIV on the gut mucosal immune response

In general, gut mucosal immunity includes inductive sites, such as Peyer's Patches and other immune aggregates that are similar to typical lymphoid tissue found in lymph nodes, as well as effector regions in the lamina propria of the gut which include effector T cells. Both regions appear dramatically affected by HIV infection. Early in HIV infection there is a substantial depletion of gut-associated CD4+ T cells, with a preferential depletion of CD4+ Th17 cells [13-14, 39]. Th17 precursor CD161+CD4+ cells are also substantially depleted, suggesting a profound immune defect [40-41]. This early depletion is a result of both direct infection and bystander apoptosis; depletion of CD4+ T cells and particularly Th17+CD4+ T cells contributes to defective barrier, loss of neutrophil recruitment, and increased translocation of microbial products [42-44]. CD4+CD25+ FoxP3+ (forkhead transcription factor) denoted regulatory T cells (Tregs) are a long lived suppressor T cell subset that modulate both the innate and adaptive immune responses to HIV infection. Both Tregs and Th17+ CD4 T cells are derived from a common progenitor. Some studies suggest that in HIV and SIV infection there is an increase in mucosal Treg frequency. The resulting alteration in the Th17/Treg balance is thought to contribute to the pathogenesis of HIV infection [45-46].

In addition, HIV-infected subjects exhibit loss of enterocytes and enterocyte tight junctions even in the absence of active opportunistic infections, which may favor microbial translocation [33, 47-49]. In experimental primate studies, Estes and coworkers demonstrated defective barrier function in the gut. They found microbial products in the lamina propria, with local IL18 and interferon alpha production that was independent of viral replication per se [50].

Consequences of HIV- induced enteropathy

The systemic consequences of HIV induced enteropathy remain uncertain. Silvestri and coworkers analyzed systemic and gastrointestinal immune activation in HIV-infected individuals and noted an inverse correlation between levels of intestinal CD4+ T cells and circulating CD4+ Ki67+ T cells, suggesting that destruction of gut immune function is associated with systemic immune activation [51]. This level of immune activation may be the result of bacterial translocation products, typically detected as lipopolysaccharide (LPS). LPS is a component of the Gram-negative cell walls and is a potent stimulator of the Toll-like receptor 4 (TLR4). The bioactive form of LPS is often reliably measured by examining soluble CD14 levels (sCD14) which is produced by monocytes upon LPS stimulation. sCD14 represents an accurate and robust marker for immune activation. Levels of LPS and bacterial 16S rDNA correlate with markers of chronic IA and predict failure of CD4 reconstitution, independent of the viral RNA levels [15]. Gabuzda and coworkers have reported a strong correlation between sCD14 levels and cognitive defects in untreated HIV-infected patients [52-53]. Marchetti and coworkers reported an association of LPS levels with disease progression [54] and Sandler et al. demonstrated that

levels of sCD14 are highly predictive of mortality in HIV infection, providing a potential link between microbial translocation and disease pathogenesis [55].

The precise relationship between the gut and progressive disease is incompletely characterized. Despite profound changes in the gastrointestinal tract after SIV infection, some studies have described functions that remain intact despite infection [56]. Similarly, in experimental SIV infections, Pandrea et al. noted profound loss of CD4 cells in the gut after experimental infections of both African green monkeys and rhesus macaques. This is interesting given that SIV infection in African green monkeys rarely progresses to AIDS, in contrast SIV infection of rhesus macaques often results in profound immunodeficiency and death from opportunistic infections [57]. Thus the long term effects of CD4 loss in the gut in experimental SIV infection are not invariably associated with progressive immune deficiency and it appears that portions of the innate immune response are intact following SIV infection.

ART and its effects on markers of microbial translocation and the gastrointestinal mucosa
HIV-infected subjects receiving ART have lower LPS levels than untreated subjects, but LPS levels in treated subjects remain higher than those detected in HIV-uninfected subjects [15]. Thus far the results of studies examining the effects of ART on sCD14 levels have reported inconsistent results. In one study despite 48 weeks of therapy no measurable declines were observed in sCD14 levels [12], while another study reported partial declines after 96 weeks of therapy [18]. In a study that examined 20 virologically suppressed HIV infected subjects and 10 HIV negative control, sCD14 levels remained elevated in the ART treated group (on average the treated group had received therapy for 5.5 years) [58]. In a smaller study that compared sCD14 levels in a group of subjects who had received ART for a median of eight or more years, sCD14 levels were similar to the levels observed in HIV- uninfected subjects, but the values were numerically higher [17].

Mucosal response to ART
Introduction of antiretroviral therapy results in substantial improvements in mucosal immune cells. Although persistent defects in gut immunity have been detected during antiretroviral therapy [51], few studies have investigated individuals after prolonged (>4 y) suppression. Sheth and coworkers [59] noted increases in CD4 cells in sigmoid colon exceeding that of peripheral blood in individuals with HIV infection undergoing suppressive antiretroviral therapy for mean of 89 months. Persistent HIV DNA levels in gut were associated with the CD8 activation marker CD69. These data suggested extensive gut reconstitution after prolonged therapy with persistent immune activation. Sereti and coworkers noted increases in the frequency of peripheral CD4 cells expressing high levels of gut homing beta7hi on CD4 cells, and a restoration of absolute numbers of CD4 cell numbers in colon and terminal ileum; the proportions of these cells in colon in terminal ileum remained lower than that detected in uninfected individuals, perhaps as a consequence of persistently elevated proportion of CD8 cells[17]. Bacterial translocation was detectable in both infected and uninfected individuals; circulating LPS levels correlated with cycling CD4+ cells (Ki67+) detected in the periphery or in the gastrointestinal tract. Marchetti and coworkers [60] also identified an association between activated cells in periphery and systemic levels of LPS in immune nonresponders. The role of LPS translocation as the cause or result of increases in cellular immune activation remains uncertain.

Persistent defects after prolonged suppression have been studied in portions of the gastrointestinal tract in addition to the colon and terminal ileum. Mavigner confirmed the observation that there were increases in gut homing (CD4+CCR9+beta7hi) cells in the peripheral blood, and found decreases in these cells in jejunal biopsies, suggesting a defect in homing to upper gastrointestinal sites. In addition, in contrast to the colon or terminal ileum, a persistent decrease in CD4 cells in jejunum of HIV infected individuals even after prolonged suppression of viremia was observed [58]. The CD4+CCR9+beta7hi population that was observed in this study included most gut homing Th17 cells, providing a possible mechanism for bacterial translocation. Levels of LPS and sCD14 were inversely correlated with levels of gut homing cells in this study. As the jejunum consists largely of effector immune cells, and not inductive centers, it is possible that effector arm in the jejunum is predominantly affected by HIV.

Yukl and coworkers [61] measured virologic and immunologic parameters in various portions of the gastrointestinal tract and found HIV nucleic acid levels were in general higher in the gut than in the periphery; highest gut levels were achieved in the ileum. Levels of HIV DNA in the gut were positively correlated with cellular immune activation measured in peripheral blood, suggesting a role for gut-associated HIV and systemic immune activation. Additional studies evaluating the effect of antiretroviral intensification using raltegravir on gut reservoirs, did not result in substantive changes in gut associated viral DNA, suggesting that in general, the gut compartment does not consist of rapidly cycling cells[62]; decreases in viral RNA in ileum after raltegravir intensification have suggested that site specific ongoing replication may occur.
Taken together these data identify that the gastrointestinal tract is critically and extensively affected by HIV infection, resulting in defects in barrier function with resulting translocation of bacterial cell products. Antiretroviral therapy results in prolonged suppression of viremia and significant increases in immune cells but it is unclear if the changes observed in the gut are completely reversed with therapy. Persistent local immune activation has been reported despite suppressive therapy.

The role of the GI tract as a latent reservoir
The gastrointestinal tract has been reported to be a sanctuary site for HIV infection, and a number of virologic studies have provided useful information regarding HIV infection in the gut. Genetic studies analyzing the sequence of HIV derived from specific sites have reported the presence of distinct populations of HIV in gastrointestinal tract-derived samples [63]. Sampling issues and technical limitations of PCR amplification and sequencing frequently complicate interpretation of these genetic studies; more recent studies using end point dilution techniques that resolve these complications have identified genetically similar HIV populations in GI biopsy derived and plasma[64]. In addition, phylogenetic analysis of HIV in a limited number of patients (N=3) interrupting antiretroviral therapy revealed rebounding virus was distinct from virus present in the gut [65], suggesting that rebound viremia was not arising from a gut reservoir. Sampling issues and a comparatively long delay after interruption in this study (9 – 12 months) may complicate interpretation of these data. These studies do illustrate the benefits of phylogenetic analyses in the setting of extensive sampling strategies to obtain representative tissue, and highlight the utility of single genome sequencing to accurately characterize HIV populations in gut tissue.

Most of the current reports are cross sectional and descriptive by nature. Additional

interventional studies will be useful to further investigate the role of the gastrointestinal tract on immune activation in during suppressive antiretroviral therapy. In the proposed study, we will characterize the effect of the antibacterial agent rifaximin on gut bacterial cell load, and investigate potential secondary effects on gut histopathology, levels of translocation of bacterial cell products, and levels of soluble and cellular measures of immune activation. In order to fully investigate the effects of rifaximin intervention, we are including in this study extensive histopathologic, immunologic, and virologic analyses. Approaches to characterize the effects of HIV on the gastrointestinal tract have included direct histopathologic studies, including immunohistochemistry and in situ hybridization, as well as virologic and immunologic analysis of cells populations derived from gut tissue. Each approach provides useful and complementary information regarding the state of gastrointestinal tract, the immune system, and the defects induced by HIV infection.

A potential role for rifaximin, a non-absorbable antibiotic

The presence of bacterial products in blood is likely related to the extent of damage to the gut barrier[58]; it is not known whether the level or type of bacteria in the GI tract may also affect the level of translocation. Investigation of other inflammatory bowel diseases, such as ulcerative colitis or Crohn's disease, and hepatic encephalopathy offer some insights. For example, in a large longitudinal study, serum LPS-binding protein levels and sCD14 correlated with Crohn's disease activity, with increases occurring in individuals experiencing disease flares, and decreases occurring as disease activity was controlled[66]. Similarly, in subjects diagnosed with hepatic encephalopathy there is increased translocation of gut-derived endotoxin. The use of antibiotics, including rifaximin, has been associated with statistically significant increases in induction of disease remission in both these states [67].

Research remains at an early stage and the relationship between disease activity and bacterial load is unclear; evidence-based review offers only a "weak" recommendation for the use of antibiotics to control disease activity [68]. The members and diversity of bacterial species present in the gut microbiome may play a key role in gastrointestinal disease. Studies using animal models have demonstrated a critical role for specific bacterial species in inducing regulatory T cell function. Several studies have demonstrated a critical role for the commensal *B. fragilis* in the induction of Foxp3 (+) regulatory T cells; a specific polysaccharide produced by *B. fragilis*, polysaccharide A (PSA), can mediate conversion of CD4 positive cells to Fox P3 (+) T reg cells through TLR 2 signaling. In animal studies, PSA can prevent and cure experimental colitis.[69] Others have reported that cell wall components from *Lactobacillus salivarius* can also cure experimental colitis and also induces Foxp3(+) cells[70]. The association between improvements in symptoms and bacterial translocation has not been investigated, and the mechanism of improvement is not completely understood. Antibiotics may improve disease activity by reducing the level of bacteria, or by altering the gut flora and changing the balance of individual bacterial species. Individual species involved in bacterial translocation have been initially investigated in HIV-infected persons. Merlini and coworkers identified diverse species by sequencing amplified 16S bacterial ribosomal DNA from plasma from individuals with HIV infection suppressed on ART for 12 months. Translocation was present in those with or without immune reconstitution (increased CD4 cell numbers), and included a number of polymicrobial species which were overwhelmingly aerobic[71].

Taken together, these observations suggest the potential for ongoing IA even after prolonged viral suppression in subjects successfully treated with ART. These collective observations have also fueled interest in examining the role of therapeutic interventions to modulate the chronic microbial translocation in HIV-infection.

HIV replication during suppressive antiretroviral therapy

Both chronic persistent IA and ongoing viral replication contribute to the chronic inflammation observed in HIV disease. One hypothesis is that IA via bacterial translocation products stimulates cells to chronically produce HIV and maintain persistent HIV infection. Despite profound and prolonged suppression of viremia, ART is not curative. There are a number of fundamental gaps in our understanding aspects of HIV infection preventing straightforward approaches to eradication [72]. In particular, the state of HIV infection during ART is uncertain. It is not known whether HIV continues to undergo complete cycles of replication despite suppressive therapy, or if HIV infection persists in long-lived chronically infected cells.

Understanding the relative contributions of active replication and chronic reservoirs to maintaining HIV infection is critical to designing new approaches to eliminate HIV infection. If persistent viremia is derived from cycles of active HIV-1 replication, improving the potency and penetration of drugs that block new cycles of replication is essential. By contrast, if new cycles of viral replication are completely suppressed by current therapy and viremia is derived from reservoirs of long-lived chronically infected cells, new strategies are necessary to cure infection. As previously reported, viral decay rates are the result of death and elimination of infected cells with short (1-1.2 d), intermediate (14 d) and prolonged (39 week) half-lives. We and others have reported substantial viral suppression and a stable low level of persistent viremia (essentially infinite half-life) following the third phase decline in subjects on suppressive therapy for >3-4 years[9]. These retrospective data suggest that all short-, and intermediate-lived HIV-1 infected cells had been eliminated after 3-4 years, and that persistent viremia was the product of long-lived cells producing virions from integrated proviruses [72-73].

To directly investigate whether ongoing cycles of HIV-1 infection continue during suppressive therapy, we conducted trials of antiretroviral intensification using inhibitors of HIV-1 reverse transcriptase (efavirenz, EFV), protease (atazanavir/ritonavir, ATV/r, or lopinavir/r, LPV/r;), and raltegravir (RAL). These studies demonstrated that using EFV, LPV/r, ATV/r, or RAL to intensify therapy did not result in decreases in HIV viremia, indicating that additional inhibition of reverse transcription, integration, or protease cleavage steps in viral replication does not further inhibit HIV-1 production [10, 74]. These studies were consistent with the hypothesis that persistent low level viremia in the presence of suppressive ART is the product of long-lived (>14 days) chronically infected cells. The nature of the cells comprising the reservoir is uncertain. The recent identification of “predominate plasma clones” of HIV circulating in the peripheral blood over weeks or months without detectable genetic variation has suggested that chronically infected cells may continue to produce virus over long periods and/or that such cells may undergo expansion [75]. It is not known whether latently infected cells with integrated proviruses are episodically activated to produce HIV in response to generalized activation or specific antigen recognition. New studies to address the relationship between IA and HIV pathogenesis are essential.

In order to investigate the role that disrupting gastrointestinal tract microflora has on bacterial translocation, IA, and persistent viremia, we have chosen rifaximin, an orally administered antibiotic with minimal systemic absorption. Even among subjects with altered gut epithelial integrity, less than 1% of the administered dose is absorbed systemically. Rifaximin is well-tolerated with a side effect profile very similar to placebo. In the United States (US) rifaximin is FDA-approved for the treatment of traveler's diarrhea and maintenance therapy for hepatic encephalopathy. However, several studies have evaluated the role of rifaximin for the treatment of irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) including pouchitis, hepatic encephalopathy, and bacterial overgrowth syndrome, disease states where the endogenous flora is thought to play an important role in the pathogenesis [19, 76-77].

Rifaximin is also currently being evaluated in subjects undergoing allogeneic bone marrow transplantation for the prevention of graft versus host disease. (http://www.clinicaltrials.gov/ct2/show/NCT00967096?term=rifaximin&rank=3) Rifaximin has been used with success in the long-term maintenance therapy of IBD, a disease characterized by defective epithelial integrity, altered adaptive and innate immune responses, and in which gut microbial translocation is thought to play an important role in the pathogenesis [76]. Rifaximin has been used successfully in the maintenance of remission in subjects with hepatic encephalopathy (HE), a disease in which gut derived neurotoxins play a central role in pathogenesis [19]. Since microbial translocation may play an important role in the pathogenesis of HIV disease, we propose to evaluate the role of a four week course of rifaximin on markers of microbial translocation, LPS bioreactivity, T cell activation, inflammation and HIV viremia (by standard and single copy assays) in ART-treated subjects with controlled viremia.
In concert with the study of cellular markers of IA, soluble markers of inflammation, and quantitative studies of HIV-1 plasma viremia, we will investigate the nature of HIV-1 genetic diversity in the GALT of suppressed and viremic subjects. We will apply single genome sequencing techniques developed for analysis of plasma HIV-1 to investigate HIV-1 diversity and recombination in GALT obtained from suppressed subjects. These studies of HIV-1 genetic diversity before and after rifaximin treatment, and comparisons of HIV-1 in GALT with those in plasma will yield useful information regarding the contribution of GALT to HIV-1 replication during suppressive ART.

1.1 Description of the Study Agent/Intervention(s)

Rifaximin is an orally administered broad-spectrum antibiotic with minimal systemic absorption. Absorption of the drug is not increased in the presence of inflammatory diarrhea [78]. The bile solubility of rifaximin far exceeds the aqueous solubility of the drug [79], thus rifaximin exerts its effect on both the terminal ileum and colon. The gut targeted localization of this antibiotic makes this agent particularly attractive in conditions where gut microbial flora play a central role in pathogenesis [20, 80]. The oral absorption of rifaximin is unaffected by the fasting state. Fecal levels after the oral administration of the drug range between 4000-8000 μg/g of stool, which is 160- 250 fold higher than the MIC_{90} for most bacterial enteropathogens. Studies in healthy volunteers suggest that less than 0.4% of the administered dose is absorbed systemically[80]. Due to the limited systemic absorption, a change in dosing is not recommended in subjects with renal or hepatic failure. Though rifaximin induces the cytochrome P450 3A4 system, no

significant drug-drug interactions have been reported, including with antiretroviral agents (Lexicomp online interaction analysis). Additionally, in studies evaluating the concomitant use of midazolam and oral contraceptives with rifaximin, no significant drug-drug interactions were observed [81-82]. Rifaximin's mechanism of action involves binding to the beta subunit of bacterial DNA-dependent RNA polymerase (RpoB), resulting in inhibition of bacterial RNA synthesis. In vitro rifaximin exhibits a broad spectrum of activity against Gram-positive, Gram-negative, aerobic, and anaerobic bacteria[83]. The antimicrobial activity of rifaximin has also been attributed to alteration in bacterial virulence and the effects of rifaximin on epithelial cells [84-86]. Much of rifaximin's activity is directed towards inhibition of bacteria, but in vitro rifaximin exhibits anti-inflammatory properties. For example, rifaximin pretreatment of Enteroaggresive *E.Coli* results in the diminished ability of the organism to induce an inflammatory response[87]. Rifaximin activates the pregnane X receptor and may exert its beneficial effects on specific gastrointestinal disorders thru this mechanism[88].

Resistance to rifaximin may occur due to single amino acid substitutions in RpoB, which is thought to mediate resistance among the rifamycin group of compounds including rifaximin. Resistance is not mediated through plasmids making the horizontal transfer of resistance unlikely with this class of compounds. In-vitro resistance to rifaximin has been observed. Selection of resistance among Gram-negative organisms is uncommon, whereas resistance in Gram-positive organisms occurs. Selection of resistance among anaerobic bacteria is uncommon. Selection of resistance is easier in an aerobic environment and in the presence of sub-inhibitory concentrations of the drug [89]. Resistance to rifaximin in fecal strains emerges within 1-2 weeks of therapy. Disappearance of resistance has been observed fairly rapidly upon discontinuation of the drug. [90] While extraintestinal resistance occurs, it is rare [91].

The clinical relevance of the emergence of resistance is not well understood. Rifaximin has been used for short durations (2 weeks) without adverse consequences.[92-93] A recently published clinical trial has examined the use of rifaximin for a longer duration.[19] In this study, a regimen of twice-daily rifaximin (550 mg) administered for 6 months was examined in subjects with HE. The proportion of infection related AE's were similar in both the rifaximin and the placebo treated subjects (30%). These rates were not thought to be greater than that expected for the severity of illness [19].

In general, the use of antibiotics has been associated with emergence of *Clostridium difficile* diarrhea. While no cases of *C. difficile* were reported in a study of IBS subjects randomized to receive a 2 week course of rifaximin, [92] three cases of *C.difficile* colitis were observed (2 in the rifaximin arm and 1 in the placebo arm) in the randomized placebo-controlled trial that examined the use of rifaximin in subjects with HE.[19] In this study, subjects who developed *C.difficile* while receiving rifaximin reported other concurrent risk factors including multiple recent hospitalizations, receipt of proton pump inhibitors, and other antibiotics[19]. Rates of *C.difficile* colitis in this study were similar between the rifaximin and placebo arm. While it is possible that rifaximin use resulted in *C. difficile* colitis in these two study participants, it is interesting to note that rifaximin has *in vitro* activity against *C. difficile*,[94] and has shown utility in refractory *C. difficile* infection in combination with vancomycin, and as sequential therapy [95-97].

Adverse effects are limited and mostly confined to the gastrointestinal tract. The following side effects have been observed in over 2% of subjects treated with rifaximin for travelers' diarrhea: flatulence, headache, abdominal pain, rectal tenesmus, defecation urgency, nausea, constipation, pyrexia, and vomiting. It is important to note that the dose and duration of rifaximin used for the treatment of travelers' diarrhea is lower and shorter than that being used in this study. Hypersensitivity to rifaximin has been rarely observed in post-marketing experience,[98] and is characterized by exfoliative dermatitis, rash, angioneurotic edema, urticaria, flushing; pruritus and anaphylaxis have been reported. Rifaximin is considered a pregnancy category C agent, and its excretion in breast milk is unknown.

1.2 Summary of Relevant Clinical Studies

To our knowledge there have been no double blind, placebo controlled randomized clinical trials evaluating the use of rifaximin in HIV-infected subjects. In a single study of 48 HIV-1 infected subjects, the use of rifaximin (600 mg three times a day for 14 days) resulted in resolution of diarrhea due to *Cryptosporidium parvum* and *Blastocystis hominis*[99]. An ongoing ACTG study of rifaximin is underway and will be enrolled by 2012 in HIV-infected individuals evaluates the effects of 4 weeks of rifaximin administered twice daily in immune nonresponder HIV-infected individuals undergoing ART. Subjects are randomized to receive 4 weeks of open label rifaximin treatment or no treatment to determine whether rifaximin administration reduces levels of translocated gut microbial products and markers of cellular immune activation. The ACTG team hypothesizes that the proportion of HLA-DR and CD38+ expressing CD8+ T-cells (%HLA-DR+CD38+CD8+ T-cells) will be lower in subjects randomized to receive 4 weeks of rifaximin than in those who do not receive rifaximin. Secondary objectives include the impact of rifaximin on levels of gut microbial translocation (LPS, bacterial 16S DNA, and soluble CD14), changes in plasma HIV-1 RNA by single copy assay (SCA), and other markers of immune activation and inflammation. Subjects are followed for 12 weeks. Our study is similar to the ACTG study in intervention and duration.

It is worth noting the following significant differences between the recently completed ACTG study and the study proposed here.

1. Study design: a) Our study will be double-blinded/placebo controlled to eliminate potential bias in the administration of the study drug, in contrast to the open-label, non-placebo controlled earlier study; b) a cross over design will be utilized to allow comparisons of the quantitative effects of rifaximin in the individual patient and across the study population.
2) Patient population: We will enroll patients at all CD4 cell levels, in contrast to the ACTG study which enrolled subjects with $\leq$ 350/mm^3. We chose this criterion because we have noted a correlation between low level viremia and cellular immune activation in patients suppressed on therapy (F. Cossarini and F. Maldarelli, unpublished observations). Since this correlation is present irrespective of immune responder status, it was logical to focus on the same broad CD4 inclusion group for this study.
3.) Study procedures: We will perform in depth and quantitative analysis of stool to characterize the effects of rifaximin, and will obtain gut tissue biopsies to explore the mechanisms of rifaximin intervention; in contrast; no stool or tissue studies are part of the ongoing ACTG study.

As a consequence of substantial differences in study design, patient selection, and study procedures, the study planned here represents a new and useful approach.

As noted above, in the US rifaximin is approved for the treatment of traveler's diarrhea due to enterotoxigenic *E. coli* [98]. In addition, rifaximin has been used successfully as maintenance therapy in subjects with HE and recently received FDA approval for this indication. The drug has also been evaluated in subjects with Crohn's Disease, IBD with pouchitis, IBS, and diverticular disease, disease states in which the gut microbial flora is thought to play an integral role in the pathogenesis [19, 76, 92].

In summary, the role of gut microbial translocation in the pathogenesis of HIV infection will be examined by studying the effects of rifaximin in virologically-suppressed HIV-infected persons receiving ART.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to compare the changes in sCD14 levels between the placebo and rifaximin phases of the study.

2.2 Secondary Objectives

1. To compare the changes in HIV-1-RNA levels (using the single copy assay or the traditional HIV RT PCR assay) between the placebo and the rifaximin phases of the study.

2. To compare changes in soluble markers of inflammation between the placebo and rifaximin phases of the study.

3. To compare the changes in cellular markers of IA (changes in the proportion of CD4+ or CD8+ T cells that express HLA-DR and/or CD38) during the rifaximin phase of the study and compare it with the changes in cellular markers of activation during the placebo phase of the study.

4. To investigate the safety profile of rifaximin in HIV-infected subjects.

2.3 Exploratory Objectives

1. To compare changes in gut mucosal biopsies between the placebo and the rifaximin phases of the study. Immunohistochemistry and routine hematoxylin & eosin stains will be used to examine gut mucosal biopsies. Gut mucosal biopsies will be examined for T-cell subtypes and for the presence of HIV-DNA.

2. To compare changes in the fecal microbiome during the placebo and rifaximin phases of the study.

3. To investigate other cellular markers of immune function and activation other than those listed in the secondary objective.

3 STUDY DESIGN

3.1 Description

The study will be conducted as a randomized, double-blind, placebo-controlled trial and utilize a case cross-over design. In such a study design each subject serves as his or her own control. Such a study design avoids issues related to the inter-individual variability associated with response to treatment. Both the primary and secondary objectives will be evaluated in ART-treated subjects who have suppressed viremia as measured by standard assays. Subjects will be initially randomized to receive a 4 week course of rifaximin or placebo. Upon completion of a 4 week course of rifaximin or placebo they will undergo a 4-6 week washout period and cross over treatment assignments to complete a 4 week course of either rifaximin or placebo. The duration of 4 weeks was chosen as studies in cirrhotic patients suggest that both plasma endotoxin levels and biomarkers of inflammation show statistically significant declines, ~50%, after 28 days of rifaximin therapy [100-101]. Both these studies also used a dosage of 1200 mg. In this study, we will be using a 1100 mg dose as the drug is approved at a 550 mg dose. A 4-6 week washout period was chosen as studies in subjects with ulcerative colitis suggest that the concentration of intestinal microbial groups return to initial values after approximately a 4 week wash-out following rifaximin therapy [101-102].

3.2 Study Population

3.3 Recruitment Plan

Subjects will be recruited at the University of Pittsburgh, National Institutes of Health (NIH), and the Walter Reed National Military Medical Center. Prior to study implementation, sites must obtain IRB approvals. Health care providers and research personnel at participating institutions will identify potentially eligible subjects. Once a subject for study entry is identified and expresses interest in learning about the study, the subject will be approached by the study team about participation in the protocol. If the subject remains interested, study details and the informed consent form will be reviewed with the candidate by the study PI/designee. If the subject is willing to participate in the study, he or she will be provided with a copy of the informed consent to review. If a subject requires more time to consider the study, the staff member may give them a copy of the informed consent along with the staff member's contact information, so that subjects may take the documents home for further review. After questions related to the study and the informed consent has been answered to the subject's satisfaction, subjects will be asked to sign the IRB-approved consent form.

3.4 Participant Inclusion Criteria

Patients who have agreed in the course of other research studies to have their records reviewed will have the following elements evaluated from their existing records: age, history of HIV-infection, ART history and viral loads prior to informed consent, or else these elements will be

assessed after informed consent. All blood draws to assess eligibility will be completed after obtaining informed consent. To participate in this study the criteria listed below will need to be met.

1. Subjects must be 18 years of age or older.
2. Able and willing to provide written informed consent
3. Must have a history of documented HIV infection.
4. HIV infection if not previously documented at host institutions will need to be documented by a plasma HIV RNA viral load, rapid HIV test or any other licensed ELISA test and confirmed by another test using a different method such as a rapid HIV test, Western Blot, HIV culture, HIV antigen, HIV pro-viral DNA at any time prior to study entry.
5. ART- treated subjects who are virologically suppressed for $\geq$ 3 years (1095 days). To meet this criteria all documented viral loads in the 3 years (1095 days) prior to the screening visit must be below the lower limit of detection [LLD] using FDA-approved standard assays (i.e. <50 copies/mL) with the following clarification: In each of the three prior years, subjects experiencing a single blip [i.e. viral loads above the lower limit of detection, LLD] may be included provided they satisfy the following criteria: the blips are below 200 copies/ml, and the blip is surrounded (i.e. the preceding and succeeding viral loads) by undetectable HIV-1 RNA level measurements. That is all viral loads must be below LLD EXCEPT for up to one 'blip'. In any 12 month period.
6. Viral RNA level < 50 c/ml at Screen 1.
7. A minimum of 2 HIV-1 RNA levels that are below the lower limit of detection using standard assays will be required during the 12 month period prior to their screening visit. As assay characteristics across the sites can vary, LLD for the assay will be used to define whether or not a subject is suppressed.
8. Stable dose of statin therapy for 6 months if receiving statin therapy.
9. No known allergy or contraindication to the use of rifamycin compounds such as rifampin, rifabutin or rifaximin.
10. The effect of rifaximin on the developing human fetus are unknown, therefore subjects must be willing to use two methods of contraception (one of which must be a barrier method) during the study period. Adequate methods of birth control include: tubal ligation, hysterectomy, condoms (male or female) with or without a spermicide; diaphragm or cervical cap with spermicide; intrauterine device; any of the methods that require a prescription (such as contraceptive pills or patch, Norplant, Depo-Provera, and others) or a male partner who has previously undergone a vasectom**y.**

The following elements will be assessed with a blood draw and after obtaining informed consent.

1. Absolute Neutrophil count (ANC) $\geq$750/mm^3
2. Hemoglobin $\geq$10.0 g/dL for women and Hemoglobin $\geq$ 11.0 g/dl for men
3. Platelet count $\geq$75,000/mm^3
4. Estimated Glomerular Filtration Rate (eGFR) >60 mL/min, eGFR will be calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

5. Confirmed serum glutamate pyruvate transaminase (SGPT)/serum glutamate oxaloacetate transferase (SGOT) ≤3times the upper limit of normal (ULN)
6. INR ≤ the ULN for the assay
7. Negative urine pregnancy test of child bearing potential at randomization
8. No evidence of active hepatitis B or hepatitis C (active hepatitis B will be defined as a positive hepatitis B surface antigen present on a single determination, whereas a positive result on hepatitis C RNA will be considered as evidence of active hepatitis C)

All routine laboratory testing used to determine safety will be completed within the 70 days prior to randomization.

Participant Exclusion Criteria

1. Known bleeding diathesis (for example a diagnosis of hemophilia or Von Willebrand disease)
2. Active drug use or alcohol abuse/dependence, which in the opinion of the investigators will interfere with the patient's ability to participate in the study
3. Serious illness requiring systemic treatment and/or hospitalization within 30 days of screening into the study
4. Evidence of active opportunistic infections or neoplasms (excluding cutaneous basal cell carcinoma and squamous cell carcinoma) in the 6 months prior to randomization
5. History of inflammatory bowel disease (Crohn's Disease, ulcerative colitis)
6. Positive urine pregnancy test at screening (of child bearing potential).
7. Breastfeeding
8. Current imprisonment
9. Concurrent immunomodulatory agents, including systemic corticosteroids in the 12 weeks prior to randomization. Topical, nasal or inhaled corticosteroid use is allowed
10. Concomitant use of probiotics except yogurt
11. Chronic antibiotic use such as tetracyclines for acne
12. Vaccinations within 6 weeks of randomization
13. Concomitant use of anticoagulants (other than aspirin and NSAIDS) is an exclusion criterion for subjects opting in for the colonoscopy. Aspirin and NSAIDs will be discontinued per each institutions requirement before the procedure.

14 Child-Pugh Class C disease

15 A prior history of *Clostridium difficile* colitis

16 Any condition that precludes the safe administration of conscious sedation for endoscopy (such as decompensated lung or heart disease) will not be able to participate in the colonoscopy aspect of the protocol

3.5 Co-enrollment Guidelines

Co-enrollment in other trials is restricted, other than for observational studies, or those that allow for co-enrollment.. Study staff should ask subjects if they are participating in other studies and inform the site principal investigator/designee.

3.6 Justification for Exclusion of Pregnant and Breastfeeding Women and Children

Pregnant women are excluded from this study because the effects of rifaximin on the developing human fetus are unknown with the potential for teratogenic or abortifacient effects. There is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with rifaximin, breastfeeding mothers will not be included in this study. Children will be excluded from this study as this study is unlikely to show any direct benefit to the child.

4 STUDY AGENT

4.1 Disposition and Dispensation

The study agent [rifaximin/placebo] will be distributed via the NIH Central Pharmacy (NIH PDS) according to standard pharmacy procedures. Both study drug and placebo will be formulated by the NIH PDS and will be supplied to the sites.

4.2 Formulation, Packaging, and Labeling

Both placebo and drug will be identical in appearance. Commercial rifaximin 550 mg tablets are purchased by the Clinical Center Pharmacy Department. They are re-formulated into a light blue, opaque, size 0, hard gelatin capsule. Each capsule contains 183.3 mg rifaximin with microcrystalline cellulose and sodium starch glycolate as filler. A matching placebo capsule will be formulated as a light blue, opaque, size 0, hard gelatin capsule containing microcrystalline cellulose and sodium starch glycolate. Capsules also contain a Colorcon® aluminum lake blend of FD&C yellow #5/tartrazine, FD&C #6/sunset yellow, and FD&C 40 allura red. They will be packaged and labeled with the study number, and will have a space for the subjects initials and randomization number to be entered. Each bottle will be individually labeled with the subject ID number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, and the subject's randomization number.

4.3 Study Products

Drug:

Description- Rifaximin capsules for oral administration
Dosing and Administration- 550 mg will consist of three capsules to be taken twice a day.
Route of Administration- Oral
Duration of Therapy- 28 days (56 doses)

Control Product:

Description: Placebo will be identical in appearance to rifaximin
Dosing and Administration: three placebo capsules twice a day
Route of Administration- Oral
Duration of Therapy- 28 days (56 doses)
Storage and Stability- Both rifaximin and placebo can be stored between 59-86 degrees Fahrenheit (i.e., room temperature)

4.4 Study Product Accountability Procedures

Study product dispensing and shipping records will be maintained centrally by the NIH PDS including shipping records. The University of Pittsburgh and Walter Reed National Military Medical Center (WRNMMC) will maintain study product dispensing and accountability records. Study products will be dispensed to the sites by NIH PDS and remaining study product will be returned to the NIH PDS at the conclusion of the study. Subjects will obtain study product per the study visit schedule.

4.5 Assessment of Subject Compliance with Study Agent/Intervention(s)

Subjects will be asked to bring their bottle of study product to each study visit. Pill counts will be performed and recorded at each visit to monitor adherence to the protocol, and subjects will be asked about their adherence to study drug.

4.6 Concomitant Medications and Procedures

All concomitant medications taken during study participation will be captured at every study visit. Medications to be reported are concomitant prescription medications, over-the-counter medications and non-prescription medications including dietary and herbal supplements. This will be assessed at enrollment and updated periodically at study visits. Prescription medication is a medication that can be prescribed only by a properly authorized/licensed clinician.

4.7 Prohibited Medications and Procedures

Treatment with immunomodulating agents including systemic steroids in the 3 months prior to screening will not be permitted as these drugs have the potential to affect the primary outcome of the study. Subjects on topical steroids will not be excluded. Concurrent statin use will be permitted if subjects have been receiving stable doses of statins for 6 or more months. The use of concurrent antibiotics/oral steroids will not be permitted while on study drug. If a subject needs to initiate antibiotic/oral steroid therapy while he/she is on the study drug, the patient will discontinue the study drug, complete the course of antibiotics/oral steroids, undergo a 4 week washout and will re-initiate the study drug starting from Day 0 of the treatment phase. Subjects initiating antibiotics/oral steroids during the screening or washout phases of the study will have these phases extended by 4 weeks after completion of the antibiotic/oral steroid course. Routine vaccinations (i.e., influenza) may be administered during the screening process or washout phase. Subjects receiving routine vaccinations during the washout phase will have their washout extended to 6 weeks.

5 STUDY SCHEDULE

5.1 Screening Visits 1 and 2

After signing the study informed consent, subjects will undergo 2 screening visits.

At the first screening visit subjects will undergo HIV testing (screening/confirmation if prior documentation of HIV status is unavailable)

Subjects found to be eligible for the study after the first screening visit will undergo the following procedures during the second screening visit:

- Complete medical history and physical examination
- Blood draw for
    - Immunophenotyping to include a CD4+ count and cellular markers of activation (including CD38+ and HLA-DR co-expression on both CD4+ and CD8+ cells)
    - Markers of bacterial translocation (including LPS, bacterial 16SrDNA or bacterial RNA and sCD14)
    - Liver function tests (including bilirubin, alkaline phosphatase (SAP), AST and ALT)
    - Renal function tests (including blood urea nitrogen (BUN), creatinine, electrolytes)
    - Complete blood count with differential (including platelets)
    - Coagulation parameters (including prothrombin time (PT) and international normalized ratio [INR])
    - Hepatitis B panel (including hepatitis B surface antigen)
    - Hepatitis C RNA assay
    - Single copy assay

Screening visits 1 and 2 must be separated by 48 hours and be no more than 65 days apart.

Screening visit 2 must be completed prior to randomization. Standard safety laboratory testing (blood counts, renal and liver function tests, coagulation studies, Hepatitis B and C panels) will be completed at the enrolling site. To minimize variability, all remaining laboratory testing will be done centrally at the NIH laboratory [Leidos Biomedical Research, Inc.].

5.2 Randomization (Day 0)

Randomization will be done using a random number table generated by the NIH pharmacy department. Twenty-two (22) subjects will be randomized initially to receive rifaximin 550 mg twice daily for 4 weeks and 22 subjects will be randomized to receive placebo one tablet twice daily for 4 weeks.

The following procedures and laboratory tests labs will be performed and/or obtained at Day 0:

General Procedures

- Targeted history and physical examination as medically indicated
- Adherence counseling and pill counts
- Assessment of signs and symptoms
- Study product dispensing (subjects will be asked to initiate study drug the day after randomization is complete)

Safety Labs

- CBC with differential, platelet count

- BUN, creatinine, electrolytes (including sodium, potassium, chloride, carbon dioxide/bicarbonate), liver function tests [including ALT, AST, bilirubin, SAP]
- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- Immunophenotyping to include a CD4 count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4 and CD8 cells)
- Single copy assay and HIV-1-RNA levels
- Soluble markers of inflammation (interleukin6 [IL6], highly sensitive C reactive protein [hsCRP], tumor necrosis factor [TNFα], D-dimer)
- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, soluble CD14 [sCD14])

Sample Storage

- Every attempt will be made to collect stool for each study visit but due to logistical constraints, samples may not be collected at each study visit. Patients may obtain samples at home and bring to the visit if preferred.
- Sample storage
- An optional baseline colonoscopy with gut biopsy will be completed in the 30 days prior to randomization
- All subjects undergoing a colonoscopy at baseline will receive a follow up phone call within 2 business days of their colonoscopy to ensure their safety.

5.3 Treatment Phase Visits

Subjects will undergo a total of eleven (11) study visits: two (2) screening visits, four (4) visits during the rifaximin dosing phase (Days 0, 7, 14, and 28); four (4) visits during the placebo dosing phase (Days 0, , 7, 14, and 28) and one (1) final visit (4 weeks after completion of both dosing phases). Between weeks 8-10 of the study (4 weeks on study treatment followed by a 4-6 week washout), all subjects will cross over to the other treatment arm. The Day 3 visits of the rifaximin and placebo phases of the study ARE OPTIONAL.

5.4 Treatment Phase 1

Day 3 (optional visit)

General Procedures

- Targeted history and directed physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- CBC with differential
- Immunophenotyping to include a CD4+ count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay & HIV-1- RNA levels
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

Sample Storage

- Stool storage for fecal microbiome analysis

Day 7 +/- 3days

General Procedures

- Targeted history and directed physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- CBC with differential
- Immunophenotyping to include a CD4+ count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay & HIV-1- RNA levels
- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, sCD14)
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

Sample Storage

- Stool storage for fecal microbiome analysis
- Sample storage

Day 14 +/- 3 days

- Targeted history and directed physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- Urine pregnancy test if applicable (of child bearing potential)
- PT/INR

Research Labs

- CBC with differential
- Immunophenotyping to include a CD4 count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay & HIV-1- RNA levels
- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, sCD14)
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

Sample Storage

- Stool storage for fecal microbiome analysis
- Sample storage

Day 28 +/- 3 days

- Targeted history and directed physical exam as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- CBC with differential, platelet count
- BUN, Creatinine, electrolytes (including sodium, potassium, chloride, carbon dioxide/bicarbonate), liver function tests [including ALT, AST, bilirubin, SAP]
- Urine pregnancy test if applicable (of child bearing potential)

Research Labs and Procedures

- Immunophenotyping to include CD4 count and cellular markers of activation (including CD4 count, HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay &HIV-1 RNA levels
- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, sCD14)
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)
- Colonoscopy with gut biopsies is optional

Sample Storage

- Stool storage for fecal microbiome analysis
- Sample storage

All subjects undergoing a colonoscopy at the end of phase 1 will receive a follow up phone call within 2 business days of their colonoscopy to ensure their safety.

5.5 Washout Phase

All participants will undergo a washout phase following phase 1. The washout period is 4 weeks in duration. If the participant requires a vaccination, the vaccine will be administered as early in the washout period as possible, and the washout period will be extended to a total of six weeks. If the patient requires antibiotics or systemic steroids during the course of the washout period, the washout period will be extended by 4 weeks after the completion of the antibiotics or steroids.

5.6 Treatment Phase 2

Subjects initially randomized to rifaximin will switch to the placebo arm, while subjects initially randomized to the placebo arm will switch to the rifaximin arm.

Day 0, Phase 2

General Procedures

- Targeted history and physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- CBC with differential, platelet count
- BUN, Creatinine, electrolytes (including sodium, potassium, chloride, carbon dioxide/bicarbonate), liver function tests [including ALT, AST, bilirubin, SAP]
- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- Immunophenotyping to include a CD4 count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4 and CD8 cells)
- Single copy assay and HIV-1- RNA levels
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)
- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, sCD14)

Sample Storage

- Stool sample for fecal microbiome
- Sample storage

Day 3, Phase 2 (optional visit)

General Procedures

- Targeted history and directed physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- CBC with differential
- Immunophenotyping to include a CD4+ count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay &HIV-1 RNA levels
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

Sample Storage

- Stool storage for fecal microbiome analysis

Day 7, Phase 2+/- 3 days
General Procedures

- Targeted history and directed physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- CBC with differential
- Immunophenotyping to include a CD4+ count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay & HIV-1 RNA levels
- Markers of bacterial translocation (LPS, bacterial 16SrDNA/bacterial RNA, sCD14)
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

Sample Storage

- Stool storage for fecal microbiome analysis
- Sample storage

Day 14, Phase 2 +/- 3 days

General Procedures

- Targeted history and directed physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- Urine pregnancy test if applicable (of child bearing potential)
- PT/INR

Research Labs

- CBC with differential
- Immunophenotyping to include a CD4+ count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4+ and CD8+ cells)
- Single copy assay & HIV-1- RNA levels
- Markers of bacterial translocation (LPS, bacterial 16SrDNA/bacterial RNA, sCD14)
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

Sample Storage

- Stool storage for fecal microbiome analysis
- Sample storage

Day 28, Phase 2, +/- 3 days

General Procedures

- Targeted history and physical examination as medically indicated
- Adherence counseling and pill counts
- Assess signs and symptoms

Safety Labs

- CBC with differential, platelet count
- BUN, Creatinine, electrolytes (including sodium, potassium, chloride, carbon dioxide/bicarbonate), liver function tests [including ALT, AST, bilirubin, SAP]
- Urine pregnancy test if applicable (of child bearing potential)

Research Labs and Procedures

- Immunophenotyping to include a CD4 count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4 and CD8 cells)
- Single copy assay & HIV-1 RNA levels
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)

- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, sCD14)
- Colonoscopy with gut biopsy is optional

Sample Storage

- Stool sample for fecal microbiome
- Storage
- All subjects undergoing a colonoscopy at the end of phase 2 will receive a follow up phone call within 2 business days of their colonoscopy to ensure their safety.

Final Visit +/- 2 weeks

General Procedures

- Targeted history and physical examination as medically indicated
- Assess signs and symptoms

Safety Labs

- CBC with differential, platelet count
- BUN, Creatinine, electrolytes (including sodium, potassium, chloride, carbon dioxide/bicarbonate), liver function tests [including ALT, AST, bilirubin, serum SAP]
- Urine pregnancy test if applicable (of child bearing potential)

Research Labs

- Immunophenotyping to include a CD4 count and cellular markers of activation (including HLA DR, CD38, co-expression of HLA DR and CD38 on CD4 and CD8 cells)
- Single copy assay & HIV-b-DNA assay)
- Soluble markers of inflammation (IL6, hsCRP, TNFα, D-dimer)
- Markers of bacterial translocation (LPS, bacterial 16S rDNA/bacterial RNA, sCD14)

Sample Storage

- Stool sample for fecal microbiome
- Sample storage

5.7 Study-Related and Non-Study Related Procedures and Vaccinations

Routine vaccinations (i.e. influenza) may be administered during the screening process or washout phase. Subjects receiving routine vaccinations during the washout phase will have their washout extended to 6 weeks. At each of these visits subjects will undergo interim evaluation of symptoms by study personnel and a targeted physical examination as deemed medically appropriate. Samples for the study are shipped from the University of Pittsburgh, NIH and Walter Reed sites to NCI for analysis. In the event of unforeseen logistical circumstances (e.g., because of a flight delay in sample shipment) resulting in a loss of samples necessary for analysis of the primary endpoint, and if the participants have already started phase 1 or 2 drug/placebo in

the interim, the participants will be asked to stop taking drug/placebo. They will undergo a four week washout and restart the respective study phase.

Gut mucosal biopsies via colonoscopy are optional and, if done, will be performed at baseline and upon completion of the rifaximin dosing and the placebo dosing phases (Days 28 of phases 1 and phase 2). If a subjects opts in for two of the three colonoscopies, these will be performed on approximately day 28 of phases 1 and 2, if a subjects opts for a single colonoscopy it will be performed on approximately day 28 of phase 2 of the study to maintain uniformity. As the relative representation of mucosal immunophenotypes and HIV-1-RNA levels can vary according to the location of the sampling site [61], multiple biopsies will be obtained during colonoscopy from the distal ileum and rectosigmoid (a total of 60 biopsies). For subjects enrolled at the NIH gut biopsies will be conducted under protocol 95-I-0027 entitled "Virologic and Immunologic Evaluation of Lymph Node, Tonsillar and Intestinal Biopsies, and Bronchoalveolar Lavage Fluid," as this protocol was designed to serve as a mechanism to obtain tissue samples.

All subjects in whom a colonoscopy is to be performed will be instructed to abstain from receptive anal intercourse for 3 days prior to rectal biopsy, and for 7 days post-biopsy to minimize risk of bleeding complications. Participants also should abstain from administration of rectal medications (including over-the-counter medications) for 3 days prior to rectal biopsies. All visits during the dosing phases will be completed on the indicated days of the protocol within a 3 day window. The washout phase may be extended from 4 to 6 weeks if necessary (i.e., immunizations, transportation, etc.). The final study visit may be completed +/- 2 weeks of anticipated study completion.

5.8 Early Termination

Subjects can be discontinued from the study for the following reasons:

- Failure to comply with the protocol requirements
- Pregnancy (followed on study but off study drugs see Section 10.5)
- Development of a serious adverse event (SAE) related to research
- Development of a Grade 3 or greater allergic reaction to rifaximin or develops signs and symptoms of an immediate hypersensitivity reaction to rifaximin
- Development of any medical or psychosocial condition that the study investigators deem would be detrimental for the subject to continue on the study
- Withdrawal of informed consent

If subjects are discontinued from the study during the treatment phases they will be replaced according to the prior randomization.

5.9 Pregnancy and Follow-up Visit

If the subject becomes pregnant while on the study, the subject will be taken immediately off study product, the blind will be broken, and the subject will be followed on-study for safety until delivery. She will be asked to return every 4 weeks for safety laboratory monitoring (including ALT, complete blood count with differential, platelet count, BUN, and creatinine) and a targeted history will be obtained and directed physical performed as warranted.

TABLE 1: Schedule of Events

Procedures	SCREENING			STUDY PHASE 1						STUDY PHASE 2					
Study Day	Screen # 1	Screen # 2	Gut Biopsy	Day 0	Day 3 (optional)	Day 7	Day 14	Day 28	Wash-out	Day 0	Day 3 (optional	Day 7	Day 14	Day 28	Final visit
Window			-30 day			+/- 3 days	+/- 3 days	+/- 3 days	+2 weeks			+/- 3 days	+/- 3 days	+/- 3 days	2 wks
Informed Consent	X														
Complete history and physical*		X													
HIV testing if needed	X														
Safety Labs **															
PT/INR		X					X						X		
CBC w/differential, platelet count		X		X	X	X	X	X		X	X	X	X	X	X
BUN, Cr, electrolytes, LFTs		X		X				X		X				X	X
Urine pregnancy test if applicable/Urine analysis[%]		X		X	X	X	X	X		X	X	X	X	X	X
Hepatitis profile[@]		X													
Research related labs															
Immunophenotyping		X		X	X	X	X	X		X	X	X	X	X	X
HIV- 1- RNA level	X	X		X	X	X	X	X		X	X	X	X	X	X
Single copy assay	X	X		X	X	X	X	X		X	X	X	X	X	X
Soluble biomarkers of inflammation[***]		X		X	X	X	X	X		X	X	X	X	X	X
Markers of bacterial translocation[#]		X		X		X	X	X		X		X	X	X	X
Colonoscopy and gut biopsy[$]			X					X						X	
General Processes															
Adherence counseling; pill counts		X		X	X	X	X	X		X	X	X	X	X	
Assessment of symptoms, targeted	X	X		X	X	X	X	X		X	X	X	X	X	X

history and physical^															
Cross-over (treatment arms)									X						
Study product dispensing				X						X					
Sample Storage															
Sample storage		X		X		X	X	X		X		X	X	X	X
Stored Stool for fecal microbiome				X	X	X	X	X		X	X	X	X	X	X

Footnotes

*Comprehensive history and physical examination will be performed at screening visit 2
** Abnormal safety labs will be confirmed before subjects are excluded
*** highly sensitive C reactive protein (hsCRP), D-dimer, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α)

^interim assessments; physical exam as warranted

Plasma LPS, bacterial 16S rDNA, sCD14

$ Colonoscopy is optional and will be performed at baseline and upon completion of the rifaximin treatment and placebo treatment phases. The baseline colonoscopy can be performed in the 30 days prior to day 0. While those being performed at the end of the treatment phases, must be completed while the subject is on study drug/placebo and can be completed in the 3 days prior to the completion of drug/placebo.

% If clinically indicated

@ Testing for Hepatitis B and Hepatitis A antibody status will only be performed in those with prior unknown or non-reactive status. Results of hepatitis C RNA testing in the 8 weeks prior to screening visit 2 are acceptable and will not be repeated.

6 STUDY EVALUATIONS

6.1 Clinical Evaluations

A medical history will be obtained and physical examination will be conducted at the screening visit. At each visit symptoms will be assessed and physical exams performed if needed.

6.2 Laboratory Evaluations

Local evaluations will include complete blood count with differential, platelet count, coagulation profile, and a metabolic profile that includes liver and kidney function tests and electrolytes. Table 1provides details of the time points at which these evaluations will be completed.

Study-related research testing will be performed primarily at the NIH or at their contract facility [Leidos Biomedical Research, Inc.]; fecal microbiome will be performed at the Uniformed Services University (USU). Study-related tests include:

- HIV-1- RNA levels by standard assay
- HIV-1- RNA level by single copy assay
- Immunophenotyping to include markers of immune activation. Immune activation will be assessed using the following parameters: co-expression of HLA-DR and CD38 on CD4 and CD8T cells, mono-expression of HLA-DR and CD38 on CD4+ and CD8+ T cells. Additional markers that may reflect cellular activation including Ki-67 expression on CD4+ CD8+ T cells will be investigated
- Soluble biomarkers of inflammation to include hsCRP, D-dimer, IL-6, and TNF-α
- Microbial translocation products will be measured using LPS levels, sCD14, and bacterial 16S rDNA or bacterial RNA.
- Stool samples for evaluation of the fecal microbiome. All stool samples for the fecal microbiome will be done prior to the bowel preps. Total bacterial load, as well as relative numbers of specific organism (such as *B. fragilis)* will be examined.
- An optional colonoscopy will be performed to sample the gut mucosal tissue. Samples will be obtained from the left colon (rectosigmoid) and terminal ileum. A maximum of 60 tissue samples will be obtained from both the rectosigmoid and terminal ileum. All procedures will be performed by trained gastroenterologists. Samples will be obtained in pre-filled tubes by the gastroenterologist. All samples will then be shipped by study personnel to a central processing laboratory housed at the Frederick National Laboratory for Cancer Research, P.O. Box B, 1050 Boyles Street, Building 535, Room 308, Frederick, MD 21702. Gut mucosal biopsies will be processed as per standard procedures at the central laboratory and not at the sites. Gut mucosal biopsies will be examined for routine hematoxylin and eosin staining (H&E), immunohistochemistry,

quantitative HIV-DNA and RNA levels, phenotyping and genotyping of HIV-1-RNA, and quantitative enumeration of T cell populations to include numbers and proportion of CD4+ and CD8+ T cells, T cell activation markers (to include CD38 and HLA DR expression on CD4 and CD8 + T cells, Ki-67 expression). Mucosal biopsies will also be analyzed for NK cell populations.

Table 1 details the time points at which these evaluations will be completed.

7 POTENTIAL RISKS AND BENEFITS

7.1 Potential Risks Associated with Rifaximin

Short term use of rifaximin is well-tolerated and frequencies of adverse events associated with this drug are similar to placebo. To date, there have been no randomized clinical trials in HIV-positive subjects. In several studies examining the effects of the drug as maintenance therapy in subjects diagnosed with decompensated liver disease and hepatic encephalopathy, as well as subjects diagnosed with irritable bowel syndrome, the emergence of treatment-emergent adverse effects (TEAEs) were similar in subjects randomized to placebo and rifaximin [19], [103]. The following adverse events have been reported in greater than 2% of subjects taking the 200 mg dose of rifaximin: flatulence, headache, abdominal pain (not otherwise specified), rectal tenesmus, defecation urgency, nausea, constipation, pyrexia, vomiting (not otherwise specified). The package insert accompanying this protocol provides information of side effects observed in less than 2% of subjects. The following events have been observed in the post-marketing experience: hypersensitivity reactions including exfoliative dermatitis, rash, angioneurotic edema, urticaria, flushing, and pruritus[98]. The 550 mg dose of rifaximin has been approved for use in patients with hepatic encephalopathy. At this dose in patients with hepatic encephalopathy [HE] the following events have been described in >5% of the patients (these include adverse events that may be attributable to the disease) peripheral edema, nausea, dizziness, fatigue, ascites, muscle spasms, pruritus, abdominal pain, abdominal distension, anemia, cough, depression, insomnia, nasopharyngitis, abdominal pain upper, arthralgia, back pain, constipation, dyspnea, pyrexia, rash. The 550 mg dose of rifaximin will be used for this study. [103]

Other Risks Associated With Rifaximin

Similar to other antibiotics, rifaximin use has been associated with *C. difficile* infection. In studies where rifaximin has been studied as maintenance therapy the incidence of *C. difficile* infection in subjects receiving rifaximin were no greater than the placebo arm and were similar to the background rates in this population. Subjects will be followed closely for the development of diarrhea, fever, abdominal cramps, or bloody stools [19]. In general, use of antimicrobial therapy may be associated with the emergence of bacterial resistance. Studies have demonstrated emergence of resistance with rifaximin therapy [80]. The consequences of this event are unknown and subjects will be monitored closely for any bacterial infection during therapy. While all risks to the subjects cannot be anticipated, all reasonable attempts will be made to ensure subject safety. This will include the evaluations outlined in Table 1.

7.2 Risks Associated with Colonoscopy and Biopsy

Diagnostic colonoscopy is generally safe and well tolerated, even in high-risk patient groups [104]. Actually, the majority of morbidity and mortality from these diagnostic procedures is related to cardiopulmonary complications of conscious sedation (aspiration or gastric contents, arrhythmia, hypoxemia) [105] with rates of attributable adverse outcomes and fatality of 0.54% and 0.03% respectively. While perforation is an exceedingly unlikely event, a risk for perforation exists, and the consequences of perforation include peritonitis, systemic sepsis, and death [106]. Bleeding and infection are other risks associated with this procedure. As a general practice, this risk is minimized by monitoring pulse, blood pressure, and oxygen saturation throughout the procedure. For colonoscopy, the reported mortality rates are 2-6 deaths/10,000 colonoscopies [107]; patients at the NIH Clinical Center are informed that the complication rates with colonoscopy are 2-4 perforations/1,000 colonoscopies, 10 perforations/1,000 colonoscopic polypectomies, 25 clinically significant bleeding episodes/1,000 colonoscopic polypectomies, 1 death/10,000 colonoscopies, and 2 deaths/10,000 colonoscopic polypectomies. Colonic perforation during lower endoscopy is almost exclusively associated with excess tension due to scope movement, inadvertent passage of an instrument (biopsy forceps, e.g.) through the wall, or over-insufflation of a segment of bowel and is reported to range from 0.03 to 0.65% for diagnostic colonoscopy and 0.073 to 2.14% in therapeutic procedure[108]. On the other hand, it is generally accepted that endoscopic biopsy-related perforation of the colon is a rare event overall, though it has been recognized to occur anecdotally in particular settings: in an area of atrophic mucosa over thinned bowel musculature [109] and in the ceca of elderly patients with bowel walls thinned by distension with air and (likely ischemia-related) mucosal ulceration and inflammation [110]. Similarly, clinically significant bleeding after colonoscopic mucosal biopsy is “exceedingly rare” [111] with no episodes of significant bleeding (requiring post-procedural evaluation or treatment) after cold biopsy in 2 series [112-113] and an estimate of minor bleeding (asymptomatic, self-limited, spotting on toilet paper or coloring toilet water) of 2.2% [112]. We could find no data relating specifically to the risk of bleeding to the number of biopsies taken per procedure. Only one published report of a single episode of major bleeding following a single cold biopsy of the cecum could be found [111]. Because of the large number of biopsies planned per endoscopy, we will inform the subjects of the potential for an increased risk of bleeding and perforation. In order to prevent complications and minimize their impact, we will avoid biopsies within ulcerated regions (where relative thinning of the gut wall may be present), avoid over-insufflation during biopsies, and provide post-procedure instructions for patients to recognize early warning signs of significant complications (abdominal pain, fever, persistent hematochezia) while providing mechanisms for early evaluation and treatment. In addition, experienced gastroenterologists will perform the colonoscopies to minimize the risk of complications related to this procedure.

7.3 Phlebotomy Risks

Risks associated with blood draw include discomfort, bleeding, bruising, and in rare cases, fainting and infection.

7.4 Breach of Confidentiality

While there is a chance that there might be a breach of confidentiality (loss of privacy), all attempts will be made to ensure that this does not happen. To ensure the utmost protection of confidentiality all laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only. The link to the subject will be maintained in a locked cabinet in a locked room. All computer entry will be done with coded numbers only.

7.5 Potential Benefits

There are no direct benefits from this study for enrolled subjects. However, the results of the study might help increase our understanding of the pathogenesis of HIV disease.

8 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS, OR DATA

Intended Use: Samples and data collected under this protocol may be used to study HIV infection, immune disorders, and interactions of HIV and host immune metabolic and genetic factors. Genetic testing will be performed.

Storage: Access to stored samples will be limited using either a locked room or a locked freezer or both. Samples and data will be stored using codes assigned by the investigators or their designees. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Stool specimens will be stored at the Uniformed Services University. All other study research-related samples will be sent to the NIH, stored, and tracked utilizing the Frederick National Laboratory for Cancer Research (formerly NCI-FCRF) Repository operated by Leidos Biomedical Research, Inc.

8.1 Disposition at the Completion of the Protocol

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior IRB approval. At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.

8.2 Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB

Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) that meets the definition of Protocol Deviation and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIAID IRB. Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what

was done to both the subject and to the IRB. This decision may not affect the subject's participation in this protocol or any other protocols at NIH.

9 REMUNERATION PLAN FOR SUBJECTS

Subjects will be reimbursed for travel and study visits per local site standards (See Appendix C).

10 ASSESSMENT OF SAFETY

10.1 Toxicity Scale

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, 2004, (Clarification August, 2009) will be utilized for grading adverse events (see Appendix B).

10.2 Specification of Safety Parameters

Definitions

Adverse Event
Any untoward or unfavorable medical occurrence in a human subject, including any abnormal symptom, sign, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Serious Adverse Event
Serious adverse events (SAEs): Any adverse event that

- results in death;
- is life threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity
- results in congenital anomaly/birth defect; or
- based upon appropriate medical judgment may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

  At the NIH site some individuals especially those travelling from some distance are admitted for convenience for study procedures. Elective admissions to the NIH will not be reported as SAEs.

Expected Adverse Events
Since rifaximin is an FDA-approved drug, adverse events described in the approved rifaximin package insert (label) will be considered to be expected.

Unexpected Adverse Events Those adverse events not described in the rifaximin

package insert, in the protocol, or in the informed consent document.

Unanticipated Problem that is not an Adverse Event (UPnonAE)
An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. These events may involve a greater risk of social or economic harm to subjects or others rather than physical/psychological harm. Such events would be considered a non-serious UP. Examples of a UPnonAE include a breach of confidentiality, accidental destruction of study records, and unaccounted-for study drug.

Protocol Deviation
Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Serious Protocol Deviation
A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance
The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as

1. Serious: Non-compliance that
    a. Increases risks, or causes harm, to participants
    b. Decreases potential benefits to participants
    c. Compromises the integrity of the NIH-HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that meets all three of the following criteria would be considered a serious UP:

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
    b. the characteristics of the subject population being studied; and

2. related or possibly related to participation in the research; and
3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Intensity or Severity of Adverse Events
Assignment of the grade of adverse events or side-effects of interventions is based on intensity of symptoms, degree of limitation of usual daily activities, or level of abnormality of objective clinical signs or laboratory parameters. All AEs that occur during the study will be assessed according to the DAIDS Toxicity Tables located in Appendix B or http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

Reporting to IRBs
For this study each institution will follow their institutions reporting requirements of their institution. Appendix D details the reporting requirements of all three institutions. Subjects enrolled in the NIAID will have their colonoscopy performed under protocol 95-I-0027, hence, colonoscopy related adverse effects observed among subjects co-enrolled in both protocols will also be reported. However, each event related to the colonoscopy (co-enrolled to 95-I-0027) procedure will be reported to the IRB under 95-I-0027. Grade 1 and 2 expected AEs will not be reported for this study. All SAE's definitely, possibly, or probably related to the drug and all unanticipated problems will be reported to all IRBs.

Each institution will follow their individual institutions reporting requirements. Appendix D details the reporting requirements of all other IRBs.

Relatedness of Adverse Event to an Intervention
The study investigator's best assessment at the time of reporting of the causal relationship between an experimental intervention and an adverse event; the degree of certainty about causality is graded as follows:

Definitely Related
- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related
- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related
- reasonable temporal relationship

- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

OR

- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

OR

- definitely due to an alternative etiology

10.3 Recording/Documentation

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations and will be immediately recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable adverse events that are identified will be recorded per local site standards [in CRIMSON at NIH, or on the appropriate case report form (CRF) at the University of Pittsburgh and WRNMMC]. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AEs relationship to the study agent/intervention will also be recorded [in CRIMSON, or on the appropriate CRF, or source document].

10.4 Reporting Procedures

Since this clinical trial is being conducted at 3 separate clinical sites, the local reporting requirements of all 3 clinical sites will be met. Adverse events will be reported per the individual institutions requirement detailed in Appendix D. The site Principal Investigator will be responsible for assuring all reporting requirements are fulfilled in a timely manner. These reporting requirements are detailed in Appendix D. Dr. Maldarelli will provide oversight to ensure study progress and the timely communication of adverse effects to all 3 IRBs. To facilitate this process, AEs occurring at any of the sites will be reported by the study personnel to the site PIs and the lead PI (namely Drs. McMahon, Maldarelli, and Ganesan) and will be submitted on CRFs and via electronic mail. In addition AEs will be discussed at team calls that will occur twice a month and ad-hoc as needed. Specifically the following will be discussed: all unexpected adverse events, all grade 3 and 4 expected adverse events, and any other adverse events in the PI's opinion that may jeopardize the subject's health, confidentiality or well-being. Unanticipated problems involving breach of confidentiality/privacy or HIPAA violation which place subjects or others at a greater risk of harm (including physical, psychological, economical, or social harm) that was not previously known or recognized will be discussed. A standardized excel spread sheet will be used to capture all AEs, Appendix F. This excel spread sheet will created using the data from CRIMSON

10.5 Reporting of Pregnancy

All unanticipated pregnancies will be reported to the IRB. All pregnant subjects will be followed on study until the completion of pregnancy.

10.6 Pausing and Halting Rules for the Protocol

The study will be paused (no new enrollments and no further administration of agent by the investigators) if the study is deemed to pose a significant risk to subjects enrolled in the study. In general, we do not anticipate significant risks requiring pausing because the interventional agent is FDA-approved and will be used at approved doses. If we experience grade 3 or 4 unexpected adverse events in two subjects thought to be related to rifaximin, we will pause the study. In this case a report will be submitted to the IRBs and the study will be reviewed by the DSMB. After the DSMB review, the study may be resumed if so recommended by the DSMB, with the approval of the IRBs.

The IRB, the NIAID, or other government agencies, as part of their duties to ensure that research subjects are protected, may discontinue the study at any time. Subsequent review of serious, unexpected and related adverse events by the DSMB, ethics review committee or IRB, the sponsor(s), and other regulatory authorities may also result in suspension of further trial interventions/administration of study agent at a site. Regulatory authorities and the study sponsor(s) retain the authority to suspend additional enrollment and Study Agent(s)/Intervention(s) administration for the entire study as applicable.

10.7 Stopping Rules for an Individual Subject

A subject will be discontinued from the study for the following reasons:

- Failure to comply with the protocol
- Develops a serious adverse event (SAE) definitely, probably or possibly related to rifaximin
- Becomes pregnant
- Develops a Grade 3 or greater allergic reaction to rifaximin/placebo or signs and symptoms of an immediate hypersensitivity reaction
- Develops any condition that the study investigators deem would be detrimental for him/her to continue on the study
- Desires to leave the study or withdraws written informed consent
- Presence or occurrence of any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- Develops any of the exclusion criteria listed in Section 3.4
- Termination of the study by the sponsor
- Requirement for excluded concomitant medications

Subjects who develop a change in condition which requires removal from the study secondary to safety, will be requested to finish protocol visits off drug to assess for safety related concerns.

10.8 Replacement of Subjects

Subjects that discontinue or are withdrawn from the study prior to completion of treatment phases 1 and 2 will be replaced according to the prior randomization.

11 CLINICAL MONITORING STRUCTURE

11.1 Site Monitoring Plan

All research data and results will be carefully recorded using data collection forms that will be saved and allow for continuous access. Further, all collected data will be entered and saved to a password-protected computer spreadsheet (Microsoft Excel™) for analysis and review. The investigator is responsible for assuring that the data collected is complete, accurate and recorded in a timely manner. Source documentation should support the data collected on the CRF and must be signed and dated by the person recording and/or reviewing the data. All data will be annotated and indexed, including all notebooks and computerized information, to facilitate detailed review of data. All data, even those of experiments not directly leading to publication, will be treated comparably. All research data will be made available to scientific collaborators and supervisors for immediate review, consistent with requirements of confidentiality. All research data, including the primary experimental results, will be retained for a minimum of 5 years to allow for analysis and repetition by others of published material resulting from the data. Demographic information and laboratory results will be collected using case report forms and then transcribed into an electronic database on an ongoing basis as participants are enrolled. At the NIH site, data will be stored in the CRIMSON database. Other experimental data, including HIV viral load results, immune activation markers, and gut translocation results, will be analyzed at NCI and reported in batches. Standard laboratory results will be accessed by the local site investigators and the study coordinators. All results, whether recorded on paper or electronically, will be maintained using only the subject's numeric code for identification. Data collection and quality assurance will be performed primarily by the site investigators and study coordinators**.** RCHSPB will complete onsite monitoring at USUHS, and the University of Pittsburgh including regulatory compliance and source verification.

11.2 Safety Monitoring Plan

Safety Review Plan by the Data Safety Monitoring Board

This is a double-blind treatment protocol. Monitoring will be performed by the site investigators. The NIAID, USUHS, and the University of Pittsburgh Institutional Review Boards will review the study at least annually. Because of the nature of the study, we plan to constitute a Data Safety Monitoring Board (DSMB) at the NIH to review the study progress and safety at least annually. This function will be provided through NIAID DSMB, and the group will be functioning as a safety monitoring committee for subjects enrolled at all the sites.

The NIAID Intramural DSMB is constituted to review the safety data of Intramural NIAID clinical studies that require DSMB oversight, and consists of experts in infectious diseases, biostatistics, and clinical research. The PI will provide the DSMB Executive Secretary with blinding codes in a sealed envelope in case the DSMB requires this information to make its recommendations. The DSMB will review the protocol prior to opening the study to enrollment and after 50% (22 subjects) complete the first dosing phase of the study and after the second dosing phase. The DSMB will also meet at least once a year to review the completeness of the study data collected and adherence to the protocol in order to evaluate safety, study conduct, and scientific validity and integrity of the study. Other reviews may occur as needed if safety issues arise. All serious adverse events and all unanticipated problems will be reported by the PI to the DSMB at the same time they are submitted to the IRB. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRBs. At the WRNMMC an independent medical monitor will also monitor the study; their role is detailed in Appendix E.

Emergency Un-blinding Procedures
In the event of a medical emergency or in the event an off-study criterion is met where knowledge of the treatment will affect medical management, the investigators can break the blind. To break the blind investigators will contact the pharmacy department by pager and via electronic mail, as they will hold the randomization code. In the rare event of such an occurrence the process will be documented in writing. The DSMB Executive Secretary will be notified of the unblinding within 1 business day. If the DSMB decides to terminate the study, the blind will be broken and study subject and their primary care physician will be informed of their drug assignment.

12 STATISTICAL CONSIDERATIONS

12.1 Study Hypotheses

This is a double-blind, randomized, crossover, placebo-controlled trial of rifaximin use in HIV-infected subjects. In this trial, ART-treated subjects with an undetectable viral load will be enrolled. The primary objective of this study is to compare the changes in sCD14 levels during the rifaximin phase of the study and compare it with the changes in sCD14 during the placebo phase of the study. Specifically the difference in sCD14 levels measured on Days 28 of treatment phase 1 and 2 will be used to assess rifaximin effect. Moreover to deal with a possible secular trend, the difference of differences (the difference of sCD14 levels measured on treatment phase 1 Days 0 and 28 and the difference measured on treatment phase 2 Days 0 and 28) will be examined as well.

12.2 Sample Size Justification

In a sub-analysis of the SMART study, baseline sCD14 levels predicted mortality; those who died in the SMART study had a baseline median sCD14 level of 2.47 pg/mL $*10^6$

(IQR 2.19 – 2.91), while matched controls had lower levels at 2.23 pg/mL *10^6 (IQR 2.01 -263). As these differences were statistically significant, we powered our study to exhibit a similar effect size [55].

With a mean of 2.45 pg/mL*10^6 and a standard deviation of 0.45 pg/mL*10^6 observed in ART treated subjects in the SMART study (personal communication Dr. Sandler) and a correlation coefficient of 0.71 (personal communication Dr. Sandler and Dr. Funderburg), a trial with a total of 40 aviremic patients has a power of 95% (with type I error of 0.05 based on a one-sample paired difference t test) for detecting a 0.20 difference in sCD14 levels between the placebo and rifaximin phases (namely Day 28 and 84) of the study. Due to possible loss to follow-up (10%), 44 patients will be accrued in this study.

12.3 Description of the Statistical Methods

This is a double-blind, randomized, crossover placebo-controlled trial of rifaximin use in aviremic HIV-infected subjects.

The primary goal of this study is to compare the sCD14 levels during the rifaximin and placebo phases of the study. Patients who receive steroids or an additional antibiotic during a treatment period will have a pause and then restart the treatment for a complete period (as described on page 29). Thus such patients will have the endpoints measured at the end of each of the two periods and will be included in the primary analysis. A sensitivity analysis will be conducted where such patients are excluded.

Forty-four aviremic subjects will be randomized to either rifaximin followed by placebo or placebo followed by rifaximin (22 per sequence). The following table displays the treatment and control sequence for the study.

Treatment phase 1 Day 0-28	Washout	Treatment phase 2 Day 0-28
22 aviremic subjects receive rifaximin initially	Washout	22 aviremic subjects switch treatment assignments and receive placebo.
22 aviremic subjects receive placebo initially	Washout	22 aviremic subjects switch treatment assignments and receive rifaximin

At the end of this trial, one sample Wilcoxon statistic will be applied to evaluate the primary end points (difference on Days 28 of treatment phase 1 and 2 of the study). The primary endpoints of interest are changes in soluble CD14 levels between the rifaximin and placebo phases of the study. A P value less than 0.05 will be used to claim significance [114]. Statistical tests and confidence intervals will be two sided.

Secondary outcomes for statistical considerations

In the following paragraphs the term paired difference refers to the difference between Days 28 of treatment phase 1 and 2. These differences will be tested using both the Wilcoxon test and the t-test.

The following paired difference will be examined between the placebo and the rifaximin phase of the study

1. Changes in HIV-1-RNA levels

2. Changes in Serum LPS levels

3. Changes in the proportion of CD8+HLADR+, CD8+CD38+, CD8+HLADR+CD38+, CD4+HLADR+, CD4+CD38+, and CD4+HLADR+CD38+ cells.

4. Changes in the soluble markers of inflammation (IL6, TNFα, D-dimer, and hsCRP)

In addition to deal with a possible secular trend, the difference of differences (for example the difference in HIV-1 RNA levels measured on Days 0 and 28 of treatment phase 1 and treatment phase 2) will be examined as well.

The proportions of different types of adverse effects as well as 95% confidence intervals will be reported.

Exploratory Objectives

Some exploratory objectives to be investigated include:
1. The comparison of changes in gut mucosal biopsies between the placebo and the rifaximin phase of studies will be performed by either paired-t test or paired Wilcoxon test.

2. The comparison of changes in fecal microbiome during the placebo and rifaximin phases of the study will be done by using either paired-t test or paired Wilcoxon test.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going

conversation between the human research subject and the researchers about the essential information about the study, which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions of essential information about the research will include the study's purpose, duration, experimental procedures, alternatives, risks, and benefits, and subjects will have the opportunity to ask questions and have them answered. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.2 Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law or other applicable law and regulation. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and 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 IRB, NIAID, OHRP, or the sponsor's designee.

14 DATA MANAGEMENT

Study data will be collected and maintained on paper Case Report Forms (CRF) at the University of Pittsburgh and WRNMMC. Case Report Forms will be sent to NIH and relevant data placed in CRIMSON. At NIH the Crimson system will be used. Finally, for data analysis all collected data (including Crimson data) will be entered and saved to a password-protected computer spreadsheet (Microsoft Excel™) for analysis and review. The study investigators will be responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of a piece of data) should support the data collected on the case report form, and be signed and dated by the person recording and/or reviewing the data. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the medical records, laboratory reports, ECG tracings, x-rays, radiologist reports, biopsy reports, ultrasound photographs, progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the protocol. Data for electronic CRFs/ source documents will be collected during study visits, phone calls with subjects and healthcare providers, and abstracted from the medical record.

14.1 Types of Data

Data will include laboratory and clinical data to include signs/symptoms, diagnoses codes, medication use, and clinical history. Data on research tests will also be collected. All data on CRF will be de-identified. A master log of all the subjects enrolled will be

maintained by the principal investigator or their designee. Access to the master log will be restricted. The master log will be kept in a locked cabinet behind locked doors, or in an electronic password protected database.

14.2 Source Documents and Access to Source Data/Documents

Study data will be collected on source documents and [case report forms (CRF) designed for the study and in an electronic data system (CRIMSON)] at the NIH. Study-specific databases will be developed at WRNNMC and the University of Pittsburgh using Excel. The Principal Investigator at each site is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner.

APPENDIX A: SCIENTIFIC REFERENCES

1. May, M.T., et al., HIV treatment response and prognosis in Europe and North America in the first decade of highly active antiretroviral therapy: a collaborative analysis. Lancet, 2006. 368(9534): p. 451-8.
2. Deeks, S.G., Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. Top HIV Med, 2009. 17(4): p. 118-23.
3. Kuller, L.H., et al., Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med, 2008. 5(10): p. e203.
4. Hunt, P.W., et al., T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis, 2003. 187(10): p. 1534-43.
5. Gupta, S.K., et al., Proteinuria, creatinine clearance, and immune activation in antiretroviral-naive HIV-infected subjects. J Infect Dis, 2009. 200(4): p. 614-8.
6. Tong-Starksen, S.E., P.A. Luciw, and B.M. Peterlin, Human immunodeficiency virus long terminal repeat responds to T-cell activation signals. Proc Natl Acad Sci U S A, 1987. 84(19): p. 6845-9.
7. Margolick, J.B., et al., Amplification of HTLV-III/LAV infection by antigen-induced activation of T cells and direct suppression by virus of lymphocyte blastogenic responses. J Immunol, 1987. 138(6): p. 1719-23.
8. Gandhi, R.T., et al., The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. PLoS Med, 2010. 7(8).
9. Palmer, S., et al., Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc Natl Acad Sci U S A, 2008. 105(10): p. 3879-84.
10. McMahon, D., et al., Short-course raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy. Clin Infect Dis, 2010. 50(6): p. 912-9.
11. Gandhi, R.T., et al., No evidence for decay of the latent reservoir in HIV-1-infected patients receiving intensive enfuvirtide-containing antiretroviral therapy. J Infect Dis, 2010. 201(2): p. 293-6.
12. Brenchley, J.M., et al., Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med, 2006. 12(12): p. 1365-71.
13. Brenchley, J.M., et al., CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med, 2004. 200(6): p. 749-59.
14. Mehandru, S., et al., Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med, 2004. 200(6): p. 761-70.
15. Jiang, W., et al., Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis, 2009. 199(8): p. 1177-85.
16. Ciccone, E.J., et al., CD4+ T cells, including Th17 and cycling subsets, are intact in the gut mucosa of HIV-1-infected long-term nonprogressors. J Virol, 2011. 85(12): p. 5880-8.
17. Ciccone, E.J., et al., Cycling of gut mucosal CD4+ T cells decreases after prolonged

anti-retroviral therapy and is associated with plasma LPS levels. Mucosal Immunol, 2010. 3(2): p. 172-81.
18. Wallet, M.A., et al., Microbial translocation induces persistent macrophage activation unrelated to HIV-1 levels or T-cell activation following therapy. AIDS, 2010. 24(9): p. 1281-90.
19. Bass, N.M., et al., Rifaximin treatment in hepatic encephalopathy. N Engl J Med, 2010. 362(12): p. 1071-81.
20. Adachi, J.A. and H.L. DuPont, Rifaximin: a novel nonabsorbed rifamycin for gastrointestinal disorders. Clin Infect Dis, 2006. 42(4): p. 541-7.
21. Neuhaus, J., et al., Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis, 2010. 201(12): p. 1788-95.
22. Giorgi, J.V., et al., Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis, 1999. 179(4): p. 859-70.
23. Ganesan, A., et al., Immunologic and virologic events in early HIV infection predict subsequent rate of progression. J Infect Dis, 2010. 201(2): p. 272-84.
24. Liu, Z., et al., Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immune Defic Syndr Hum Retrovirol, 1997. 16(2): p. 83-92.
25. Benito, J.M., et al., Differential upregulation of CD38 on different T-cell subsets may influence the ability to reconstitute CD4+ T cells under successful highly active antiretroviral therapy. J Acquir Immune Defic Syndr, 2005. 38(4): p. 373-81.
26. Hunt, P.W., et al., 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): p. 126-33.
27. French, M.A., et al., Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells. J Infect Dis, 2009. 200(8): p. 1212-5.
28. Abraham, C. and J.H. Cho, Inflammatory bowel disease. N Engl J Med, 2009. 361(21): p. 2066-78.
29. Mattapallil, J.J., et al., Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature, 2005. 434(7037): p. 1093-7.
30. Shacklett, B.L., Mucosal immunity to HIV: a review of recent literature. Curr Opin HIV AIDS, 2008. 3(5): p. 541-7.
31. Lavreys, L., et al., Primary human immunodeficiency virus type 1 infection: clinical manifestations among women in Mombasa, Kenya. Clin Infect Dis, 2000. 30(3): p. 486-90.
32. Sterling, T.R., Chaisson, R.E., General Manifestations of Human Immunodeficiency Virus Infection (Including Acute Retroviral Syndrome, and Oral, Cutaneous, Renal, Ocular, Metabolic, and Cardiac Diseases, in Mandell, Douglass, and Bennett's Principles and Practice of Infectious Diseases, G.L. Mandell, Bennett, J.E., Dolin, R., Editor. 2010. p. 1705-1725.

33. Kotler, D.P., et al., Enteropathy associated with the acquired immunodeficiency syndrome. Ann Intern Med, 1984. 101(4): p. 421-8.
34. Kotler, D.P., HIV infection and the gastrointestinal tract. AIDS, 2005. 19(2): p. 107-17.
35. Ullrich, R., et al., Small intestinal structure and function in patients infected with human immunodeficiency virus (HIV): evidence for HIV-induced enteropathy. Ann Intern Med, 1989. 111(1): p. 15-21.
36. Kotler, D.P., Characterization of intestinal disease associated with human immunodeficiency virus infection and response to antiretroviral therapy. J Infect Dis, 1999. 179 Suppl 3: p. S454-6.
37. Cello, J.P. and L.W. Day, Idiopathic AIDS enteropathy and treatment of gastrointestinal opportunistic pathogens. Gastroenterology, 2009. 136(6): p. 1952-65.
38. Budhraja, M., et al., Duodenal mucosal T cell subpopulation and bacterial cultures in acquired immune deficiency syndrome. Am J Gastroenterol, 1987. 82(5): p. 427-31.
39. Brenchley, J.M., et al., Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood, 2008. 112(7): p. 2826-35.
40. Prendergast, A., et al., HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells. AIDS. 24(4): p. 491-502.
41. Cecchinato, V., et al., Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. Mucosal Immunol, 2008. 1(4): p. 279-88.
42. Ford, E.S., C.E. Puronen, and I. Sereti, Immunopathogenesis of asymptomatic chronic HIV Infection: the calm before the storm. Curr Opin HIV AIDS, 2009. 4(3): p. 206-14.
43. Raffatellu, M., et al., Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. Nat Med, 2008. 14(4): p. 421-8.
44. Liu, J.Z., M. Pezeshki, and M. Raffatellu, Th17 cytokines and host-pathogen interactions at the mucosa: dichotomies of help and harm. Cytokine, 2009. 48(1-2): p. 156-60.
45. Shaw, J.M., et al., Increased frequency of regulatory T cells accompanies increased immune activation in rectal mucosae of HIV-positive noncontrollers. J Virol, 2011. 85(21): p. 11422-34.
46. Nigam, P., et al., Expansion of FOXP3+ CD8 T cells with suppressive potential in colorectal mucosa following a pathogenic simian immunodeficiency virus infection correlates with diminished antiviral T cell response and viral control. J Immunol, 2010. 184(4): p. 1690-701.
47. Lim, S.G., et al., Intestinal permeability and function in patients infected with human immunodeficiency virus. A comparison with coeliac disease. Scand J Gastroenterol, 1993. 28(7): p. 573-80.
48. Gillin, J.S., et al., Malabsorption and mucosal abnormalities of the small intestine in the acquired immunodeficiency syndrome. Ann Intern Med, 1985. 102(5): p. 619-22.

49. Stockmann, M., et al., Mechanisms of epithelial barrier impairment in HIV infection. Ann N Y Acad Sci, 2000. 915: p. 293-303.
50. Estes, J.D., et al., Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. PLoS Pathog. 6(8): p. e1001052.
51. Gordon, S.N., et al., Disruption of intestinal CD4+ T cell homeostasis is a key marker of systemic CD4+ T cell activation in HIV-infected individuals. J Immunol. 185(9): p. 5169-79.
52. Lyons, J.L., et al., Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIV infection. J Acquir Immune Defic Syndr. 57(5): p. 371-9.
53. Ancuta, P., et al., Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. PLoS One, 2008. 3(6): p. e2516.
54. Marchetti, G., et al., Microbial translocation predicts disease progression of HIV-infected antiretroviral-naive patients with high CD4+ cell count. AIDS. 25(11): p. 1385-94.
55. Sandler, N.G., et al., Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis, 2011. 203(6): p. 780-90.
56. Zaragoza, M.M., et al., Persistence of gut mucosal innate immune defenses by enteric alpha-defensin expression in the simian immunodeficiency virus model of AIDS. J Immunol. 186(3): p. 1589-97.
57. Pandrea, I.V., et al., Acute loss of intestinal CD4+ T cells is not predictive of simian immunodeficiency virus virulence. J Immunol, 2007. 179(5): p. 3035-46.
58. Mavigner, M., et al., Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest.
59. Sheth, P.M., et al., Immune reconstitution in the sigmoid colon after long-term HIV therapy. Mucosal Immunol, 2008. 1(5): p. 382-8.
60. Marchetti, G., et al., Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy. AIDS, 2008. 22(15): p. 2035-8.
61. Yukl, S.A., et al., Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. J Infect Dis. 202(10): p. 1553-61.
62. Yukl, S.A., et al., Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. AIDS. 24(16): p. 2451-60.
63. Poles, M.A., et al., Despite high concordance, distinct mutational and phenotypic drug resistance profiles in human immunodeficiency virus type 1 RNA are observed in gastrointestinal mucosal biopsy specimens and peripheral blood mononuclear cells compared with plasma. J Infect Dis, 2001. 183(1): p. 143-8.
64. Imamichi, H., et al., Lack of compartmentalization of HIV-1 quasispecies between the gut and peripheral blood compartments. J Infect Dis. 204(2): p. 309-14.
65. Lerner, P., et al., The gut mucosal viral reservoir in HIV-infected patients is not the major source of rebound plasma viremia following interruption of highly active antiretroviral therapy. J Virol. 85(10): p. 4772-82.
66. Lakatos, P.L., et al., Serum lipopolysaccharide-binding protein and soluble CD14

are markers of disease activity in patients with Crohn's disease. Inflamm Bowel Dis, 2011. 17(3): p. 767-77.
67. Khan, K.J., et al., Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol, 2011. 106(4): p. 661-73.
68. Talley, N.J., et al., An evidence-based systematic review on medical therapies for inflammatory bowel disease. Am J Gastroenterol, 2011. 106 Suppl 1: p. S2-25; quiz S26.
69. Round, J.L. and S.K. Mazmanian, Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A, 2010. 107(27): p. 12204-9.
70. Macho Fernandez, E., et al., Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. Gut, 2011.
71. Merlini, E., et al., Evidence for polymicrobic flora translocating in peripheral blood of HIV-infected patients with poor immune response to antiretroviral therapy. PLoS One, 2011. 6(4): p. e18580.
72. Margolis, D.M., Mechanisms of HIV latency: an emerging picture of complexity. Curr HIV/AIDS Rep, 2010. 7(1): p. 37-43.
73. Bailey, J., et al., Mechanisms of HIV-1 escape from immune responses and antiretroviral drugs. Curr Opin Immunol, 2004. 16(4): p. 470-6.
74. Dinoso, J.B., et al., Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proc Natl Acad Sci U S A, 2009. 106(23): p. 9403-8.
75. Bailey, J.R., et al., Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4+ T cells. J Virol, 2006. 80(13): p. 6441-57.
76. Shafran, I. and P. Burgunder, Adjunctive antibiotic therapy with rifaximin may help reduce Crohn's disease activity. Dig Dis Sci, 2010. 55(4): p. 1079-84.
77. Koo, H.L. and H.L. DuPont, Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases. Curr Opin Gastroenterol, 2010. 26(1): p. 17-25.
78. Taylor, D.N., et al., Systemic pharmacokinetics of rifaximin in volunteers with shigellosis. Antimicrob Agents Chemother, 2008. 52(3): p. 1179-81.
79. Darkoh, C., et al., Bile acids improve the antimicrobial effect of rifaximin. Antimicrob Agents Chemother, 2010. 54(9): p. 3618-24.
80. Scarpignato, C. and I. Pelosini, Rifaximin, a poorly absorbed antibiotic: pharmacology and clinical potential. Chemotherapy, 2005. 51 Suppl 1: p. 36-66.
81. Pentikis, H.S., et al., The effect of multiple-dose, oral rifaximin on the pharmacokinetics of intravenous and oral midazolam in healthy volunteers. Pharmacotherapy, 2007. 27(10): p. 1361-9.
82. Trapnell, C.B., et al., Absence of effect of oral rifaximin on the pharmacokinetics of ethinyl estradiol/norgestimate in healthy females. Ann Pharmacother, 2007. 41(2): p. 222-8.
83. Ojetti, V., et al., Rifaximin pharmacology and clinical implications. Expert Opin Drug Metab Toxicol, 2009. 5(6): p. 675-82.
84. Mencarelli, A., et al., Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells.

Biochem Pharmacol, 2010. 80(11): p. 1700-7.
85. Debbia, E.A., et al., Effects of rifaximin on bacterial virulence mechanisms at supra- and sub-inhibitory concentrations. J Chemother, 2008. 20(2): p. 186-94.
86. Cheng, J., et al., Therapeutic role of rifaximin in inflammatory bowel disease: clinical implication of human pregnane X receptor activation. J Pharmacol Exp Ther, 2010. 335(1): p. 32-41.
87. Jiang, Z.D., S. Ke, and H.L. Dupont, Rifaximin-induced alteration of virulence of diarrhoea-producing Escherichia coli and Shigella sonnei. Int J Antimicrob Agents, 2010. 35(3): p. 278-81.
88. Ma, X., et al., Rifaximin is a gut-specific human pregnane X receptor activator. J Pharmacol Exp Ther, 2007. 322(1): p. 391-8.
89. Maccaferri, S., et al., Rifaximin modulates the colonic microbiota of patients with Crohn's disease: an in vitro approach using a continuous culture colonic model system. J Antimicrob Chemother, 2010. 65(12): p. 2556-65.
90. De Leo, C., C. Eftimiadi, and G.C. Schito, Rapid disappearance from the intestinal tract of bacteria resistant to rifaximin. Drugs Exp Clin Res, 1986. 12(12): p. 979-81.
91. Valentin, T., et al., Rifaximin intake leads to emergence of rifampin-resistant staphylococci. J Infect, 2011. 62(1): p. 34-8.
92. Pimentel, M., et al., Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med, 2011. 364(1): p. 22-32.
93. DuPont, H.L., et al., A randomized, double-blind, placebo-controlled trial of rifaximin to prevent travelers' diarrhea. Ann Intern Med, 2005. 142(10): p. 805-12.
94. Jiang, Z.D., et al., In vitro susceptibility of Clostridium difficile to rifaximin and rifampin in 359 consecutive isolates at a university hospital in Houston, Texas. J Clin Pathol, 2010. 63(4): p. 355-8.
95. Patrick Basu, P., et al., Rifaximin therapy for metronidazole-unresponsive Clostridium difficile infection: a prospective pilot trial. Therap Adv Gastroenterol, 2010. 3(4): p. 221-5.
96. Johnson, S., et al., Rifaximin Redux: treatment of recurrent Clostridium difficile infections with rifaximin immediately post-vancomycin treatment. Anaerobe, 2009. 15(6): p. 290-1.
97. Johnson, S., et al., Interruption of recurrent Clostridium difficile-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. Clin Infect Dis, 2007. 44(6): p. 846-8.
98. Incorporated, S.P., XIFAXAN product labeling.
99. Amenta, M., et al., Intestinal protozoa in HIV-infected patients: effect of rifaximin in Cryptosporidium parvum and Blastocystis hominis infections. J Chemother, 1999. 11(5): p. 391-5.
100. Vlachogiannakos, J., et al., Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. Aliment Pharmacol Ther, 2009. 29(9): p. 992-9.
101. Kalambokis, G.N., et al., Rifaximin improves thrombocytopenia in patients with alcoholic cirrhosis in association with reduction of endotoxaemia. Liver Int, 2011.
102. Brigidi, P., et al., Effects of rifaximin administration on the intestinal microbiota in patients with ulcerative colitis. J Chemother, 2002. 14(3): p. 290-5.
103. Inc, S.P., XIFAXAN (rifaximin) Tablets, 550 mg, NDA 22-54. Briefing Document

for Gastrointestinal Advisory Committee Meeting.
104. Cappell, M.S., Gastrointestinal endoscopy in high-risk patients. Dig. Dis., 1996. 14: p. 228-244.
105. Hart, R. and M. Classen, Complications of diagnostic gastrointestinal endoscopy. Endoscopy, 1990. 22: p. 229-233.
106. Yao, M.D., et al., Multiple endoscopic biopsies in research subjects: safety results from a National Institutes of Health series. Gastrointest Endosc, 2009. 69(4): p. 906-10.
107. Froehlich, F., et al., Appropriateness of gastrointestinal endoscopy: risk of complications. Endoscopy, 1999. 31: p. 684-686.
108. Damore, L.J., et al., Colonoscopic perforations: etiology, diagnosis, and management. Dis. Colon Rectum, 1996. 39: p. 1308-1314.
109. Eckardt, V.F., C. Gaedertz, and C. Eidner, Colonic perforation with endoscopic biopsy. Gastrointest. Endosc., 1997. 46: p. 560-562.
110. Foliente, R.L., et al., Endoscopic cecal perforation: mechanisms of injury. Amer. J. Gastroenterol., 1996. 91: p. 705-708.
111. Raijman, I., J. Sellin, and A. Glombicki, Gastrointestinal bleeding after cold biopsy [Letter]. Gastrointest. Endosc., 1992. 38: p. 636.
112. Shiffman, M.L., M.T. Farrel, and Y.S. Yee, Risk of bleeding after endoscopic biopsy or polypectomy in patients taking aspirin or other NSAIDs. Gastrointest. Endosc., 1994. 40: p. 458-462.
113. Macrae, F.A., K.G. Tan, and C.B. Williams, Towards safer colonoscopy: a report on the complications of 5000 diagnostic or therapeutic colonoscopies. Gut, 1983. 24: p. 376-383.
114. Friendman, L.M., Furberg, C. D. and DeMets, D. L. (1983). Fundamentals of Clinical trials. John Wright. PSG Inc.

APPENDIX B: TOXICITY

The toxicity grading scale for this protocol is the "Table for Grading the Severity of Adult and Pediatric Adverse Events; Version 1.0 - December 2004 (Clarification dated August 2009)."

It can be found at:
http://rsc.techres.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

APPENDIX C: COMPENSATION PLAN

University of Pittsburgh-- Participants will be compensated $25-$100 per blood draw depending upon distance traveled and $200 for each colonoscopy.

NIAID- Participants will be compensated $40 per blood draw and $350 for each colonoscopy.

WRNMMC- Participants will be compensated $40 per blood draw only. There will not be colonoscopy compensation at the WRNMMC site.

Appendix D: Reporting Requirements:

Individual requirements for all three IRBs have been included in the package.

NIAID Reporting Requirements

Expedited Reporting to the NIAID IRB
Serious and non-serious Unanticipated Problems, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. Serious Adverse Events that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

Annual Reporting to the NIAID IRB
The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All grade 3/4 adverse events.
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

Appendix E

As per the USU ID IRB requirements all greater than minimal risk will require an independent medical monitor, the duties of the medical monitor at WRNMMC are detailed below
Duties as the Medical Monitor include:

1) Monitoring the conduct of the protocol per the approval plan and ensuring protection of human subjects. This may involve periodic review of medical records of enrolled subjects and the research files being maintained by the PI.
2) Reviewing and keeping abreast of protocol reported adverse events and protocol deviations that occur during the research (all adverse events, including deaths and serious or unexpected side effects, are reported to the Medical Monitor via the PI).
3) If there is concern about the welfare of enrolled subjects, the Medical Monitor has the authority to stop a research study in progress, remove individual subject from a study, and take whatever steps necessary to protect the safety and well being of research subjects until the IRB can assess the Medical Monitor's report. Notification of such actions must be forwarded to the IRB staff within one (1) working day of receipt of knowledge prompting human subject welfare concerns.
4) Medical Monitors will be required to co-sign all adverse event reports, protocol deviation memoranda, Annual Progress Reports, and addendum.
5) The Medical Monitor must keep current the required research ethics Human Subjects Training every year.
6) If the Medical Monitor is expected to be away for more than 14 days but less than 30, the PI or Medical Monitor must designate an acting Medical Monitor and document such action.
7) If a Medical Monitor leaves for greater than 30 days then the PI must be informed to designate a new Medical Monitor and report such change to the IRB via a memorandum for a change of Medical Monitor.