A5350

Safety, Tolerability, and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

DAIDS ES # 12040

This file contains the current ACTG A5350 protocol, which includes the following document:

- Letter of Amendment #1, dated 15 April 2016
- Clarification Memorandum #1, dated 22 February 2016
- Protocol Version 1.0, dated 5 January 2016

Letter of Amendment #1 for:

A5350

Safety, Tolerability and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study

A Limited Center Trial of the AIDS Clinical Trials Group (ACTG)

DAIDS ES # 12040

Letter of Amendment Date: 15 April 2016

ACTG Network Coordinating Center

Social & Scientific Systems, Inc.
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910

Phone: *(301) 628-3000*
Fax: *(301) 628-3302*

LETTER OF AMENDMENT

DATE: April 15, 2016

TO: ACTG CTU Principal Investigators, CRS Leaders, and CTU/CRS Coordinators

FROM: A5350/A5352s Protocol Team

SUBJECT: Letter of Amendment #1 for Protocol A5350 Version 1.0, 01/05/16, entitled, "Safety, Tolerability, and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study," and the substudy A5352s, Version 1.0, 01/05/16, entitled, "Effects of the Probiotic Visbiome Extra Strength on Epithelial Barrier Function and Inflammation in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Substudy of A5350."

The following information impacts the A5350 study and the A5352s substudy and must be forwarded to your institutional review board (IRB) as soon as possible for their information and review. This Letter of Amendment (LOA) **must be approved by your IRB before implementation.**

The following information may also impact the Sample Informed Consent. Your IRB is responsible for determining the process of informing participants of the contents of this LOA.

Upon receiving final IRB and any other applicable regulatory entity approvals for this LOA, sites should implement the LOA immediately. Sites are still required to submit an LOA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center. Sites will receive a registration notification for the LOA once the DAIDS PRO verifies that all the required LOA registration documents have been received and are complete. An LOA registration notification from the DAIDS PRO is not required prior to implementing the LOA. A copy of the LOA registration notification along with this letter and any IRB correspondence should be retained in the site's regulatory files.

The following are changes to A5350, Version 1.0, 01/05/16 (changes are noted in bold or strikethrough):

1. Section 4.1.8 is revised to:

   For females of reproductive potential, negative serum or urine pregnancy test **(latter with a sensitivity of ≤25 mIU/mL)** within 45 days prior to entry by any US clinic or laboratory that has a CLIA certification or its equivalent, or is using a point-of-care (POC)/ CLIA-waived test, or at any network-approved non-US laboratory or clinic that operates in accordance with Good Clinical Laboratory Practices (GCLP) and participates in appropriate external quality assurance programs.

   Note: Reproductive potential is defined as girls who have reached menarche and pre-menopausal women who have not had a sterilization procedure (eg hysterectomy, bilateral oophorectomy, tubal ligation, or salpingectomy), Women are considered menopausal if they have not had a menses for at least 12 months and have a FSH of greater than 40 IU/L, or if FSH testing is not available, they have had amenorrhea for 24 consecutive months. Acceptable documentation of a sterilization procedure and menopause is patient-reported history.

The above information will be incorporated into the next protocol version as necessary if the protocol is amended.

The following are changes to A5352s, Version 1.0, 01/05/16 (changes are noted in bold or strikethrough):

1. Section 6.1 Schedule of Events is revised to remove the hematology evaluation from the week 24 visit.

Evaluation	Screening (within 45 days prior to substudy entry)	Entry	Week 1 (±4 days)	Post-Entry	
				On Treatment	
				Week 24 (at least 14 days prior to week 26) (±7 days)	Week 26 (±7 days)
Hematology	X			~~X~~	

2. Section 6.3.2 Laboratory Evaluations is revised to:

   At screening, ~~and week 24,~~ all hematology laboratory values are recorded in the source document only.

   Hematology
   INR and PT/PTT

3. APPENDIX II, SAMPLE INFORMED CONSENT
   - The "During the Study" section is revised to:

     You will remain on your assigned A5350 treatment. You will have a total of 2 sigmoidoscopies done while on study, one at entry and then again at week 24. ~~Before each procedure you will have blood drawn to see if it is safe for you to have the sigmoidoscopy.~~ You will also undergo a test of the permeability of your intestines using a cocktail of sugars that you will drink at least 4 days after the first sigmoidoscopy and again at week 26.

   - Table 1, "Evaluations performed during the study", is revised to remove blood collection from the week 24 visit.

Evaluation or Test	Screening	Entry	Week 1	Post-Entry Visits	
				Week 24	Week 26
Blood Collected	✓			~~✓~~	
Colonic Biopsies for Immunologic Assessments		✓		✓	
Sugar Absorption Test / Urine Collected			✓		✓

The above information will be incorporated into the next protocol version as necessary if the protocol is amended.

Clarification Memorandum #1 for:

A5350

Safety, Tolerability and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study

A Limited Center Trial of the AIDS Clinical Trials Group (ACTG)

DAIDS ES # 12040

Clarification Memorandum Date: 22 February 2016

ACTG Network Coordinating Center

Social & Scientific Systems, Inc.
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910

Phone: *(301) 628-3000*
Fax: *(301) 628-3302*

CLARIFICATION MEMO

DATE: February 22, 2016

TO: ACTG CTU Principal Investigators, CRS Leaders, and CTU/CRS Coordinators

FROM: A5350/A5352s Protocol Team

SUBJECT: Clarification Memo # 1 to the A5350 Protocol, Version 1.0, 01/05/16 entitled, "Safety, Tolerability, and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study," and the substudy A5352s, Version 1.0, 01/05/16 entitled, "Effects of the Probiotic Visbiome Extra Strength on Epithelial Barrier Function and Inflammation in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Substudy of A5350."

This clarification memo does not result in a change in the protocol informed consent document. The Division of AIDS does not require you to forward it to your IRB; however, as always, you must follow your IRB's policies and procedures. If IRB review of clarification memos is required at your site, please submit this document for review.

Each site should file a copy of this clarification memo with the protocol for reference.

The protocol clarification contained in this memo should be implemented immediately. This update will be included in the next version of the A5350 protocol if it is amended at a future date.

The following clarification to protocol A5350, Version 1.0, 01/05/16 and the substudy A5352s, Version 1.0, 01/05/16, is intended to clarify that all A5350 on-treatment visits must be fasting, add to items that were inadvertently left out of the protocol, and to confirm that fasting is required for the lactulose/mannitol visits.

A5350

1. Section 6.1 Schedule of Events
   Add "if pregnancy is suspected" to the Week 2 visit in the Schedule of Events (SOE).

Evaluation	Screening (Within 45 days prior to entry)	Entry Week 0	Post-Entry							Conf. of Virologic Failure	Prem. Study DC
			Week 2 ±7days	On Treatment (Weeks)					Post Treat. (Weeks)		
				4	6	14	25	26	38		
				±7 days					±7 days		
Pregnancy Testing	X	X	**If pregnancy is suspected**								**X**

2. Section 6.2.2 Entry Evaluations
   Entry evaluations must occur at least 24 hours after screening. **This visit for blood draws must be a fasting visit. If the study participant is not fasting, the participant should be rescheduled. (See section 6.3.14 for a definition of fasting.)**

3. Section 6.2.3 Post-Entry Evaluations
   Pre-Treatment Evaluations
   Participants must begin treatment within 72 hours after the week 2 visit. **The week 2 visit must be a fasting visit. If the study participant is not fasting, the participant should be rescheduled within 3 days. (See section 6.3.14 for a definition of fasting).**

A5352s

1. Section 6.2.1 Screening and Pre-Treatment Evaluations
   Week 1
   Week 1 evaluations must be completed at least 4 days after entry evaluations unless otherwise specified.

   NOTE: At the time of the **Week 1** sugar absorption test visit, the participant must have avoided drinking alcohol for 3 days prior to the test, avoided taking nonsteroidal anti-inflammatory medications, including aspirin or any blood thinners for 7 days prior to the test, and have abstained from exercise the day before and the morning of the test. **If a**

participant has not abstained from alcohol, refrained from taking NSAIDS, and avoided exercise, the site should contact the core team at actg.corea5350@fstrf.org to determine whether to proceed with the test. This visit must be a fasting visit. If the study participant is not fasting, the participant should be rescheduled within 3 days. (See A5350 section 6.3.14 for a definition of fasting). Additional details about the lactulose/mannitol test are included in the study MOPS.

2. Section 6.2.2 Post-Entry Evaluations

   On-Treatment Evaluations

   All on-treatment evaluations must be scheduled as per section 6.1 with a ±7 day window.

   NOTE: At the time of the week 26 sugar absorption test visit, the participant must have avoided drinking alcohol for 3 days prior to the test, avoided taking nonsteroidal anti-inflammatory medications, including aspirin or any blood thinners for 7 days prior to the test, and have abstained from exercise the day before and the morning of the test. If a participant has not abstained from alcohol, refrained from taking NSAIDS, and avoided exercise, the site should contact the core team at actg.corea5350@fstrf.org to determine whether to proceed with the test. This visit must be a fasting visit. If the study participant is not fasting, the participant should be rescheduled within 3 days. (See A5350 section 6.3.14 for a definition of fasting). Additional details about the lactulose/mannitol test are included in the study MOPS.

A5350

Safety, Tolerability, and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

Sponsored by:

The National Institute of Allergy and Infectious Diseases

Industry Support Provided by:

Exegi Pharma, LLC

Non-IND Protocol

The ACTG End-Organ Disease/Inflammation Transformative Science Group:	**Peter W. Hunt, MD, Chair**
Protocol Co-Chairs:	**Adriana Andrade, MD, MPH** **Turner Overton, MD**
Protocol Co-Vice Chairs:	**Jeffrey M. Jacobson, MD** **Cara C. Wilson, MD**
DAIDS Clinical Representative:	**Karin L. Klingman, MD**
Clinical Trials Specialist:	**Linda Boone, BS**

Final Version 1.0
January 5, 2016

CONTENTS

Page

CONTENTS (Cont'd)

Page

SITES PARTICIPATING IN THE STUDY

A5350 is open to all US AIDS Clinical Trials Group (ACTG) clinical research sites (CRSs).

SITES PARTICIPATING IN THE SUBSTUDY

A5352s is open to select US ACTG CRSs participating in A5350. Selected sites will be listed on the Protocol-Specific Web Page (PSWP).

PROTOCOL TEAM ROSTER

Co-Chairs
Adriana Andrade, MD, MPH
Division of Infectious Diseases
Johns Hopkins Adult AIDS CRS
1830 East Monument Street, Suite 8074
Baltimore, MD 21205
Phone: 410-614-4036
Fax: 410-614-9978
E-mail: aandrade@jhmi.edu

Edgar Turner Overton, MD
Alabama CRS
908 20th St South
CCB 330A
Birmingham, AL 35294
Phone: 205-934-5191
Fax: 205-975-6027
E-mail: toverton@uab.edu

Co-Vice Chairs
Jeffrey M. Jacobson, MD
Division of Infectious Diseases Clinical Research Center
Drexel University CRS
245 North 15th Street, MS461
Room 6302, New College Building
Philadelphia, PA 19102
Phone: 215-762-6555
Fax: 267-507-6927
E-mail: jeffrey.jacobson@drexelmed.edu

Cara C. Wilson, MD
Medicine - Infectious Diseases
University of Colorado Hospital CRS
Mail Stop #B168
Building P15 Research 2, Room #11011
P.O. Box 6511
12700 East 19th Avenue
Aurora, CO 80045
Phone: 303-724-4601
Fax: 303-724-4926
E-mail: cara.wilson@ucdenver.edu

DAIDS Clinical Representative
Karin L. Klingman, MD
HIV Research Branch
TRP, DAIDS, NIAID, NIH
5601 Fishers Lane, Rm 9E40A, MSC 9830
Bethesda, MD 20892-9830
Phone: 240-627-3067
E-mail: kklingman@niaid.nih.gov

Clinical Trials Specialist
Linda Boone, BS
ACTG Network Coordinating Center
Social & Scientific Systems, Inc.
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910-3714
Phone: 301-628-3363
Fax: 301-628-3302
E-mail: lboone@s-3.com

Statistician
Douglas Kitch, MS
Harvard T.H. Chan School of Public Health
651 Huntington Avenue
FXB Building, Room 504
Boston, MA 02115-6017
Phone: 617-432-3281
Fax: 617-432-3163
E-mail: dkitch@sdac.harvard.edu

Data Manager
David Nichols, BS
Frontier Science & Technology Research Foundation, Inc.
4033 Maple Road
Amherst, NY 14226-1056
Phone: 716-834-0900 Ext. 7359
Fax: 716-834-8432
E-mail: nichols@fstrf.org

DAIDS Pharmacist
Bijal Patel, PharmD
Pharmaceutical Affairs Branch
DAIDS, NIAID, NIH
5601 Fishers Lane
Room 9D35
Rockville, MD 20852
Phone: 240-421-8445
Fax: 240-627-3112
E-mail: bijal.patel@nih.gov

Immunologists

Jason M. Brenchley, MA, PhD
NIAID, NIH
4 Memorial Drive
Room 201
9000 Rockville Pike
Bethesda, MD 20892
Phone: 301-196-1498
E-mail: jbrenchl@mail.nih.gov

Alan Landay, PhD
Immunology/Microbiology
Rush University Medical Center
1725 West Harrison Street, Suite 306 POB1
Chicago, IL 60612
Phone: 312-942-6554
E-mail: alanday@rush.edu

Virologist

Robert W. Coombs, MD, PhD
Retrovirology Laboratory
University of Washington AIDS CRS
Research and Training Building, 7th Floor
325 9th Avenue
Seattle, WA 98104-2420
Phone: 206-897-5205
Fax: 206-897-5203
E-mail: bcoombs@u.washington.edu

Investigators

Michael P. Dubé, MD
Division of Infectious Diseases
University of Southern California
Keck School of Medicine
1300 N. Mission Road, Room 349
Los Angeles, CA 90033
Phone: 323-343-8293
Fax: 323-226-2083
E-mail: mdube@usc.edu

Carl J. Fichtenbaum, MD
University of Cincinnati CRS
Mail Location 405
3223 Eden Avenue, Room 3114
Cincinnati, OH 45267-0405
Phone: 513-584-6361
Fax: 513-584-6040
E-mail: carl.fichtenbaum@uc.edu

Investigators (Cont'd)

F. Parker Hudson, MD
Prevention and Treatment CTU
University of North Carolina Global HIV
130 Mason Farm Road
CB #7030
Chapel Hill, NC 27599
Phone: 919-966-6714
Fax: 919-966-6714
E-mail: fphudson@email.unc.edu

Rachel Presti, MD, PhD
Infectious Disease
Washington University CRS
P.O. Box 8051
660 South Euclid Avenue
St. Louis, MO 63110
Phone: 314-286-0345
Fax: 314-510-3374
E-mail: rpresti@dom.wustl.edu

Netanya Sandler Utay, MD
Division of Infectious Diseases
Galveston Department of Internal Medicine
University of Texas Medical Branch
301 University Blvd, Rte. 0435
Galveston, TX 77555-0435
Phone: 409-747-0240
E-mail: neutay@utmb.edu

Valdiliea Veloso, MD
Instituto de Pesquisa Clinica Evandro
Chagas CRS
Avenida Brazil, 4361
Manguinhos
Rio de Janeiro, 21040-360
BRAZIL
Phone: 55-21-22904532
E-mail: valdilea.veloso@ipec.fiocruz.br

Brett Williams, MD
Rush University CRS
600 South Paulina Street Suite 140
Chicago, IL 60612
Phone: 312-942-2292
E-mail: brett_williams@rush.edu

Investigators (Cont'd)
Kevin E. Yarasheski, PhD
Division of Endocrinology/Metabolism
Washington University School of Medicine
660 South Euclid Ave, BOX 8127
St. Louis, MO 63110
Phone: 314-362-8173
Fax: 314-362-8188
E-mail: key@wustl.edu

Field Representative
Suzanne Fiorillo, MSPH
University of Colorado Hospital CRS
Academic Office 1, MS 8205
12631 East 17th Avenue
P.O. Box 6511
Aurora, CO 80045
Phone: 303-724-5931
Fax: 303-724-0802
E-mail: suzanne.fiorillo@ucdenver.edu

Laboratory Technologist
Amy James Loftis, BS
School of Medicine
University of North Carolina, Chapel Hill
UNC AIDS CRS
710 Mary Ellen Jones Building
116 Manning Drive
Chapel Hill, NC 27599
Phone: 919-966-6963
Fax: 919-966-9872
E-mail: amy_james@med.unc.edu

Community Scientific Subcommittee (CSS) Representative
Angel L. Hernandez, BA
Puerto Rico-AIDS CRS
Barrio Saltos
Road 566 Int Road 593, KM 0.2
Orocovis, Puerto Rico 00720
Phone: 787-919-6145
E-mail: angelhdz2863@gmail.com

Industry Representative
Marc Tewey, MBA
Exegi Pharma, LLC
312 Main St, Suite 200
Gaithersburg, MD 20898
Phone: 240-888-1294
E-mail: marc.tewey@exegipharma.com

Laboratory Data Manager
Sarah Strobino
Frontier Science & Technology Research Foundation, Inc.
4033 Maple Rd.
Amherst, NY 14226
Phone: 716-834-0900
E-mail: strobino@fstrf.org

STUDY MANAGEMENT

All questions concerning this protocol should be sent to actg.teamA5350@fstrf.org via e-mail. The appropriate team member will respond with a "cc" to actg.teamA5350@fstrf.org. A response should generally be received within 24 hours (Monday-Friday).

Protocol E-mail Group
Sites should contact the Computer Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the actg.protA5350 e-mail group. Include the protocol number in the e-mail subject line.

- Send an e-mail message to actg.user.support@fstrf.org

Clinical Management
For questions concerning entry criteria, toxicity management, concomitant medications, and co-enrollment, contact the protocol team.

- Send an e-mail message to actg.teamA5350@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory
For questions specifically related to immunologic or virologic laboratory tests, contact the protocol Immunologists or Virologist.

- Send an e-mail message to actg.teamA5350@fstrf.org (ATTN: Jason Brenchley or Alan Landay [Immunologists] or Robert Coombs [Virologist]).

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, case report forms (CRF), the CRF schedule of events, randomization/registration, delinquencies, and other data management issues, contact the Data Manager. CRFs can be downloaded from the FSTRF website at www.fstrf.org.
- For transfers, reference the Patient Transfer from Site to Site SOP 119, and contact David Nichols directly.
- For other questions, send an e-mail message to actg.teamA5350@fstrf.org (ATTN: David Nichols).
- Include the protocol number, PID, and a detailed question.

Randomization/Participant Registration
For randomization/participant registration questions or problems and study identification number SID lists.

- Send an e-mail message to rando.support@fstrf.org. Call the Statistical and Data Analysis Center (SDAC)/DMC Randomization Desk at 716-834-0900 extension 7301.

Computer and Screen Problems
Contact the SDAC/DMC programmers.

- Send an e-mail message to actg.support@fstrf.org or call 716-834-0900 x7302.

Protocol Document Questions
For questions concerning the protocol document, contact the Clinical Trials Specialist.
- Send an e-mail message e-mail message to actg.teamA5350@fstrf.org (ATTN: Linda Boone).

Copies of the Protocol
To request a hard copy of the protocol, send a message to ACTGNCC@s-3.com (ATTN: Diane Delgado) via e-mail. Electronic copies can be downloaded from the ACTG Web site (https://www.actgnetwork.org).

Product Package Inserts and/or Investigator Brochures
To request copies of product package inserts, product information sheets, or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration
For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

Protocol Activation
For questions related to protocol activation, contact the Clinical Trials Specialist Linda Boone at lboone@s-3.com or ACTG Site Coordination group at actgsitecoordination@s-3.com.

Study Product
For questions or problems regarding study product, dose, supplies, records, and returns, call Bijal Patel, Protocol Pharmacist, at 240-421-8445.

Study Product Orders
Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

Expedited Adverse Event (EAE) Reporting/Questions
Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Phone Calls
Sites are responsible for documenting any phone calls made to A5350 team members.
- Send an e-mail to actg.teamA5350@fstrf.org.

Protocol-Specific Web Page
Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

aGLP-1	glucagon-like peptide-1 receptor agonists
ART	antiretroviral therapy
BMI	body mass index
CLIA	Clinical Laboratory Improvement Amendments
COX-2	cyclooxygenase-2
DSS	dextran sulfate sodium
FXR	farnesoid X receptor
GALT	gut-associated lymphoid tissue
GLP-1	glucagon-like peptide-1
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment
hsCRP	high-sensitivity c-reactive protein
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
ICAM-1	intercellular adhesion molecule 1
IFABP	intestinal fatty acid binding protein
IFNα	interferon alpha
IFNγ	interferon gamma
IHC	immunohistochemistry
IL-6	interleukin 6
IL-10	interleukin 10
INR	international normalized ratio
IP-10	interferon gamma-induced protein 10
iNOS	inducible nitric oxide synthase
IVIG	intravenous immunoglobulin
LBP	lipopolysaccharide-binding protein
LDL	low-density lipoprotein cholesterol
LPS	lipopolysaccharide
MIP-1α	macrophage inflammatory protein 1 alpha
MPO	myeloperoxidase
NASH	nonalcoholic steatohepatitis
OI	opportunistic infection
POC	point of care
PPARγ	peroxisome proliferator-activated receptor gamma
PTT	partial thromboplastin time
RANTES	regulated on activation, normal T-cell expressed and secreted
RCT	randomized controlled trial
sCD14	soluble CD14
sCD163	soluble CD163
SCFA	short-chain fatty acid
SIV	simian immunodeficiency virus
SOE	schedule of events
sTNF-RI	soluble tumor necrosis factor receptor I

Th1	t-helper cell type 1
TLR-2	toll-like receptor 2
TLR-4	toll-like receptor 4
TNFα	tumor necrosis factor alpha
UC	ulcerative colitis
VDR	vitamin D receptor

SCHEMA

A5350

Safety, Tolerability, and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study

DESIGN

A5350 is a phase II, randomized, double-blind, two-arm study to evaluate whether there is a significant change in sCD14 after 24 weeks of probiotic Visbiome Extra Strength therapy, and to determine the safety and tolerability of this agent in HIV-infected participants on stable antiretroviral therapy (ART). Participants will be followed for an additional 12 weeks off study product.

A substudy, A5352s will be conducted in a subset of participants to collect colonic biopsies via flexible sigmoidoscopy, and to evaluate intestinal permeability and inflammation at two time points.

DURATION

Participants will be on study for 38 weeks.

SAMPLE SIZE

90 participants (45 in Arm A and 45 in Arm B)

POPULATION

HIV-infected participants (≥18 years of age) who have been on stable ART for at least 24 weeks prior to study entry, and have a CD4+ T-cell count >200 cells/mm^3 prior to study entry, and plasma HIV-1 RNA <50 copies/mL for 48 weeks prior to study entry.

STRATIFICATION

By intent and eligibility to enroll in the substudy A5352s.

REGIMEN

Participants will be randomized in a 1:1 ratio to either Arm A (Visbiome Extra Strength + stable ART; treatment arm) or Arm B (placebo for Visbiome Extra Strength + stable ART; control arm).

Arm A: Visbiome Extra Strength
2-week lead-in period: 1 Visbiome Extra Strength sachet per day.
Then increase to 1 sachet twice daily for 22 weeks.
Followed by 12 weeks of additional follow-up.

Arm B: Placebo for Visbiome Extra Strength
2-week lead-in period: 1 placebo sachet per day.
Then increase to 1 sachet twice daily for 22 weeks.
Followed by 12 weeks of additional follow-up.

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Primary Hypothesis

After administration of Visbiome Extra Strength, systemic levels of sCD14 will decrease.

1.2 Secondary Hypotheses

1.2.1 After administration of Visbiome Extra Strength, other markers of systemic inflammation interleukin (IL)-6, interferon gamma-induced protein (IP)-10, soluble tumor necrosis factor receptor I (sTNF-RI), soluble CD163 (sCD163), oxidized low-density lipoprotein (LDL), kynurenine to tryptophan (KT) ratio, and coagulopathy (D-dimer) will decrease.

1.2.2 After administration of Visbiome Extra Strength, markers of microbial translocation lipopolysaccharide (LPS) and lipopolysaccharide-binding protein (LBP) will decrease.

1.2.3 After administration of Visbiome Extra Strength, peripheral CD4+ lymphocyte counts and CD4+/CD8+ ratio will increase.

1.2.4 After administration of Visbiome Extra Strength, markers of lymphocyte and monocyte activation and senescence will decrease.

1.2.5 Visbiome Extra Strength administration will increase gastrointestinal microbial diversity in fecal samples.

1.2.6 After administration of Visbiome Extra Strength, markers of enterocyte death as measured by I-FABP (intestinal fatty acid binding protein) will decrease.

1.2.7 Visbiome Extra Strength will be safe and well tolerated.

1.2.8 The gut microbiome changes will persist after completion of Visbiome Extra Strength administration.

1.3 Exploratory Hypotheses

1.3.1 After administration of Visbiome Extra Strength, insulin sensitivity, as measured by homeostatic model assessment (HOMA-IR), will improve.

1.3.2 After administration of Visbiome Extra Strength, fasting lipid parameters will improve.

1.3.3 After administration of Visbiome Extra Strength, changes in gut microbiome will be associated with changes in markers of systemic inflammation and coagulation as well as changes in cellular immune activation markers.

1.3.4 After administration of Visbiome Extra Strength, changes in markers of microbial translocation will be associated with changes in systemic markers of inflammation and coagulation as well as changes in cellular immune activation markers.

1.3.5 The improvements in markers of systemic inflammation and microbial translocation will persist for 12 weeks after completion of 24 weeks of Visbiome Extra Strength.

1.3.6 At baseline, the composition of the microbiome will differ by antiretroviral class including protease inhibitors (PIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and integrase inhibitors (InSTIs).

1.3.7 After administration of Visbiome Extra Strength, gastrointestinal symptom scores will improve.

1.3.8 The composition of the microbiome will be influenced by dietary intake as reported through the dietary assessments.

1.4 Primary Objective

To assess changes in sCD14 in participants treated with Visbiome Extra Strength compared to placebo.

1.5 Secondary Objectives

1.5.1 To assess changes in additional markers of systemic inflammation (IL-6, IP-10, sCD163, sTNF-RI, oxidized LDL), KT ratio, and coagulation (D-dimer) after Visbiome Extra Strength administration.

1.5.2 To assess changes in markers of microbial translocation (LPS, LBP) after Visbiome Extra Strength administration.

1.5.3 To assess changes in peripheral CD4+ lymphocyte counts and CD4+/CD8+ ratio after Visbiome Extra Strength administration.

1.5.4 To assess markers of monocyte and lymphocyte activation and senescence after Visbiome Extra Strength administration.

1.5.5 To assess change in gastrointestinal microbial diversity from fecal samples after Visbiome Extra Strength administration.

1.5.6 To assess measures of enterocyte death (I-FABP) after administration of Visbiome Extra Strength.

1.5.7 To assess safety and tolerability of Visbiome Extra Strength.

1.5.8 To assess diversity of the gut microbiome 12 weeks after completion of 24 weeks of Visbiome Extra Strength.

1.6 Exploratory Objectives

1.6.1 To assess changes in a marker of insulin sensitivity (HOMA-IR) after administration of Visbiome Extra Strength.

1.6.2 To assess changes in fasting lipid parameters (LDL, high-density lipoprotein [HDL], non-HDL cholesterol, and triglycerides) after administration of Visbiome Extra Strength.

1.6.3 To explore the correlations between the gut microbiome and soluble and cellular markers performed in the study.

1.6.4 To explore the correlations between the microbial translocation markers and other soluble and cellular markers performed in the study.

1.6.5 To assess durability of the probiotic effect on inflammatory and microbial translocation markers 12 weeks after discontinuation of Visbiome Extra Strength therapy.

1.6.6 To assess the differences in composition of the baseline microbiome based on ART.

1.6.7 To assess changes in chronic gastrointestinal symptom score after administration of Visbiome Extra Strength.

1.6.8 To assess the relationship between reported dietary intake and composition of the microbiome.

2.0 INTRODUCTION

2.1 Background

The efficacy of combination antiretroviral therapy (ART) has produced significant gains in survival, resulting in an aging HIV-infected population [1-6]. This demographic shift has been accompanied by changing morbidity and mortality patterns, with a decline in AIDS-defining diseases and concomitant increase in aging-related diseases (malignancies, cardiovascular disease [CV] disease, diabetes, osteoporosis, frailty, cognitive decline) [2-4, 7-12]. Numerous factors contribute to the development of these comorbidities, including a persistent state of inflammation that contributes to HIV disease progression. The alterations in the gut-associated lymphoid tissue (GALT) and subsequent changes in the gut microbiome to a less diverse, more pathogenic bacterial community (termed dysbiosis), contribute to increased microbial translocation with consequent heightened systemic inflammation and end-organ disease [13-20].

Due to the constant surveillance of the intestinal flora, the GALT serves as the largest reservoir for lymphocytes in the body [21-22]. The profound depletion of CD4+ T-cells from the GALT during acute HIV infection causes catastrophic changes in the gut mucosal integrity, facilitating bacterial translocation, augmenting trafficking of inflammatory cells to the gastrointestinal (GI) tract, and promoting chronic immune activation [23-25]. With ART initiation, the level of peripheral T-cell activation decreases but fails to return to levels seen in HIV-uninfected persons [26, 27]. Interestingly, T-cell activation in the GALT is not significantly reduced with ART, leading to ongoing inflammation in the gut mucosa [25]. Following HIV-1 infection, individuals also have a shift in their gut microbiome to one that consists of a greater proportion of gram negative bacteria with enhanced potential to induce systemic inflammation [28, 29]. Several recent studies have shown alterations in both stool and mucosal microbial communities that variably include increases in Prevotella family members and Proteobacteria, and decreases in Bacteroides family members and Firmicutes [14, 30-32]. Importantly these microbiome changes, especially of mucosa-associated bacteria, have been associated with markers of microbial translocation, inflammation, and immune activation [14, 31].

The link between HIV-infection, changes in the GALT, and the development of a chronic inflammatory state impairing immune function and exacerbating chronic disease risk has been reported extensively in the literature. HIV-related intestinal dysbiosis contributes to CD4+ T-cell depletion, and chronic inflammation [14, 32, 33]. Notably, it is not only the dysbiosis that contributes to persistent inflammation but also the downstream subsequent effects on gut permeability that likely facilitate excess bacterial translocation and heightened systemic inflammation. For example, Chung et al recently demonstrated that intestinal epithelial damage manifested by decreased colonic epithelial tight junction proteins, enhances colonic permeability and thus, microbial translocation despite suppressive ART [34]. Therefore, interventions are needed to improve the gut integrity beyond the effects of ART. Previous studies using antibiotics, such as rifaximin, or intraluminal binding agents, such as sevelamer, have failed to improve markers of systemic inflammation, potentially because they failed to effectively alter the microbiome or they failed to restore the gut mucosal barrier [35, 36]. Herein, we propose an innovative intervention to establish whether the dysbiosis reported with HIV infection can be reversed with Visbiome Extra Strength administration, with the downstream effects of reducing chronic immune activation and inflammation.

Administration of this probiotic will facilitate reconstitution of a healthy, diverse microbiome, and have significant potential to reduce chronic inflammation and immune activation. Identifying whether this approach restores a healthy, diverse microbiome in HIV-infected persons and positively impacts chronic immune activation, will greatly enhance our understanding of the interface between the microbiota, the immune system, and overall health.

Probiotics

Probiotics are organisms, such as yeast or bacteria that are available in foods and supplements and are used as a modality to improve overall gut health [37, 38]. Various probiotics have been studied in disease states associated with a gut dysbiosis, including inflammatory bowel disease (IBD) and infectious diarrheas. The striking results seen in these dysbiotic conditions provides rationale to pursue probiotics in the setting of HIV

infection to increase the diversity of the gut microbiome, improve the gut barrier function, and ultimately reduce systemic inflammation which characterizes chronic HIV infection.

Probiotics impact health through numerous mechanisms including changes in the microbiome composition and diversity, as well as alterations in the metabolic environment of the intestinal microbiome [39]. For example, the probiotic bacterial strains often compete with pathogenic bacterial for mucosal cell surface binding sites and mucus layer [40, 41]. Furthermore, repopulating the microbiome with microbes that produce short-chain fatty acids, including butyrate, likely has several positive benefits: reduction in luminal pH inhibits the growth of pathogenic bacteria; butyrate serves as an important nutrient for mucosal cells and thus, improves mucosal integrity; additionally butyrate decreases transcription of the NF-KB genes, thereby reducing local inflammation [42]. Furthermore, butyrate has been demonstrated to down-regulate indoleamine 2,3-dioxgenase (IDO), an interferon-inducible enzyme that has been linked to excess inflammation and immune activation in chronic HIV infection [43-45]. The effect of probiotics on the immune system can be put into two distinct categories: immuno-stimulatory properties and anti-inflammatory effects [46].

A number of studies investigated the potential therapeutic effects of probiotic products on gastrointestinal diseases [47-49]. Visbiome, a combination of four strains of *Lactobacilli*, three strains of *Bifidobacteria*, and one strain of *Streptococcus thermophila,* had curative effects on ulcerative colitis (UC) and improved gastrointestinal integrity [50, 51]. Similar benefits were also observed in patients with IBD treated with *Lactobacillus rhamnosus* [52]. Visbiome has proven to be a particularly safe and well-characterized probiotic for evaluation in the setting of HIV infection. It meets the requirements for a probiotic: the bacteria included are known to the level of genus, species, and strains. The agent has proven to be extremely safe; it has been demonstrated to be viable after storage as well as after passage through the hostile environment of the upper GI tract; and it is also efficacious in both animal models of colitis and colorectal cancer, as well as in human disease states, including decompensated cirrhosis and IBD.

Visbiome Extra Strength Quality Assurance

Visbiome Extra Strength is classified by the Food and Drug Administration (FDA) as a "Medical Food," which is different than prescription drugs. Unlike prescription drugs, medical foods do not have to undergo premarket review and approval, nor can they make any health benefit claims. Medical foods must comply with all FDA requirements for foods, including, food Good Manufacturing Practices (GMPs) and other applicable regulations. (See http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/MedicalFoods/)

Medical foods must be manufactured under the FDA's compliance program for medical foods. This is a manufacturing inspection program with the main objective of assuring the safety and integrity of the medical food products. (http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/ComplianceEnforcement/ucm073339.pdf)

The FDA does not require a written or oral prescription for medical foods, but does

require that a medical food be consumed or administered enterally under the supervision of a physician.

The PSWP contains a GMP statement document from the Visbiome Extra Strength manufacturer, DuPont, and a Product Description document from DuPont /Danisco. The Product Description document includes the composition, storage, microbiological testing specifications, and other descriptive information.

Animal Studies of Visbiome

In animal models of colorectal cancer, dextran sulfate sodium (DSS)-induced colitis, and mice at risk for nonalcoholic steatohepatitis (NASH) and atherosclerosis, Visbiome has consistently demonstrated benefit in outcomes that are mediated through anti-inflammatory properties. In a rat model of DSS-induced colitis, rats treated with Visbiome prior to administration of DSS were protected from the development of colitis with marked reduction in disease activity index, as well as decreased neutrophil infiltration and myeloperoxidase (MPO) levels in colonic tissue (controls 8.6 + 0.8 mU/mg; DSS 21.9 + 1.2 mU/mg; DSS with Visbiome 12.2 + 0.9 mU/mg, $p<0.05$) [53]. With DSS administered alone, colonic protein expression was shifted to a pro-inflammatory milieu (increased inducible nitric oxide synthase (iNOS), COX-2, TNF-α, and IL-6 levels but decreased IL-10 levels). With co-administration of Visbiome and DSS, the colonic expression of these proteins was similar to levels seen in the control animals. Systemic markers of inflammation demonstrated similar results:

Group	TNF-α	IL-6	IL-10
Control	22.2 (2.3)	8.3 (1.3)	280.4 (2.5)
DSS	33.3 (2.0)*	44.1 (2.5)*	101.8 (2.3)*
DSS + Visbiome	24.9 (2.2)ǂ	14.5 (1.4)ǂ	240.8 (1.9)ǂ

Data presented as mean (SE of each group). * denotes $p< 0.05$ compared to control group.
ǂ denotes $p<0.05$ compared to DSS group.

Given that IL-6, TNF-α, and IL-10 are produced by macrophages and lymphocytes, these data indicate that Visbiome likely has important immunomodulatory effects on cells from both the innate and adaptive immune systems. Furthermore, reductions in iNOS and COX-2 expression indicate that Visbiome mediates its effect through antioxidant effects as well.

These data were supported by another study in which a mouse model of colitis-associated colorectal cancer was used to assess the impact of Visbiome [54]. This colitis model was similar to that in the above study with the addition of chemically induced cancer or by infection with *Helicobacter typhlonius*. As above, Visbiome reduced disease activity scores, as well as decreased development of adenomas and adenocarcinomas. Mice receiving Visbiome actually demonstrated increased TNFα expression, which the authors suggested was related to antitumor and enhanced epithelial healing. Visbiome-treated mice demonstrated increased expression of CD36 and PPARγ while upregulating angiotensin mRNA levels. PPARγ has been demonstrated to improve insulin sensitivity as well as block iNOS-related inflammation. Angiotensin expression likely limits the process of angiogenesis, which is a key component to early cancer development. Finally, mesenteric lymph nodes demonstrated greater IL-17 expressing CD4+ cells, Treg cells (CD4+FoxP3+ and CD4+CD44+CD62L+), and CD4+ memory

cells, cell populations that are depleted with HIV infection. This polarization of the lymphocyte population toward IL17 and T regulatory cells likely indicates a shift to an anti-inflammatory composition of the adaptive immune system. In summary, the data highlight the potent anti-inflammatory and immunomodulatory properties of Visbiome.

Another study utilizing the mouse DSS-induced colitis model went one step further to look at the impact of Visbiome on atherosclerosis, [55]. This study was based on the premise that atherosclerosis is an inflammatory process and persons with persistent inflammation (including HIV-infection, IBD, NASH, autoimmune and collagen vascular disorders) are at increased risk for more rapid progression of atherosclerotic lesions. Increased gut permeability in the setting of colitis contributes to increased systemic inflammation and thus, accelerates the process of atherosclerosis. The authors hypothesized that by repairing the gut mucosa and reducing gut permeability, Visbiome would reduce systemic inflammation and slow the atherosclerosis progression. In the animal model Visbiome restored gut permeability to that seen in DSS-naïve mice as evidenced by assessing plasma DXA-4000-FITC levels after an oral DSS challenge, by histopathological assessment with decreased inflammatory cell infiltration, and finally, by reducing the expression of inflammatory biomarkers (TNFα and RANTES). With improvements in gut permeability, there was less systemic inflammation as measured by levels of TNFα, RANTES, intracellular adhesion molecule-1 (ICAM-1), and macrophage inflammatory protein 1 alpha (MIP-1α) in the liver, and levels of RANTES, ICAM-1, and MIP-1α expressed in the aorta. Additionally, after Visbiome administration, circulating T-cells expressed lower IFNγ and higher IL-10 thus, attenuating the DSS-induced shift to a Th1 inflammatory profile. Finally, as noted in the previous trial, intestinal expression of certain nuclear receptors was increased with Visbiome administration, including PPARγ, FXR, and VDR. These receptors are involved in improving insulin sensitivity and modulating immune responses. In addition, in a recent study in SIV-macaques, Visbiome decreased the plasma KT ratio, a marker of IDO activity, an enzyme that has been demonstrated to be linked to HIV-associated pathogenesis in the GALT [56-59].

Human Studies of Visbiome

In summary, these animal colitis models highlight that Visbiome improves outcomes mediated through both improved gut permeability and anti-inflammatory and immunomodulatory effects. Human studies in IBD and cirrhosis have provided similar readouts. A recent study assessed changes in microbiome in 10 persons with irritable bowel syndrome (IBS) who were given Visbiome for 4 weeks [60]. At baseline, the IBS participants had lower diversity than control subjects with increased *Bacteroides*, *Eggerthella*, and *Cloasibacillus sp* and decreased *Flavobacterium*, *Actinomyces*, *Veillonella, Dorea*, and *Roseburia sp* ($p<.05$ for all). Clinically, 6/10 IBS participants had reduced symptoms at the time of evaluation (4 weeks). With probiotic therapy, the subjects had increased diversity with significant increases in *Bifidobacteria*, *Lactobacillus*, and *Streptococcus sp* in the mucosal biopsy specimen. Thus, Visbiome clearly alters the microbiome to one that is more similar to the healthy controls after only 4 weeks of administration.

From an ongoing randomized controlled trial (RCT) randomizing persons with UC to receive either Visbiome or placebo for 8 weeks, Ng et al reported on a subset of participants who underwent additional testing to evaluate the mechanism of Visbiome

action [61]. In this small cohort, 10/14 Visbiome recipients (71%) versus 4/14 placebo recipients (29%) had a clinical response to therapy (p =0.068). Notably, Visbiome significantly downregulated toll-like receptor 2 (TLR-2) expression with similar trends in TLR-4, CD40, and CD86 expression. Furthermore, IL-10 production was increased while IL-12 was decreased with administration of Visbiome. These data indicate that Visbiome impacts UC disease severity by modulating immune responses.

Another RCT assessed the potential for Visbiome as an adjuvant to beta blocker therapy to improve portal hypertension in cirrhotic participants with severe esophageal varices [62]. The authors hypothesized that improving gut permeability would reduce bacterial translocation and hepatic inflammation and thus, reduce portal pressures. After 2 months of Visbiome administration, portal pressures and large varices were reduced in 15/25 (60%) of Visbiome recipients versus only 7/27 (27%) of propranolol with placebo (p=0.025). TNF-α concentrations significantly declined in peripheral blood and hepatic venous blood with Visbiome when compared to placebo. No difference was detected with regards to IL-6 or serum nitrate levels. Nevertheless, there was clinical benefit with a signal suggesting that decreased inflammation was a potential mechanism of action.

Studies have also evaluated the effect of Visbiome in the setting of obesity, an epidemic comorbidity in the US and one that is becoming more common among HIV-infected patients. In an RCT of 60 overweight participants, persons with lipid abnormalities, insulin resistance, and high-sensitivity c-reactive protein (hsCRP) >3 mg/L had lower diversity at baseline, specifically with decreased *Lactobacilli, Bifidobacteria,* and *Streptococcus* yet higher *E. coli* and *Bacteroides sp*. [63]. These data indicate that dysbiosis is associated with a poorer overall health status. After Visbiome administration, microbial diversity was significantly increased compared to persons who received placebo, including total aerobes, total anaerobes, *Lactobacillus*, *Bifidobacteria*, and *Streptococcus sp*. After administration of Visbiome, insulin sensitivity improved and hsCRP declined ($p<0.05$ within group and between groups for both). Furthermore, there were modest declines in IL-1β, TNFα, and IL-6 with Visbiome administration.

A similar trial in obese children has recently been reported [64] in which 44 obese children with NASH were randomized to Visbiome or placebo. After 4 months of therapy, both groups experienced improvements in insulin resistance but the Visbiome group had improvements in glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-1 receptor agonists (aGLP-1) levels. Furthermore, liver inflammation and steatosis decreased and body mass index (BMI) declined significantly with Visbiome administration. The authors concluded that the restoration of a normal gut flora led to increased short-chain fatty acid (SCFA) production, which resulted in the production of anorexogenic gut hormones (GLP-1 and aGLP-1) and improved insulin sensitivity with BMI reduction.

A small study of critically ill participants evaluated whether Visbiome would improve inflammation and lipid parameters [65]. In this study, 40 participants were randomized to 7 days of Visbiome or placebo. Despite the short course of therapy and high severity of illness scores at baseline, the Visbiome recipients experienced significant declines in hsCRP and TG levels and increases in HDL-c levels ($p<0.05$ for all). While no data were collected regarding microbiome or more sophisticated analyses of immunity, the data support the hypothesis that Visbiome will reduce inflammatory parameters.

Visbiome in SIV-infected Macaques

The promising effects of probiotics on gut dysbiosis and inflammation were also evaluated in SIV-infected macaques [66]. In this study, eleven macaques treated with ART were studied; four received ART alone and seven also received Visbiome with inulin for 5 months. Interestingly, there were no microbiome changes seen with SIV infection and only modest changes seen with administration of Visbiome. Peripheral CD4+ counts increased similarly in both groups but there were striking differences in certain cell populations and in gene expression in the cells isolated from the GI mucosa at necropsy. A significantly greater number of antigen presenting cells (APC), and specifically HLA-DR+ APCs, were measured from the Visbiome animals; these cells had increased gene expression for HLA-G, HLA-DB3, HLA-H, CD14, and CD68. As a consequence, IL-23 levels were significantly higher among the macaques receiving Visbiome. Furthermore, colonic CD4+ T-cells were reconstituted to near normal levels with Visbiome administration despite confirmed massive depletion with SIV infection. Notably, these CD4+ T-cells were more often multifunctional (defined as producing at least two of the following cytokines: IL-17, TNF-α, or IL-2). Additionally, T-cell activation was reduced in the colonic mucosa, as measured by Ki67 expression. While none of the systemic markers of immune activation were significantly decreased, this likely reflects that the sample size of 11 individuals prohibits the ability to assess differences in these highly variable measures. In summary, this research demonstrates that Visbiome facilitated a reconstitution of colonic CD4+ cells, and enhanced gastrointestinal immune function in this highly relevant SIV model.

Probiotics in HIV-infected Persons

The therapeutic effects of probiotic products have also been studied in the setting of HIV infection [66-81]. Results from these studies have been mixed for various reasons. One approach that consistently fails to demonstrate benefit is the use of probiotics in the setting of uncontrolled HIV infection [76, 77, 82]. Without control of viremia, the impact of probiotics is negligible at best. However, when given in the setting of virologically suppressive ART, probiotics have demonstrated significant benefit. In an RCT of 17 virologically suppressed participants, Yang et al showed that *Bacillus coagulans* GBI-306086 capsule probiotic demonstrated no benefit on CD4+ count but significantly improved CD4 percentages and reduced sCD163 levels. An alternative probiotic study of *Lactobacillus rhamnosus* in a cohort of virologically suppressed individuals demonstrated a significant increase in total CD4+ T-cells [73]. In recently presented data on Visbiome probiotic supplementation in a cohort of 20 HIV-infected, ART-suppressed individuals and 11 seronegative controls [59], CD4+ and CD8+ T-cell activation, characterized by CD38+/HLA-DR+ co-expression, was decreased after 3 months of probiotic supplementation to levels seen in the seronegative controls. sCD14 and D-dimer levels were relatively stable over the brief intervention, but lipopolysaccharide-binding protein (LBP) was reduced to levels of the control participants, suggesting that bacterial translocation was reduced with the probiotic. While this study was small in size, the data are consistent with the results previously reported in SIV-infected macaques. Therefore, further investigations in appropriately powered human trials are needed.

These results suggest that Visbiome is a promising adjunctive probiotic therapeutic approach with ART to improve gastrointestinal damage and inflammation in HIV-infected

patients. We hypothesize that administration of Visbiome will alter the gut microbiome, positively impact gut mucosal integrity, decrease systemic markers of inflammation and immune activation, and improve other metabolic parameters.

2.2 Rationale

We propose to conduct a phase II, randomized two-arm study with Visbiome Extra Strength and matching placebo in HIV-infected participants on stable ART with CD4+ count >200 c/mm^3 and plasma HIV-1 RNA <50 copies/mL to better understand the effects of this probiotic product on reduction in bacterial translocation and systemic immune activation.

The selection of the CD4+ cell count cutoff level was guided primarily by safety issues [83]. According to a 2002 report released by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0282-tab-03-ref-19-joint-faowho-vol219.pdf),there are four potential safety concerns for probiotics: 1) systemic infection, 2) metabolic complications, 3) excessive immune activation, and 4) gene transfer especially of antimicrobial resistance. Of these four, only systemic infection has been reported in the literature, including several cases of bacteremia, sepsis, endocarditis, and abscess from organisms present in probiotic preparations. While reports of infections related to probiotic bacteria have been published, these cases have primarily occurred in persons with short gut syndrome, recent gastrointestinal procedure, or severe immunosuppression (recent surgery; bone marrow, stem cell, or solid organ transplantation; uncontrolled diabetes; cancer; and advanced AIDS [ie, CD4+ count <200 cells/mm^3]. Population-based studies in Sweden and Finland are reassuring as they showed no increase in the rate of bacteremia of common probiotic bacteria such as *Lactobacillus sp.*, despite dramatic increases in the use of probiotics [84, 85]. In addition to the potential safety risk for persons with low CD4+ cell counts, potential participants with a CD4+ ≤200 cells/mm^3 would require prophylactic antibiotics for opportunistic infections (OIs); thus, these participants would be excluded since antibiotics will alter the intestinal microbiome. Therefore, to maximize participant safety and limit potential confounding from OI prophylactic antibiotics, this study will only enroll HIV-infected participants with CD4+ cell count >200 cells/mm^3.

The rationale for only enrolling participants with a plasma HIV-1 RNA <50 copies/mL is based on knowledge that probiotic products consistently fail to demonstrate benefit in the setting of uncontrolled HIV infection [77, 80, 82]. Without controlled viremia, the impact of probiotics is negligible at best. However, when given in the setting of virologically suppressive ART, probiotics have demonstrated benefit.

The rationale for excluding persons with significant weight change (defined as 25 pounds) in the past 24 weeks is related to the fact that previous research has demonstrated a strong association between the gut microbiome and BMI [86-88]. If participants have had a recent change in their weight, the gut microbiome may not be stable at the time of entry.

Rationale for Selection of sCD14 as the Primary Study Outcome

The premise of this study is that HIV infection alters the mucosal integrity of the intestinal mucosa leading to translocation of bacterial products and ultimately persistent systemic inflammation. Therefore, it is crucial to identify a primary endpoint biomarker that is both involved in the mechanistic pathway, and is recognized as a relevant marker of persistent inflammation (ie, it has previously been associated with end organ disease and non-AIDS endpoints [56,89]). While direct measurement of bacterial products, such as LPS, may serve as an accepted measure of microbial translocation, there are concerns about the reliability and accuracy of that assay, as well as the lack of direct relationship between LPS levels and meaningful clinical outcomes in HIV disease. We ultimately concluded sCD14 is the most plausible primary marker for a number of reasons including:

- CD14 is a monocyte/macrophage surface marker that recognizes both pathogen- and damage-associated molecular patterns (PAMP and DAMP) and is a coreceptor for LPS.
- CD14 is expressed at low levels by intestinal epithelial cells but with marked increased expression in response to intestinal damage to prevent microbial translocation.
- HIV-associated immune activation is mediated, at least in part, through sCD14 transfer of LPS to the Toll-like receptor 4, and stimulation of both the innate and adaptive immune systems [90, 91].
- sCD14 has been associated with meaningful clinical endpoints in previous studies of HIV-infected persons, particularly in the setting of controlled viremia [56, 89].
- sCD14 levels do not normalize with successful ART.
- Several groups have reported that immunologic nonresponders have higher levels of sCD14 than immunologic responders [92-94]. One postulate is that the failure of immune reconstitution is most profound in the GALT and therefore, an intervention that reconstitutes the GALT has the potential to reduce both local mucosal and systemic inflammation as manifested by decreased sCD14 levels.

Two potential alternative biomarkers that have shown promise in one published probiotic study in HIV-infected persons are lipopolysaccharide binding protein (LBP) and IL-6 [37]. LBP is produced by hepatocytes in response to the presence of LPS and other bacterial products in the portal venous system. LBP facilitates the presentation of LPS to monocytes via CD14. IL-6 is an alternative inflammatory biomarker that has been associated with relevant clinical endpoints, and has been demonstrated to respond to probiotics in the setting of HIV-infection in a single trial [37]. However, IL-6 levels have significant variability in a single individual. Furthermore, IL-6 is a very non-specific inflammatory biomarker and not specific to microbial translocation. Therefore, the available literature supports the selection of sCD14 as the best marker of the systemic inflammation that arises as a consequence of microbial translocation. In addition to sCD14, other inflammatory markers of cellular activation, inflammation, gut permeability, and microbial translocation, will also be measured in this trial including IP-10, TNF-RI, sCD163, oxidized LDL, D-dimer, LPS, LBP, kynurenine to tryptophan ratio, and I-FABP. Thus, while the trial is powered to evaluate changes in sCD14, we will evaluate a number of relevant biomarkers related to inflammation, oxidative stress, and microbial translocation.

Rationale for Selection of the Probiotic Product Visbiome Extra Strength
Visbiome Extra Strength is a combination of four strains of *Lactobacilli* (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei,* and *Lactobacillus delbrueckii ssp. Bulgaricus*), three strains of *Bifidobacteria* (*Bifidobacterium breve, Bifidobacterium infantis,* and *Bifidobacterium longum),* and one strain of *Streptococcus salivarius ssp. thermophilus*. This mixture of bacteria is associated with a superior performance of Visbiome Extra Strength when compared to other probiotic products, particularly in IBD, which serves as a close model for HIV-related changes in the mucosal immune response and mucosal inflammation that characterizes HIV disease and IBD. For instance, Visbiome Extra Strength has been associated with induction and maintenance of remission from UC in previous randomized controlled trials [95, 96].

- Visbiome is well characterized and has been studied in numerous animal models and human studies of diseases with altered gut permeability and gut inflammation, including studies for UC and Crohn's disease.
- Visbiome has been evaluated by one of the A5350 team members [59] in a macaque SIV model in which this probiotic restored functional gut immunity and decreased local and systemic inflammation.
- There are also data on Visbiome safety profile as being well tolerated and not associated with clinically important adverse events (AEs).
- Visbiome has also been evaluated in HIV-infected participants [59]. Data from a pilot study with Visbiome in a cohort of 20 HIV-infected, ART-suppressed individuals and 11 seronegative controls showed that CD4+ and CD8+ T-cell activation, characterized by CD38+/HLA-DR+ co-expression, was decreased after 3 months of probiotic supplementation to levels seen in the seronegative controls. In addition, sCD14 and D-dimer levels were relatively stable over the brief intervention, but LBP was reduced to levels of the control participants, suggesting that bacterial translocation was reduced with the probiotic product. While this study was small in size, the data are consistent with the results previously reported in SIV-infected macaques and warranted further investigation in larger trials.

Rationale for Proposed Visbiome Extra Strength Dose
900–1800 billion colony forming units (CFU) (1-2 packets per day) is an extra strength formulation of Visbiome generally used in patients with pouchitis (Visbiome Extra Strength product information sheet). This dose is based on data from several studies demonstrating excellent tolerability and responses seen in IBD [82], as well as significant improvement in IBS symptoms and increases in microbiome diversity in adult patients with IBS [97].

3.0 STUDY DESIGN

A5350 is a phase II, randomized, double-blind, two-arm study that will enroll 90 HIV-infected participants ≥18 years of age, who have been on stable ART for at least 24 weeks prior to study entry, and have a CD4+ T-cell count >200 cells/mm^3 prior to study entry and plasma HIV-1 RNA <50 copies/mL for 48 weeks prior to study entry. One blip ≤500 copies/mL within 48 weeks prior to study entry is allowed providing the preceding

and subsequent determinations are below 50 copies/mL. Randomization will be stratified by intent and eligibility to enroll in the substudy A5352s.

The primary objective of the study is to assess 24-week changes in sCD14 in participants treated with Visbiome Extra Strength compared to placebo.

A total of 90 participants will be randomized at 1:1 ratio to either Arm A (Visbiome Extra Strength + stable ART; treatment arm) or Arm B (placebo for Visbiome Extra Strength + stable ART; control arm). Participants will be on study for a total of 38 weeks. Probiotic Visbiome Extra Strength or placebo will be administered for 24 weeks after initiation at week 2. Participants will be followed for an additional 12 weeks after completing probiotic Visbiome Extra Strength treatment or placebo to determine whether the restoration to pre-intervention levels of gut microbial translocation products after discontinuation of the probiotics is associated with corresponding increases in levels of markers of immune activation, inflammation, and coagulation. Blood samples will be collected per the Schedule of Events (SOE) to measure markers of cellular activation, inflammation, and microbial translocation. Safety assessments will be performed per the SOE.

A substudy (A5352s) will also be conducted to evaluate whether there is a significant change in intestinal permeability and mucosal inflammation after 24 weeks of probiotic Visbiome Extra Strength. In the substudy, colonic biopsies collected by flexible sigmoidoscopy will be obtained per the A5352s SOE in 40 participants (20 from each arm) to assess tissue-specific effects related to immunologic outcomes, inflammation, microbial translocation, and gut integrity. Participants will also undergo evaluations of the mucosal barrier function. To optimize expeditious enrollment in the substudy, one of every three participants at an individual site enrolled in the parent study must also agree to participate in the substudy. Additional details about the substudy activities and requirements are included in the substudy protocol.

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

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

NOTE: The term “licensed” refers to a US FDA-approved kit.

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an

E/CIA that is based on a different antigen preparation and/or different test principle (eg, indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

4.1.2 Currently on continuous ART for ≥48 weeks prior to study entry with no change in the ART regimen within the 24 weeks prior to study entry except as noted below.

NOTE A: Continuous ART is defined as continuous ART for the 48-week period prior to study entry with no ART interruption longer than 7 consecutive days.

NOTE B: Modifications of ART during the 24 weeks prior to study entry are permitted in certain circumstances. For example, the change in formulation (eg, from standard formulation to fixed-dose combination including ART modifications switching from ritonavir- to cobicistat-boosted protease inhibitors or from tenofovir disoproxil fumarate to tenofovir alafenamide) is allowed within 24 weeks prior to study entry. A within-class, single-drug substitution (eg, switch from nevirapine to efavirenz or from atazanavir to darunavir) is allowed within 24 weeks prior to study entry, with the exception of a switch between any other NRTI to/from abacavir. No other changes in ART within the 24 weeks prior to study entry are permitted.

4.1.3 No plan to change ART regimen for the study duration.

4.1.4 Screening CD4+ cell count >200 cells/mm^3 obtained within 45 days prior to study entry by any US laboratory that has a CLIA certification or its equivalent.

4.1.5 Screening HIV-1 RNA levels <50 copies/mL using a FDA-approved assay performed by any laboratory that has a CLIA certification or its equivalent within 45 days prior to study entry.

4.1.6 HIV-1 RNA levels below the limit of quantification using a FDA-approved assay with a quantification limit of 50 copies/mL or lower for at least 48 weeks prior to study entry performed by any laboratory that has a CLIA certification or its equivalent.

NOTE: Single determinations that are between the assay quantification limit and 500 copies/mL (ie, “blips”) are allowed as long as the preceding and subsequent determinations are below the level of quantification. The screening value may serve as the subsequent undetectable value following a blip.

4.1.7 The following laboratory values obtained within 45 days prior to entry by any US laboratory that has a CLIA certification or its equivalent:

- Absolute neutrophil count (ANC) ≥1000/mm^3
- Hemoglobin ≥10.0 g/dL for men and 9.0 g/dL for women
- Platelet count ≥50,000/mm^3
- Aspartate aminotransferase (AST) (SGOT) ≤5 x upper limit normal (ULN)
- Alanine aminotransferase (ALT) (SGPT) ≤5 x ULN

- Alkaline phosphatase ≤5 x upper limit normal ULN
- Total bilirubin ≤2.5 x ULN (if on atazanavir ≤5 x ULN)
- Calculated creatinine clearance (CrCl) >60 mL/min, as estimated by the Cockcroft-Gault equation.
  NOTE: Calculator for the Cockcroft-Gault equation is available at https://www.fstrf.org/apps/cfmx/apps/common/Portal/index.cfm.

4.1.8 For females of reproductive potential, negative serum or urine pregnancy test within 45 days prior to entry by any US clinic or laboratory that has a CLIA certification or its equivalent, or is using a point-of-care (POC)/ CLIA-waived test, or at any network-approved non-US laboratory or clinic that operates in accordance with Good Clinical Laboratory Practices (GCLP) and participates in appropriate external quality assurance programs.

4.1.9 If participating in sexual activity that could lead to pregnancy, the female study participant must be willing to use a contraceptive while receiving protocol-specified medication. At least one of the following methods MUST be used:
- Condoms (male or female), with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive

4.1.10 Men and women age ≥18 years.

4.1.11 Ability and willingness of participant or legal guardian/representative to provide informed consent.

4.2 Exclusion Criteria

4.2.1 Initiation of ART during acute HIV infection.

NOTE: Participants who initiate ART within 6 months of HIV seroconversion are considered to have been initiated during acute infection and are excluded.

4.2.2 Receipt of antibiotic therapy within 60 days prior to study entry.

NOTE: Antibiotics for OI prophylaxis are exclusionary.

4.2.3 Known allergy/sensitivity or any hypersensitivity to components of Visbiome Extra Strength or its formulation.

4.2.4 Use of investigational therapies or investigational vaccines within 90 days prior to study entry.

4.2.5 Non-investigational vaccinations within 2 weeks prior to study entry.

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

4.2.7 Serious illness requiring systemic treatment and/or hospitalization within 30 days prior to entry.

4.2.8 History of positive HCV antibody with detectable HCV RNA in plasma within 48 weeks prior to study entry.

NOTE: Persons with positive HCV Ab but negative plasma HCV RNA are allowed to participate. Sites must document negative HCV RNA within 24 weeks of study entry.

4.2.9 History of positive HBsAg within 48 weeks prior to study entry.

4.2.10 Liver cirrhosis, history of inflammatory bowel disease, total colectomy, colon or rectal anastomosis, bowel resection, or current colostomy.

4.2.11 Current diagnosis of diabetes.

4.2.12 Either breastfeeding or pregnant within 24 weeks prior to study entry.

4.2.13 OIs within 45 days prior to study entry.

4.2.14 Use of any of the following medications/products for more than 3 consecutive days within the 60 days prior to study entry:

- Immunosuppressives (eg, azathioprine, corticosteroids greater than 20 mg per day [physiologic replacement doses are allowed], cyclosporine, mycophenolate, intravenous immunoglobulin (IVIG), interferon, sirolimus, sulfasalazine, tacrolimus).
- Immune modulators (eg, cytokines [eg, IL-2], granulocyte colony stimulating factor, growth hormone, tumor necrosis factor antagonists, thalidomide).
- Antineoplastic agents (except for topical agents for skin cancer).
- Probiotics and prebiotics (supplements and products).

NOTE: Yogurt with live cultures is allowed.

4.2.15 History of lactose intolerance or milk allergy.

4.2.16 Any episode of acute or persistent diarrhea within 60 days prior to study entry.

NOTES:

1. Diarrhea is defined as three or more stools per day that are liquid/loose/watery and will take the shape of a container. If the duration of loose stools meeting this criterion definition is greater than 30 days, this is chronic diarrhea and is not exclusionary.
2. Acute diarrhea is defined as 3–14 day duration.
3. Persistent diarrhea is defined as 15–30 day duration.

4.2.17 Weight loss or gain of more than 25 pounds in the 24 weeks prior to study entry.

4.3 Study Enrollment Procedures

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

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

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

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

Once a candidate for study entry has been identified, details will be carefully discussed with the participant. The participant (or, when necessary, the legal representative if the participant is under guardianship) will be asked to read and sign the approved protocol consent form.

For participants from whom a signed informed consent has been obtained, an ACTG Screening Checklist must be entered through the Data Management Center (DMC) Participant Enrollment System.

To optimize expeditious enrollment in the substudy, one of every three participants at an individual site enrolled in the parent study must also agree to participate in the substudy. Additional details about the substudy activities and requirements are included in the substudy protocol.

4.3.2 Randomization/Participant Registration

For participants from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database. Participants who meet the enrollment criteria will be registered to the study according to the standard DMC procedures.

4.4 Co-enrollment Guidelines

- US sites are encouraged to co-enroll participants in A5128, "Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses."
- Co-enrollment in A5128 does not require permission from the A5350 protocol chairs.
- For specific questions and approval for co-enrollment in other studies, sites should first check the PSWP or contact the protocol team via e-mail as described in the Study Management section.

5.0 STUDY TREATMENT

Study treatment for A5350 is defined as Visbiome Extra Strength and placebo for Visbiome Extra Strength.

5.1 Regimens Administration, and Duration

A total of 90 participants will be randomized in 1:1 ratio to either Arm A or Arm B (45 participants per arm).

5.1.1 Regimens

Duration	Arm A (Treatment)	Arm B (Control)
Week 2 to week 4 (total 2 weeks)	Visbiome Extra Strength One sachet orally daily	PLACEBO for Visbiome Extra Strength One sachet orally daily
Week 4 to week 26 (total 22 weeks)	Visbiome Extra Strength One sachet orally twice daily	PLACEBO for Visbiome Extra Strength One sachet orally twice daily
Week 26 to week 38 (total 12 weeks)	Off study product	Off study product

5.1.2 Administration

Study product should be taken at the same time every day. Visbiome Extra Strength and placebo for Visbiome Extra Strength can be mixed into cold water or any cold, noncarbonated beverage and consumed. If a participant forgets to take the study product, they should take the missed dose as soon as possible

during the same day. If an entire day has gone by, then the missed dose should be skipped, and the normal dosing schedule should be resumed. Participants should not double the next dose of study product in order to "make up" what had been missed.

5.1.3 Duration

Study treatment for Arm A participants is Visbiome Extra Strength, starting at week 2, for 24 weeks, followed by 12 weeks off study treatment. Study treatment for Arm B participants is placebo for Visbiome Extra Strength, starting at week 2, for 24 weeks, followed by 12 weeks off study treatment. All participants will be followed for 38 weeks after enrollment.

5.2 Study Product Formulation and Preparation

5.2.1 Visbiome Extra Strength is a high-potency probiotic medical food for oral administration. This product compares to ingredients in VSL#3 DS probiotic blend. Visbiome Extra Strength contains one strain of *Streptococcus thermophiles*, three strains of *Bifidobactera,* and four strains of *Lactobacilli* in defined ratios. Each sachet contains at least 900 billion lyophilized lactic acid bacteria per sachet. Visbiome Extra Strength and placebo for Visbiome Extra Strength are soluble in water. Visbiome Extra Strength and placebo for Visbiome Extra Strength may also contain maltodextrose, maltose, silicon dioxide, trace amounts of lactose (less than 0.1g per 100g) and dehydrated skim milk or milk protein (casein and beta---lacto globulin of less than 2mg/Kg).

5.2.2 Storage

Visbiome Extra Strength and placebo for Visbiome Extra Strength should be refrigerated between 4 to 8°C (39 to 46°F). Refrigerator temperature from 2 to 8^0C (36 to 46°F) is acceptable. Do not freeze.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

Visbiome Extra Strength and Placebo for Visbiome Extra Strength is manufactured by DuPont/Danisco for Exegi Pharma., LLC. Exegi Pharma., LLC will supply Visbiome Extra Strength and Placebo for Visbiome Extra Strength for this study. Study products will be available through the NIAID Clinical Research Products Management Center (CRPMC).

The site pharmacist should obtain the study product(s) for this protocol by following the instructions in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* in the section *Study Product Management Responsibilities.*

ART will not be provided through the study.

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All unused study products in US CRSs must be returned to the NIAID CRPMC (or as otherwise directed by the sponsor) after the study is completed or terminated. The procedures to be followed are in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.*

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent product information sheet, Investigator's Brochure, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

Additional drug information may be found on the updated ACTG Drug Interactions Database located at: http://tprc.pharm.buffalo.edu/home/di_search/.

5.4.1 Required Medications

See inclusion criteria (section 4.1) for specifications about ART. Participants must be on ART (not study provided) as specified in the inclusion criteria.

Discontinuation of ART regimen will result in permanent discontinuation of the study treatment. Participants may change ART regimens under the supervision of their physicians as long as they continue to meet all inclusion and exclusion criteria.

5.4.2 Prohibited Medications

The following medications/products are prohibited while participants are taking the study agents:

- Immunosuppressives (eg, azathioprine, corticosteroids greater than 20 mg per day [physiologic replacement doses are allowed], cyclosporine, mycophenolate, IVIG, interferon, sirolimus, sulfasalazine, tacrolimus).
- Immune modulators (eg, cytokines (eg, IL-2), granulocyte colony stimulating factor, growth hormone, tumor necrosis factor antagonists, thalidomide).
- Antineoplastic agents (except for topical agents for skin cancer).
- Prebiotics and probiotics other than the study medication.

5.4.3 Precautionary Medications

Antibiotics. Some strains of bacteria in Visbiome Extra Strength may be inactivated by certain antibiotics. Do not consume the study products within 4 hours after taking antibiotics.

5.5 Unblinding

Unblinding of study products (Visbiome Extra Strength and the placebo for Visbiome Extra Strength) will occur in accordance with the ACTG Unblinding Subjects Standard Operating Procedure (SOP) 123, located at: https://member.actgnetwork.org/cms/dl/10466.

Individual requests for unblinding will be handled on a case-by-case basis by the A5350 team leadership. The site pharmacists are not to reveal unblinded treatment information. More information regarding the site pharmacist role is in the "Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks."

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events

Evaluation	Screening (Within 45 days prior to entry)	Entry Week 0	Post-Entry							Conf. of Virologic Failure	Prem. Study DC
			Week 2 ±7days	On Treatment (Weeks)					Post Treat. (Weeks)		
				4	6	14	25	26	38		
				±7 days					±7 days		
Documentation of HIV	X										
Medical History/ Medication History	X		X								
Targeted Physical Exam	X		X		X	X		X	X		X
Height	X										
Weight	X							X	X		
Clinical Assessments			X		X	X		X	X		X
Dietary Assessment			X		X	X		X	X		
Sexual Activity Assessment			X		X	X		X	X		
Bowel Symptom Assessment			X		X			X			
Fatigue Symptom Assessment			X					X			
Dispense Study Products			X			X					
Hematology	X		X			X		X	X		
Liver Function Tests	X		X			X		X			
Blood Chemistries	X		X			X		X			
Calculated CrCl	X										
Fasting Lipid Profile			X			X		X			
Pregnancy Testing	X	X			If pregnancy is suspected						
Hepatitis Serology	X										
CD4+/CD8+	X		X			X		X	X		
Stored Plasma for sCD14		X	X		X	X	X	X	X		X

Evaluation	Screening (Within 45 days prior to entry)	Entry Week 0	Post-Entry							Conf. of Virologic Failure	Prem. Study DC
			Week 2 ±7days	On Treatment (Weeks)					Post Treat. (Weeks)		
				4	6	14	25	26	38		
				±7 days					±7 days		
Stored Plasma for Soluble Markers of Inflammation, Coagulation, Immune Activation, and Gut Epithelial Barrier Integrity		X	X		X	X	X	X	X		
Stored Plasma for Insulin Resistance			X			X		X			
Stored Plasma for Kynurenine to Tryptophan Ratio			X					X			
Stored peripheral blood mononuclear cells (PBMCs) for Monocyte and T-cell Phenotyping			X			X		X	X		
Stored Plasma for Enterocyte Death (I-FABP)			X		X	X	X	X	X		
Stored PBMC for Future Analysis			X		X	X		X	X		
Stored Plasma for Future Analysis			X		X	X		X	X		
Plasma HIV-1 RNA	X		X			X		X		X	X
Fecal Sample and Rectal Swabs			X		X	X		X	X		
Telephone Assessment				X							
Adherence Assessment					X	X		X			X
Empty Sachet Count					X	X		X			X

6.2 Timing of Evaluations

6.2.1 Screening Evaluations

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

Screening
Screening evaluations to determine eligibility must be completed within 45 days prior to study entry unless otherwise specified.

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

6.2.2 Entry Evaluations

Entry evaluations must occur at least 24 hours after screening.

6.2.3 Post-Entry Evaluations

Pre-Treatment Evaluations
Participants must begin treatment within 72 hours after the week 2 visit.

On-Treatment Evaluations
All on-treatment evaluations must be scheduled as per section 6.1 with a ± 7 day window. Week 6, 14, 25, and 26 visits for blood draws must be fasting visits. If the study participant is not fasting, the participant should be rescheduled within 3 days. (See section 6.3.14 for a definition of fasting.)

NOTE: The week 26 visit must occur at least 24 hours after and up to 7 days after the week 25 visit.

Post-Treatment Evaluations
All post-treatment evaluations must be scheduled as per section 6.1 with a ± 7 day window.

Study Completion Evaluations
The study completion evaluations will take place at week 38. This visit for blood draws must be a fasting visit. If the study participant is not fasting, the participant should be rescheduled within 3 days. (See section 6.3.14 for a definition of fasting)

Visit to Confirm a Suspected Virologic Failure
Confirmed virologic failure is defined as two consecutive HIV-1 RNA levels ≥200 copies/mL by real-time HIV-1 RNA testing. Participants with a plasma HIV-1 RNA ≥200 copies/mL at any visit will have a confirmatory viral load obtained as soon as possible but within 14 days after the first sample was drawn, if possible. If this visit coincides with a regularly scheduled visit, the evaluations should be combined. If the consecutive measurement of HIV-1 RNA is also ≥200

copies/mL, the participant will be considered to have confirmed virologic failure and the protocol core team must be notified via e-mail actg.corea5350@fstrf.org within 48 hours.

If a participant is confirmed to have virologic failure, then the participant must discontinue study treatment and return to the clinic within 14 days after discontinuing study treatment to complete the premature treatment/study discontinuation evaluations. The participant will then continue to be followed on study/off study treatment as per section 6.1.

6.2.4 Discontinuation Evaluations

Evaluations for Randomized or Registered Participants Who Do Not Start Study Treatment
All CRFs must be completed and keyed for the period up to and including week 2.

Premature Treatment Discontinuation Evaluations
Participants who prematurely permanently discontinue study treatment will be followed on study/off study treatment per the SOE.

Site personnel should notify the protocol core team via e-mail (actg.corea5350@fstrf.org) within 48 hours of any participant who prematurely discontinues study product.

Premature Study Discontinuation Evaluations
Participants who prematurely discontinue from the study will have the premature study discontinuation evaluations performed as soon as possible prior to being taken off the study.

If premature discontinuation from the study occurs at a scheduled study visit, complete the scheduled study visit evaluations and any additional required premature study discontinuation evaluations per the SOE.

Site personnel should notify the protocol core team via e-mail (actg.corea5350@fstrf.org) within 48 hours of any participant who prematurely discontinues the study.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS web site for information about what must be included in the source document: http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/sourcedocappndx.pdf.

At screening and entry, diagnoses and signs and symptoms should be entered onto the medical history forms. After entry, there are several separate logs that are to be

maintained throughout this study: an Adverse Event Log, Study Medication Log, Concomitant Medication Log, and ARV Log. There are also specific visit logs for lab reporting. The sections below will refer to reporting using these logs.

6.3.1 Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease having been absent at baseline, or, if present at baseline, appears to worsen AND is temporally associated with medical treatment/study product/device or procedure, REGARDLESS of the attribution (ie, relationship of event to medical treatment/ study product/device or procedure).

All AEs must be reported on the AE log if it meets the reporting requirements for this study including the diagnoses described in section 6.3.1.2, signs and symptoms (as described in section 6.3.1.3), and laboratory evaluations (as described in section 6.3.1.4) during the conduct of this study. AEs that meet the definition of a serious adverse events (SAEs) (as defined in section 6.3.1.1), or Expedited Adverse Events (EAEs), as defined in Section 11.4 of this protocol must also be entered onto the AE log. All AEs recorded on the AE log must have their severity (grade) defined.

To grade diagnoses, signs and symptoms, and laboratory results, sites must refer to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014, which can be found on the DAIDS RSC Web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

6.3.1.1 SAEs are adverse events that result in one or more of the following outcomes:

- Result in death
- Are life-threatening
- Require inpatient hospitalization or prolongation of existing hospitalization
- Result in persistent or significant disability/incapacity
- Are congenital anomaly/birth defect
- Are other important medical events (that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the events listed above).

NOTE: SAEs should be entered into the AE log and into the DAERS system as indicated.

6.3.1.2 Diagnoses

Post-entry, record any of the following diagnoses that are newly diagnosed or participant reported since the last visit on the AE log:

- CDC category B/C diseases, diabetes mellitus, hypertension, myocardial infarction (MI), coronary artery disease (CAD) (not MI), congestive heart failure (not HIV cardiomyopathy), stroke, malignancy (excluding basal cell cancer of the skin), renal insufficiency, liver disease, bleeding or coagulation disorders, and any inflammatory condition including, but not limited to, systemic illness or genital infections requiring antimicrobial therapy, immune reconstitution inflammatory syndromes, lupus, rheumatoid arthritis, or inflammatory bowel disease.

  NOTE: Cutaneous non-inflammatory conditions not requiring antimicrobial therapy do not need to be reported as an AE on the AE log.

6.3.1.3 Signs and Symptoms

Post-entry, all signs and symptoms Grade ≥3 and all signs and symptoms that led to a change in study treatment, regardless of grade, must be recorded on the AE log.

6.3.1.4 Laboratory Evaluations

For post-entry assessments, Grade ≥3 laboratory toxicities and all laboratory toxicities that led to a change in treatment, regardless of grade, must be recorded on the AE log.

6.3.2 Documentation of HIV-1

Section 4.1.1 specifies assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the CRF.

6.3.3 Medical History

The medical history must include all diagnoses identified by the ACTG criteria for clinical events and other diagnoses. In addition to reporting all diagnoses within the past 30 days, the following diagnoses should be reported regardless of when the diagnosis was made:

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

Any allergy to any medication and its formulation must also be documented in the source documentation only and is not recorded on the CRF.

At week 2, medical history will include a smoking and alcohol status.

6.3.4 Medication History

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

Medication Category	Complete History or Timeframe	Record on CRF (Yes/No)
ART	Cumulative overall ART duration	Yes
Current ART therapy	Current	Yes
Blinded study treatment	Current	Yes
Antihypertensive medications	Current	Yes
Statin therapy	Current	Yes
Aspirin therapy (ongoing regular therapy) and anticoagulant medications	Current	Yes
Hormonal contraceptives or hormone replacement therapy	Current	Yes
Acid reducing agents (H2 blockers and PPIs)	Current	Yes
OTC therapies/dietary supplements (eg, fish oil, vitamin D, vitamin A, ginseng)	Current	Yes
Antimicrobials	Current	Yes

6.3.5 Targeted Physical Exam

A targeted physical examination is to include auscultation of the chest; cardiac exam; abdominal exam, vital signs (temperature, pulse, respiration rate, and blood pressure), and is to be driven by any previously identified or new signs or symptoms and diagnoses that the participant has experienced within 30 days prior to entry/since the last visit.

6.3.6 Height

Height will be recorded at the screening visit only.

6.3.7 Weight

Weight will be recorded per the SOE.

6.3.8 Clinical Assessments

At the screening and week 2 visits, diagnoses and signs and symptoms should be recorded on the medical history forms. At all post-entry visits with a clinical assessment (see SOE), record all reportable AEs (see section 6.3.1) on the AE log.

Signs and Symptoms
At week 2, all grades of signs and symptoms that occurred 7 days before entry must be recorded on the medical history form. For post-entry signs and symptoms, please refer to section 6.3.1.3 for reporting requirements using the AE log.

Concomitant Medications
Post entry, record new or discontinued concomitant prescription and nonprescription medications listed in section 6.3.4.

Study Treatment Modifications
All study product modifications, including initial doses, participant-initiated and/or protocol-mandated modifications, inadvertent and deliberate interruptions will be recorded at each study visit. Any permanent discontinuation of treatment should be recorded.

ART Modifications
Post entry, record new or discontinued ART medications.

6.3.9 Dietary Assessment

The dietary assessment will be completed per the SOE. Additional details about the dietary evaluations are included in the study MOPS.

6.3.10 Sexual Activity Assessment

The sexual activity assessment will be completed per the SOE and MOPS. Details about the sexual activity evaluations are included in the study MOPS.

6.3.11 Bowel Symptom Assessment

The bowel symptom assessment will be completed per the SOE. Additional details about the bowel symptom assessment are included in the study MOPS.

6.3.12 Fatigue Symptom Assessment

The fatigue symptom assessment will be completed per the SOE. Additional details about the fatigue symptom assessment are included in the study MOPS.

6.3.13 Dispense Study Product

The study product will be dispensed per the SOE.

6.3.14 Laboratory Evaluations

At screening, and week 2 all laboratory values must be recorded on the laboratory findings log. For post-entry assessments, record the specific values for all hemoglobin, creatinine, AST, ALT, glucose, and platelet counts regardless of grade on the laboratory findings log for that visit. In addition, any post-entry Grade ≥3 laboratory toxicities or results that led to a change in treatment, regardless of grade, must be recorded on both the AE log (Refer to section 6.3.1.4) and the laboratory findings log.

Hematology
Hemoglobin, hematocrit, white blood cell count (WBC), differential WBC, absolute neutrophil count (ANC), and platelet count, will be performed in real time at the local laboratory.

Liver Function Tests
Total bilirubin, AST (SGOT), ALT (SGPT), alkaline phosphatase, and indirect bilirubin will be performed in real time at the local laboratory.

Blood Chemistries
Electrolytes (sodium, potassium, chloride, and bicarbonate), glucose, phosphate, creatinine, total protein, and albumin will be performed in real time at the local laboratory.

Calculated Creatinine Clearance
Calculated CrCl is required as estimated by the Cockcroft-Gault equation. This requires the recording of all serum creatinine values regardless of grade.

NOTE: Calculator for the Cockcroft-Gault equation is available at https://www.fstrf.org/apps/cfmx/apps/common/Portal/index.cfm

Fasting Lipid Profile

- To be performed batched at a central laboratory utilizing stored serum. For storage and shipping information, please see the A5350 LPC.
- Standard lipid profile (total cholesterol, calculated LDL cholesterol, HDL cholesterol, and triglycerides).

Fasting is defined as nothing to eat and drink except for prescription medications and water for at least 8 hours. If participants are in a non-fasting state, they should be asked to return to the clinic within 3 days of the original scheduled study visit for fasting evaluations.

Pregnancy Testing
For women with reproductive potential: Serum or urine β-HCG (urine test must have a sensitivity of ≤25mIU/mL) tests are acceptable. Negative serum or urine pregnancy test must be obtained within 45 days prior to study entry and again 24 hours prior to study entry. After entry, perform a pregnancy test when pregnancy is suspected.

Hepatitis Serology
For participants with known HBV immunity, prior documentation of positive HBsAb is acceptable. If documentation is not available, HBsAg must be obtained at screening or within 48 weeks of entry from standard of care (SOC). Results must be available prior to study entry so that participants who have active HBV can be excluded from participating in the study.

HCV antibody test (and HCV RNA if the HCV Ab test is positive) must be obtained at screening or within 48 weeks of entry for HCV Ab or within 24 weeks of entry for HCV RNA from SOC. Results must be available prior to study entry.

6.3.15 Immunologic Studies

Because of the diurnal variation in biomarkers that may be measured as part of the study (eg, CD4+ and CD8+ T-cell counts and other biomarkers that may be measured on stored samples), blood draws for individual participants should be performed consistently in either the morning or the afternoon throughout the study, if possible.

NOTE: If a participant experiences an inflammatory condition (ie, infection requiring hospitalization, a systemic viral illness such as an influenza-like illness, a severe drug hypersensitivity reaction, or a fever on the day of visit defined as T° >38°C), receives a vaccine or experiences a concurrent illness, wait 7 days for blood draw.

CD4+/CD8+
At screening, obtain absolute CD4+/CD8+ count and percentages within 45 days prior to entry from a laboratory that possesses a CLIA certification or equivalent.

During the study, all laboratories must possess a CLIA certification or equivalent.

Stored Plasma for sCD14
Plasma specimens will be collected and stored for batch analysis at the end of the study. For storage and shipping, please see the A5350 LPC.

Stored Plasma for Soluble Markers of Inflammation, Coagulation, Immune Activation, and Gut Epithelial Barrier Integrity
Plasma specimens for measurement of IL-6, IP10, sCD163, TNF-RI, D-dimer, LPS, LBP, and oxidized LDL will be collected and stored for batch analysis at the end of the study. For storage and shipping, please see the A5350 LPC.

Stored Plasma for Insulin Resistance
Plasma specimens will be collected and stored for batch analysis at the end of the study. For storage and shipping, please see the A5350 LPC.

Stored Plasma for Kynurenine to Tryptophan Ratio
Plasma specimens will be collected and stored for batch analysis at the end of the study. For storage and shipping, please see the A5350 LPC.

Stored PBMCs for Monocyte and T-Cell Phenotyping
PBMC specimens will be collected and stored for batch analysis at the end of the study.

For PBMC cryopreservation during the study, all laboratories must be certified for protocol testing by the DAIDS Immunology Quality Assurance (IQA) Program.

Enterocyte Death Assessment
Plasma specimens for measurement of I-FABP will be collected and stored for batch analysis at the end of the study. For storage and shipping, please see the A5350 LPC.

Stored Specimens
PBMCs and plasma will be stored for future analysis. For storage and shipping, please see the A5350 LPC.

Specimens will be stored for possible future genetic testing.

6.3.16 Virologic Studies

Plasma HIV-1 RNA
Screening HIV-1 RNA must be performed within 45 days prior to study entry by a laboratory that possesses a CLIA certification or equivalent. Eligibility will be determined based on the screening value.

Post-entry evaluations will be performed at Quest Diagnostics per the SOE.

6.3.17 Fecal Sample and Rectal Swabs

Fecal sample and rectal swabs will be collected and stored per the SOE and MOPS.

6.3.18 Telephone Assessment

A telephone assessment will be performed at week 4 to confirm that the participant increased his or her study product dose.

6.3.19 Adherence Assessment and Sachet Count

A standardized assessment by self-report to monitor adherence to study product will be performed. Empty sachets will be counted to monitor adherence to study product per the SOE and recorded on the CRF. At week 26, a final sachet count will be performed and recorded on the CRFs.

7.0 CLINICAL MANAGEMENT ISSUES

7.1 Toxicity

Only toxicities related to Visbiome Extra Strength will be considered in the toxicity management section. The grading system for drug toxicities is located in the DAIDS AE Grading Table, Version 2.0, November 2014, which can be found on the DAIDS RSC Web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

7.1.1 Gastrointestinal Symptoms

Mild abdominal bloating has been reported in the first few days of consuming Visbiome Extra Strength. This is generally a physiological adaptation of the microflora, which usually resolves within 3–4 days. If bloating persists, the site staff should contact the protocol core team (actg.corea5350@fstrf.org) to consider whether the study agent dose should be reduced for a few days. Other GI symptoms have been reported and include nausea, abdominal pain, and flatulence. These symptoms are usually mild and resolve within a few days.

7.1.2 Infections

Multiple studies of probiotics in a variety of settings have shown that they appear to be safe, even in patients with compromised immune systems, such as transplant patients. However, there are case reports of bacteremia, sepsis, endocarditis, and abscess due to microorganisms that are present in probiotic preparations [72]. The site staff should contact the protocol core team (actg.corea5350@fstrf.org) if an infection due to one of the microorganisms found in the probiotic occurs in a participant.

7.2 Pregnancy

Pregnant women will discontinue study product and will be encouraged to continue on study and complete the evaluations included in the post-treatment evaluation section. At the end of the pregnancy, outcome and AEs for participant and infant will be recorded on the outcome CRF. If a woman has completed the study or chooses to discontinue from the study before the end of the pregnancy, then site staff should request permission to contact her regarding pregnancy outcomes at the end of pregnancy. If the information is obtained, pregnancy outcomes will be submitted on a CRF at the end of the pregnancy.

Obstetric history, and/or pregnancy outcome will be recorded on the CRFs. Pregnancies

that occur on study should be reported prospectively to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Phone: 800-258-4263; Fax: 800-800-1052.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Permanent and Premature Treatment Discontinuation

- Treatment-related toxicity (see section 7.1 Toxicity).
- Requirement for prohibited concomitant medications (see section 5.4).
- Discontinuation of ART regimen (see section 5.4.1).
- Confirmed virologic failure (see section 6.2.3).
- Completion of treatment as defined in the protocol.
- Request by participant to terminate treatment.
- Clinical reasons believed life-threatening by the physician, even if not addressed in the toxicity section of the protocol.
- Other reasons at the discretion of the physician.
- Pregnancy.

8.2 Premature Study Discontinuation

- Failure by the participant to attend three consecutive study visits.
- Request by the participant to withdraw.
- Request of the primary care provider who thinks the study is no longer in the best interest of the participant.
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the IRB NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, investigator, or industry supporter.

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design

A5350 is a phase II, randomized, double-blind, two-arm study to evaluate 24 weeks of Visbiome Extra Strength therapy compared with matching placebo on markers of inflammation and bacterial translocation among HIV-infected participants on ART with plasma HIV-1 RNA <50 copies/mL and CD4+ T-cell >200 cells/mm^3. Participants will be randomized at 1:1 ratio to either Arm A (Visbiome Extra Strength + stable ART; treatment arm) or Arm B (placebo for Visbiome Extra Strength + stable ART; control arm). Participants will be on study for a total of 38 weeks. Probiotic therapy will be administered for 24 weeks initiating at week 2. Participants will be followed for an additional 12 weeks after completing probiotic therapy to determine whether the restoration to pre-intervention levels of gut microbial translocation products after

discontinuation of the probiotics is associated with corresponding increases in levels of immune activation, inflammation, and coagulation. The total sample size will be 90 participants (45 in Arm A, 45 in Arm B).

9.2 Outcome Measures

9.2.1 Primary Outcome Measure

Change in sCD14 from baseline to week 26.

9.2.2 Secondary Outcome Measures

9.2.2.1 Change from baseline to week 26 in systemic inflammation markers (IL-6, IP10, sCD163, TNF-RI, oxidized LDL), kynurenine to tryptophan (KT) ratio, and coagulation marker (D-dimer).

9.2.2.2 Change from baseline to week 26 in microbial translocation markers (LPS, LBP).

9.2.2.3 Change from baseline to week 26 in CD4+ lymphocyte counts and CD4+/CD8+ ratio.

9.2.2.4 Change from baseline to week 26 in markers of monocyte and lymphocyte activation and senescence.

9.2.2.5 Change from baseline to week 26 in gastrointestinal microbial diversity from fecal samples.

9.2.2.6 Change from baseline to week 26 in enterocyte death (I-FABP).

9.2.2.7 Occurrence of Grade ≥3 AEs related to study product.

9.2.2.8 Diversity of gut microbiome at week 38.

9.2.3 Exploratory Outcome Measures

9.2.3.1 Change from baseline to week 26 in HOMA-IR.

9.2.3.2 Change from baseline to week 26 in LDL, HDL, non-HDL cholesterol, and triglycerides.

9.2.3.3 Change from week 26 to week 38 in systemic inflammation markers (IL-6, IP10, sCD163, sTNF-RI, oxidized LDL, and KT ratio).

9.2.3.4 Gastrointestinal microbial diversity from fecal samples at entry and post-entry visits.

9.2.3.5 Change in gastrointestinal symptoms from baseline to 26 weeks.

9.2.3.6 Assess diversity of gut microbiome based on dietary recall.

9.3 Randomization and Stratification

After eligibility is confirmed, participants will be randomized at a 1:1 ratio (Visbiome Extra Strength: placebo) using permuted blocks without institutional balancing with stratification by intent and eligibility to enroll in the substudy A5352s. Because the stratification is to ensure balance between the treatment arms within A5352s and is not felt to affect A5350 outcomes, the stratification will not be taken into account in analyses.

9.4 Sample Size and Accrual

The primary objective of this study is to compare sCD14 change over 24 weeks of treatment in participants randomized to Visbiome Extra Strength compared to placebo. The sample size calculations are based on the primary hypothesis testing H_o: $\mu_{\text{Visbiome Extra Strength}} = \mu_{\text{placebo}}$ targeting 90% power in the comparison of changes in $\log_{10}$-transformed sCD14 between Visbiome Extra Strength and placebo.

Our estimate of the standard deviation for the change in plasma sCD14 levels over 24 weeks of treatment comes from a recent placebo-controlled trial of maraviroc intensification among ART-suppressed, HIV-infected individuals [98]. Among the 20 placebo-treated participants, the mean change in plasma sCD14 levels from baseline to week 16 was +0.003 $\log_{10}$ ug/ml (SD: 0.11) when analyzing a single measurement at each time point and -0.005 $\log_{10}$ ug/ml (SD: 0.06) when taking the average of two measures at each time point. Although we plan to take the average of two sCD14 levels at both baseline and week 26, we have conservatively powered the study assuming the SD is 0.09 $\log_{10}$ ug/ml.

A 0.07 $\log_{10}$ sCD14 between-arm difference was associated with a 23% decreased odds of a non-AIDS event or non-accidental death at the pre-event time point [56] and is considered as clinically significant, and therefore was chosen as the effect size for this study.

A total sample of 90 participants (45 per arm) is needed to detect a difference of 0.07 $\log_{10}$ in sCD14 levels between Visbiome Extra Strength and placebo with 90% power and a 0.05 two-sided type I error rate, under the assumption that the SD of the changes in $\log_{10}$ sCD14 from baseline to week 26 is 0.09 for both arms.

Although it is expected that Visbiome Extra Strength will be well tolerated in this study, it is anticipated that there will be some missing data due to laboratory complications and loss to follow-up. The sample size is inflated by 20% to account for missing data.

The following table summarizes the calculation of the sample sizes corresponding to the detection of various effect sizes under various assumed standard deviations, with the effect size, standard deviation, and adjusted sample size selected for this study shown in bold.

Effect size in log_{10} sCD14 (difference between Visbiome Extra Strength and placebo)	Std Dev of Δ Baseline to Week 26	Total Sample size (90% power, alpha=0.05)	Adjusted Sample Size (20% inflation)
0.03	0.05	60*2=120	75*2=150
	0.07	116*2=232	145*2=290
	0.09	191*2=382	239*2=478
0.05	0.05	23*2=46	29*2=58
	0.07	43*2=86	54*2=108
	0.09	70*2=140	88*2=176
0.07	0.05	12*2=24	15*2=30
	0.07	23*2=46	29*2=58
	0.09	**36*2=72**	**45*2=90**

For each continuous secondary endpoint, change from baseline to week 26 will be compared between treatment arms. The following table summarizes the power to detect various effect sizes as well as the precision of the effect size estimate.

Comparison of 2 arms (36 vs. 36 evaluable participants)		
Effect size	Power	Width of 95% CI
0.77*SD	90%	± 0.47*SD
0.67*SD	80%	± 0.47*SD
0.59*SD	70%	± 0.47*SD

Accrual

We expect to enroll an average of two study participants per month at each site and expect to complete enrollment within 9 months (2 participants x 5 sites over 9 months = 90 participants) once all sites are registered. Should more sites register for the trial, enrollment will occur more rapidly.

9.5 Monitoring

Accrual, a summary of AEs, and sample/data availability will be reviewed monthly by the protocol core team. This summary will be pooled over the study arms. In addition, baseline characteristics as well as early study product and study discontinuations (and reasons) will be reviewed regularly by the protocol core team. The Statistical and Data Analysis Center (SDAC) will also provide a report of all reported AEs by blinded treatment arm, to be reviewed quarterly by the DAIDS clinical representative or designee.

An ACTG-appointed Study Monitoring Committee (SMC) will review accrual, adverse event summaries, off-product and off-study rates and reasons, and sample/data availability, broken down by study arm. The first SMC review will occur 6 months after

the first participant is enrolled and then every 6 months as long as participants remain in follow-up. In addition, longitudinal changes in CD4+ T-cell count and HIV-1 RNA levels will be reviewed. Note that biomarker measurements will be run in batches after follow-up is concluded, and, therefore these data are not expected to be available at interim reviews. The SMC may also be convened if a reason is identified by the DAIDS clinical representative, study chairs, or study statistician in consultation with the team.

9.6 Analyses

All statistical tests will be two-sided with a nominal alpha level of 0.05. Because this is a phase II study and all potential biologic activities of the intervention are of interest, analyses will be as-treated, and limited to participants who 1) have baseline and week 26 sCD14 measurements, 2) remain on study product through week 26 (participants with more than 50% on average of missed study product may be excluded), 3) have not used prohibited medications, 4) do not have a confirmed virologic failure (as defined per section 6.2.3 at or prior to week 26, and 5) do not experience inflammatory conditions, receive vaccines, or have concurrent illness (as defined per section 6.2.3).

NOTE: Participants experiencing inflammatory conditions at the time of a visit are instructed to wait 7 days before measurements are obtained. Measurements obtained while inflammatory conditions are present will not be included in the analysis.
A supplemental intent-to-treat analysis will also be performed and will include all randomized participants. No adjustment for multiple testing will be performed. A summary of the number of participants experiencing study product-related AEs and the types of AEs will be reported, as will the proportion of participants who discontinue study product after entry.

To reduce intra-participant variability, two blood draws will be performed at baseline (entry and week 2) and week 26 (week 25 and week 26) and the measurements will be the average of two for sCD14. If repeat measurements are not available at either of these time points, a single measurement will be used rather than the average.

9.6.1 Primary Analysis

The primary objective of the study is to assess the effect of Visbiome Extra Strength on sCD14 in HIV-infected participants well-controlled on ART. To address this, changes in sCD14 from baseline to week 26 will be compared between the Visbiome Extra Strength arm and the placebo arm by linear regression. For this model each participant will have a single outcome measure of sCD14 change from baseline to week 26. The only predictor variable will be study arm.

Two supplemental linear regression analyses will be performed. The first will additionally adjust for baseline sCD14 values (continuous) while the second will assess differential Visbiome Extra Strength effects by baseline sCD14 tertile by additionally adjusting for the sCD14 tertile main effect and the study arm by sCD14 tertile interaction.

9.6.2 Secondary Analyses

Similar to the initial primary analysis regression model, changes in other outcome measures will be compared between the Visbiome Extra Strength and placebo arms by linear regression. For these models each participant will have a single outcome measure of change from baseline to week 26. The only predictor variable will be study arm.

The weeks 26 and 38 levels of the primary, secondary, and exploratory outcome measures will be compared with baseline levels through linear regression.

Spearman correlations will be used to evaluate correlations between changes in select outcome measures. In addition, longitudinal changes in these measures will be examined using graphical approaches, descriptive statistics, and possibly repeated measures modeling.

Safety will be evaluated by summarizing the nature and rate of AEs within each study arm for all participants initiating study product. Comparisons of overall and AE-specific grades between study arms will be evaluated with an exact test for ordered categorical data (Wilcoxon rank-sum test of the maximum grade for each participant). Study product tolerability will be evaluated by summarizing study product modifications and discontinuations by study arm.

10.0 PHARMACOLOGY PLAN

Not applicable

11.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

11.1 Records to Be Kept

Case report forms (CRFs) will be provided for each participant. Participants must not be identified by name on any CRFs. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon randomization/registration.

11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

11.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

11.3 Clinical Site Monitoring and Record Availability

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

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

11.4 Expedited Adverse Event Reporting to DAIDS

11.4.1 Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

The DAIDS Adverse Events Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact DAIDS-ES at DAIDS-ESSupport@niaid.nih.gov. Site queries may also be sent from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

11.4.2 Reporting Requirements for this Study

- The SUSAR Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agents for which expedited reporting are required is: Visbiome Extra Strength and the placebo for Visbiome Extra Strength.
- Grade 2 and higher adverse events related to the flexible sigmoidoscopy and rectal biopsy procedures performed in A5352s.

11.4.3 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014 must be used and is available on the DAIDS RSC Web site at http://rsc.tech-res.com/safetyandpharmacovigilance/.

11.4.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study is as per the EAE manual.
- After the protocol-defined AE reporting period, unless otherwise noted, only suspected, *unexpected* serious adverse reactions (SUSARs), as defined in Version 2.0 of the EAE Manual, will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

12.0 PARTICIPANTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Appendix I) and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from the participant (or person with power of attorney for participants who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, or legal guardian, and this fact will be documented in the participant's record.

12.2 Participant Confidentiality

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

12.3 Study Discontinuation

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

13.0 PUBLICATION OF RESEARCH FINDINGS

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

14.0 BIOHAZARD CONTAINMENT

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

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

15.0 REFERENCES

1. Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. Lancet. 2008;372:293-9.

2. Weber R, Ruppik M, Rickenbach M, et al. Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. HIV Med. 2013;14:195-207.

3. Data Collection on Adverse Events of Anti-HIV drugs (D:A:D) Study Group. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study. AIDS. 2010;24:1537-48.

4. Palella FJ Jr, Baker RK, Moorman AC, et al. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr. 2006;43:27-34.

5. Effros RB, Fletcher CV, Gebo K, et al. Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. Clin Infect Dis. 2008;47:542-53.

6. High KP, Brennan-Ing M, Clifford DB, et al. HIV and aging: state of knowledge and areas of critical need for research. A report to the NIH Office of AIDS Research by the HIV and Aging Working Group. J Acquir Immune Defic Syndr. 2012;60 Suppl 1:S1-18.

7. Wada N, Jacobson LP, Cohen M, et al. Cause-specific life expectancies after 35 years of age for human immunodeficiency syndrome-infected and human immunodeficiency syndrome-negative individuals followed simultaneously in long-term cohort studies, 1984-2008. Am J Epidemiol. 2013;177:116-25.

8. Neuhaus J, Angus B, Kowalska JD, et al. Risk of all-cause mortality associated with nonfatal AIDS and serious non-AIDS events among adults infected with HIV. AIDS. 2010;24:697-706.

9. Antiretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clin Infect Dis. 2010;50:1387-96.

10. Marin B, Thiébaut R, Bucher HC, et al. Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy. AIDS. 2009;23:1743-53.

11. French AL, Gawel SH, Hershow R, et al. Trends in mortality and causes of death among women with HIV in the United States: a 10-year study. J Acquir Immune Defic Syndr. 2009;51:399-406.

12. Lewden C, May T, Rosenthal E, et al. Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalité 2000 and 2005" surveys (ANRS EN19 and Mortavic). J Acquir Immune Defic Syndr. 2008;48:590-8.

13. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. Clin Microbiol Rev. 2013;26:2-18.

14. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med. 2013;5:193ra91.

15. Mutlu EA, Keshavarzian A, Losurdo J, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog. 2014;10:e1003829.

16. Yu G, Fadrosh D, Ma B, et al. Anal microbiota profiles in HIV-positive and HIV-negative MSM. AIDS. 2013. [Epub ahead of print].

17. Marchetti G, Bellistrì GM, Borghi E, 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;22:2035-8.

18. Merlini E, Bai F, Bellistrì GM, 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:e18580.

19. Klatt NR, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease. Trends Microbiol. 2013;21:6-13.

20. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12:1365-71.

21. Mowat AM and Viney JL The anatomical basis of intestinal immunity. Immunol Rev. 1997;156:145-166.

22. Scholer, A., Hugues, S., Boissonnas, A., et al. Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine CD8+ T cell memory. Immunity. 2008;28:258-270.

23. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12:1365-1371.

24. Gordon SN, Cervasi B, Odorizzi P, 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. 2010;185:5169-5179.

25. Li Q, Estes JD, Duan L, et al. Simian immunodeficiency virus-induced intestinal cell apoptosis is the underlying mechanism of the regenerative enteropathy of early infection. J Infect Dis. 2008;197:420-429.

26. Lederman MM, Calabrese L, Funderburg NT, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. J Infect Dis. 2011; 204:1217-1226.

27. Robbins GK, Spritzler JG, Chan ES, et al. Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. Clin Infect Dis. 2009;48:350-361.

28. Ellis CL, Ma ZM, Mann SK, et al. Molecular characterization of stool microbiota in HIV-infected subjects by panbacterial and order-level 16S ribosomal DNA (rDNA) quantification and correlations with immune activation. J Acquir Immune Defic Syndr.. 2011;57:363-370.

29. Gori A, Tincati C, Rizzardini G, et al. Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. J Clin Microbiol. 2008;46:757-758.

30. Lozupone CA, Li M, Campbell TB, Flores SC, Linderman D, Gebert MJ, Knight R, Fontenot AP, Palmer BE. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe 2013;14(3):329-39.

31. Dillon SM, Lee EJ, Kotter CV, Austin GL, Dong Z, Hecht DK, Gianella S, Siewe B, Smith DM, Landay AL, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 2014;7(4):983-94.

32. Mutlu EA, Keshavarzian A, Losurdo J, Swanson G, Siewe B, Forsyth C, French A, Demarais P, Sun Y, Koenig L, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog 2014;10(2):e1003829.

33. Yu G, Fadrosh D, Ma B, et al. Anal microbiota profiles in HIV-positive and HIV-negative MSM. AIDS. 2013. [Epub ahead of print].

34. Chung CY, Alden SL, Funderburg NT, Fu P, Levine AD. Progressive proximal-to-distal reduction in expression of the tight junction complex in colonic epithelium of virally-suppressed HIV+ individuals. PLoS Pathog 2014;10(6):e1004198.

35. Tenorio AR, Wilson CC, Chan ES, et al. Rifaximin has marginal impact on immune activation in immune non-responders to ART- ACTG 5286. 21st Conference on Retroviruses and Opportunistic Infections 2014, Abstract #339.

36. Sandler NG, Zhang X, Bosch RJ, et al. Sevelamer does not decrease plasma LPS or sCD14 but does decrease soluble tissue factor and LDL. 21st Conference on Retroviruses and Opportunistic Infections 2014, Abstract #337.

37. Marteau PR, de Vrese M, Cellier CJ, Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr. 2001; 73(2 suppl):430S–436S.

38. Madsen KL. The use of probiotics in gastrointestinal disease. Can J Gastroenterol. 2001;15:817–822.

39. Mack DR. Probiotics in inflammatory bowel diseases and associated conditions. Nutrients 2011;3(2):245-64.

40. Collado MC, Meriluoto J, Salminen S. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Lett Appl Microbiol. 2007;45(4):454-60.

41. Veerappan GR, Betteridge J, Young PE. Probiotics for the treatment of inflammatory bowel disease. Curr Gastroenterol Rep. 2012;14(4):324-33.

42. Kanauchi O, Mitsuyama K, Andoh A. The therapeutic impact of manipulating microbiota in inflammatory bowel disease. Curr Pharm Des. 2009;15(18):2074-86.

43. Vyboh K, Jenabian MA, Mehraj V, Routy JP. HIV and the gut microbiota, partners in crime: breaking the vicious cycle to unearth new therapeutic targets. J Immunol Res. 2015;2015:614127.

44. Jenabian MA, El-Far M, Vyboh K, Kema I, Costiniuk CT, Thomas R, Baril JG, LeBlanc R, Kanagaratham C, Radzioch D, Allam O, Ahmad A, Lebouché B, Tremblay C, Ancuta P, Routy JP; Montreal Primary infection and Slow Progressor Study Groups. Immunosuppressive Tryptophan Catabolism and Gut Mucosal Dysfunction Following Early HIV Infection. J Infect Dis. 2015;212(3):355-66.

45. Chen J, Shao J, Cai R, Shen Y, Zhang R, Liu L, Qi T, Lu H. Anti-retroviral therapy decreases but does not normalize indoleamine 2,3-dioxygenase activity in HIV-infected patients. PLoS One. 2014;9(7):e100446.

46. Macho Fernandez E, Pot B, Grangette C. Beneficial effect of probiotics in IBD: are peptidogycan and NOD2 the molecular key effectors? Gut Microbes. 2011;2(5):280-6.

47. Song M, Xia B, Li J. Effects of topical treatment of sodium butyrate and 5-aminosalicylic acid on expression of trefoil factor 3, interleukin 1beta, and nuclear factor kappaB in trinitrobenzene sulphonic acid induced colitis in rats. Postgrad Med J 2006;82(964):130-5.

48. Vernia P, Annese V, Bresci G, d'Albasio G, D'Inca R, Giaccari S, Ingrosso M, Mansi C, Riegler G, Valpiani D, et al. Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: Results of a multicentre trial. Eur J Clin Invest 2003;33(3):244-8.

49. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients 2011;3(10):858-76.

50. Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem 2013;288(35):25088-97.

51. Garcia Vilela E, De Lourdes De Abreu Ferrari,M., Oswaldo Da Gama Torres H, Guerra Pinto A, Carolina Carneiro Aguirre A, Paiva Martins F, Marcos Andrade Goulart E, Sales Da Cunha A. Influence of saccharomyces boulardii on the intestinal permeability of patients with crohn's disease in remission. Scand J Gastroenterol 2008;43(7):842-8.

52. Liu Z, Qin H, Yang Z, Xia Y, Liu W, Yang J, Jiang Y, Zhang H, Yang Z, Wang Y, et al. Randomised clinical trial: The effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery - a double-blind study. Aliment Pharmacol Ther 2011;33(1):50-63.

53. Dai C, Zheng CQ, Meng FJ, Zhou Z, Sang LX, Jiang M. VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. Mol Cell Biochem. 2013;374(1-2):1-11.

54. Bassaganya-Riera J, Viladomiu M, Pedragosa M, De Simone C, Carbo A, Shaykhutdinov R, Jobin C, Arthur JC, Corl BA, Vogel H, Storr M, Hontecillas R. Probiotic bacteria produce conjugated linoleic acid locally in the gut that targets macrophage PPAR γ to suppress colitis. PLoS One. 2012;7(2):e31238.

55. Mencarelli A, Distrutti E, Renga B, D'Amore C, Cipriani S, Palladino G, Donini A, Ricci P, Fiorucci S. Probiotics modulate intestinal expression of nuclear receptor and provide counter-regulatory signals to inflammation-driven adipose tissue activation. PLoS One. 2011;6(7):e22978.

56. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis. 2014;210:1248-59.

57. Huengsberg M, Winer JB, Gompels M, Round R, Ross J, Shahmanesh M. Serum kynurenine-to-tryptophan ratio increases with progressive disease in HIV-infected patients. Clin Chem. 1998;44(4):858-62.

58. Byakwaga H, Boum Y 2nd, Huang Y, Muzoora C, Kembabazi A, Weiser SD, Bennett J, Cao H, Haberer JE, Deeks SG, Bangsberg DR, McCune JM, Martin JN, Hunt PW. The kynurenine pathway of tryptophan catabolism, CD4+ T-cell recovery, and mortality among HIV-infected Ugandans initiating antiretroviral therapy. J Infect Dis. 2014;210(3):383-91.

59. d'Ettorre G, Ceccarelli G, Giustini N, Serafino S, Calantone N, De Girolamo G, Bianchi L, Bellelli V, Ascoli-Bartoli T, Marcellini S, Turriziani O, Brenchley JM, Vullo V. Probiotics Reduce Inflammation in Antiretroviral Treated, HIV-Infected Individuals: Results of the "Probio-HIV" Clinical Trial. PLoS One. 2015;10(9):e0137200. PMID: 26376436.

60. Ng SC, Lam EF, Lam TT, Chan Y, Law W, Tse PC, Kamm MA, Sung JJ, Chan FK, Wu JC. Effect of probiotic bacteria on the intestinal microbiota in irritable bowel syndrome. J Gastroenterol Hepatol. 2013;28(10):1624-31.

61. Ng SC, Plamondon S, Kamm MA, Hart AL, Al-Hassi HO, Guenther T, Stagg AJ, Knight SC. Immunosuppressive effects via human intestinal dendritic cells of probiotic bacteria and steroids in the treatment of acute ulcerative colitis. Inflamm Bowel Dis. 2010;16(8):1286-98.

62. Gupta N, Kumar A, Sharma P, Garg V, Sharma BC, Sarin SK. Effects of the adjunctive probiotic VSL#3 on portal haemodynamics in patients with cirrhosis and large varices: a randomized trial. Liver Int. 2013;33(8):1148-57.

63. Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP. Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial. Mediators Inflamm. 2014;2014:348959.

64. Alisi A, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, Giammaria P, Reali L, Anania F, Nobili V. Randomised clinical trial: The beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2014;39(11):1276-85.

65. Sanaie S, Ebrahimi-Mameghani M, Mahmoodpoor A, Shadvar K, Golzari SE. Effect of a Probiotic Preparation (VSL#3) on CardiovascularRisk Parameters in Critically-Ill Patients. J Cardiovasc Thorac Res. 2013;5(2):67-70.

66. Klatt NR, Canary LA, Sun X, et al. Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. J Clin Invest. 2013;123:903-7.

67. Irvine SL, Hummelen R, Hekmat S, et al. Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS. J Clin Gastroenterol. 2010;44:e201–e205.

68. Hummelen R, et al. Effect of 25 weeks probiotic supplementation on immune function of HIV patients. Gut Microbes. 2011;2:80–85.

69. Irvine SL, Hummelen R, Hekmat S. Probiotic yogurt consumption may improve gastrointestinal symptoms, productivity, and nutritional intake of people living with human immunodeficiency virus in Mwanza, Tanzania. Nutr Res. 2011;31:875–881.

70. Schunter M, et al. Randomized pilot trial of a symbiotic dietary supplement in chronic HIV-1 infection. BMC Complement Altern Med. 2012;12:84.

71. González-Hernández LA, Jave-Suarez LF, Fafutis-Morris M, et al. Synbiotic therapy decreases microbial translocation and inflammation and improves immunological status

in HIV-infected patients: a double-blind randomized controlled pilot trial. Nutr J. 2012;11:90.

72. Wilson NL, Moneyham LD, Alexandrov AW. A systematic review of probiotics as a potential intervention to restore gut health in HIV infection. J Assoc Nurses AIDS Care. 2013;24:98-111.

73. Hemsworth JC, Hekmat S, Reid G. Micronutrient supplemented probiotic yogurt for HIV-infected adults taking HAART in London, Canada. Gut Microbes. 2012;3:414-9.

74. Schunter M, Chu H, Hayes TL, et al. Randomized pilot trial of a synbiotic dietary supplement in chronic HIV-1 infection. BMC Complement Altern Med. 2012;12:84.

75. Cunningham-Rundles S, Ahrné S, Johann-Liang R, et al. Effect of probiotic bacteria on microbial host defense, growth, and immune function in human immunodeficiency virus type-1 infection. Nutrients. 2011;3:1042-70.

76. Hummelen R, Hemsworth J, Changalucha J, et al. Effect of micronutrient and probiotic fortified yogurt on immune-function of antiretroviral therapy naive HIV patients. Nutrients. 2011;3:897-909.

77. Hummelen R, Changalucha J, Butamanya NL et al. Effect of 25 weeks probiotic supplementation on immune function of HIV patients. Gut Microbes. 2011;2:80-5.

78. Reid G. The potential role for probiotic yogurt for people living with HIV/AIDS. 2010;1:411-4.

79. Hummelen R, Vos AP, van't Land B, et al. Altered host-microbe interaction in HIV: a target for intervention with pro- and prebiotics. Int Rev Immunol. 2010;29:485-513.

80. Hummelen R, Hemsworth J, Reid G. Micronutrients, N-acetyl cysteine, probiotics and prebiotics, a review of effectiveness in reducing HIV progression. Nutrients. 2010;2:626-51.

81. Irvine SL, Hummelen R, Hekmat S, et al. Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS. J Clin Gastroenterol. 2010;44:e201-5.

82. Wolf BW, Wheeler KB, Ataya DG, Garleb KA. Safety and tolerance of Lactobacillus reuteri supplementation to a population infected with the human immunodeficiency virus. Food Chem Toxicol. 1998;36(12):1085-94.

83. Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis. 2015;60 Suppl 2:S129-34.

84. Salminen MK, Tynkkynen S, Rautelin H, Saxelin M, Vaara M, Ruutu P, Sarna S, Valtonen V, Järvinen A. Lactobacillus bacteremia during a rapid increase in probiotic use of Lactobacillus rhamnosus GG in Finland. Clin Infect Dis. 2002;35(10):1155-60.

85. Sullivan A, Nord CE. Probiotic lactobacilli and bacteraemia in Stockholm. Scand J Infect Dis. 2006;38(5):327-31.

86. López-Cepero AA, Palacios C. Association of the Intestinal Microbiota and Obesity. P R Health Sci J. 2015;34(2):60-4.

87. Koleva PT, Bridgman SL, Kozyrskyj AL. The infant gut microbiome: evidence for obesity risk and dietary intervention. Nutrients. 2015;7(4):2237-60.

88. Cénit MC, Matzaraki V, Tigchelaar EF, Zhernakova A. Rapidly expanding knowledge on the role of the gut microbiome in health and disease. Biochim Biophys Acta. 2014;1842(10):1981-1992.

89. Hunt PW, Sinclair E, Rodriguez B, et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. J Infect Dis. 2014;15;210:1228-38.

90. da Silva B, Singer W, Fong IW, et al. In vivo cytokine and neuroendocrine responses to endotoxin in human immunodeficiency virus-infected subjects. J Infect Dis. 1999;180:106-115.

91. Latz E, Visintin A, Lien E, et al. Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the Toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. J Biol Chem. 2002;277:47834-47843.

92. Merlini E, Bai F, Bellistri GM, et al. Evidence for polymicrobic flora translocating in peripheral blood of HIV-infected patients with poor immune response to antiretroviral therapy. PLoS ONE [Electronic Resource]. 2011;6:e18580.

93. Lederman MM, Calabrese L, Funderburg NT, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. J Infect Dis. 2011;204:1217-1226.

94. Massanella M, Negredo E, Perez-Alvarez N, et al. CD4 T-cell hyperactivation and susceptibility to cell death determine poor CD4 T-cell recovery during suppressive HAART. AIDS. 2010;24:959-968.

95. Miele E, Pascarella F, Giannetti E, Quaglietta L, Baldassano RN, Staiano A. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. Am J Gastroenterol. 2009;104(2):437-43.

96. Jonkers D, Penders J, Masclee A, Pierik M. Probiotics in the management of inflammatory bowel disease: a systematic review of intervention studies in adult patients. Drugs. 2012;72(6):803-23.

97. Ng SC, Lam EF, Lam TT, Chan Y, Law W, Tse PC, Kamm MA, Sung JJ, Chan FK, Wu JC. Effect of probiotic bacteria on the intestinal microbiota in irritable bowel syndrome. J Gastroenterol Hepatol. 2013;28(10):1624-31.

98. Hunt PW, Lederman MM, Deeks SG. Response: Maraviroc intensification and microbial translocation. Blood. 2013;122(13):2283-4.

SUBSTUDY A5352s

Effects of the Probiotic Visbiome Extra Strength on Epithelial Barrier Function and Inflammation in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Substudy of A5350

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

Sponsored by:

The National Institute of Allergy and Infectious Diseases

Industry Support Provided by:

Exegi Pharma, LLC

Non-IND Protocol

The ACTG End-Organ Disease/Inflammation Transformative Science Group:	**Peter W. Hunt, MD, Chair**
Protocol Co-Chairs:	**Rachel Presti, MD, PhD** **Brett Williams, MD**
Protocol Vice-Chair	**Adriana Andrade, MD, MPH**
DAIDS Clinical Representative:	**Karin L. Klingman, MD**
Clinical Trials Specialist:	**Linda Boone, BS**

Final Version 1.0
January 5, 2016

PROTOCOL TEAM ROSTER

Co-Chairs
Rachel Presti, MD, PhD
Infectious Disease
Washington U CRS
P.O. Box 8051
660 South Euclid Avenue
St. Louis. MO 63110
Phone: 314-286-0345
Fax: 314-510-3374
E-mail:rpresti@dom.wustl.edu

Brett Williams, MD
Rush University CRS
600 South Paulina Street Suite 140
Chicago, IL 60612
Phone: 312-942-2292
E-mail: brett_williams@rush.edu

Vice-Chair
Adriana Andrade, MD, MPH
Division of Infectious Diseases
Johns Hopkins Adult AIDS CRS
1830 East Monument Street, Suite 8074
Baltimore, MD 21205
Phone: 410-614-4036
Fax: 410-614-9978
E-mail: aandrade@jhmi.edu

DAIDS Clinical Representative
Karin L. Klingman, MD
HIV Research Branch
TRP, DAIDS, NIAID, NIH
5601 Fishers Lane, Rm 9E40A, MSC 9830
Bethesda, MD 20892-9830
Phone: 240-627-3067
E-mail: kklingman@niaid.nih.gov

Clinical Trials Specialist
Linda Boone, BS
ACTG Network Coordinating Center
Social & Scientific Systems, Inc.
8757 Georgia Avenue, 12th Floor
Silver Spring, MD 20910-3714
Phone:301-628-3363
Fax: 301-628-3302
E-mail: lboone@s-3.com

Statistician
Douglas Kitch, MS
Harvard T.H. Chan School of Public Health
651 Huntington Avenue
FXB Building, Room 504
Boston, MA 02115-6017
Phone: 617-432-3281
Fax: 617-432-3163
E-mail: dkitch@sdac.harvard.edu

Data Manager
David Nichols, BS
Frontier Science & Technology Research
Foundation, Inc.
4033 Maple Road
Amherst, NY 14226-1056
Phone: 716-834-0900 Ext. 7359
Fax: 716-834-8432
E-mail: nichols@fstrf.org

Immunologists
Jason M. Brenchley, MA, PhD
NIAID, NIH
4 Memorial Drive
Room 201
9000 Rockville Pike
Bethesda, MD 20892
Phone: 301-196-1498
E-mail: jbrenchl@mail.nih.gov

Alan Landay, PhD
Immunology/Microbiology
Rush University Medical Center
1725 West Harrison Street, Suite 306 POB1
Chicago, IL 60612
Phone: 312-942-6554
E-mail: alanday@rush.edu

Cara C. Wilson, MD
Medicine - Infectious Diseases
University of Colorado Hospital CRS
Mail Stop #B168
Building P15 Research 2, Room #11011
P.O. Box 6511
12700 East 19th Avenue
Aurora, CO 80045
Phone: 303-724-4601
Fax: 303-724-4926
E-mail: cara.wilson@ucdenver.edu

Virologist
Robert W. Coombs, MD, PhD
University of Washington Retrovirology Laboratory
University of Washington AIDS CRS
Research and Training Building, 7th Floor
325 9th Avenue
Seattle, WA 98104-2420
Phone: 206-897-5205
Fax: 206-897-5203
E-mail: bcoombs@u.washington.edu

Investigators
F. Parker Hudson, MD
University of North Carolina Global HIV Prevention and Treatment CTU
130 Mason Farm Road
CB #7030
Chapel Hill, NC 27599
Phone: 919-966-6714
Fax: 919-966-6714
E-mail: fphudson@email.unc.edu

Jeffrey M. Jacobson, MD
Division of Infectious Diseases Clinical Research Center
Drexel University CRS
245 North 15th Street, MS461
Room 6302, New College Building
Philadelphia, PA 19102
Phone: 215-762-6555
Fax: 267-507-6927
E-mail: jeffrey.jacobson@drexelmed.edu

Edgar Turner Overton, MD
Alabama CRS
908 20th St South
CCB 330A
Birmingham, AL 35294
Phone: 205-934-5191
Fax: 205-975-6027
E-mail: toverton@uab.edu

Investigators (Cont'd)
Netanya Sandler Utay, MD
Division of Infectious Diseases
Galveston Department of Internal Medicine
University of Texas Medical Branch
301 University Blvd, Rte. 0435
Galveston, TX 77555-0435
Phone: 409-747-0240
E-mail: neutay@utmb.edu

Kevin E. Yarasheski, PhD
Division of Endocrinology/Metabolism
Washington University School of Medicine
660 South Euclid Ave, BOX 8127
St. Louis, MO 63110
Phone: 314-362-8173
Fax: 314-362-8188
E-mail: key@wustl.edu

Field Representative
Suzanne Fiorillo, MSPH
University of Colorado Hospital CRS
Academic Office 1, MS 8205
12631 East 17th Avenue
P.O. Box 6511
Aurora, CO 80045
Phone: 303-724-5931
Fax: 303-724-0802
E-mail: suzanne.fiorillo@ucdenver.edu

Laboratory Technologist
Amy James Loftis, BS
University of North Carolina at Chapel Hill School of Medicine
UNC AIDS CRS
710 Mary Ellen Jones Building
116 Manning Drive
Chapel Hill, NC 27599
Phone: 919-966-6963
Fax: 919-966-9872
E-mail: amy_james@med.unc.edu

Community Scientific Subcommittee (CSS) Representative
Angel L. Hernandez, BA
Puerto Rico-AIDS CRS
Barrio Saltos
Road 566 Int Road 593, KM 0.2
Orocovis, Puerto Rico 00720
Phone: 787-919-6145
E-mail: angelhdz2863@gmail.com

Industry Representative
Marc Tewey, MBA
Exegi Pharma, LLC
312 Main St, Suite 200
Gaithersburg, MD 20898
Phone: 240-888-1294
E-mail: marc.tewey@exegipharma.com

Laboratory Data Manager
Sarah Strobino
Frontier Science & Technology Research Foundation, Inc.
4033 Maple Rd.
Amherst, NY 14226
Phone: 716-834-0900
E-mail: strobino@fstrf.org

STUDY MANAGEMENT

All questions concerning this protocol should be sent to actg.teamA5352s@fstrf.org via e-mail. The appropriate team member will respond with a "cc" to actg.teamA5352s@fstrf.org. A response should generally be received within 24 hours (Monday-Friday).

Protocol E-mail Group
Sites should contact the Computer Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the actg.protA5352s e-mail group. Include the protocol number in the e-mail subject line.

- Send an e-mail message to actg.user.support@fstrf.org

Clinical Management
For questions concerning entry criteria, toxicity management, concomitant medications, and co-enrollment, contact the protocol team.

- Send an e-mail message to actg.teamA5352s@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory
For questions specifically related to immunologic or virologic laboratory tests, contact the protocol Immunologist or Virologist.

- Send an e-mail message to actg.teamA5352s@fstrf.org (ATTN: Jason Brenchley or Alan Landay [immunologists] or Robert Coombs [Virologist]).

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, case report forms (CRF), the CRF schedule of events, randomization/registration, delinquencies, and other data management issues, contact the Data Manager. CRFs can be downloaded from the FSTRF website at www.fstrf.org.
- For transfers, reference the Patient Transfer from Site to Site SOP 119, and contact David Nichols directly.
- For other questions, send an e-mail message to actg.teamA5352s@fstrf.org (ATTN: David Nichols).
- Include the protocol number, PID, and a detailed question.

Participant Registration
For participant registration questions or problems and study identification number SID lists.

- Send an e-mail message to rando.support@fstrf.org. Call the Statistical and Data Analysis Center (SDAC)/DMC Randomization Desk at 716-834-0900 extension 7301.

Computer and Screen Problems
Contact the SDAC/DMC programmers.

- Send an e-mail message to actg.support@fstrf.org or call 716-834-0900 x7302.

Protocol Document Questions
For questions concerning the protocol document, contact the Clinical Trials Specialist. Send an e-mail message to actg.teamA5352s@fstrf.org (ATTN: Linda Boone).

Copies of the Protocol
To request a hard copy of the protocol, send a message to ACTGNCC@s-3.com (ATTN: Diane Delgado) via e-mail. Electronic copies can be downloaded from the ACTG Web site (https://www.actgnetwork.org).

Protocol Registration
For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

Protocol Activation
For questions related to protocol activation, contact the Clinical Trials Specialist Linda Boone at lboone@s-3.com or ACTG Site Coordination group at actgsitecoordination@s-3.com.

Expedited Adverse Event (EAE) Reporting/Questions
Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Phone Calls
Sites are responsible for documenting any phone calls made to A5352s team members.
- Send an e-mail to actg.teamA5352s@fstrf.org.

Protocol-Specific Web Page
Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

ART	antiretroviral therapy
CLIA	Clinical Laboratory Improvement Amendments
COX-2	cyclooxygenase-2
CVD	cardiovascular disease
DSS	dextran sulfate sodium
FXR	farnesoid X receptor
GALT	gut-associated lymphoid tissue
GLP-1	glucagon-like peptide-1
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment
hsCRP	high-sensitivity c-reactive protein
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
ICAM-1	intercellular adhesion molecule 1
IFNα	interferon alpha
IFNγ	interferon gamma
IHC	immunohistochemistry
IL-6	interleukin 6
IL-10	interleukin 10
MIP-1α	macrophage inflammatory protein 1 alpha
iNOS	inducible nitric oxide synthase
LBP	lipopolysaccharide-binding protein
LDL	low-density lipoprotein cholesterol
LMR	lactulose/mannitol ratio
LPS	lipopolysaccharides
MPO	myeloperoxidase
NASH	nonalcoholic steatohepatitis
OI	opportunistic infection
POC	point of care testing
PPARγ	peroxisome proliferator-activated receptor gamma
RANTES	regulated on activation, normal T-cell expressed and secreted
RCT	randomized controlled trial
sCD14	soluble CD14
sCD163	soluble CD163
Th1	t helper cell 1
TLR-2	toll-like receptor 2
TLR-4	toll-like receptor 4
TMA	trimethylamine
TMAO	trimethylamine N-oxide
TNFα	tumor necrosis factor alpha
UC	ulcerative colitis
VDR	vitamin D receptor

SCHEMA

A5352s
Effects of the Probiotic Visbiome Extra Strength on Epithelial Barrier Function and Inflammation in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Substudy of A5350

DESIGN	A5352s is a substudy of A5350 to evaluate whether there is a significant change in intestinal permeability and mucosal inflammation after 24 weeks of probiotic Visbiome Extra Strength therapy.
DURATION	Participants will be on the substudy for 26 weeks.
SAMPLE SIZE	40 participants: 20 participants from Arm A (Visbiome Extra Strength) and 20 participants from Arm B (placebo).
POPULATION	A5350 participants who commit to undergo two flexible sigmoidoscopies and biopsies and who have no contraindications for sigmoidoscopy.
INTERVENTION	Participants will undergo a flexible sigmoidoscopy as part of the entry visit, prior to starting Visbiome Extra Strength treatment. A second sigmoidoscopy will be performed at week 24. Participants will also undergo two lactulose/mannitol tests to evaluate change in gut permeability.

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Primary Hypothesis

After administration of Visbiome Extra Strength, CD4+ T-cells in the colon will increase.

1.2 Secondary Hypotheses

1.2.1 After administration of Visbiome Extra Strength, CD8+ T-cells in the colon will increase.

1.2.2 After the administration of Visbiome Extra Strength, neutrophil infiltration in the colon will decrease.

1.2.3 After administration of Visbiome Extra Strength, increased numbers of CD4+ T-cells in the colon will express the cytokine IL-17.

1.2.4 After administration of Visbiome Extra Strength, there will be a reduction in microbial translocation.

1.2.5 After administration of Visbiome Extra Strength, markers of gut permeability as measured by the Lactulose/Mannitol test will improve.

1.2.6 Visbiome Extra Strength administration will increase gastrointestinal microbial diversity from colonic mucosa samples.

1.2.7 Visbiome Extra Strength administration will change mucosal microbial metabolism of phosphatidylcholine, resulting in decreased trimethylamine (TMA) and trimethylamine N-oxide (TMAO).

1.3 Primary Objective

To assess total numbers of CD4+ T-cells per area by immunohistochemistry (IHC) from colonic tissue biopsies after Visbiome Extra Strength administration.

1.4 Secondary Objectives

1.4.1 To assess total numbers of CD8+ T-cells per area by IHC from colonic tissue biopsies after Visbiome Extra Strength administration.

1.4.2 To assess changes in neutrophil infiltration quantified by myeloperoxidase staining per area in colonic mucosa by IHC after Visbiome Extra Strength administration.

1.4.3 To assess changes in expression of cytokines IL17 by CD4+ T-cells per area by IHC after Visbiome Extra Strength administration.

1.4.4 To assess changes in microbial translocation by enumerating bacterial products (LPS and 16sRNA) per area within the lamina propria by IHC after Visbiome Extra Strength administration.

1.4.5 To assess measures of gut permeability after administration of the probiotic with the Lactulose/Mannitol Test after Visbiome Extra Strength administration.

1.4.6 To assess change in microbial diversity via 16S rRNA sequencing from the colonic mucosal samples after Visbiome Extra Strength administration.

1.4.7 To assess changes in mucosal microbial metabolism of phosphatidylcholine, resulting in decreased TMA and TMAO after Visbiome Extra Strength administration.

1.5 Exploratory Objectives

1.5.1 To assess changes in mucosal microbial metabolism by metabolomics and transcriptional analysis.

2.0 INTRODUCTION

2.1 Background

HIV-related intestinal dysbiosis contributes to CD4+ T-cell depletion, and chronic inflammation [1-3]. While the depletion of CD4+ T-cells occurs in the peripheral blood, it is most profound and persistent in the gut-associated lymphoid tissue (GALT). In nonhuman primate models, 90% of CD4+ T-cells are depleted from the GALT within 2 weeks of acute simian immunodeficiency virus (SIV) infection [4]. Similar findings have been reported with HIV infection [5]. Notably, ART initiation leads to incomplete reconstitution of intestinal mucosal CD4+ cells [6-9]. This failure to reconstitute the GALT leaves an individual susceptible to invasion of bacterial pathogens, enhanced local innate immune cell activation, and microbial translocation with consequent persistent systemic inflammation [10]. The study by Mehandru et al is particularly informative [7]. The research team identified persons with acute or early HIV infection and demonstrated the massive depletion of mucosal CD4+ T-cells early in infection. Even after 1-7 years of ART, 70% of HIV-infected individuals maintained a 50-60% depletion of mucosal lymphocytes despite complete viral suppression. The table below demonstrates the limited reconstitution of mucosal CD4 T-cells in different groups of individuals based on duration of ART treatment:

Participant Group	HIV Treatment	PBMC CD4%	MMC CD4%
HIV negative (n=18)	NA	59.6% ± 14.3%	56.4% ± 8.8%
HIV infected (n=32)	Before treatment	41.5% ± 12.9%	19.3% ± 8.8%
HIV infected (n=7)	After ~1 year of ART	46.9% ± 10.1%	27.8% ± 14.5%
HIV infected (n=7)	After 3-7 years ART	58.1% ± 12.3%	42.3% ± 3.1%

These data indicate that ART alone will not reconstitute the mucosal CD4+ lymphocytes and that the identification of adjunctive therapies that restore these cells may have the potential to also restore mucosal immune health in HIV-infected persons.

Notably, it is not only the dysbiosis that contributes to persistent inflammation, but also the downstream subsequent effects on gut permeability that likely facilitates excess bacterial translocation and heightened systemic inflammation. For example, Chung et al recently demonstrated that intestinal epithelial damage, manifested by decreased colonic epithelial tight junction proteins enhances colonic permeability and thus, microbial translocation despite suppressive ART [11]. Several studies have demonstrated a shift from Bacteroidales dominant microbiome to more Prevotella in HIV-infected individuals, potentially leading to a decrease in production of short chain fatty acids such as butyrate [12-15]. Butyrate appears to be important as both an energy source for colonic epithelial cells and also as a downregulator of inflammation through inhibition of histone deacetylase and activation of G-protein coupled receptors [16, 17]. One potential alternative to intestinal antibiotics would be to utilize probiotics to repair intestinal damage through increased production of butyrate to improve mucosal immune function and to decrease microbial translocation. Indeed, topical butyrate has been demonstrated to improve outcomes in humans with refractory UC and rats with sulfonic acid induced colitis [18, 19]. Visbiome in particular has been demonstrated to increase levels of butyrate in mice [20].

In A5350, we propose an innovative intervention to reduce the chronic immune activation and inflammation by reversing the dysbiosis seen with HIV infection, by Visbiome Extra Strength administration. In this substudy (A5352s), we will perform flexible sigmoidoscopies before and after Visbiome Extra Strength administration to assess for changes in the gut mucosa and GALT. We hypothesized that administration of this probiotic will facilitate reconstitution of a healthy, diverse microbiome and have significant potential for repairing mucosal integrity and immune function via reconstitution of mucosal CD4+ T-cells and APCs, repair of intestinal damage, and improved epithelial tight junctions. The functionality of these histochemical changes will be tested via two methods. First, we will administer non-absorbed sugars to determine the level of gut permeability via the Lactulose/Mannitol test. Second, we will assess for recovery of CD4+ and CD8+ T-cells and reduction in mucosal inflammation via colonic biopsies. Identifying whether this approach restores a healthy microbiome in HIV-infected persons and positively impacts mucosal function to reduce chronic immune activation will greatly enhance our understanding of the interface between the microbiota, the immune system, and overall health.

2.2 Rationale

We propose to conduct a phase II, randomized two-arm substudy with Visbiome Extra Strength and matching placebo in HIV-infected participants on stable ART with CD4+ count >200 cells/mm^3 and plasma HIV RNA <50 copies/mL, to better understand the effects of this probiotic product on colonic mucosal histopathology, immune function, and barrier function. This is a substudy of A5350, in which participants will undergo sigmoidoscopy to obtain colonic biopsy tissue, as well as additional testing of mucosal barrier function.

3.0 STUDY DESIGN

A5352s is a substudy of A5350 that will enroll HIV-infected participants ≥18 years of age, who have been on stable ART for at least 24 weeks prior to study (A5350) entry, and have a CD4+ T-cell >200 cells/mm^3 prior to study entry and plasma HIV RNA <50 copies/mL for 48 weeks prior to study entry.

The primary objective of the substudy is to determine whether there is a significant change in colonic CD4+ T-cell count per area after administration of probiotic Visbiome Extra Strength therapy.

A total of 40 participants from A5350 will be included, 20 from Arm A (Visbiome Extra Strength + stable ART; treatment arm) and 20 from Arm B (placebo for Visbiome Extra Strength + stable ART; control arm). Participants will be on the substudy for a total of 26 weeks. Probiotic Visbiome Extra Strength or placebo will be administered from week 2 through week 26. Colonic biopsies collected by flexible sigmoidoscopy will be obtained per the SOE to assess tissue-specific effects related to immunologic outcomes, inflammation, bacterial translocation, and gut integrity. Any incidental clinical findings will be followed up at the time of the procedure if possible (eg, removal of incidental polyps or biopsy of abnormal lesions). All abnormal findings will be referred for further appropriate care under the direction of the site gastroenterologist. Participants will also undergo two Lactulose/Mannitol tests to evaluate gut permeability. Further details about these procedures are included in the study MOPS.

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

4.1.1 Enrollment in A5350

4.1.2 The following laboratory values obtained within 45 days prior to entry by any US laboratory that has a CLIA certification or its equivalent:

- Platelet count ≥100,000/mm^3
- INR ≤1.3
- Partial thromboplastin time (PTT) <2x upper limit of normal (ULN)

4.1.3 Ability and willingness of participant or legal guardian/representative to provide informed consent.

4.2 Exclusion Criteria

4.2.1 Diagnosis with gastrointestinal disease such as inflammatory bowel disease or irritable bowel syndrome.

4.2.2 Bleeding diathesis or requirement for anticoagulation that would increase the risk of sigmoidoscopy and biopsy.

4.2.3 History of anal, rectal, or colon cancer.

4.2.4 Any prior surgical procedure requiring repair, excision, or bypass of the intestinal tract.

NOTE: Surgery for excision of condyloma is allowed.

4.2.5 Use of clopidrogel (Plavix), warfarin (Coumadin), or other blood thinners that cannot be stopped for clinical reasons for 5 days before and 5 days after the sigmoidoscopy with colonic biopsies.

4.2.6 Use of aspirin and /or nonsteroidal anti-inflammatory drugs (NSAIDs) that cannot be stopped for clinical reasons for a minimum of 7 days before and after the sigmoidoscopy with colonic biopsies, and at least 7 days before the lactulose/mannitol gut permeability test.

4.3 Substudy Enrollment Procedures

As specified in the main study, A5350. To optimize expeditious enrollment in the substudy, one of every three participants at an individual site enrolled in the parent study must also agree to participate in the substudy.

5.0 SUBSTUDY TREATMENT

There will be no study treatment provided by A5352s. Participants will continue to take their randomized A5350 regimens. See section 5.0 of A5350 for study product requirements.

5.1 Prohibited Medications

Use of the following medications during the substudy are prohibited:

- Aspirin, antiplatelet agents, NSAIDs which cannot be held as above for flexible sigmoidoscopy and lactulose/mannitol gut permeability test.
- Medications as specified in the main study, A5350.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events

Evaluation	Screening (Within 45 days prior to substudy entry)	Entry	Week 1 (±4 days)	Post-Entry	
				On Treatment	
				Week 24 (at least 14 days prior to Week 26) (±7 days)	Week 26 (±7 days)
Hematology	X			X	
Urine for Gut Permeability Assessment by LMR			X		X
Sigmoidoscopy with Colonic Biopsies		X		X	

6.2 Timing of Evaluations

6.2.1 Screening and Pre-Treatment Evaluations

Screening
Screening evaluations to determine eligibility must be completed within 45 days prior to substudy entry unless otherwise specified.

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

Entry
Entry evaluations must be completed at least 24 hours after screening evaluations.

NOTE: At the time of a sigmoidoscopy visit, the participant must avoid drinking alcohol or taking nonsteroidal anti-inflammatory medications, including aspirin or any blood thinners for 7 days prior to the test.

Week 1
Week 1 evaluations must be completed at least 4 days after entry evaluations unless otherwise specified.

NOTE: At the time of a sugar absorption test visit, the participant must have avoided drinking alcohol for 3 days prior to the test, avoided taking nonsteroidal

anti-inflammatory medications, including aspirin or any blood thinners for 7 days prior to the test, and have abstained from exercise the day before and the morning of the test.

6.2.2 Post-Entry Evaluations

On-Treatment Evaluations
All on-treatment evaluations must be scheduled as per section 6.1 with a ±7 day window.

6.2.3 Discontinuation Evaluations

Evaluations for Registered Participants Who Do Not Start Study Intervention
All CRFs must be completed and keyed for the period up to and including week 1.

No follow-up evaluations are required for registered participants who do not have the first biopsy.

Premature Discontinuation Evaluations
Participants who prematurely discontinue the substudy (ie, elect not to have the second biopsy) will be taken off the substudy. No further substudy evaluations are required.

Site personnel should notify the protocol core team via e-mail (actg.corea5352s@fstrf.org) within 48 hours after a participant indicates his/her intention to prematurely discontinue the substudy.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS Web site for information about what must be included in the source document: http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/sourcedocappndx.pdf

6.3.1 Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease having been absent at baseline, or, if present at baseline, appears to worsen AND is temporally associated with medical treatment/study product/device or procedure, REGARDLESS of the attribution (ie, relationship of event to medical treatment/ study product/device or procedure).

To grade diagnoses, signs and symptoms, and laboratory results, sites must refer to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014, which can be

found on the DAIDS RSC Web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

6.3.1.1 SAEs are adverse events that result in one or more of the following outcomes:

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

NOTE: SAEs should be entered into the AE log and into the DAERS system as indicated.

6.3.1.2 Any adverse event related to the sigmoidoscopy with biopsies or the Lactulose/Mannitol testing must be reported regardless of grade as an AE on the A5350 AE log.

6.3.2 Laboratory Evaluations

At screening and week 24, all hematology laboratory values are recorded in the source document only.

Hematology
INR and PT/PTT

6.3.3 Urine for Gut Permeability Assessment by LMR

Urine will be collected for 5 hours after administration of a standard oral solution containing lactulose and mannitol. Gut permeability assessment will be performed at least 7 days after flexible sigmoidoscopy. Additional details about the lactulose/mannitol test are included in the study MOPS.

6.3.4 Sigmoidoscopy with Colonic Biopsies

Colonic biopsies collected by flexible sigmoidoscopy will be obtained per the SOE to assess tissue-specific effects related to immunologic outcomes, inflammation, bacterial translocation, and gut integrity. Immunohistochemistry will be used to assess the total numbers of CD4+ and CD8+ T-cells, neutrophil infiltration, IL-17 expression, and markers of microbial translocation. Additional details about the flexible sigmoidoscopy procedure and colonic biopsy are included in the study MOPS.

Colonic biopsy specimens will be stored for possible future genetic testing.

6.3.5 Immunologic Assays on Colonic Biopsy Specimens

Colonic biopsy specimens will be collected per section 6.1 and evaluated for:

- Total numbers of CD4+ and CD8+ T-cells
- Neutrophil infiltration
- IL-7 expression
- Markers of microbial translocation

Colonic biopsy specimens will be processed and stored according to the LPC.

7.0 CLINICAL MANAGEMENT ISSUES

7.1 Sigmoidoscopy and Colonic Biopsies

On extremely rare occasions, a sigmoidoscopy may cause pain, infection, bleeding, or perforation of the GI tract (occurs about once out of every 1000 procedures and may require hospitalization and surgical management).

Any incidental clinical findings will be followed up at the time of the procedure if possible (eg, removal of incidental polyps or biopsy of abnormal lesions). All abnormal findings will be referred for further appropriate care under the direction of the site gastroenterologist.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Permanent and Premature Discontinuation

- Procedure-related complication, that in the opinion of the local investigator, prohibits the participant from continuing in the substudy (see section 7.1)
- Discontinuation of ART on A5350
- At the discretion of the IRB, NIAID, Office for Human Research Protections (OHRP), or other government agencies as part of their duties
- Request by participant to terminate the substudy
- Clinical reasons believed life-threatening by the physician, even if not addressed in the toxicity section of the protocol

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

A5352s is a substudy of A5350 that will evaluate administration of Visbiome Extra Strength therapy compared to placebo on change in rectal lamina propria CD4+ T-cell

numbers among HIV-infected participants ≥18 years of age who have been on stable ART for at least 24 weeks prior to study entry, and have CD4+ T-cell >200 cells/mm3 prior to study entry and plasma HIV RNA <50 copies/mL for 48 weeks prior to study entry. All participants will be enrolled in A5350 where they will be randomized to either Arm A (Visbiome Extra Strength + stable ART; treatment arm) or Arm B (placebo for Visbiome Extra Strength + stable ART; control arm). Participants will be on the substudy for a total of 26 weeks. Probiotic therapy will be administered from week 2 to week 26. Colonic biopsies collected by flexible sigmoidoscopy will be obtained at entry and week 24 to assess tissue-specific effects related to immunologic outcomes, inflammation, bacterial translocation, and gut integrity. The lactulose/mannitol test will be administered at visits week 1 and week 26. The total sample size will be 40 participants (20 in Arm A, 20 in Arm B).

9.2 Outcome Measures

9.2.1 Primary Outcome Measure

Change from entry to week 24 in CD4+ T-cell count/area by IHC from colonic tissue biopsies.

9.2.2 Secondary Outcome Measures

9.2.2.1 Change from entry to week 24 in CD8+ T-cell count/area by IHC from colonic tissues biopsies.

9.2.2.2 Change from entry to week 24 in neutrophil infiltration quantified by myeloperoxidase staining/area in colonic mucosa by IHC.

9.2.2.3 Change from entry to week 24 in expression of cytokines IL17 by CD4+ T-cells/area by IHC.

9.2.2.4 Change from entry to week 24 in microbial translocation by enumerating bacterial products (LPS and 16sRNA)/area within the lamina propria by IHC.

9.2.2.5 Change from week 1 to week 26 in measures of gut permeability after administration of the probiotic with the Lactulose/Mannitol Test.

9.2.2.6 Change from entry to week 24 in microbial diversity via 16s rRNA sequencing from the colonic mucosal samples after Visbiome Extra Strength administration.

9.2.2.7 Change from entry to week 24 in mucosal microbial metabolism of phophatidylcholide as measured by TMA and TMAO.

9.2.3 Exploratory Outcome Measure

Change from entry to week 26 in mucosal microbial metabolism by metabolomics and transcriptional analysis.

9.3 Randomization and Stratification

Treatment regimens will be assigned in A5350.

9.4 Sample Size and Accrual

The primary objective of this substudy is to compare change in total numbers of CD4+ + T-cells per area in colonic tissue from entry to week 24 in participants on Visbiome Extra Strength compared to placebo. The sample size calculations are based on the primary hypothesis testing H_o: $\mu_{VisbiomeExtraStrength} = \mu_{placebo}$ targeting 90% power in the comparison of changes in total numbers of CD4+ T-cells per area between Visbiome Extra Strength and placebo.

Our estimate of the standard deviation for the change in CD4+ frequency in colonic tissue over 24 weeks was derived from Mehandru et al [7]. Cross sectional CD4+ frequency for various populations (HIV negative, HIV-infected ART-naïve, HIV-infected 1 year of ART, HIV-infected 3-7 years of ART) had standard deviations of 8.8% (n=18), 8.8% (n=32), 14.5% (n=7), and 3.1% (n=7), respectively. Due the large range of standard deviations, we conservatively used the median value (11.6%) for sample size calculations. Given that we are performing repeated measurements and evaluating a change in CD4+ T-cells rather than a cross sectional evaluation, the standard deviation of change was conservatively estimated to be 16.4% (√(〚11.6〛^2+ 〚11.6〛^2)).

A total sample size of 40 participants (20 per arm) is needed to detect a difference of 20.1% in CD4+ T-cells between Visbiome Extra Strength and placebo with 90% power and a 0.05 two-sided type I error rate, under the assumption that the SD of the changes in CD4+ T-cells from entry to week 24 is 16.4% in both arms and accounting for ~20% missing endpoints. Under the same assumptions, 40 participants will provide 80% power to detect a difference of 17.4% between the two arms.

The following table summarizes the calculation of the sample sizes corresponding to the detection of various effect sizes under various assumed standard deviations, with the effect size, standard deviation, and adjusted sample size selected for this substudy shown in bold.

Effect size in CD4+ T-cells (difference between Visbiome Extra Strength and placebo)	Std Dev of Δ entry to week 24	Total Sample size (90% power, alpha=0.05)	Adjusted Sample Size (20% inflation)
18.1	13.4	14*2=28	18*2=36
	16.4	20*2=40	25*2=50

Effect size in CD4+ T-cells (difference between Visbiome Extra Strength and placebo)	Std Dev of Δ entry to week 24	Total Sample size (90% power, alpha=0.05)	Adjusted Sample Size (20% inflation)
	19.4	28*2=56	35*2=70
20.1	13.4	12*2=24	15*2=30
	16.4	**16*2=32**	**20*2=40**
	19.4	22*2=44	28*2=56
22.1	13.4	10*2=20	13*2=26
	16.4	14*2=28	18*2=36
	19.4	19*2=38	24*2=48

The effect size of 20.1% is thought to be clinically meaningful based on available data. Compared to HIV negative subjects (56.4%), Mehandru et al [7] found decreased CD4+ frequency for HIV-infected naïve (19.3%), HIV-infected 1 year of ART (27.8%), and HIV-infected 3-7 years of ART (42.3%) populations [7]. In the SIV-macaques study, Klatt et al [56] found that the probiotic/prebiotic + ARV group had 46.13% CD4+ T-cells in the colon compared to 26.12% in the ARV alone group.

For each continuous secondary endpoint, change from entry to week 24 (and change from week 1 to week 26 for measures of gut permeability) will be compared between study arms. The following table summarizes the power to detect various effect sizes as well as the precision of the effect size estimate.

Comparison of 2 arms (16 vs. 16 evaluable participants)		
Effect size	Power	Width of 95% CI
1.18*SD	90%	± 0.72*SD
1.02*SD	80%	± 0.72*SD
0.91*SD	70%	± 0.72*SD

NOTE: If enrollment into the substudy is slow and proves to be prohibitive, we will alter the enrollment plan to a lower number of participants and perform analyses that are exploratory in nature.

9.5 Monitoring

Accrual, a summary of AEs related to biopsy, and sample/data availability will be reviewed monthly by the protocol core team. This summary will be pooled over the substudy arms. In addition, baseline characteristics as well as early substudy discontinuations (and reasons) will be reviewed regularly by the protocol core team.

An ACTG-appointed Study Monitoring Committee (SMC) will review accrual, biopsy-related adverse event summaries, and off-study rates and reasons, and sample/data

availability, broken down by study arm. The first SMC review will occur 6 months after the first participant is enrolled and then every 6 months as long as participants remain in follow-up. Note that evaluation of biopsies for outcome measures will occur after follow-up is concluded, and, therefore these data are not expected to be available at interim reviews. The SMC may also be convened if a reason is identified by the DAIDS clinical representative, study chairs, or study statistician in consultation with the team.

9.6 Analyses

All statistical tests will be two-sided with a nominal alpha level of 0.05. Because this is a phase II substudy and all potential biologic activities of the intervention are of interest, analyses will be as-treated, and limited to participants who 1) have entry and week 24 biopsies, 2) remain on study product and ART through week 24 (participants with more than 50% on average of missed study product may be excluded), 3) have not used prohibited medications, 4) do not have a confirmed virologic failure (as defined per A5350 section 6.2.3) at or prior to week 24, and 5) do not experience inflammatory conditions, receive vaccines, or have concurrent illness (as defined per A5350 section 6.3.7).

NOTE: Participants experiencing inflammatory conditions at the time of a visit are instructed to wait 7 days before measurements are obtained. Measurements obtained while inflammatory conditions are present will not be included in the analysis.

A supplemental intent-to-treat analysis will also be performed and will include all enrolled participants. No adjustment for multiple testing will be performed.

9.6.1 Primary Analysis

The primary objective of the substudy is to assess the effect of Visbiome Extra Strength on total numbers of CD4+ T-cells per area of colonic tissue in HIV-infected participants well-controlled on ART. To address this, changes in CD4+ T-cells from entry to week 24 will be compared between the Visbiome Extra Strength arm and the placebo arm by the Wilcoxon rank-sum test due to the small sample size.

9.6.2 Secondary Analyses

Changes in other outcome measures will be compared between the Visbiome Extra Strength and placebo arms by the Wilcoxon rank-sum test.

10.0 PHARMACOLOGY PLAN

Not applicable

11.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

11.1 Records to Be Kept

Case report forms (CRF) will be provided for each participant. Participants must not be identified by name on any CRFs. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon randomization/registration.

11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

11.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

11.3 Clinical Site Monitoring and Record Availability

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

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

11.4 Expedited Adverse Event Reporting to DAIDS

11.4.1 Adverse Event Reporting to DAIDS

See section 11.4.1 in the main study, A5350 for adverse event reporting to DAIDS.

11.4.2 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014, must be used and is available on the DAIDS RSC Web site at http://rsc.tech-res.com/safetyandpharmacovigilance/.

11.4.3 Expedited AE Reporting Period

See section 11.4.2 in the main study, A5350, for EAE reporting requirements.

12.0 PARTICIPANTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Appendix II) and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from the participant (or legal guardian, or person with power of attorney for participants who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant or legal guardian, and this fact will be documented in the participant's record.

12.2 Participant Confidentiality

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

12.3 Study Discontinuation

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

13.0 PUBLICATION OF RESEARCH FINDINGS

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

14.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and

handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

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

15.0 REFERENCES

1. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med. 2013;5:193ra91.

2. Mutlu EA, Keshavarzian A, Losurdo J, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog. 2014;10:e1003829.

3. Yu G, Fadrosh D, Ma B, et al. Anal microbiota profiles in HIV-positive and HIV-negative MSM. AIDS. 2013. [Epub ahead of print].

4. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature. 2005;434(7037):1093-7.

5. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12:1365-71.

6. Kotler DP, Gaetz HP, Lange M, Klein EB, Holt PR. Enteropathy associated with the acquired immunodeficiency syndrome. Ann Intern Med. 1984;101(4):421-8.

7. Mehandru S, Poles MA, Tenner-Racz K, Jean-Pierre P, Manuelli V, Lopez P, Shet A, Low A, Mohri H, Boden D, Racz P, Markowitz M. Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med. 2006;3(12):e484.

8. Costiniuk CT, Angel JB. Human immunodeficiency virus and the gastrointestinal immune system: does highly active antiretroviral therapy restore gut immunity? Mucosal Immunol. 2012;5(6):596-604.

9. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, Dandekar S. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol. 2003;77(21):11708-17. PMID:14557656

10. Klatt NR, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease. Trends Microbiol. 2013;21(1):6-13a.

11. Chung CY, Alden SL, Funderburg NT, Fu P, Levine AD. Progressive proximal-to-distal reduction in expression of the tight junction complex in colonic epithelium of virally-suppressed HIV+ individuals. PLoS Pathog 2014;10(6):e1004198.

12. Mutlu EA, Keshavarzian A, Losurdo J, Swanson G, Siewe B, Forsyth C, French A, Demarais P, Sun Y, Koenig L, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog 2014;10(2):e1003829.

13. Lozupone CA, Li M, Campbell TB, Flores SC, Linderman D, Gebert MJ, Knight R, Fontenot AP, Palmer BE. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe 2013;14(3):329-39.

14. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, Anton P, Braun J. HIVinfection is associated with compositional and functional shifts in the rectal mucosal microbiota. Microbiome 2013;1(1):26,2618-1-26.

15. Dillon SM, Lee EJ, Kotter CV, Austin GL, Dong Z, Hecht DK, Gianella S, Siewe B, Smith DM, Landay AL, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 2014;7(4):983-94.

16. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients 2011;3(10):858-76.

17. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci U S A 2014;111(6):2247-52.

18. Vernia P, Annese V, Bresci G, et al. Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: Results of a multicentre trial. Eur J Clin Invest 2003;33(3):244-8.

19. Song M, Xia B, Li J. Effects of topical treatment of sodium butyrate and 5-aminosalicylic acid on expression of trefoil factor 3, interleukin 1beta, and nuclear factor kappaB in trinitrobenzene sulphonic acid induced colitis in rats. Postgrad Med J 2006;82(964):130-5.

20. Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem 2013;288(35):25088-97.

APPENDIX I SAMPLE INFORMED CONSENT

DIVISION OF AIDS
AIDS CLINICAL TRIALS GROUP (ACTG)

For protocol: A5350

Safety, Tolerability and Effects of the Probiotic Visbiome Extra Strength on Gut Microbiome and Immune Activation Markers in HIV-infected Participants on Suppressive Antiretroviral Therapy: A Phase II Study

Final Version 1.0, 01/05/16

SHORT TITLE FOR THE STUDY: Visbiome Extra Strength, HIV, and the Gut

INTRODUCTION

You are being asked to take part in this research study because you are infected with the human immunodeficiency virus (HIV), the virus that causes AIDS, and because you are taking anti-HIV drugs that are controlling your HIV infection. This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: (insert name of Principal Investigator). Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

Since HIV-infected people started taking HIV medications, illness from AIDS has decreased, but other serious diseases like heart disease, cancer, kidney, and liver disease have increased. HIV causes inflammation (irritation) inside the body that cannot be felt but can be measured by blood tests. Inflammation can lead to diseases that have become some of the leading causes of death in people with HIV. HIV therapy can partly lower levels of inflammation measured in blood; however, levels of inflammation in people who have HIV may remain high compared with those found in people not infected with HIV, even when the virus level is not detected in blood tests (viral load). We can check the level of inflammation in the body by measuring certain proteins in the blood. In this study, we will measure several tests of inflammation, including one called “soluble CD14”. This test is increased with HIV infection and in diseases that alter normal intestinal health.

The purpose of the study that you are being asked to take part in is to evaluate whether the probiotic Visbiome Extra Strength reduces inflammation in HIV-infected men and women when

compared to a placebo (inactive medication like a dummy pill). The study will evaluate whether taking Visbiome Extra Strength by mouth for 24 weeks is safe and well-tolerated for HIV-infected persons on antiretroviral therapy (ART). Probiotics are germs such as yeast or bacteria that are found in food and supplements that are used to improve the health of your digestive system. Many people refer to probiotics as "helpful bacteria." These bacteria live in our body and help our bodies work normally. In some medical conditions, including HIV infection, helpful bacteria are replaced with bacteria that can change the normal intestinal function and increase inflammation. We will test whether giving a probiotic can restore normal intestinal function and decrease inflammation.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

Screening
If you agree to join this study, you will be asked to sign this consent form. Before signing it, ask your study nurse or doctor to explain anything that you do not fully understand. After you have signed the form, you will be asked some questions and will undergo some tests to see if it is safe for you to join the study (this visit is called the screening visit).

The evaluations performed at the screening visit are listed in Table 1 of this consent form.

You will receive the results of the HIV viral loads, CD4+ cell counts, hepatitis virus testing, routine safety blood tests, and pregnancy tests.

If you do not enroll into the study
If you decide not to take part in this study or if you do not meet the eligibility requirements, we will still use some of your information. As part of this screening visit, some demographic (for example, age, gender, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, CD4+ cell count, HIV viral load, hepatitis test results) information is being collected from you so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

You will be required to come to most visits fasting
- Fasting means that you should not eat or drink anything for at least 8 hours before your visit.
- You may drink only water and take your prescription medications during this time.
- The study staff will remind you about fasting before study visits.

Entry
If you have met all of the requirements to enter the study you will come to the clinic at least 24 hours after the screening evaluations for the entry evaluations. The evaluations performed at the entry visit are listed in Table 1 of this consent form.

Week 2
At this visit, you will begin taking the provided study product (Visbiome Extra Strength) or placebo (like a dummy pill). You will be randomized into the study by chance (like the flip of a coin) to one of two treatment groups:

Group 1: Visbiome Extra Strength

- 2-week lead-in period: 1 Visbiome Extra Strength sachet per day. (The sachet can be mixed into cold water or any cold, noncarbonated beverage and consumed).
- Then increase to 1 sachet twice daily for 22 weeks.
- Followed by 12 weeks off study product.

Group 2: Placebo for Visbiome Extra Strength

- 2-week lead-in period: 1 placebo sachet per day. (The sachet can be mixed into cold water or any cold, noncarbonated beverage and consumed).
- Then increase to 1 sachet twice daily for 22 weeks.
- Followed by 12 week off study product.

This is a double-blind study, which means neither you nor the study staff will know which treatment group you are in. You will not be told which group you are in until all participants have finished the study.

The probiotic product, Visbiome Extra Strength, and placebo are provided by the study and will be given to you at this visit. Your other HIV medications will not be provided by the study; you will need to continue to obtain them through your HIV care doctor or provider.

It is very important that you continue taking all of your regular anti-HIV medications as well as the study medicine. If you miss doses of the anti-HIV medicines, your virus may become resistant to the medicines, which means that the treatment may no longer work to control your HIV. In addition, you may have to stop taking the study product.

On-study Evaluations after Treatment Initiation

You will be asked to come to the clinic 5 times in 38 weeks at weeks 6, 14, 25, 26, and 38. The evaluations performed during these visits are listed in Table 1 of this consent form. At week 4 you will be contacted by telephone to see how well you are taking the study product and to remind you to increase dosing to twice daily.

Premature Study Discontinuation Evaluations

If you stop taking part in the study before the end of the study, you will be asked to come into the clinic and have a physical exam performed, have blood collected, and answer questions about how you take your medications.

Virologic Failure

If, at any point during the study, you are found to have an HIV viral load value that is higher than expected (more than 200 copies/mL), you will be asked to come back in for another HIV viral load test to make sure the first one was correct. If your second viral load is still more than 200 copies/mL, your study doctor will take you off the probiotic or placebo, and you will be followed on study, off study treatment for the remaining weeks of the study.

Use of Your Samples Required for this Study

Some of your blood samples will be stored and used for testing that is required for this study. No one will know just from looking at the labels of your stored samples that they came from you.

Use of Your Stored Samples

If you agree, some of your blood, urine, and stool samples that are left over after all required study testing is done may be stored for future research that is not yet planned, including future ACTG-approved HIV-related studies. Some of these tests may include tests that involve how your genes function or how their function is changed by the Visbiome Extra Strength. We may also perform tests for genetic markers that might affect HIV disease course, as well as other related diseases. These tests will be used to identify human genes or how genes function which may be important to human disease. The data from sequencing will be submitted to a large national database (GenBank or dbGaP) of genetic information. We will not share any of your identifying information with this database, and so you will not personally be linked to this genetic information. Some information about your medical condition will be linked to this genetic information, so that other investigators who have patients with similar conditions to yours will be able to test for similar genetic differences which may cause these problems.

Risks of Genetic Testing

The genetic tests are used for research purposes only. We will not be able to get information that could be used for your regular medical care or treatment. You and your doctor will not be given your genetic test results, and this information will not become part of your medical record. You may experience some concern or anxiety over maintaining confidentiality of study information. All of your specimens and information will be labeled with your study identification number. Information about taking part in a genetic study may influence insurance companies and/or employers regarding your health. If you do not share information about taking part in this study, you will reduce these risks. Every effort will be made to keep your part in this study confidential, although we cannot guarantee absolute confidentiality.

No one will know just from looking at the labels of your stored samples that they came from you. Although researchers will not be given your name or any other personally identifying information about you, some information about your medical condition, your race, ethnicity, gender, and age may be shared.

These samples will be kept frozen for an indefinite length of time. We cannot ensure that you will be told of the results of the research done on these samples.

Allowing your samples to be stored for this use is optional. Please indicate below if you agree to this storage for later use. No matter what you decide, it will not affect your participation in the study.

_____ (initials) YES, I agree _____ (initials) NO, I do not agree

If you decide now that your samples can be stored for research to be done at a later date, you may change your mind at any time. If you change your mind, you must contact your study doctor or nurse and let them know that you do not want your samples used for research to be done at a later date. Every effort will then be made to destroy your leftover samples

Table1. Evaluations performed during the study

Evaluation or Test	Screening	Entry	Week 2	Other Post-Entry Visits Most Visits	Early Discontinuation
Consent	✓				
Medical/Medication History	✓		✓		
Physical Exam	✓		✓	✓	✓
Dietary Assessment			✓	✓	
Sexual Activity Assessment			✓	✓	
Bowel Symptom Assessment			✓	Weeks 6 and 26	
Fatigue Symptom Assessment			✓	Week 26	
Dispense Study Products			✓	Week 14	
Blood Collected	✓	✓	✓	✓	✓
Hepatitis Testing	✓				
Fecal Sample and Rectal Swab			✓	✓	
Phone Assessment				Week 4	
Adherence Assessments				✓	✓
Empty Sachet Counts				✓	✓

Description of Evaluations

Medical/Medication History

- You will be asked about any new medical issues or symptoms that have occurred since your last study visit, especially possible side effects to the medicine you are taking.
- You will be asked about any changes in the type or amount of medication or nutritional supplements (prescription and over the counter) you have taken since your last visit.

Physical Exam

Your temperature, blood pressure, pulse, and breathing rate will be measured.

Dietary Assessment

You will be asked to complete both a detailed and an automated dietary recall assessment.

Sexual Activity Assessment
You will be asked a few questions about recent sexual activity.
Bowel Symptom Assessment
You will be asked to complete a questionnaire describing your bowel habits.

Fatigue Symptom Assessment
You will be asked to complete a questionnaire describing your level of fatigue.

Blood Tests – Safety
- Routine tests of your liver, your kidneys, and your blood counts.
- You will be given the results of this test as soon as they become available.

Blood Test – Pregnancy
- If you are a woman able to become pregnant, you will have a urine or blood test at screening, entry, and whenever pregnancy is suspected to make sure that you are not pregnant.
- You will be given the results of this test as soon as they become available.

Blood Test – CD4+/CD8+ T-cell Count
- This is a measure of your immune system; T-cells help the immune system fight infections.
- You will be given the results of these blood tests as soon as they become available.

Blood Test – HIV-1 RNA (Viral Load)
- This measures the amount of HIV in your blood.
- You will be given the results of this test as soon as they become available.

Blood Test – Immunology Assays and Virologic Studies
- These blood samples will be used to measure inflammation inside your body and to check your T-cells for how well they are working.
- Your blood will be stored and will be tested after the study is over. You will not be given the results.

Blood Test – Hepatitis
You will have blood drawn to test for irritation of your liver. You will be given the test results when they are available.

Fecal Sample and Rectal Swab
A fecal sample and rectal swab to study the different kind of bacteria will be collected. These samples will be stored and will be tested after the study is over. You will not be given the results.

Phone Assessment
At week 4 you will be contacted by phone and asked about how well you take your study-provided medications and to remind you to increase dosing to twice daily.

Adherence Assessments
You will be asked about how well you take your study-provided medications. The study staff will

give you information and encouragement to help you take your medications as prescribed.

Used Sachet Count
You will be asked to keep and bring in the empty used sachets. The study staff will count the number of used sachets.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 90 people will take part in this study

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 38 weeks (10 months).

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:
- the study is stopped or cancelled
- you are not able to attend the study visits as required by the study
- you have confirmed virologic failure

The study doctor may also need to take you off the study product without your permission if:
- continuing the study product may be harmful to you
- you need a treatment that you may not take while on the study
- you are not able to take the study product(s) as required by the study
- you stop taking your ART medication
- you become pregnant

If you must stop taking the study product(s) before the study is over, the study doctor will ask you to continue to be part of the study and return for some study visits and procedures.

<u>If I have to permanently stop taking study-provided product, or once I leave the study, how would study product be provided?</u>

During the study:

If you must permanently stop taking study-provided product before your study participation is over, the study staff will discuss other options that may be of benefit to you.

After the study:

After you have completed you study participation, the study will not be able to continue to provide you with the study product you received on the study. If continuing to take these or

similar drugs/agents would be of benefit to you, the study staff will discuss how you may be able to obtain them.

WHAT ARE THE RISKS OF THE STUDY?

The study product used in this study may have side effects, some of which are listed below. Please note that this list does not include all the side effects seen with this product. This list includes the more serious or common side effects with a known or possible relationship. If you have questions concerning the additional potential side effects please ask the medical staff at your site.

There is a risk of serious and/or life-threatening side effects when non-study medications are taken with the study product. For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study and also before starting any new medications while on the study. Also, you must tell the study doctor or nurse before enrolling in any other clinical trials while on this study.

The following common side effect has been associated with the use of Visbiome Extra Strength:

- Stomach bloating or discomfort

Risks of Blood Draws
A needle will be used to take blood from a vein in your arm. This may lead to brief pain from the needle stick, bruising, and rarely, infection. Some people may become light-headed. The risk of taking blood includes low red blood counts, which can make you feel tired, weak, and dizzy.

Risks of Rectal Swab
You may have mild discomfort when the swab is performed, particularly if you are already suffering from sores or hemorrhoids. In some cases, a very small amount of bleeding may occur. If you are already having pain in the rectal area, be sure to let the study team know.

Risks of Social Harm
Although the study site will make every effort to protect your privacy and confidentiality, it is possible that your involvement in the study as a participant could become known to others if it is not already and that social harms may result (because you could become labeled as being infected with HIV). For example, you could be treated unfairly or discriminated against by family members, friends, and/or the community.

Risk of Embarrassment
The sensitive nature of the information obtained from the Sexual Activity and Bowel Symptom Assessment may be embarrassing.

ARE THERE RISKS RELATED TO PREGNANCY?

If you are a woman having sex that could lead to pregnancy, you must agree not to become pregnant. At least one of the following methods MUST be used:

- Condoms (male or female) with or without a spermicidal agent

- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive

If you become pregnant while on study you will discontinue the study product and will be encouraged to continue to come to study visits. The study staff would like to obtain information from you about the outcome of the pregnancy (even if it is after your participation in the study ends). If you are taking anti-HIV drugs when you become pregnant, your pregnancy will be reported to an international database that collects information about pregnancies in women taking anti-HIV drugs. This report will not use your name or other information that could be used to identify you.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If you take part in this study, you should expect no direct benefit. Information learned from this study may help others who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:
- treatment with prescription drugs available to you
- treatment with experimental drugs, if you qualify
- no treatment

Please talk to your doctor about these and other choices available to you. Your doctor will explain the risks and benefits of these choices.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

People who may review your records include the ACTG, Office for Human Research Protections (OHRP) or other government agencies as part of their duties, (insert name of site) institutional review board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, the drug company supporting this study, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by US law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE THE COSTS TO ME?

Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are taking part in a research study.

WILL I RECEIVE ANY PAYMENT?

[Sites: Please indicate whether you will provide payment to participants. If so, please describe the amount to be paid or reimbursed, the payment schedule, and any prorated schedule should the participant decide to withdraw or is withdrawn early by the investigator.]

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health. You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research participant, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Participant's Legal Guardian (print) (As appropriate)	Legal Guardian's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date

APPENDIX II SAMPLE INFORMED CONSENT

DIVISION OF AIDS
AIDS CLINICAL TRIALS GROUP (ACTG)

For protocol: A5352s

Effects of the Probiotic Visbiome Extra Strength on Epithelial Barrier Function and Inflammation in HIV-Infected Participants on Suppressive Antiretroviral Therapy: A Substudy of A5350

Final Version 1.0, 01/05/16

SHORT TITLE FOR THE STUDY: Epithelial Barrier Substudy of A5350

INTRODUCTION

You are being asked to take part in this research substudy because you are planning to enroll into A5350.

This substudy is sponsored by the National Institutes of Health (NIH).The doctor in charge of this substudy at this site is: (insert name of Principal Investigator). Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

This purpose of this substudy is to look at how the probiotic Visbiome Extra Strength affects the inner lining of the gastrointestinal tract (intestines) in people taking Visbiome Extra Strength compared to people taking the placebo for Visbiome Extra Strength.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

If you agree to participate in the substudy, you will be asked to sign this consent form. You will be seen 5 times in 7 months for blood and urine collections, sigmoidoscopy (exam of the large intestines and rectum), and biopsy. A biopsy is a procedure to remove a small piece of intestinal tissue for examination.

At the screening visit, approximately 2 teaspoons of blood will be collected for safety tests. The test results from this visit will determine whether it is safe for you to have a sigmoidoscopy.

If You Do not Enroll Into the Study
If you decide not to take part in this study or if you do not meet the eligibility requirements, we will still use some of your information. As part of this screening visit, some demographic (for example, age, gender, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, CD4+ T-cell count, HIV viral load) information is being collected from you so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

During the Study
You will remain on your assigned A5350 treatment. You will have a total of 2 sigmoidoscopies done while on study, one at entry and then again at week 24. Before each procedure you will have blood drawn to see if it is safe for you to have the sigmoidoscopy. You will also undergo a test of the permeability of your intestines using a cocktail of sugars that you will drink at least 4 days after the first sigmoidoscopy and again at week 26.

Sigmoidoscopy and Biopsy
You will have a sigmoidoscopy performed by the site gastroenterologist. You will be asked to avoid drinking alcohol or taking nonsteroidal anti-inflammatory medications, including aspirin or any blood thinners for 7 days prior to the test. A sigmoidoscopy biopsy is a procedure to remove small pieces of intestinal tissue for examination. To do this, the doctor will insert a long, flexible, lighted tube into your rectum and slowly guide it into your large intestine. The doctor will insert an instrument into this tube and remove up to 12 small samples (biopsies) for examination. The procedure may cause some discomfort.

The site gastroenterologist will discuss findings with you after the procedure and a copy of the sigmoidoscopy report will be mailed to you.

[Note to sites: Insert specifics regarding how this procedure is performed at your institution and whether an additional consent form is required at the clinic where it will be performed].

Sugar Absorption Test
At a visit separate from the sigmoidoscopy visit you will be asked to avoid taking nonsteroidal anti-inflammatory medications, including aspirin for 7 days before the test, avoid alcohol for 3 days before the test, and avoid exercise the day before and morning of the test. You will be asked to come to the visit fasting. Fasting means you should not eat or drink anything for at least 8 hours before your visit. You may drink only water and take your medications during this time. You will be asked to give a urine sample. You will then be asked to drink a combination of sugars that are not digested by your body and then continue to fast for another 2 hours before eating. When you are allowed to eat, you should avoid sweets and artificial sweeteners. All of your urine will be collected throughout the 5 hours following consumption of the sugar mix.

WHAT ARE THE RISKS OF THE PROCEDURE?

Sigmoidoscopy and Biopsy
- You may experience some mild discomfort and feel like you have a “bloated stomach.”
- Even though the risk is low, you may experience infection, mild rectal irritation, and urgency. It is important that you do not put anything in your rectum for 5 days after the biopsies,

because you may be at higher risk for getting or spreading an infection until the biopsy site(s) have healed.
- You may experience limited rectal bleeding (1 to 2 days after the procedure) related to the biopsies.
- You may experience low blood pressure.
- Even though the risk is very rare, there is a very small chance that you may have a hole or a tear in the intestine. The risk of this complication is estimated to be about 1 in a 1,000 people who have a flexible sigmoidoscopy with biopsies. If this happens, surgery to repair the tear may be necessary.
- Any abnormal findings will be followed up at the time of the procedure if possible (for example, removal of incidental polyps or biopsy of abnormal lesions). All abnormal findings will be referred to the site gastroenterologist for further care.

Sugar Absorption Test

You may experience some temporary bloating or loose stools following administration of the sugar mix but this should resolve within several hours. You may also experience some discomfort from hunger as you will need to avoid eating for 8 hours before the test and 2 hours after the test begins.

Use of Your Stored Samples

Some of your tissue that is left over after all required study testing is done may be stored and used for ACTG-approved HIV-related research. Some of these tests may include tests that involve how your genes function or how their function is changed by the Visbiome Extra Strength. We may also perform tests for genetic markers that might affect HIV disease course, as well as other related diseases. These tests will be used to identify human genes or how genes function which may be important to human disease. The data from sequencing will be submitted to a large national database (GenBank or dbGaP) of genetic information. We will not share any of your identifying information with this database, and so you will not personally be linked to this genetic information. Some information about your medical condition will be linked to this genetic information, so that other investigators who have patients with similar conditions to yours will be able to test for similar genetic differences which may cause these problems.

Risks of Genetic Testing

The genetic tests are used for research purposes only. We will not be able to get information that could be used for your regular medical care or treatment. You and your doctor will not be given your genetic test results, and this information will not become part of your medical record. You may experience some concern or anxiety over maintaining confidentiality of study information. All of your specimens and information will be labeled with your study identification number. Information about taking part in a genetic study may influence insurance companies and/or employers regarding your health. If you do not share information about taking part in this study, you will reduce these risks. Every effort will be made to keep your part in this study confidential, although we cannot guarantee absolute confidentiality.

No one will know just from looking at the labels of your stored samples that they came from you. Although researchers will not be given your name or any other personally identifying information

about you, some information about your medical condition, your race, ethnicity, gender, and age may be shared.

These samples will be kept frozen for an indefinite length of time. We cannot ensure that you will be told of the results of the research done on these samples.

Allowing your samples to be stored for this use is optional. Please indicate below if you agree to this storage for later use. No matter what you decide, it will not affect your participation in the study.

_____ (initials) YES, I agree _____ (initials) NO, I do not agree

If you decide now that your samples can be stored for research to be done at a later date, you may change your mind at any time. If you change your mind, you must contact your study doctor or nurse and let them know that you do not want your samples used for research to be done at a later date. Every effort will then be made to destroy your leftover samples.

Table1. Evaluations performed during the study

Evaluation or Test	Screening	Entry	Week 1	Post-Entry Visits	
				Week 24	Week 26
Blood Collected	✓			✓	
Colonic Biopsies for Immunologic Assessments		✓		✓	
Sugar Absorption Test / Urine Collected			✓		✓

Description of Evaluations

Blood Collected
- These blood samples will be used to determine whether it is safe for you to have a sigmoidoscopy.
- You will be given the results of these tests.

Sugar Absorption Test/Urine Collected
- These urine samples will be used to evaluate the lining of the intestines.
- You will not be given the results of these tests.

Colonic Biopsies for Immunologic Assessments
- These samples will be used to measure inflammation inside your intestines.
- You will not be given the results of these tests.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 40 people will take part in this study.

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 26 weeks (7 months).

WHY WOULD THE DOCTOR TELL YOU NOT TO HAVE THE SECOND PROCEDURE?

The study doctor may need to take you off the study early without your permission if:

- the study is stopped or cancelled
- you are not able to attend the study visits as required by the study
- you have procedure-related complications during your first procedure

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If you take part in this study, there is not likely to be any benefit to you. Information learned from this study may help others who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Taking part in these procedures is optional. You may choose not to participate.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

People who may review your records include the ACTG, Office for Human Research Protections (OHRP) or other government agencies as part of their duties, (insert name of site) institutional review board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, the drug company supporting this study, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

Your records may be reviewed by the ACTG, OHRP, insert name of site) IRB, National Institutes of Health (NIH), study staff, study monitors, and the drug company supporting this study.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by US law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE THE COSTS TO ME?

Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are taking part in a research study.

WILL I RECEIVE ANY PAYMENT?

[Sites: Please indicate whether you will provide payment to participants. If so, please describe the amount to be paid or reimbursed, the payment schedule, and any prorated schedule should the participant decide to withdraw or is withdrawn early by the investigator.]

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health. You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:

- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research participant, contact:

- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Participant's Legal Guardian (print) (As appropriate)	Legal Guardian's Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date