A Study to Investigate Gastrointestinal Epithelial Integrity and Arterial Inflammation in Individuals With and Without HIV

November 19, 2018

NCT02431325

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Detailed Protocol
Version 22: November 19, 2018

I. Background and Significance

Cardiovascular disease is an important cause of morbidity and mortality in patients with HIV infection. Survival of HIV-infected patients worldwide remains lower compared to the general population despite the remarkable improvement highly active anti-retroviral combination therapy (HAART) has provided. Excess mortality occurs now in large part due to non-infectious illnesses including cardiovascular disease (CVD)[1,2]. We have previously shown that coronary atherosclerosis is highly prevalent among HIV-infected patients, even at a relatively young age[3]. Myocardial infarction rates are also higher in HIV-infected patients[4,5] compared to uninfected individuals. While traditional risk factors for CVD are highly prevalent among the HIV population[6-8], a number of studies demonstrate that these traditional risk factors account for only a portion of the increased CVD risk in this population[4,5]. There exists a gap in knowledge of what the specific key factors are in driving this increased risk. Improved understanding of the specific underlying mechanisms is critical to identify specific prevention and treatment strategies for atherosclerotic disease in the HIV patient population.

Cognitive impairment and depression is a cause of morbidity in 20-50% of HIV-1-infected (HIV+) adults on antiretroviral therapy (ART), and may be related to atherosclerotic disease and chronic inflammation [9-13]. In animal models, lipopolysaccharide (LPS) induces carotid atheroma formation and intimal thickening [14-16]. In cohort studies, LPS and monocyte activation predict carotid atherosclerosis progression in HIV-infection [17]. While a recent study suggested a relationship between microbial products, and microglial activation in HIV+ adults [18], the relationship between microbial translocation, neurovascular inflammation, and brain function are not established.

Immune activation in HIV disease may activate inflammatory pathways of atherosclerosis development. Atherosclerosis is an inflammatory disease in which immune mechanisms, particularly of the innate immune system, are clearly established as key contributors in atherosclerotic disease development[19-22]. Monocytes play a very central role in the pathogenesis of atherosclerosis. Immune activation and systemic inflammation are hallmarks of HIV disease. Although chronic inflammation is implicated in the excess cardiovascular risk attributable to HIV disease[23-25], little data exists on the immunologic mechanisms of atherosclerosis in the HIV-infected population.

Given the known central role of monocytes in the initiation of atherosclerosis, we investigated the role of monocyte activation in atherosclerotic heart disease in HIV infection. First, we demonstrated an independent relationship between monocyte chemoattractant protein-1 (MCP-1) and coronary atherosclerotic plaque burden in HIV-infected patients[3]. Corroborating this finding, very recently, Shikuma *et al.* also showed plasma MCP-1 predicted presence of coronary artery calcium independent of traditional CVD risk factors in HIV-infected patients[26]. Second, we showed that soluble CD163 (sCD163), a marker of monocyte and macrophage activation, to be elevated in patients with chronic HIV infection compared to HIV-negative controls, and to be positively associated with amount of coronary atherosclerosis, particularly noncalcified plaque [27] and with arterial inflammation in HIV-infected patients[28]. Among patients with chronic HIV infection and even with suppressed viremia, we and others have previously demonstrated that monocyte/macrophage activation may distinctively contribute to increased atherosclerotic disease and arterial inflammation[27-31]. ***Given these findings, we hypothesize that***

activation of the innate immune system plays a major role in the pathogenesis of coronary artery disease in HIV-infected patients.

What drives innate immune activation in HIV disease? While the causes are likely multifaceted, we hypothesize that increased microbial translocation across the gastrointestinal (GI) mucosa is a major driving force of monocyte and macrophage activation into proatherogenic phenotypes leading to cardiovascular disease in this patient population. We hypothesize that HIV-associated damage to mucosal barrier function to be key in this process. ***We additionally suggest innate immune activation plays a major role in cognitive impairment and depression in virally suppressed HIV-infected patients and is related to coronary and carotid disease.*** *We propose to test this hypothesis in the proposed study.*

Loss of GI mucosal epithelial integrity and GI tract CD4+ T cells in HIV-infected patients impair the host defense system from intestinal microbes and is proposed to be a cause of immune activation in HIV disease[32,33]. Structural breakdown of the tight epithelial barrier of the GI tract in HIV and SIV infection leads to translocation of microbes from the GI lumen into the body[33,34]even among ART-treated HIV patients[32]. GI tract structural integrity is dependent on the health of the mucosal immune system. One of the hallmarks of HIV infection is a massive depletion of CD4+ T cells in the gut associated lymphoid tissue (GALT). This depletion is accompanied by vigorous mucosal inflammation that impairs gut epithelial barrier function. This loss of epithelial integrity results in the translocation of luminal bacterial products into the systemic circulation which drives systemic immune activation, progressive immune dysfunction, and ultimately disease progression. Thus, HIV immunity within the GALT is central to our understanding of HIV pathogenesis and critical to our efforts to better manage the consequences of chronic infection. In HIV and SIV infection, depletion of Th17 cells, which normally respond to bacterial products, is associated with microbial translocation[35,36]. HIV viral infection itself may cause epithelial damage due to production of proinflammatory cytokines that can directly induce apoptosis of epithelial cells[34]. Markers of bacterial translocation into the circulation, plasma lipopolysaccharide (LPS), a bacterial cell wall component, and soluble CD14 (sCD14) are both significantly elevated in chronic HIV infection and plasma LPS levels are found to be related to T cell activation[32]. Bacterial LPS binds through the CD14 receptor on monocytes and macrophages[37] and stimulates activation of the host innate immune system. Furthermore, plasma levels of sCD14 have been demonstrated to independently predict mortality in HIV-infected patients in the SMART trial[38] and sCD14 correlated with increases in carotid intimal media thickness[17].

To further test our central hypothesis, we propose to study an intervention to improve the integrity of the intestinal mucosa using glucagon-like peptide-2 (GLP-2) as a strategy to decrease microbial translocation and, therefore, potentially decrease immune activation and its downstream cardiovascular effects. GLP-2 is an intestinal-specific trophic factor which promotes mucosal healing, reduces bacterial translocation and exerts anti-inflammatory effects in the gut via enteric neuroendocrine pathways[39,40]. Teduglutide, a dipeptidyl peptidase-IV resistant analog of GLP-2, is FDA-approved for use in short bowel syndrome and has been shown to be well-tolerated in clinical trials and to improve clinical outcomes including reducing parenteral support requirements in patients with short bowel syndrome[41].

Identifying distinct mechanisms that underlie the atherosclerotic disease process in HIV patients is crucial to develop appropriate strategies to treat and prevent CVD in this patient population. The proposed studies aim to determine the role of intestinal microbial translocation in driving inflammatory atherosclerosis via innate immune system activation. Taking this further, we propose to study a therapeutic intervention aimed to restore epithelial integrity as a means to decrease immune activation driven cardiovascular disease in HIV-infected patients. If microbial translocation from breakdown of the tight epithelial barrier of the GI tract is identified as a cause of chronic inflammation and atherosclerosis disease development, this can have implications for other disease states where there is damage to the GI epithelial barrier such as inflammatory bowel disease or celiac disease.

II. Specific Aims

Among patients living with HIV infection, cardiovascular disease (CVD) is increasingly prevalent and has become a significant cause of mortality. Identifying the mechanisms that are distinctive to the atherosclerotic disease process in HIV-infected patients is crucial to develop appropriate strategies to treat and prevent CVD in this patient population. Activation of the innate immune system may stimulate inflammatory mechanisms of atherosclerosis development. Loss of gastrointestinal (GI) mucosal epithelial integrity and loss of CD4+ T-lymphocytes in the intestinal lamina propria occur in HIV-infected patients. Translocation of microbial products from the intestinal lumen into the systemic circulation has been demonstrated to be increased in HIV-infected patients and we hypothesize to be a key driver of monocyte and macrophage activation. In turn, these pro-inflammatory monocytes and macrophages can induce atherosclerotic disease development. However, the respective roles of microbial translocation and the gut mucosal immunity on cardiovascular inflammation in HIV infection are not yet known. Furthermore, no treatment strategy as yet exists to effectively target proatherogenic immune activation as an important mechanism of CVD in HIV-infected patients.

Our central hypothesis is that increased microbial translocation from impaired epithelial integrity and impaired mucosal immunity activates the innate immune system, causing proatherogenic monocyte and macrophage activation and atherosclerotic disease development in HIV disease. Furthermore, we hypothesize that intervening to enhance the intestinal epithelial barrier using a glucagon-like peptide 2 (GLP-2) analog will decrease intestinal permeability to microbial products and thus ameliorate monocyte/macrophage pathways that lead to downstream decrease in arterial macrophage activity, inflammation and atherosclerotic disease risk. *We will test our hypotheses in studies proposed in the following specific aims:*

Aim 1: To determine the role of innate immune system activation in atherosclerosis development among HIV-infected patients, focusing on microbial translocation from impaired GI mucosal barrier as the hypothesized inciting event for activation of monocytes and macrophages.

A) To determine how intestinal epithelial integrity (assessed via characterization of intestinal tissue tight junctions and also via GI permeability probe studies) and microbial translocation relate to measures of monocyte and macrophage activation and to measures of CVD risk utilizing functional imaging (by ^{18}fluorodeoxyglucose positron emission tomography (FDG-PET) and structural (cardiac CTA) cardiac imaging to assess arterial inflammation and coronary atherosclerotic disease.

B) Trancriptome analysis of circulating monocytes using RNA-Seq to identify distinctive genomic signatures in monocytes of HIV-infected patients with greatest amount of coronary atherosclerosis detected on CTA compared to HIV-infected patients without atherosclerosis to elucidate novel biological pathways involved in atherogenesis in HIV disease. These pathways will also be compared to dysregulated pathways in HIV patients with greatest degree of intestinal permeability/microbial translocation compared to patients with the least. We hypothesize that biological pathways in monocytes stimulated by microbial translocation will be similar to pathways activated in monocytes of patients with greatest degree of CVD.

Aim 2: Determine the effects of a glucagon-like peptide-2 analog, teduglutide, on intestinal epithelial integrity, on microbial translocation across the gut lumen, on markers of innate immune system activation including the monocyte transcriptome, and on arterial inflammation in a 6-month randomized, double-blind placebo-controlled proof of concept trial in HIV-infected individuals.

Exploratory Aim: An additional exploratory aim is to study the interrelationships between cerebral glucose metabolism measured by FDG-PET of the brain (a proxy for neural activity), cognitive performance and arterial inflammation as well as gut microbial translocation in patients living with HIV.

To achieve these goals, this proposal comprises a multidisciplinary partnership of investigators from endocrinology, infectious diseases, cardiology, radiology, neurology and gastroenterology at Massachusetts General Hospital as well as the immunology laboratories at the Ragon Institute of MGH, MIT and Harvard to investigate the relationship of the gut epithelial integrity and mucosal immunity to atherosclerotic plaque inflammation in HIV patients. Data generated from the studies proposed are expected to address whether the altered gastrointestinal epithelial integrity from HIV infection is closely linked to atherosclerosis via innate immune activation and whether treatment to improve intestinal epithelial integrity can prevent further atherosclerotic disease development and decrease vascular inflammation by curtailing pathologic activation of the innate immune system. Answers to these critical questions will impact the development of novel therapies and preventative strategies for proatherogenic immune activation and CVD in HIV-infected patients.

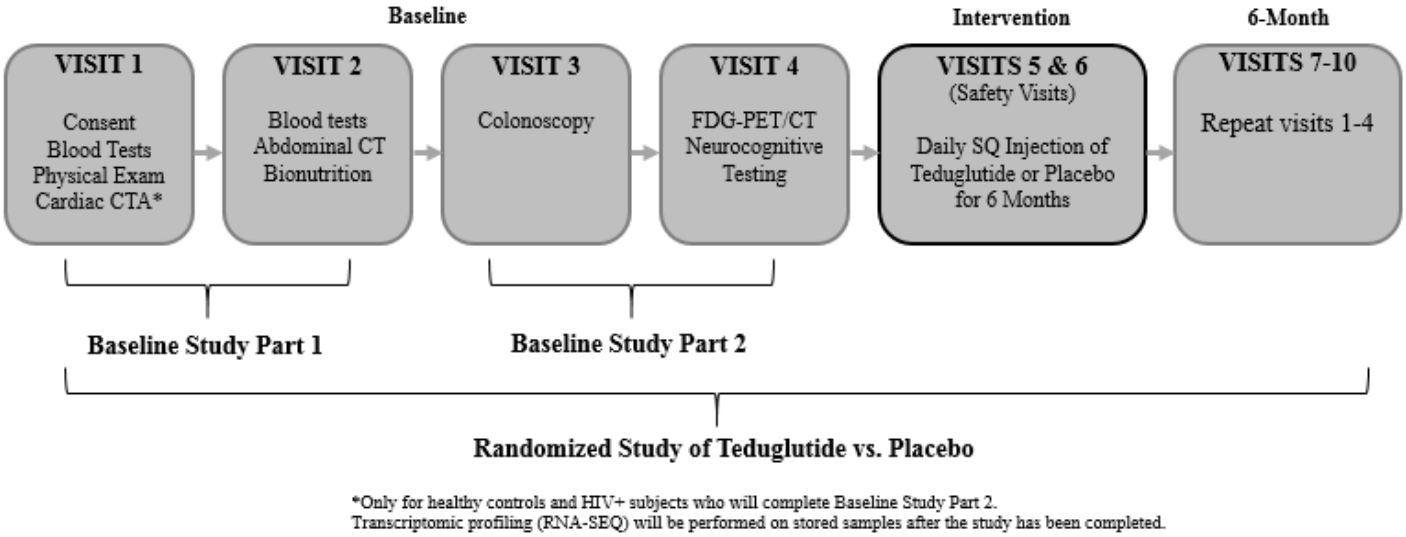
Research Design

To answer Aim 1A, we propose an observational cross-sectional study to investigate the relationships of epithelial integrity (assessed by intestinal biopsy), depletion of GI tract T cells, circulating markers of enterocyte damage (I-FABP[38]) and microbial translocation (LPS[32], sCD14[38]) to arterial inflammation and coronary artery atherosclerosis and markers of monocyte/macrophage activation. The procedures for the cross-sectional study can be divided into two groups, which we will call **Baseline Study Part 1: Biomarkers, Immunology and Intestinal Permeability Studies** (or simply "Baseline Study Part 1") and **Baseline Study Part 2: Colonoscopy, Cardiac CTA and FD-PET/CT Studies** (or simply "Baseline Study Part 2").

To answer Aim 1B, transcription profiling by RNA-Seq will be performed on a subset of participants. Seven patients with greatest degree of coronary atherosclerosis vs. 7 patients with the least amount of coronary atherosclerosis and 7 patients with greatest degree of microbial translocation vs. 7 patients with the least amount of microbial translocation. Both groups will be matched by age and traditional cardiovascular risk factors. As Verdugo *et al* recently found that smoking can affect the monocyte transcriptome[42], we will exclude patients who are active smokers in Aim 1B. Transcription profiling of monocytes by RNA-Seq will also be performed in a subset of 7 participants in the active teduglutide arm who have the greatest amount of coronary atherosclerosis before and after their teduglutide treatment in the Randomized Study of Teduglutide vs. Placebo.

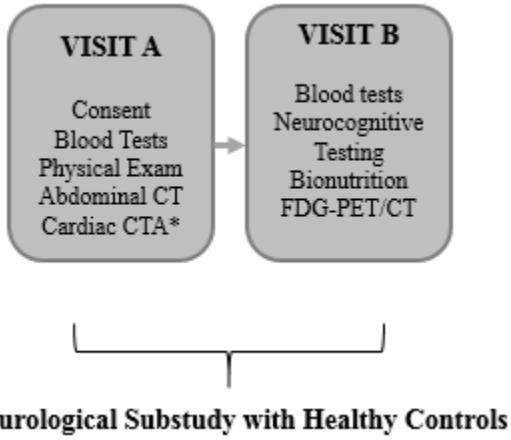
To answer Aim 2, we propose a randomized, double-blind, placebo-controlled trial to assess the effects of treatment with teduglutide 0.05 mg/kg/day for 6 months duration compared to placebo. Recruitment for this interventional trial will occur concurrently with the cross-sectional study in Aim 1A. Participants who are recruited for the randomized study will complete Baseline Studies Part 1 and Part 2 as their baseline for the randomized study (see Figure 1a). Participants who are recruited for the neurological substudy will complete Visit A and B (see Figure 1b). *This design is intended to minimize risk of study procedures to patients by maximizing use of data and also to improve efficiency in recruitment.* Thus, the participants in the randomized study will also complete the cross-sectional study. These participants undergo the same baseline procedures again at the end of the 6-month intervention for their end-of-study procedures. We will refer to the procedures involved in the randomized study as the **Randomized Study of Teduglutide vs. Placebo.**

Figure 1a.



*Only for healthy controls and HIV+ subjects who will complete Baseline Study Part 2.
Transcriptomic profiling (RNA-SEQ) will be performed on stored samples after the study has been completed.

Figure 1b.



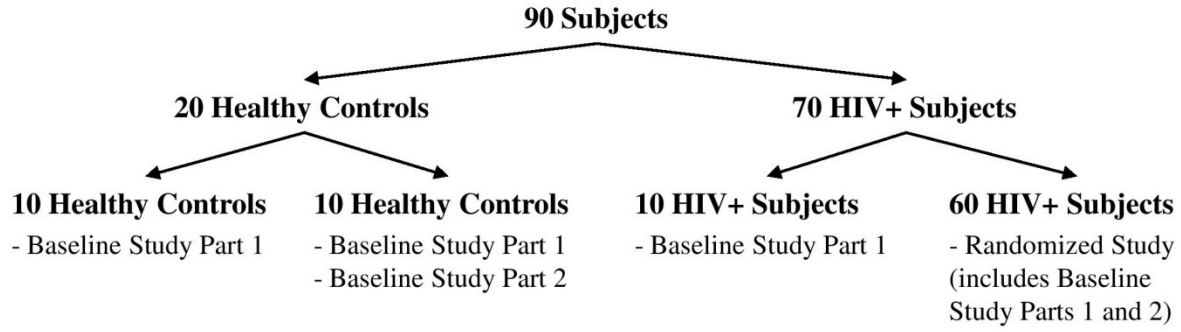
III. Subject Selection

Overview of the Approach*:* A total of 90 subjects (70 subjects with chronic HIV-1 infection and 20 HIV-negative control subjects) will be recruited (See Figure 2). For the neurological substudy, an additional 15 HIV-negative control subjects will be recruited and complete Visit A and Visit B (see Figure 1b, not shown in Figure 2 flowchart). Ninety subjects will complete **Baseline Study Part 1** of the cross-sectional study. **Baseline Study Part 2** will be performed in a subset of 60 HIV-infected patients and 10 HIV-negative controls. The 60 HIV-positive patients who complete Baseline Studies Part 1 and Part 2 will enter the **Randomized Study of Teduglutide vs. Placebo**. The tests and procedures obtained during the Baseline Studies Part 1 and Part 2 for these 60 HIV-positive participants will serve as the baseline measurements for the randomized study (they are not repeated) in order to minimize risk from exposure to study procedures for the participants and to maximally utilize the study data.

Having the control group will help us establish whether assessed relationships between GI epithelial integrity, microbial translocation, immune activation, and cardiovascular imaging markers are HIV-specific. Adding the neurological substudy control group (n=15) will help us potentially identify a HIV-

specific, cardiovascular and inflammatory link to cognitive impairment and depression**.** The control group can also serve as a comparison group with which to compare the HIV patients.

Figure 2



After all subjects have completed the study, transcriptomic profiling by RNA-Seq will be performed in 7 HIV+ participants with greatest degree of coronary disease seen on CTA vs. 7 HIV+ participants without and 7 HIV+ participants with the greatest degree of bacterial translocation vs. 7 HIV+ participants with the least amount of microbial translocation.

Participants will be recruited from the Boston area from infectious disease clinics, HIV community centers and also via IRB-approved advertisements. Patients will also be recruited via referral by primary care providers, infectious disease providers, newspaper advertisement, posted flyers, and Partners subject recruitment broadcast. After obtaining informed consent, baseline measurements and studies will be performed.

Inclusion criteria for HIV-infected participants:

1. Men and women age 21-65 with previously diagnosed HIV disease
2. Stable anti-retroviral therapy (ART), as defined by no changes in ART regimen for >6 months
3. HIV viral load < 200 copies/mL
4. To be eligible for colonoscopy procedure, laboratory values that meet the following criteria:
    a. Hemoglobin > 9.0 g/dL
    b. Absolute neutrophil count ≥ 1000/mm3
    c. Platelet count ≥ 100,000/mm3
    d. Prothrombin time (PT) < 1.2 x upper limit of normal (ULN)
    e. Partial thromboplastin time (PTT) < 1.5 x ULN
5. Ability and willingness to give written informed consent and to comply with study requirements

Exclusion criteria:

1. History of clinically significant gastrointestinal disease including but not limited to: colon cancer, intestinal obstruction, ulcerative colitis, Crohn's disease, or history of C. difficile within the past 3 months

2. First-degree relative with history of colon cancer

3. Active gall bladder, biliary or pancreatic disease
4. Female subject who is pregnant, nursing or less than 8 weeks post partum.
5. Use of any immunomodulatory agents within 30 days prior to study enrollment
6. History of intolerance, sensitivity, allergy or anaphylaxis to benzodiazepines or other narcotics to be used during the colonoscopy procedure
7. Contraindication to beta-blocker (including moderate to severe asthma or heart block) or nitroglycerin use as these drugs are given as part of the standard cardiac CT protocol. Previous allergic reaction to beta blocker or nitroglycerin.
8. Patients with previous allergic reactions to iodine-containing contrast media
9. Renal disease or creatinine >1.5 mg/dL (contrast will be administered during CT angiography of the heart)
10. History of requiring antibiotic prophylaxis for invasive procedures
11. History of myocardial infarction, decompensated cirrhosis, or any other condition that in the opinion of the investigator will compromise ability to participate in the study
12. Currently taking anticoagulants including but not limited to: heparin (Hep-Lock, Hep-Pak), Hep-Pak CVC, Heparin Lock Flush), warfarin (Coumadin), tinzaparin (Innohep), enoxaparin (Lovenox), danaparoid (Orgaran), dalteparin (Fragmin), and clopidogrel (Plavix).
13. Subject taking any of the following medications: systemic steroids (inhaled or nasal steroid therapy is permitted), interleukins, systemic interferons (e.g. local injection of interferon alpha for treatment of HPV is permitted), systemic chemotherapy including oral chemotherapeutic agents, methotrexate, octreotide, growth hormone, antiarrhythmics including digoxin, antiepileptics, immunosuppressants, vancomycin, rifampin, aminoglycosides, clonidine, prazosin, lithium and ritonavir-boosted lopinavir (Kaletra).
14. Subjects receiving high dose statin therapy (a stable low or moderate dose of statin is permitted)
15. Initiation of statin therapy or change in statin dose <90 days prior to entry
16. Subject has had two or more of the same endoscopy procedures (sigmoidoscopy, or colonoscopy) within the past 12 months for clinical purposes or other research studies.
17. Body weight greater than 350 lbs
18. Active illicit drug use
19. Patients who report any significant radiation exposure over the course of the year prior to randomization. Significant exposure is defined as:
    a) More than 2 percutaneous coronary interventions (PCI) within 12 months of randomization
    b) More than 2 myocardial perfusion studies within the past 12 months
    c) More than 2 CT angiograms within the past 12 months
    d) Any subjects with history of radiation therapy
20. Patients already scheduled or being considered for a procedure or treatment
    a) requiring significant radiation exposure (e.g., radiation therapy, PCI, or catheter
    b) ablation of arrhythmia) within 12 months of randomization
21. History of malignancy in the last 5 years, with the exception of surgically cured non-metastatic

non-melanoma skin cancers

22. Prior recipient of a HIV vaccine

Inclusion/Exclusion Criteria for HIV-negative Controls

For the HIV-negative control group in the parent study and the neurological substudy, we will recruit a total of 35 men and women without known HIV disease with the same inclusion and exclusion criteria other than the inclusion criteria of presence of HIV infection and stable antiretroviral therapy.

The Baseline Study Part 1 includes 10 patients with HIV and 10 patients without HIV and does not involve a colonoscopy, CT scan, or PET scan. Some of the exclusion criteria listed above are only relevant for participants undergoing a colonoscopy, CT scan, or PET scan. Patients participating only in the Baseline Study Part 1 will be excluded based on the following criteria only:

1. History of clinically significant gastrointestinal disease including but not limited to: colon cancer, intestinal obstruction, ulcerative colitis, Crohn's disease, or history of C. difficile within the past 3 months
2. First-degree relative with history of colon cancer
3. Active gall bladder, biliary or pancreatic disease
4. Female subject who is pregnant, nursing or less than 8 weeks post partum.
5. Use of any immunomodulatory agents within 30 days prior to study enrollment
6. Renal disease or creatinine >1.5 mg/dL (contrast will be administered during CT angiography of the heart)
7. History of myocardial infarction, decompensated cirrhosis, or any other condition that in the opinion of the investigator will compromise ability to participate in the study
8. Currently taking anticoagulants including but not limited to: heparin (Hep-Lock, Hep-Pak), Hep-Pak CVC, Heparin Lock Flush), warfarin (Coumadin), tinzaparin (Innohep), enoxaparin (Lovenox), danaparoid (Orgaran), dalteparin (Fragmin), clopidogrel (Plavix)
9. Subject taking any of the following medications: systemic steroids (inhaled or nasal steroid therapy is permitted), interleukins, systemic interferons (e.g. local injection of interferon alpha for treatment of HPV is permitted), systemic chemotherapy including oral chemotherapeutic agents, methotrexate, octreotide, growth hormone, antiarrhythmics including digoxin, antiepileptics, immunosuppressants, vancomycin, rifampin, aminoglycosides, clonidine, prazosin, lithium and ritonavir-boosted lopinavir (Kaletra).
10. Subjects receiving high dose statin therapy (a stable low or moderate dose of statin is permitted)
11. Initiation of statin therapy or change in statin dose <90 days prior to entry
12. Body weight greater than 350 lbs
13. Active illicit drug use
14. History of malignancy in the last 5 years, with the exception of surgically cured non-metastatic non-melanoma skin cancers

15. Prior recipient of a HIV vaccine

16. Currently receiving pre-exposure prophylaxis (PrEP)

IV. Subject Enrollment

Attempts will be made to recruit patients who will be undergoing endoscopy and/or colonoscopy as part of their routine care (e.g., patients undergoing screening colonoscopies).

In many cases, the patient's primary/specialist health care provider will initially inform the patient of the study, if it appears that the subject would be an appropriate candidate for the study. Individuals enrolled in other studies at the MGH Program in Nutritional Metabolism or at the Ragon Institute who have indicated a willingness to participate in additional studies for which they may be eligible may also be contacted regarding this study. Subjects may also be recruited using the Partners' Research Patient Data Registry (RPDR). Additionally, some subjects may be self referred in response to IRB approved recruitment tools such as flyers, posters, website postings, or advertisements.

After referral is received regarding a potential subject or a potential subject contacts the study staff, the study will be thoroughly explained and a pre-screening eligibility questionnaire will be administered by a member of the study staff during a pre-screening phone call. A consent form (containing information about the procedures) will be sent to the potential subject for review if they wish to receive this information beforehand. The information in the completed phone eligibility screening form will be reviewed for approval by the study physician before the various appointments and accommodations are scheduled. All subjects will be given adequate time to consider participation and ask questions about the study.

Written informed consent will be obtained by a licensed physician investigator at the initiation of the screening visit. The study investigator will review the consent form with the potential subject in detail. After all questions are answered to the satisfaction of the subject, the subject and investigator will sign the consent form. A copy of the signed consent form will be given to the subject to keep. The consent form will be in accordance with the guidelines of and approved by the Partners Human Research Committee. Eligible participants who consent to participate in the Randomized Study of Teduglutide vs. Placebo will undergo randomization to active study drug vs. placebo.

Intervention in Randomized Study of Teduglutide vs. Placebo: Teduglutide 0.05mg/kg body weight subcutaneously once a day vs. placebo of identical appearance from baseline to 6-month final visit. Dosing of teduglutide is based on published randomized placebo-controlled trial of teduglutide in patients with short bowel syndrome[41]. At initiation of study medication, half of the dose (0.025mg/kg body weight subcutaneously once a day) will be administered in the first week. If participant tolerates the study medication, the dose will be increased to the full dose (0.05mg/kg body weight subcutaneously once a day). If the participant develops gastrointestinal side effects while on the full dosage of the study drug, the study team will instruct the participant to return to the half dose of 0.025mg/kg.

Method of Randomization: Permuted blocks randomization with randomly varying block sizes will be implemented. All investigators and patients will remain blinded to drug assignment.

Only HIV-positive participants will enter the **Randomized Study of Teduglutide vs. Placebo. HIV-negative controls will only participate in the baseline cross-sectional study (part 1 and 2 of baseline).**

V. Study Procedures

All potential subjects will complete a brief telephone screen followed by a clinical evaluation to determine eligibility for the study. The first clinical evaluation will be performed in the Clinical Research Center (CRC) at Massachusetts General Hospital (MGH) during the screening visit. Written informed consent will be obtained from each subject by a physician investigator upon arrival to the CRC during the first screen visit and prior to testing.

Visit 1 (Screen Visit):

1. Participant will meet with study investigator to review the consent form and signing of informed consent
2. Detailed medical history, including assessment of history of anti-retroviral use, hepatitis status, nadir CD4 count, gastrointestinal disease, prior history of CNS opportunistic infections and risk factors for cardiovascular disease, including smoking history, family history, and history of recreational drug use
3. Physical examination
4. Fasting blood will be obtained for glucose, comprehensive metabolic panel, amylase (only for subjects who are enrolled in the randomized study), lipase (only for subjects who are enrolled in the randomized study), creatinine, insulin, hemoglobin A1c, Prothrombin Time / INR, Partial thromboplastin time (PTT), CBC plus differential, lipids, high-sensitivity C-reactive protein, T-cell subset, and HIV RNA (for HIV-infected patients) and HIV ELISA test (for HIV-negative controls). Additional blood will be collected for isolation of peripheral blood mononuclear cells for immunological studies, serum and plasma to be frozen and stored.
5. Urine HCG for women
6. Cross-sectional computed tomography (CT) scan: non-contrast enhanced 1cm cross-sectional CT scan of the abdomen to determine visceral adipose tissue (VAT) area and abdominal subcutaneous adipose tissue (SAT) area (only for participants also participating in **Baseline Study Part 2**)
7. Cardiac CTA (only for participants also participating in **Baseline Study Part 2**). Subjects who have had a cardiac CTA less than 3 months prior to visit 1 will not have a cardiac CTA at visit 1.

Visit 2:

1. Intestinal Permeability Study (sugar probe solution drink and urine collection)
2. Bionutrition:
   - -Anthropometry (weight, height, waist and hip circumference measurements)
   - -4-day food record form to be explained and given to patient
   - -Bouchard activity questionnaire administered (bionutrition visit at the MGH CRC will be done at same time while patient is undergoing the urine collections for intestinal permeability study on the CRC)
3. Twenty-six milliliters of blood (3 ACD tubes) will be collected for isolation of peripheral blood monocytes.
4. The bowel preparation kit will be given to the patient, and the preparation for the colonoscopy will be explained.
5. Subject will meet with nursing to learn how to self-administer teduglutide. Subjects will not begin taking the study drug until after visit 4.

Bowel preparation: After eligibility is confirmed the subject will prepare for the procedures. To maximize the thoroughness and safety of the procedure, the colon must be completely empty prior to colonoscopy. The procedure preparation will be as per the GI Procedure Unit SOP.

Medical Record Review: Medical records will be reviewed for relevant clinical data for subjects who are currently followed at MGH or BWH for clinical care. Subjects who receive clinical care from external providers will be asked to sign a Release of Information form. We will request and/or review relevant medical history, laboratory results and treatment history for HIV and/or Hepatitis B and C.

Visit 3 (Colonoscopy)

1. Colonoscopy will be performed in the MGH GI endoscopy suite and performed according to standard MGH clinical colonoscopy protocol.
2. Blood draw for isolation of cells to be used for immunological studies.

Follow-up Phone call:

The study coordinator or a study investigator will have a follow up phone call with subjects the day after the colonoscopy procedure. The purpose of the call will be to assess how the participant tolerated the procedures and whether an adverse event occurred. If an adverse event occurred, a study physician will speak with the participant. If a study coordinator calls the subject, she/he will inform the subject during the call that a study doctor will call them if they wish.

If eligible, subject will be randomized to either teduglutide or placebo and return for**:**

Visit 4 (Baseline visit):

1. Meet with investigator for dispensing of study drug (including administration of first dose and 30 minute observation)
2. Serum HCG will be obtained STAT as a pregnancy test for women prior to FDG-PET
3. Blood drawing for RNA-sequencing, and for sera and plasma to be frozen for assays to be run in batch using kits.
4. FDG-PET/CT
5. Neurocognitive Test Battery administered between 18F-FDG tracer injection and prior to FDG PET/CT scan.
6. Center for Epidemiological Studies-Depression Scale (CES-D).
7. Cognitive Function Instrument (CFI), and Patient's Assessment of Own Functioning Inventory (PAOFI) Questionnaire.

Visit 4 may be conducted before Visit 3, depending on scheduling availability.

Follow up safety visit 1 month after randomization and then at month 3:

1. Blood draw for safety labs, comprehensive metabolic panel, amylase, lipase, CBC with differential, and blood samples will also be stored for biomarkers to be run in batch (sCD14, LPS, I-FABP, sCD163)
2. Assess for side effects
3. Assess for change in medical problems or medications
4. Urine HCG for women
5. PAOFI and CES-D at month 3

Final visits at month 6:

1. Repeat all procedures performed at Visits 1, 2, 3 and 4 in four separate visits

Fecal occult blood testing at 1 year:
Participants who participated in the Randomized Study of Teduglutide vs. Placebo will be given the fecal immunochemical test kit to be performed at home at 1 year from the starting of study medication teduglutide or placebo. They will be asked to return this to the laboratory for processing. If the result returns abnormal, we would recommend follow-up with their primary care physician and also offer to the participant to perform a colonoscopy using study funds if their primary care physician agrees.

For Participants in Baseline Study Part 1 (Biomarkers, Immunology and Intestinal Permeability Studies) only:
All potential subjects will complete a brief telephone screen followed by a clinical evaluation to determine eligibility for the study. The first clinical evaluation will be performed in the Clinical Research Center (CRC) at Massachusetts General Hospital (MGH) during the screening visit. Written informed consent will be obtained from each subject upon arrival to the CRC during the first screen visit and prior to testing. The consent form will be discussed with the subject by a licensed investigator (MD or NP).

Visit 1:
1. Participant will meet with study investigator (M.D. or N.P.) to review the consent form and signing of informed consent
2. Detailed medical history, including assessment of history of anti-retroviral use, hepatitis status, nadir CD4 count, gastrointestinal disease, prior history of CNS opportunistic infections and risk factors for cardiovascular disease, including smoking history, family history, and history of recreational drug use
3. Physical examination
4. Fasting blood will be obtained for glucose, comprehensive metabolic panel, CBC plus differential, high-sensitivity C-reactive protein, insulin, hemoglobin A1C, lipids, T-cell subset, and HIV RNA (for HIV-infected patients) and HIV ELISA test (for HIV-negative controls). Additional blood will be collected for isolation of peripheral blood mononuclear cells for immunological studies, serum and plasma to be frozen and stored.

Visit 2:
1. Intestinal Permeability Study (sugar probe solution drink and urine collection)
2. Twenty-six milliliters of blood (3 ACD tubes) will be collected for isolation of peripheral blood monocytes.
3. Bionutrition:
   -Anthropometry (weight, height, waist and hip circumference measurements)
   -4-day food record form to be explained and given to patient
   -Bouchard activity questionnaire administered (bionutrition visit at the MGH CRC will be done at same time while patient is undergoing the urine collections for intestinal permeability study on the CRC)
4. Cognitive Function Instrument (CFI), and Patient's Assessment of Own Functioning Inventory (PAOFI) Questionnaire.

For HIV-Negative Control Participants in Neurological Substudy only:

Visit A (Screen Visit):
1. Participant will meet with study investigator to review the consent form and signing of informed consent
2. Detailed medical history, including assessment of history of anti-retroviral use, hepatitis status, nadir CD4 count, gastrointestinal disease, prior history of CNS opportunistic infections and risk factors for cardiovascular disease, including smoking history, family history, and history of recreational drug use
3. Physical examination

4. Fasting blood will be obtained for glucose, comprehensive metabolic panel, amylase (only for subjects who are enrolled in the randomized study), lipase (only for subjects who are enrolled in the randomized study), creatinine, insulin, hemoglobin A1c, Prothrombin Time / INR, Partial thromboplastin time (PTT), CBC plus differential, lipids, high-sensitivity C-reactive protein, T-cell subset, and HIV RNA (for HIV-infected patients) and HIV ELISA test (for HIV-negative controls). Additional blood will be collected for isolation of peripheral blood mononuclear cells for immunological studies (60 mL of blood), serum and plasma to be frozen and stored.
5. Urine HCG for women.
6. Food diary and nutrition.
7. Cross-sectional computed tomography (CT) scan: non-contrast enhanced 1cm cross-sectional CT scan of the abdomen to determine visceral adipose tissue (VAT) area and abdominal subcutaneous adipose tissue (SAT) area.
8. Cardiac CTA. Subjects who have had a cardiac CTA less than 3 months prior to visit A will not have a cardiac CTA at visit A.

Visit B:
1. Serum HCG will be obtained STAT as a pregnancy test for women prior to FDG-PET
2. Blood drawing for RNA-sequencing, and for sera and plasma to be frozen for assays to be run in batch using kits.
3. Food diary will be collected from Visit A 4. FDG-PET/CT
5. Neurocognitive Test Battery administered between 18F-FDG tracer injection and prior to FDG PET/CT scan.
6. Center for Epidemiological Studies-Depression Scale (CES-D).
7. Cognitive Function Instrument (CFI), and Patient's Assessment of Own Functioning Inventory (PAOFI) Questionnaire.

Data Collection/Study Procedures:

Multidetector Cardiac CT and CT Coronary Angiography (CCTA): Image acquisition will take place at MGH using the "SOMATOM Definition Flash", a second generation dual source 128 detector-row CT scanner (Siemens Medical Solutions, Forchheim, Germany). Based on a 280 millisecond gantry rotation speed this allows for a temporal resolution of 75 milliseconds, independent of heart rate. The study protocol employs several state-of-the-art technologies to minimize radiation dose. Semiautomated reference tube current selection and exposure control (CAREDose 4D, Siemens AG) are tailored to the subject's body size, in conjunction with semiautomated reference tube potential (voltage) selection (CARE kV, Siemens, AG), with individually adapted exposure settings on a per-patient basis. Prospective ECG-triggered axial-sequential or retrospective ECG-gated spiral with adaptive pulsing scan modes are used based on the heart rate and rhythm. Iterative image reconstruction algorithms allow for further reductions in radiation dose. These techniques allow for an up to 50% reduction in radiation dose compared with previous generation protocols.

The study protocol is based on the standard clinical CCTA protocol at MGH. Intravenous access will be obtained, most commonly in the right antecubital vein. The subject's blood pressure will be taken, and ECG leads will be attached to monitor heart rate and rhythm. At the supervising physician's discretion, for heart rates over 65 beats per minute or with significant variability, an intravenous dose of 5 mg metoprolol tartrate will be administered, and repeated once if insufficiently effective. Sublingual nitroglycerin (0.6 mg) will also be administered as per standard protocol for cardiac CT scans. Contraindications to metoprolol include hypotension (systolic blood pressure < 90 mm Hg), active asthma requiring inhalers on the day of scan, first degree heart block, or narrow-angle glaucoma. Contraindications to nitroglycerin include hypotension, severe aortic stenosis, or phosphodiesterase type 5 inhibitor (e.g. sildenafil, tadalafil, vardenafil) use within the preceding 5 days. A prospectively-ECG triggered noncontrast CT of the heart is obtained to measure coronary and aortic calcium. The test bolus

method will be used to plan the CTA timing, via a test bolus injection of contrast medium (20 ml), followed by a saline flush (40 ml). Contrast-enhanced CT acquisition: the contrast bolus is injected: 60-100 ml of nonionic iodinated contrast based on subject size (iopamidol 370 g/cm^3, Isovue 370, Bracco Diagnostics, Princeton, NJ), followed by a flush of 40 ml of saline. After a delay based on the test bolus, a CT acquisition is performed from the carina to the diaphragm: tube voltage 80 - 120 kVp, tube current-time product reference 320 mAs, detector collimation 2 x 128 x 0.6 mm. A number of high-resolution images of the heart at slightly different time-positions within the cardiac cycle will be reconstructed, of which the best phase will be used to evaluate the coronary artery lumen, plaque distribution and morphology.

18-FDG PET/CT: An approach to detecting macrophage activity in atherosclerotic plaques uses FDG as the contrast radiotracer for positron emission tomography (PET). FDG competes with glucose for uptake into metabolically active cells, including macrophages in atherosclerotic plaques, and is trapped inside cells after phosphorylation. FDG-PET can image metabolic activity (as a marker of plaque inflammation) quantitatively in atherosclerotic plaques[43,44], has been shown to have high reproducibility[45], and correlates with histologic data[44]. Taking advantage of the myocardium's preference for free fatty acids over glucose as an energy source, to minimize myocardial glucose uptake in order to better visualize coronary plaque activity, patients will be asked to adhere to an Atkins style diet for the evening prior to imaging and then to fast overnight. Cardiac PET/CT imaging will be performed two hours following intravenous administration of approximately 10mCi of 18-FDG. Imaging will be performed using a Siemens Biograph PET/CT system (Siemens Medical Solutions, Forchheim, Germany**)** Once co-registered, the standardized uptake value (SUV) of FDG will be measured in various arteries. The SUV is the decay-corrected tissue concentration of FDG (in kBq/ml) divided by the injected dose per body weight (kBq/g). Target-to-background ratio (TBR) will be calculated for each vascular segment as the segment SUV divided by vena cava or atrial blood SUV. FDG uptake in the brain will also be assessed.

Gastrointestinal Integrity, Microbial Translocation Assessments: Intestinal fatty acid binding protein (I-FABP), LPS and sCD14 are planned to be measured in plasma samples from 70 HIV-infected patients and 20 HIV-negative controls. Histologic analyses of intestinal tissue discussed in intestinal biopsy section below.

Metabolic and HIV-related parameters**:** Fasting lipids, fasting glucose and insulin, chem 7, CBC, and liver function tests are planned to be measured in plasma samples from 70 HIV-infected patients and 20 HIV-negative controls. HIV RNA and T cell subsets (CD4+ and CD8+ T-cells) will also be measured in HIV patients.

Plasma Markers of Monocyte/Macrophage Activation**:** MCP-1, sCD163 and sCD14 will be measured in all.

Immunologic Assessments: Peripheral blood mononuclear cells (PBMCs) will be isolated from blood by ficoll density centrifugation. Mucosal mononuclear cells (MMC) will be purified from colonoscopic biopsies using a combination of mechanical disaggregation and collagenase digestion as previously described (*Isolating Mucosal Lymphocytes from Biopsy Tissue for Cellular Immunology Assays.* Barbara L. Shacklett, J. William Critchfield, Donna Lemongello). Cells will be stained with markers for cell viability as well as lineage markers for T cells (CD3, CD4, CD8), antigen presenting cells (CD14, CD16, CD68, CD11, CD123, HLA-DR) and cellular activation (CD80, CD86, CD38, CD69). CD14+ monocytes will be assessed for surface markers associated with atherosclerosis (CCR2[46], CCR5[46], CX3CR1[46], CD11c, CD16 and tissue factor[30,31]). CD14+ monocytes will be sorted from blood and MMCs using a FACS Aria III sorter (BD Biosciences) into trizol for subsequent isolation of nucleic acid. Immunology studies will be performed in Dr. Kwon's laboratory at the Ragon Institute.

Genetic Studies: Tests for genetic factors that may contribute to HIV disease course, cardiometabolic risk, gastrointestinal health, and other related disorders will be performed on blood samples.

Colonoscopies with intestinal biopsies will be performed in the MGH GI endoscopy suite by Dr. Richter and his colleagues. **Intestinal tissue from the terminal ileum and the transverse colon** will be obtained to assess epithelial integrity including evaluation of **tight junction proteins (claudins, zonula occludens-1, occludin)**, intraepithelial **adherens junction protein E-cadherin**, staining for **LPS within the mucosa and in the lamina propia for evidence of microbial translocation**, and characterization of **intestinal T cells and macrophages** using flow cytometry and *in situ* staining of cytokines associated with macrophage activation. The Ragon Tissue Platform was established to provide necessary infrastructure to conduct patient-based research investigating tissue specific immune responses against HIV.

Bowel preparation: After eligibility is confirmed the subject will prepare for the procedures. To maximize the thoroughness and safety of the procedure, the colon must be completely empty prior to colonoscopy. The procedure preparation will be as per the GI Procedure Unit SOP.

Sedation for colonoscopy: Administration of sedative drugs at colonoscopy and has drawbacks, including an increased rate of complications, higher cost, and longer recovery periods for patients. Some studies have demonstrated that routine use of conscious sedation does not seem to be necessary because some participants found the examination to be only modestly or not at all uncomfortable. However, some investigators have proposed that without conscious sedation, the rate of intubation of the cecum may decrease and the risk of missing adenomas and cancer may increase. The decision to administer sedative drugs prior or during colonoscopy will be made by the physician, taking into account the patient's wishes and his/her medical conditions.

Intravenous benzodiazepines are the usual premedications used for colonoscopy, either alone or with a narcotic. Midazolam (2-5 mg) and Fentanyl (50 – 150 mcg) are commonly used. The combination of benzodiazepines and narcotics may achieve sedation more smoothly but is associated with a greater risk of respiratory depression. All procedures will be performed in the MGH Endoscopy Unit and patients will be monitored (eg, blood pressure, pulse, oxygen saturation) both during and after the procedure. Subjects with a history of adverse reactions to conscious sedation will be excluded from the study.

Colonoscopy: With the patient in left lateral decubitus position, a long, flexible, lighted viewing tube (colonoscope) will be inserted through the rectum into the colon. The scope is advanced and maneuvered while the lumen and walls of the colon are visualized by projections onto a television screen. The colonoscope has channels through which instruments can be passed in order to the perform biopsies or to cauterize potential bleeding. Air, water, and suction can be applied to help provide a clearer visual field for inspection. The goal for this procedure is to reach the cecum and the terminal ileum, and to collect specimens from throughout the colon as well as terminal ileum. Landmarks that may help to determine if this has been achieved include visualization of the appendiceal orifice and the ileocecal valve. No more than 28 intestinal biopsies will be obtained for research. In the event that the gastroenterologist identifies an abnormal finding in the colon, additional biopsies may be obtained as appropriate. In the event that the gastroenterologist identifies polyps on colonoscopy prior to initiation of study medication or at the end of the randomized treatment study, the polyps will be removed.

Intestinal Alkaline Phosphatase (IAP) Measurement: During the colonoscopy procedure, luminal fluid will be collected to measure IAP. Luminal fluid is collected by injecting about 5 ml of saline

through the operating channel of the scope into the terminal ileum and then aspirating approximately 3-5ml of fluid into a specimen trap.

Abdominal CT scan: Each subject will also have a non-contrast enhanced CT scan of the abdomen. A 1cm cross-sectional CT scan of the abdomen at L4 will be obtained to determine visceral adipose tissue (VAT) area and abdominal subcutaneous adipose tissue (SAT) area as previously described[47]

Anthropometric Measurements: Subjects' height and weight will be obtained and plotted on standard weight charts to determine percent of ideal body weight. Body mass index will be calculated. Anthropometric measurements of waist to hip ratio, leg circumference, arm circumference and neck circumference will be performed using a standardized technique[48]. Waist-to-hip ratio will be determined from the circumferential measurements of the iliac waist and the hips at the level of the iliac crest taken with the patient standing.

Nutritional Status and Exercise Activity: Food records will be analyzed for protein, carbohydrate, and fats (Nutritional Data Systems, Minneapolis, MN). The Bouchard 3-Day Physical Activity Record will also be administered.

Transcriptomic profiling of circulating monocytes: RNA from sorted CD14+ monocytes from the blood will be converted into a cDNA library using Illumina TrueSeq RNA sample preparation kit followed by paired end 50 cycles sequencing by HiSeq. Data will be analyzed using TopHat and Cufflinks and visualized using CummRbund as described by Trapnell *et al*[49]. These software tools will allow us to analyze the data sets for differences in transcriptional splice variants. For analysis, we will specifically examine differential expression of transcripts as well as perform pathway analysis using IPA Transcriptional Analysis (Ingenuity Systems).

Medical Record Review: Medical records will be reviewed for relevant clinical data for subjects who are currently followed at MGH or BWH for clinical care. Subjects who receive clinical care from external providers will be asked to sign a Release of Information form. We will request and/or review relevant medical history, laboratory results and treatment history for HIV and/or Hepatitis B and C.

Fecal Occult Blood Testing (FOBT) Using the Fecal Immunochemical Test (FIT): Participants in the Randomized Study of Teduglutide vs. Placebo will be given a FIT kit to perform the test at home 1 year after starting the study medication (6 months after the study is over) to screen for colorectal cancer. Participants will be asked to asked to collect a very small stool sample using a brush so that they do not directly handle the fecal material. Using the brush, subjects will place the sample on the collection card, place the card in an envelope provided and mail to the laboratory.

Functional Assessment:
Functional assessments/scales are required to differentiate HIV-associated neurocognitive disorders into asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorders (MND), per Frascati guidelines for HAND[50]. Subjects will complete the Cognitive Function Instrument (CFI) which is designed to detect early changes in cognitive and functional abilities in individuals without clinical impairment and is a sensitive measure in tracking longitudinal changes in cognitive function among older individuals [51]. The CFI contains 14 questions; the participant version will be used in this study. The Patient's Assessment of Own Functioning Inventory (PAOFI) questionnaire which is complementary to the CFI will be used (33 questions) [52]. Questionnaires will be administered through REDCap using a Partners Encrypted iPad.

Mood Assessment

Mood will be assessed using the Centre for Epidemiologic Studies Depression scale (CES-D [53]); a score of ≥ 16 is indicative of high depressive symptoms [13,54,55]. This scale will be important to test if the Teduglutide affects depressive symptoms and will be needed to identify possible confounding to cognitive performance. The scale will be administered through REDCap using a Partners Encrypted iPad.

Neurocognitive Assessment (administered by neurologist or neuropsychologist):
The neurocognitive battery will include tests assessing:

a. Premorbid intelligence: WRAT 4 reading
b. Attention/Working Memory: WAIS IV Digit Span; CalCAP Abbreviated Test Battery
c. The speed of information Processing: WAIS III Digit Symbol, Trail making test-part A, Victoria Stroop Test Word and Color; CalCAP Abbreviated Test Battery[56].
d. Delayed Verbal Learning and Recall: Hopkins verbal learning test 1-3 and delayed
e. Motor Skills: Grooved Pegboards for dominant and non-dominant hands
f. Executive function: Trail making test-part B; Victoria Stroop Test Interference

These tests have been previously proven to be sensitive to neurocognitive disturbances in HIV infection[57] and have standardized norms. The complete neurocognitive assessment is designed to last <1 hour, and will be performed at visit 4 in all subjects and also repeated at the final 6 month visit following completion of the interventional study. We will determine the z-score for each test using normative data, average z-scores from all tests, and create a composite cognitive deficit score for subjects at each time-point. The data will be scored per tests' instructions, and scores will be entered into REDCap database.

Remuneration

Transportation to and from visits will also be additionally subsidized and a meal ticket as well as parking will be provided at each visit.

Participants who participate in only Baseline Study Part 1 (2-visit cross-sectional substudy) will receive $75.

Participants who undergo Baseline Study Part 1 and Part 2, (4-visit cross-sectional substudy including colonoscopy, cardiac CTA and FDG-PET) will receive $375.

Participants in the interventional Randomized Study of Teduglutide vs. Placebo study will receive up to $825 for completion of study.

HIV-negative control participants who undergo testing for neurological substudy (2-visits cross-sectional substudy including cardiac CTA and FDG-PET) will receive $125.

Pro-rated remuneration

- In addition to providing parking and assistance with transportation when needed, participants will receive up to $825 for the Randomized Study of Teduglutide vs. Placebo:
- $50 after the completion of screen visit 1
- $25 after the completion of screen visit 2 (bionutrition).
- $225 after completion of screen visit 3 (colonoscopy)
- $75 after the completion of the baseline visit
- $25 after each safety visit (1, 3 month)
- $400 at the completion of the final study visits

Participants in the cross-sectional study will receive up to $375:

- $50 after the completion of screen visit 1

- $25 after the completion of screen visit 2 (bionutrition)
- $225 after completion of screen visit 3 (colonoscopy)
- $75 after the completion of visit 4

Participants in the neurological substudy will receive up to $125:
- $50 after the completion of visit A
- $75 after the completion of visit B

Costs
There will be no cost to the subject for participation in the study.

Research Team: The Principal Investigator, Dr. Janet Lo, will lead and coordinate all collaborating groups for the projects. Human subjects research will take place at the MGH in the MGH Clinical Research Center. Recruitment of participants and the conduct of the clinical studies for both Specific Aims will be done by a clinical investigative team of nurse practitioner and research coordinator supervised by Dr. Lo. Intestinal biopsies will be obtained during colonoscopies and upper endoscopies taking place in the MGH GI endoscopy suite and will be performed by Dr. James Richter and other clinical gastroenterologists in the MGH gastroenterology division. Laboratory work including processing of intestinal biopsy samples, immunohistochemistry, ELISA assays, flow cytometry and transcriptional profiling of monocytes by RNA-Seq will take place in Dr. Douglas Kwon's laboratory in the Ragon Institute of MGH, MIT and Harvard. Dr. Kwon, Co-Investigator, has extensive scientific expertise in mucosal immunology. He is the PI on six active protocols examining the mucosal immune response to HIV. Cardiac CT imaging and nuclear imaging will take place in the MGH Radiology department. Interpretation and analyses of cardiac CT imaging as well as analyses of cardiac FDG-PET and co-registration of CT with PET imaging will be performed in the MGH Section of Cardiac Imaging. Dr. Wendy Henderson, Chief of the Digestive Disorders Unit at the NIH, leads a laboratory whose focus is on gastrointestinal function, permeability and inflammation as it relates to abdominal symptoms in various disease states. Measurement of gut permeability using sugar probes will be directed through Dr. Henderson's laboratory utilizing existing set-up and resources at the NIH. Urine samples sent to Dr. Henderson's laboratory for analysis will be deidentified. Dr. Steven Grinspoon will serve as Co-Investigator and assist Dr. Lo with the clinical studies proposed in this grant application. As a pioneering leader in physiological studies of metabolic and cardiovascular diseases in HIV, he has vast experience to provide expert advice for specific questions that may arise during the course of the study and to provide additional clinical research infrastructural support to assist with conduct of the clinical studies proposed. Dr. Hang Lee of the MGH Biostatistics Center will serve as study statistician.

VI. Biostatistical Analysis

Dr. Hang Lee of the MGH Biostatistics Center has provided biostatistical consultation regarding power calculations and design for the proposed studies. Dr. Lee's biostatistical support will continue to be readily available for randomization, data analysis, and manuscript preparation.

Statistical Analysis Plan:
Baseline Study Part 1: Biomarkers, Immunology and Intestinal Permeability Studies and **Baseline Study Part 2: Colonoscopy, Cardiac CTA and FDG-PET/CT Studies (Aim 1A):** The statistical approach to assess correlations between intestinal permeability and microbial translocation with innate immune activation and cardiovascular disease. For mechanistic modeling, we will perform bivariate exploratory analysis followed by multivariate linear regression models to explain the impact of microbial translocation on cardiovascular inflammation.

Univariate analysis: To identify relationships between GI epithelial integrity, markers of innate immune activation and markers of microbial translocation with cardiovascular markers of vascular inflammation (TBR measured by FDG-PET) and atherosclerosis (segments with plaque) in HIV-infected patients, we plan to use linear correlation coefficients for continuous predictor variables (Pearson correlation coefficients if variables are normally distributed or Spearman correlation coefficients if their distributions are non-normal) to assess bivariate relationships between cardiovascular imaging measurements and measured covariates hypothesized to be associated with atherosclerosis development and vascular inflammation.

Multivariate modeling: For outcomes TBR and plaque, to control for potential confounders, multivariate linear regression modeling will be used to identify factors independently associated with increasing TBR and increasing number of atherosclerotic plaque lesions. Similar methods will be applied for neurocognitive score and mood assessment to cerebral FDG-PET/CT. Model variable selection will be based on "change-in-estimate" method[58]. Candidate covariates to be chosen for entry into our model include all covariates significant on individual univariate models ($p<0.05$), potential confounders, and biologically plausible covariates with established relationships to atherosclerosis. Colinearity among covariates will be identified and redundant covariates will be excluded from the model. Confounders will be identified by observing changes in effect estimate β; colinearity will be identified by observing changes in standard error without substantial changes in β as well as by using Variance Inflation Factor criterion. Regression diagnostics will be used to check if model assumptions are met[58]. We will control for age, gender, race, smoking, lipids, diabetes mellitus, WRAT 4 reading versus educational years, and hypertension in addition to other potential confounders identified by methods above.

Between group comparisons: We will also assess differences in microbial translocation, mucosal immunity and GI permeability markers among those with coronary atherosclerosis vs. those without atherosclerosis and in those with elevated TBR (>1.8) vs. those without elevated TBR. 2 group comparisons will be performed using Student's T-test if the outcome variable is normally distributed. Distributions will be tested for normality using the Wilk-Shapiro test and examination of the histogram distributions. If the outcome variable is not normally distributed, we will consider appropriate transformation to approximate a normal distribution. Alternatively, if distributions are non-normal despite the applied transformation, we will use nonparametric Wilcoxon rank sum test to compare the 2 groups. All tests will be 2-sided, and a $p<0.05$ will be considered statistically significant.

Sample Size Determination: With 70 HIV-infected patients and 20 uninfected controls, we can detect a bivariate correlation Pearson's coefficients of at least 0.58, 0.33, and 0.29 can be detected at a 5% significance level with 80% power within uninfected, infected, and both the uninfected and infected subjects respectively. The detectable effect sizes of markers between elevated - and without elevated TBR groups will depend on the distribution of the observed TBR and the markers' distributions. If the rate of elevated TBR is 50% among the whole cohort of 70 patients, then an effect size with Cohen's effect size $d>0.68$ can be detected by a two-sided independent samples t-test at a 5% significance level with 80% power.

RNA Sequencing Subset (Aim 1B):

Statistical Analysis Plan: The BioConductor package LIMMA will be used to identify genes which are differentially expressed between the two compartments. Holm's and Benjamin-Hochberg methods will be applied for correcting multiple testing. ***Sample Size Determination***: For *RNA-Seq* analysis using 7 patients with CVD and 7 without, there will be 27,000 genes to be included in the initial testing of which about 140 genes are expected to be differentially expressed significantly with a two to three times of the standard deviation of the fold difference in the expression levels. With a targeted false discovery rate (FDR) of 20% or less, the power to detect at least 112 differentially expressed genes significantly with an effect size of 2.7×SD with $p<0.0011$ (i.e., multiple testing adjusted alpha=0.0011) will be at least 80%.

Randomized Study of Teduglutide vs. Placebo (Aim 2):

Statistical Analysis Plan: For the primary outcome assessment, intention to treat principle will be applied in the analysis of this randomized placebo-controlled trial. For analysis of our primary outcome of arterial inflammation, we will compare the mean changes in arterial inflammation as assessed by FDG-PET measured as target to background ratio (TBR) of standardized uptake value in the group is randomized to teduglutide to the group that is randomized to placebo using independent samples t-test. Secondarily, we will also examine whether or not the mean of within group changes during the intervention period is zero using paired sample t-test. For secondary endpoints including change in markers of intestinal permeability, innate immune activation and microbial translocation, we will use the independent samples t-test to compare the means of the changes between the teduglutide group and the placebo group. In addition, longitudinal linear mixed effects modeling will be applied. The model will include random subject level intercept, fixed discrete time and group, and time × group interaction term to characterize the within-group longitudinal means and to compare the between group difference in those longitudinal means. Effects of the potential confounders, (lipids, age, gender, smoking, diabetes mellitus, hypertension and other cardiovascular risk factors that may be different between the groups), will be adjusted by including them in the model as the covariates. We will utilize all available observations for this modeling even if there are some patients who have missing data at any time. If necessary, we will also perform Markov Chain Monte-Carlo (MCMC) based multiple imputation procedure.

Sample Size Determination: With 30 patients in each group and up to 10% attrition (i.e. evaluable n=27/group), this trial can detect at least a between group difference of 0.2244 in the two within person TBR mean changes at a 5% significance level with 85% power by a two-sided independent samples t-test. This assumed that the standard deviation of the within subject TBR change is 0.27, which was estimated from our prior study at MGH study of similar patient population[28] of which the observed mean ± (standard deviation) of the cross sectional TBR data was 2.24 (±0.35) and the assumed pre- to post intervention longitudinal correlation was 0.7. This detectable effect size is close to 10% of the baseline mean 2.24, and we anticipate that the placebo group will show no change (or even increase its mean value). The effect size of 10% is of clinical significance as it is similar to the amount of decrease seen with statin treatment for 3 months[59], the most effective medical therapy we have to date for treatment of atherosclerotic disease and prevention of MI.

VII. Risks and Discomforts

Colonoscopy: Colonoscopy are generally safe procedures and complications are rare. Given the underlying HIV infection of the study participants, there is potential for the sequellae of the endoscopy complications outlined below to be more serious and more difficult to treat.

***Perforation*:** The risk of perforation of the colon is 0.2-0.4% after diagnostic colonoscopy and 0.3-1.0% with polypectomy. A higher rate (4.6%) is associated with hydrostatic balloon dilatation of colonic strictures.. Perforation is more common (1) in patients who are oversedated or under general anesthesia, (2) in the presence of poor bowel preparation, or (3) with acute bleeding, and generally results from mechanical or pneumatic pressure or from biopsy techniques. Mechanical perforation by the tip of the instrument occurs at sites of weakness of the intestinal wall (eg, diverticula, transmural inflammation) and proximal to obstructing points (eg, neoplasms, strictures). Pneumatic perforation of the colon or ileum results from distension by insufflated air. Perforation from polypectomy is an electrosurgical injury. Free perforation into the peritoneal cavity may be recognized during the procedure if abdominal viscera become visible. A laceration so large that it can be observed directly through the colonoscope is a surgical emergency. In less severe situations, marked persistent abdominal distension or pain should prompt the ordering of radiographs; this imagery may reveal free air in the peritoneum. These symptoms may be delayed for several days if the leak is tiny and well localized. Retroperitoneal perforation, usually a pneumatic injury, can give rise to subcutaneous emphysema. Fever and leukocytosis may eventually

develop with any of these perforations. When plain abdominal or chest radiographs show pneumoperitoneum, gross extravasation should be assessed; if present, surgical intervention is required. In the absence of leakage, treatment with intravenous antibiotics and close observation may be considered. This is a clinical determination.

***Bleeding*:** Bleeding complicates approximately 1 of every 1000 colonoscopic procedures. Most cases resolve spontaneously. Following polypectomy, bleeding may occur immediately, but, in 30-50% of cases, it is delayed from 2-7 days until the eschar sloughs. Immediate bleeding can be treated by resnaring the remaining stalk and tightening the snare for 10-15 minutes, usually without further electrocoagulation. Another procedure that may be helpful is the injection of 5-10 mL of a 1:10,000 epinephrine solution into the stalk or the submucosa to achieve vasoconstriction. Delayed bleeding usually stops spontaneously, although transfusions, endoscopic therapy, angiography, and even laparotomy may be required in more severe cases.

***Infection*:** Documented instances of transmission of infection from one patient to another or to endoscopic personnel are extremely rare. Bacteria reported to have spread include *Salmonella* species, *Pseudomonas* species, and *Escherichia coli*. Transmission of hepatitis B or C or HIV is extremely rare and has been linked to improper disinfection of scopes and accessories. With proper disinfection, there is no evidence that endoscopy presents a risk of hepatitis or HIV transmission.

***Abdominal distension*:** Intestinal distension during endoscopy can cause notable discomfort and may also impair mucosal blood flow. Carbon dioxide rather than air insufflation during colonoscopy may offer some advantages, i.e., it is absorbed from the colon, it is nonexplosive, and mucosal blood flow is less affected, thus decreasing the risk of colonic ischemia.

***Splenic rupture*:** Although a very uncommon complication, the presumed mechanisms of splenic rupture during colonoscopy include direct trauma to the spleen, marked angulation of the splenic flexure, excessive splenocolic ligament traction, and decrease in the relative mobility between the spleen and the colon. Hemodynamic instability, clinical features of acute abdomen, leukocytosis, and/or acute anemia in patients with persistent abdominal pain after colonoscopy demand immediate attention. Intestinal perforation or bleeding must first be excluded, after which CT scans can be used for further evaluation.

***Small bowel obstruction*:** Small bowel obstruction is another rare complication of colonoscopy, although it is perhaps more common in patients who have a history of abdominal surgery and postoperative adhesions. The mechanism is uncertain, but it may occur secondary to air insufflation into the small bowel as a result of an incompetent ileocecal valve causing distension and entrapment of the small bowel by adhesions. Colonoscopists should be aware of this possible complication, particularly as skills improve and the ileum is intubated more frequently. Patients with a history of abdominal surgery or bowel obstruction should be informed of this complication when consent is given.

***Medication effects*:** Sedatives used during colonoscopy may cause complications from allergic reactions or, more importantly, from doses that may be excessive for certain individuals and lead to respiratory depression. Serious events may complicate up to 0.5% of procedures. More than 50% of deaths associated with endoscopy are related to cardiopulmonary events. Adverse effects of benzodiazepines, other than respiratory depression, include anxiety and occasional injection-site reaction; the latter are more frequent with diazepam than with midazolam. Other adverse effects of narcotics include nausea, vomiting, and hypotension. Naloxone and flumazenil readily reverse the adverse effects of narcotics and benzodiazepines, respectively, within minutes. The proper technique and sequence of administration of these drugs, together with continuous monitoring of the sedated patient, can help minimize complications.

Simethicone: Simethicone is available over the counter and is generally considered safe. Simethicone is not absorbed into the body. Side effects are rare and include mild diarrhea, regurgitation, nausea, vomiting, bloating, heartburn, and constipation.

Magnesium Hydroxide: Magnesium Hydroxide is available over the counter and is generally considered safe. Side effects are rare and include diarrhea, upset stomach, stomach cramps, and vomiting.

Teduglutide:

In a published randomized placebo-controlled trial of teduglutide for 24 weeks in patients with short bowel syndrome, teduglutide was well-tolerated, safe and facilitated reductions in parenteral support in these patients[41]. In the package insert for Gattex (teduglutide), the most common adverse reactions across all studies are abdominal pain, injection site reactions, nausea, headaches, abdominal distension, and upper respiratory tract infection. The package insert also includes the following statement: "In the clinical studies, 13 subjects were diagnosed with polyps of the G.I. tract after initiation of study treatment. In the SBS placebo controlled studies, 1/59 (1.7%) of subjects on placebo and 1/109 (0.9%) of subjects on GATTEX 0.05 mg/kg/day were diagnosed with intestinal polyps (inflammatory stomal and hyperplastic sigmoidal after 3 and 5 months, respectively). The remaining 11 polyp cases occurred in the extension studies – 2 colorectal villous adenomas (onset at 6 and 7 months in GATTEX 0.10 and 0.05 mg/kg/day dose groups, respectively), 2 hyperplastic polyp (onset 6 months in GATTEX 0.10 mg/kg/day dose group and 24 months in GATTEX 0.05 mg/kg/day dose group), 3 colorectal tubular adenomas (onset between 24 and 29 months in GATTEX 0.05 mg/kg/day dose group), 1 serrated adenoma (onset at 24 months in GATTEX 0.05 mg/kg/day dose group), 1 colorectal polyp biopsy not done (onset at 24 months in GATTEX 0.05 mg/kg/day dose group), 1 rectal inflammatory polyp (onset at 10 months in the GATTEX 0.05 mg/kg/day dose group, and 1 small duodenal polyp (onset at 3 months in GATTEX 0.05 mg/kg/day dose group)."

As teduglutide was approved by the FDA for short gut syndrome in December 2012, it is still fairly new to market, so there may be yet some unknown side effects outside of those reported in the clinical trials, but data is being collected in an FDA-required postmarketing study of patients with short bowel syndrome treated with teduglutide in the clinical setting to further evaluate the drug's long-term safety. In our proposed randomized study, we will monitor patients at each visit for possible symptomatic side effects. In addition, we will also follow the necessary recommended safety testing at baseline and at safety interim visits in the study. Patients with known malignancy within the last 5 years, history of colon cancer, or first degree relative with colon cancer will be excluded from the study. Screening colonoscopy will identify participants with previously undiagnosed colon cancer, whom we will exclude from the interventional study. All interventional study participants will undergo a colonoscopy at the end of the study. Women who are pregnant, planning on becoming pregnant, or are breastfeeding will be excluded from the study.

Radiation Risks:

The estimated median dose of CCTA is 4 mSv. Individual dose will vary, as each exam is tailored to the subject's size, heart rate, and rhythm. This tailored approach along with the state-of-the-art dose reduction techniques described above allow the dose to be substantially lower than historically reported median CCTA doses (please see references from MGH below).

References for CCTA dose at MGH:
Ghoshhajra, B. B., Engel, L.-C., Major, G. P., Goehler, A., Techasith, T., Verdini, D., et al. (2012). Evolution of coronary computed tomography radiation dose reduction at a tertiary referral center. The American journal of medicine, 125(8), 764–772. doi:10.1016/j.amjmed.2011.10.036

Engel, L.-C., Lee, A. M., Seifarth, H., Sidhu, M. S., Brady, T. J., Hoffmann, U., & Ghoshhajra, B. B. (2013). Weekly dose reports: the effects of a continuous quality improvement initiative on coronary computed tomography angiography radiation doses at a tertiary medical center. Academic Radiology, 20(8), 1015–1023. doi:10.1016/j.acra.2013.04.012

Ghoshhajra, B. B., Engel, L.-C., Károlyi, M., Sidhu, M. S., Wai, B., Barreto, M., et al. (2012). Cardiac Computed Tomography Angiography With Automatic Tube Potential Selection: Effects on Radiation Dose and Image Quality. Journal of thoracic imaging, 1–9. doi:10.1097/RTI.0b013e3182631e8a

Radiation exposure for a low dose CT scan of the abdomen is about 0.10 mSV per slice.

For FDG-PET/CT, the administered dose from F-18 FDG for a typical cardiac study is 370 MBq (10 mCi) and the effective dose for an attenuation correction scan of head/neck/chest/upper abdomen is about 2.8 mSv. The total effective dose from two FDG-PET/CT studies would be 20 mSv.

The total radiation dose associated with this study is approximately 28 mSv and is about the same as one would normally receive in approximately 9 years from background radiation.

Blood drawing:
The maximum total blood drawn for a patient completing the 6 month long interventional study is approximately 502 ml for participants with HIV and 226 ml for participants without HIV. There will be minimal risk and discomfort associated with blood drawing and IV placement. The risks of these procedures are minor bruising or bleeding at the site of the blood draw or IV catheter, nausea from the glucose infusion, and lightheadedness from the blood draws.

The maximum total blood drawn for a patient completing only the cross-sectional substudy is approximately 150 mL.

For each participant, blood will not be drawn more than twice per week and the total amount will not exceed 550mL in an 8-week period. Blood sampling exceeding 200mL will not be drawn at any one point in time.

Genetic Research Studies:
Genetic research studies may create special risks to human subjects and their relatives. These involve medical, psychosocial and economic risks, such as the possible loss of privacy, insurability and employability, change in immigration status and limits on education options, and may create a social stigma. Knowledge of one's genetic make-up may also affect one's knowledge of the disease risk status of family members. To minimize the risks associated with genetic testing, no results will be filed in the subject's medical record and no research results will be given to subjects or healthcare providers.

CT contrast medium:
This procedure requires the injection of an iodinated contrast medium. These contrast media have been known to cause renal failure, particularly in patients with a pre-existing renal impairment. The contrast dose in cardiac CT has decreased with the introduction of faster scanners. The dose required in 128-slice CT will be between 60 and 120 ml. Anaphylactoid (allergic-type) reactions to iodinated contrast media may case skin reactions or in very rare occasions result in breathing difficulty and hypotension (shock). Patients with previous reactions to iodine-containing contrast media will be excluded from participation.

Participants with signs of renal failure will not be included in the study. Typical contrast dose during a cardiac CT exam translates into ~ 1 ml/kg in an average patient.

In participants with normal renal function the elimination half-life of iodine-containing contrast media is 2 hours and 99% is excreted after 24 hours. This is irrespective of the total amount of contrast. Unless the patient has elevated creatinine levels, we can safely assume that all contrast is eliminated after one day.

The following additional safety criteria will be implemented. Participants will be excluded if:
- Serum creatinine > 1.5 mg/ml
- The estimated creatinine clearance < 60 ml/min

Rarely, complication of IV contrast extravasation may occur.

Beta-blockers:
Beta-blockers are used by millions of Americans and are generally considered safe. The side-effects and risks of incidental (incremental) use of beta-blockers include bradycardia (which is the purpose of the administration), hypotension, and in rare occasions wheezing (bronchospasm) and dyspnea. Allergic reactions to beta-blockers are rare.

Nitroglycerin:
Nitroglycerin is routinely used in clinical CT coronary angiography and the treatment of angina pectoris, and is generally considered safe. The side-effects and risks of nitroglycerin are generally mild and of short duration and include hypotension, tachyarrhythmia, headache, lightheadedness, and visual disturbance. Subjects who are on sildenafil, tadalafil, or vardenafil will need to stop these drugs at least 5 days prior to receiving nitroglycerin on the day of the cardiac CT scan. Nitroglycerin will not be administered to subjects who have taken sildenafil, tadalafil, or vardenafil within 5 days of the cardiac CT scan. If baseline systolic blood pressure is below 90, nitroglycerin will not be given. If a participant's blood pressure were to drop precipitously or if the participant develops symptoms, standard clinical resuscitative care will be given. For hypotension, intravenous boluses of normal saline will be administered and the subject will be closely monitored. If there is no response to intravenous volume resuscitation, then pressor agents will be administered as per standard clinical practice.

Pregnancy and urine tests:
Urine pregnancy test will be performed in female participants at each visit. Female participants will be cautioned carefully about the risks of the study and pregnancy. If a female participant were to become pregnant at any time point during the study, the study drug will be immediately stopped and no radiologic procedures will be performed.

Questionaires and Neurocognitive Testing:
Subjects will be instructed to complete the questionnaires to the best of their ability, but will have the option to leave any question(s) blank. In the unlikely event that evidence of a new physical or psychological disorder is found, with the individual's permission, the information will be shared with his or her primary care physician who can direct care as needed. There are minimal risks associated with completing questionnaires and neurocognitive testing and include subject fatigue and the possibility of minor psychological distress associated with answering sensitive questions regarding psychological functioning. As detailed, the investigators will be careful regarding the protection of confidentiality, and multiple procedures are in place to reduce the likelihood of a breach of confidentiality.

VIII. Potential Benefits

There may be no direct benefit to the individual participant. As part of the study, participants may undergo: 1) the examination of the colon which might reveal polyps, cancers, vessel abnormalities, lymphomas, or other disorders that need treatment; and 2) determination of coronary calcium score, coronary anatomy and pathology. Participants and their healthcare provider (with the participants' permission) will be informed of any abnormal findings of clinical relevance in a timely fashion. In the event that neurocognitive impairment or high depressive symptoms are found, with the participant's permission, the information will be shared with his or her primary care physician who can direct care as needed, and will include information to neurology or psychiatry-infectious disease specialists for appropriate medical care and counseling. Patients who are initially screened for the HIV uninfected control group who are found to have a positive HIV ELISA test at study entry, will be immediately contacted and will be referred to an infectious disease specialist for appropriate medical care and counseling.

Potential benefits to society include an increased understanding of the mechanisms of atherosclerotic disease in the HIV-infected population and immune or intestinal factors that may be associated with atherosclerosis in this patient population. Additional potential benefits to society include an increased understanding of the mechanisms of neurocognitive impairment and depression in the HIV-infected population and immune or intestinal factors that may be associated with these conditions.

IX. Monitoring and Quality Assurance

The principal investigator (PI) will continually monitor the study. She will ensure that adverse events are reviewed promptly and reported to the Partners Human Research Committee (PHRC) as per PHRC policy. A data and safety report will be included in the annual report to the PHRC. Any serious adverse events will be reported within 24 hours to the PHRC.

The data and safety monitoring board (consisting of a gastroenterologist, a HIV specialist, and a cardiologist) will meet regularly every 6 months to review all adverse events and will be called upon at other times also as significant adverse events arise.

The PI and study team will review all adverse events on a case-by-case basis. The PI and study team will also be reviewing all adverse events with the DSMB every 6 months and all serious adverse events when they arise.

Unanticipated problems including adverse events will be reported to the Partners Human Research Committee (PHRC) as described in the PHRC policy on Unanticipated Problems Involving Risks to Subjects or Others including adverse events:
http://healthcare.partners.org/phsirb/Guidance/Reporting_Unanticipated_Problems_including_Adverse_Events.1.11.pdf

The PI, Dr. Lo, will report to the PHRC any of the following unanticipated problems and adverse events that occur: 1) during the conduct of the study, 2) after study completion, or 3) after subject withdrawal or completion. Reports are to be submitted within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem.
- Internal adverse events that are unexpected, and related or possibly related to the research and that indicate there are new or increased risks to subjects;
- External adverse events that are serious, unexpected, and related or possibly related to the research and that indicate there are new or increased risks to subjects that require some action (e.g., modification of the protocol, consent process, or informing subjects);
- Unanticipated adverse device effects that are serious and caused by, or associated with, the device;
- Deviation from the approved research protocol or plan without IRB approval in order to eliminate apparent immediate hazard to subjects or harm to others;
- Deviation from the approved research protocol or plan that placed subjects or others at an increased risk of harm regardless of whether there was actual harm to subjects or others;
- Any event that requires prompt reporting according to the research protocol or investigational plan or the sponsor;
- Breach of confidentiality or violation of HIPAA (e.g., lost or stolen laptop);
- Medication, procedural or laboratory error (e.g., errors in drug administration or dosing, surgical or other procedure, or testing of samples or test results) regardless of whether subjects experienced any harm;
- Interim analysis, safety monitoring report, publication in a peer-reviewed journal, or other finding that indicates that there are new or increased risks to subject or others or that subjects are less likely to receive any direct benefits from the research;

- Change in FDA labeling (e.g., black box warning), withdrawal from market, manufacturer alert from the sponsor, or recall of an FDA-approved drug, device, or biologic used in the research;

Reporting of unanticipated problems that are adverse events: Any unanticipated untoward or unfavorable medical occurrence, including abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, that indicates that the research places subjects at increased risk of physical or psychological harm than previously known or recognized will be reported to the Partners IRB within 5 working days/7 calendar days of the date the principal investigator first becomes aware of the problem according to Partners Human Research Committee (PHRC) Policy.

Subjects will be informed about any incidental findings found on any of the study procedures obtained in the study. If the subject grants permission, the study investigator will also inform the subject's primary care physician (PCP). If the finding requires that the subject be referred to a specialist, we will make a recommendation to the subject and the PCP and offer to facilitate the referral, but will defer to the PCP to make the final decision.

In an effort to maximize the safety of subjects, those subjects who are taking anticoagulants, that require antibiotic prophylaxis for invasive procedures, and who have an allergy/intolerance/sensitivity to benzodiazepines have been excluded from participation.

Colonoscopies will be performed in the GI Endoscopy Unit at MGH by the gastroenterologist co-investigator. A member of the study staff will be present at the procedures.

Subjects will be discontinued from the study if they develop elevated creatinine >2 mg/dl, rise in AST or ALT > 5x upper limit of normal, pancreatitis, malignancy or become pregnant. Subjects will be instructed to immediately report significant symptoms including, but are not limited to abdominal pain, nausea, injection site reactions, and swelling. These symptoms will be carefully monitored and subjects with intolerable side effects may be discontinued. In additional, subjects who develop generalized hypersensitivity will also be discontinued from the study.

The investigators and study staff will take measures to protect the privacy of patients. Study-specific Data Collection/Case Report Forms will be completed for all subjects who consent to study participation. In order to further protect the privacy and confidentiality of subjects, subject data and case report forms will be labeled with an alphanumeric code and will not contain the subject's name, initials, medical record number, date of birth or social security number. The key to the code which links subject's identity to the code will be kept in a secure location separate from the case report forms, subject data and documents. Subject clinical source documents include: laboratory reports, procedure reports, and radiologic reports. All study records will be maintained in a secure fashion with access limited to essential study personnel only.

The PI will review the case report forms and source documents quarterly and periodically as the need arises. An internal audit will be conducted annually for both subject and regulatory study files. Accuracy of data entered into the database will be verified with source documents. The PI and study team will discuss and review research results and corresponding data on a regular basis at laboratory meetings.

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