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A Phase I/II Pilot Study of Dipyridamole as a Modulator of Immune Activation and Systemic Inflammation in HIV-1-Infected Subjects on Antiretroviral Therapy

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Non-IND Study

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GLOSSARY OF TERMS

ADO	Adenosine
AE	Adverse Event
ART	antiretroviral therapy
ASA	Aspirin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATS	American Thoracic Society
CrCL	calculated creatinine clearance
CRHC DC	Center for Research on Health Care Data Center
CLIA	Clinical Laboratory Improvement Amendments
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-reactive protein
CVD	Cardiovascular disease
DHHS	Division of Health and Human Services
DP	Dipyridamole
EAE	Expedited adverse event
E/CIA	Enzyme or chemiluminescence immunoassay
FEV_1	Forced exhaled volume in 1 second
FMD	Flow-mediated dilation
FVC	Forced vital capacity
HAART	Highly active antiretroviral therapy
hsCRP	High Sensitivity C-Reactive Protein
IDS	Investigational Drug Service
IDSMB	Institutional Data Safety and Monitoring Board
LFT	Liver function test
MOPS	Manual of Procedures
NNRTI	Nonnucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
PI	Protease inhibitor
SAE	Serious adverse event
SUSAR	Suspected, unexpected, serious, adverse reaction

PROTOCOL SUMMARY

A Phase I/II Pilot Study of Dipyridamole as a Modulator of Immune Activation and Systemic Inflammation in HIV-1-Infected Subjects on Antiretroviral Therapy

Short Title: Dipyridamole for immune activation in HIV

DESIGN — This is a Phase 1/2, randomized, double blind, two-arm, partial cross-over, single-site, pilot study evaluating the effect of oral dipyridamole (DP) on immune activation and inflammation in HIV-1-infected, ART-treated subjects with HIV-1 RNA less than 50 copies/mL. The change from baseline to week 12 in the plasma levels of sCD163 and sCD14, and IL-6 will be assessed within and compared between study arms, as will secondary endpoints related to immune activation, inflammation, gut mucosal changes, pulmonary function and brachial artery flow-mediated dilation (FMD).

Subjects will be randomized 1:1 to DP 100 mg 4 times a day or matching placebo. Following week 12, subjects in the DP arm will continue DP for an additional 12 weeks (for total 24 weeks) and subjects in the placebo arm will cross over to DP 100 mg 4 times a day for 12 weeks.

Evaluations will be scheduled at Entry (week 0) and Weeks 2, 4, 8, 12, 14, 16, 20, 24, and 28.

DURATION — Subjects will be in the study for 28 weeks.

SAMPLE SIZE — 40 subjects, 20 per arm. Rectal Tissue Subset: 20 subjects, 10 per arm.

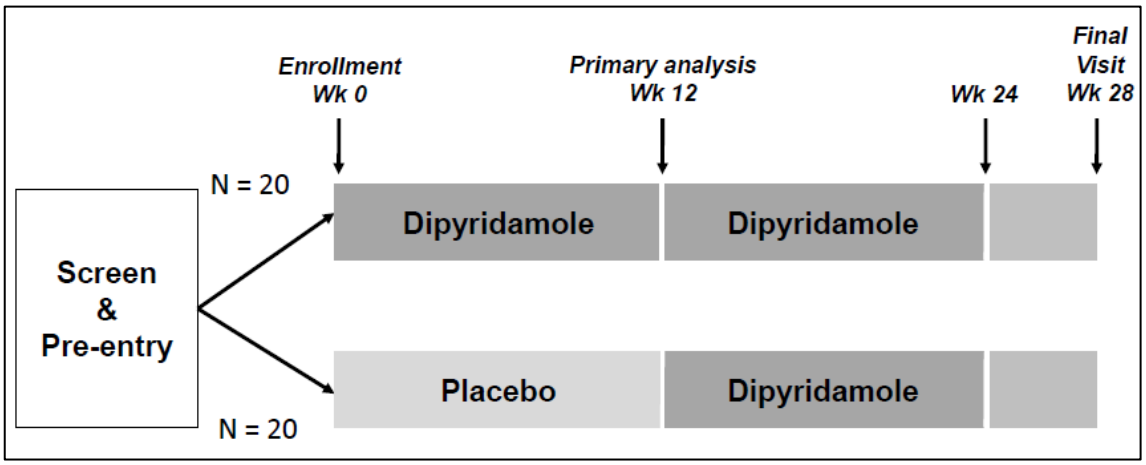
POPULATION — HIV-1-infected male and female subjects, 18 years of age or older, on ART with HIV-1 RNA <50 copies/mL for a minimum of 12 months.

STRATIFICATION — Subjects will be stratified by CD4+ T-cell counts (≤ 500 versus > 500 cells/mm^3, and by enrollment into the Rectal Tissue Subset)

REGIMEN

Arm A: Dipyridamole 100 mg 4 times daily for 24 weeks
Arm B: Dipyridamole placebo 1 capsule 4 times daily for 12 weeks, then Dipyridamole 100 mg 4 times daily for 12 weeks.

STUDY SCHEMA:



1.0 HYPOTHESES AND STUDY OBJECTIVES

1.1 Hypotheses

Dipyridamole (DP) will decrease the levels of monocyte and macrophage activation (as measured by the plasma levels of sCD163 and sCD14) and systemic inflammation (as measured by plasma IL-6) in antiretroviral treated HIV-1-infected individuals with HIV-1 RNA <50 copies/mL.

1.2 Primary Objective

1.2.1 To compare changes in monocyte and macrophage activation and systemic inflammation, as measured by plasma levels of sCD163, sCD14, and IL-6 after 12 weeks of DP treatment to placebo.

1.3 Secondary Objectives

1.3.1 To compare changes in the level of T cell immune activation as measured by the proportion of CD8+ and CD4+ T cells co-expressing HLA-DR and CD38 and the proportion of CD8+ and CD4+ T cell co-expressing CD69 and CD25, after 12 weeks of DP treatment to placebo.

1.3.2 To compare changes in cellular markers of monocyte and macrophage activation, as measured by tissue factor and CD16 expression in monocytes after 12 weeks of DP treatment to placebo.

1.3.3 To compare changes in the levels of systemic inflammatory biomarkers (sTNFαR, TNFα, hsCRP) and levels of coagulation (D-dimer, tissue factor) after 12 weeks of DP treatment to placebo.

1.3.4 To compare changes in brachial artery flow-mediated dilation (FMD) 12 weeks after treatment with DP to placebo.

1.3.5 To evaluate the safety and tolerability of DP administered for up to 24 weeks in HIV-1-infected adults.

1.3.6 To assess persistence of changes in immune activation and levels of systemic inflammation, including the primary endpoint measures, after 24 weeks of DP treatment and 4 weeks after discontinuation of therapy.

1.3.7 To assess whether DP reduces the proportion of cycling CD4+ and CD8+ T cells as measured by Ki-67 expression at baseline and after treatment with DP.

1.3.8 To correlate plasma DP levels to urine adenosine, plasma IL-6, and cellular and soluble markers of immune activation.

1.3.9 To evaluate changes in residual plasma HIV-1 RNA using single copy assay (SCA) before and after DP treatment.

1.4 Exploratory Objectives

1.4.1 To evaluate the changes in T cell activation in rectosigmoid tissue by measuring the proportion of CD4+ and CD8+ T cells co-expressing CD38 and HLA-DR at baseline and after treatment with DP.

1.4.2 To explore the frequency of gut-homing CD4+ T cells by measuring blood and tissue CCR9+ β7high T cells at baseline and after treatment with DP.

1.4.3 To explore the frequency and phenotype of T cells, B cells, and monocytes expressing CD73 in peripheral blood as well as T cells and B cells expressing CD73 in gut tissue at baseline and after DP treatment.

1.4.4 To measure levels of purines involved in the adenosine (ADO) suppression pathway before and after DP treatment.

1.4.5 To correlate expression of CD73 in the different immune cells with levels of ADO and other purines involved in the ADO suppression pathway as well as with levels of cellular immune activation and inflammation.

1.4.6 To correlate the levels of ADO and other purines in the ADO suppression pathway with levels of cellular immune activation and inflammation.

1.4.7 To explore correlations between CD73-expressing immune cells, Th17 cells, and regulatory T cells.

1.4.8 To explore levels of residual viral expression before and after DP treatment by through single copy assays and measurement of cell-associated HIV-1 RNA and DNA in blood and GALT.

1.4.9 To explore the effect DP treatment has on pulmonary function by comparing change in pre- and post-bronchodilator forced expired volume in 1 second (FEV_1) after 12 and 24 weeks of DP treatment.

2.0 INTRODUCTION

2.1 Background

Although effective antiretroviral therapy (ART) has significantly increased the life expectancy of individuals with HIV-1 infection, mortality rates are still higher compared to the HIV-1 uninfected population.[1-3] In HIV-1 infection, despite virologic suppression, the levels of cellular immune activation and inflammation rarely return to levels observed in HIV-1 seronegative individuals.[4,5] It is believed that the non-AIDS-associated morbidity and mortality is due, in part, to the consequences of this persistent immune activation and elevated levels of systemic inflammation.[6] Furthermore, the levels of inflammation are higher in ART-treated individuals with incomplete immune reconstitution compared to those with good $CD4^+$ T cell recovery.[7,8] Although persistent viremia has been proposed to be the major cause of immune activation and inflammation in HIV-1 infection, in virally suppressed individuals, chronic inflammation has also been hypothesized to be due to a number of different factors including depletion of $CD4^+$ T cells in the gut mucosa leading to the continued translocation of microbial products into

the circulation,[9,10] reactivation of other viruses such as cytomegalovirus,[11,12] or dysregulation of T cell homeostasis.[13] A number of clinical trials are currently assessing these possible causes, as well as evaluating various immunotherapeutic agents as strategies to decrease the inflammation.

In increased inflammatory states, adenosine (ADO) is produced by both intracellular and extracellular mechanisms, the latter being the more significant source of ADO generation.[14] ADO limits cellular damage by activating the adenosine receptors, A2A/2BR, leading to increased intracellular cAMP and a subsequent anti-inflammatory effect.[15] ADO is a highly potent immunoregulatory nucleoside that is produced by cells during conditions of stress such as during trauma, hypoxia, and inflammation in order to limit the tissue damage,[15-17] with levels increasing from nano-molar to micro-molar concentrations during inflammatory states.[14,16,18] Extracellular generation of ADO is facilitated by the ectoenzymes CD39 and CD73. ATP released by cells during inflammation is hydrolyzed by CD39 to 5'-AMP, which in turn is hydrolyzed by CD73 to ADO.[19] Once ADO is generated, it binds to specific cell receptors in order to mediate its effects. Of these receptors, the $A_{2A}R$ is expressed on most lymphoid cells where ligation causes an increase in cAMP production thereby allowing the lymphocytes to regulate the immune response.[15,20] ADO is then transported intracellularly down its concentration gradient by nucleoside transporters where it is degraded by ADO deaminase or rephosphorylated by ADO kinase.[21] During T cell activation, $A_{2A}R$ signaling may allow T cell proliferation but cytokine production is strongly inhibited.[22] *In vitro* studies show that $A_{2A}R$ signaling also protects $CD4^+$ T cells from activation-induced cell death by blocking the extrinsic apoptotic pathway.[23] Apart from regulating T lymphocytes, ADO through the $A_{2A}R$ signaling, prevents excessive macrophage activation leading to a decrease in the expression of TNFα and nitric oxide[24] and a switch to the production of the anti-inflammatory cytokine IL-10.[25] Indeed, recent *in vitro* studies using T cells from both normal controls (NC) and HIV-1^+ subjects demonstrated the ability of exogenous ADO to inhibit both T cell secretion of TNFα and IL-2 secretion as well as T cell immune activation.

It has been shown that the levels of CD73-expressing CD4+ T cells are markedly reduced in the peripheral blood of HIV-1-infected patients regardless of viral suppression, and that ADO decreases T cell immune activation in a concentration dependent manner.[26] The decreased levels of ADO, due to the low numbers of CD4+CD73+ T cells, fails to counter HIV-1-associated persistent immune activation and subsequent increased inflammation. It is hypothesized that an intervention increasing extracellular ADO will lead to decreased immune activation in HIV-1-infected subjects.

2.2 Rationale

As the HIV-1 infected population ages, there is increased risk for the development of non-AIDS-related chronic diseases. Identifying specific immunologic pathway(s) that regulates the elevated levels of immune activation and inflammation is paramount in the prevention of these chronic diseases and in increasing the life expectancy of those living with HIV-1. Furthermore, identification of specific pathways will lead to the development of more effective immunotherapeutic strategies that target and regulate these pathways.

Dipyridamole (DP) is an FDA-approved medication used as an adjunct to anticoagulants in preventing post-operative thromboembolic complications following heart surgery, and has been studied with aspirin in stroke prevention.[27,28] It increases extracellular ADO by

blocking equilibrative nucleoside transporters which facilitate the transport of ADO down its concentration gradient.[29,30] Moreover, it is able to further increase cAMP levels by inhibiting phosphodiesterase that prevents the breakdown of cAMP.[29,31] In a clinical study by Gamboa et al., DP was shown to enhance the delivery of ADO into the interstitium, allowing ADO to exert is effects.[32] In another clinical study, pretreatment with DP potentiated the vasodilatory effects of ADO, with the magnitude of ADO-induced vasodilation directly correlating with plasma DP concentrations.[33] The anti-inflammatory effect of DP is evident in an earlier study by Brozna et al., which showed DP inhibiting LPS-induced superoxide anion release and tissue factor (TF) expression in peripheral blood monocytes.[34] In a more recent study, DP blocked the generation of monocyte chemotactic protein-1 by LPS-treated monocytes.[35]

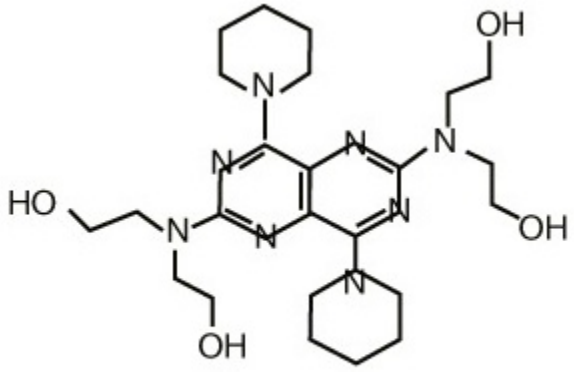
Animal studies on the anti-inflammatory effect of DP were validated in a clinical trial by Ramakers et al.[30] In this randomized, double-blind study, healthy male subjects received either extended-release DP (200mg twice daily) or placebo for 7 days. The subjects then received a 2ng/kg dose of endotoxin. DP treatment caused an 89% reduction in nucleoside transporter activity as well as an increase in endogenous ADO concentration, with the DP levels strongly correlating with peak ADO concentrations ($r=0.82$; $P<0.01$). DP augmented the LPS-induced increase in levels of the anti-inflammatory cytokine, IL-10. Furthermore, the decline of TNFα and IL-6 levels was accelerated in DP-treated subjects.

Chronic obstructive lung disease (COPD) is an important co-morbidity in HIV that may also be the consequence of immune activation and systemic inflammation. COPD, characterized by irreversible airflow obstruction and inflammation[36], is the fourth leading cause of death in the United States. COPD is defined by the Global Initiative for Obstructive Lung Diseases as "a preventable and treatable disease…characterized by airflow obstruction that is not fully reversible" and is generally defined by forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC)<0.70, with the severity of airflow obstruction measured by degree of decrease in FEV_1[36]. HIV is an independent risk factor for COPD, and there has been a three-fold increase in obstructive lung disease deaths in HIV since the introduction of antiretroviral therapy (ART)[37-39]. We see an average rate of decline in FEV_1 in our cohorts of stable HIV-infected outpatients (including non-smokers and those without any lung disease) of approximately 75-98ml/year, which is far greater than that seen in both non-smoking and smoking HIV-uninfected individuals who typically experience a 20-60ml/year decline. In addition, FEV_1 is an independent predictor of mortality in the HIV-uninfected population,[40] and we have similarly found that FEV_1 is related to mortality in our cohort of 237 HIV+ individuals followed for an average of 3.2 years, with those dying having FEV_1/FVC of 0.69 (SD 0.15) compared to 0.77 (SD 0.10) in survivors ($p=0.031$). Decline in FEV_1 in HIV may be related to local and systemic inflammation as we see increases in lung and blood cytokines, markers of immune activation, and co-infections in HIV+ individuals with lung dysfunction. The inflammation may be exaggerated by smoking, but does not depend on smoke exposure. We have recently shown that worse airflow obstruction is associated with increased immune activation and inflammation including increases in IL-6, hsCRP, and activated T-cells.[41] In a separate analysis, we have also found correlations of FEV_1 with IL-8 and endothelin-1 (ET1), and confirmed associations with IL-6. Based on these observations, inflammation is important in HIV pulmonary dysfunction. We will measure FEV_1 in participants as an exploratory outcome to see if DP may be a potential therapeutic option for HIV-1 infected individuals where few therapeutic options exist.

Administration of DP in HIV-1-infected individuals will be ideal in further investigating whether deficiencies in ADO signaling pathway play an important role in the persistent immune activation and systemic inflammation in chronic HIV-1 infection. This pilot clinical trial will evaluate the effect of DP, an FDA-approved drug with a known mechanism of action, on immune activation and inflammation of HIV-1 infected individuals on effective ART.

2.3 Description of Study Product

Dipyridamole[42] is an antiplatelet agent chemically described as 2,2',2",2"'-[(4,8-Dipiperidinopyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]-tetraethanol. It has the following structural formula:



$C_{24}H_{40}N_8O_4$ Mol. Wt. 504.63

Mechanism of Action:
DP inhibits the uptake of adenosine into platelets, endothelial cells and erythrocytes in vitro and in vivo; the inhibition occurs in a dose-dependent manner at therapeutic concentrations (0.5-1.9 µg/mL). This inhibition results in an increase in local concentrations of adenosine which acts on the platelet A2-receptor thereby stimulating platelet adenylate cyclase and increasing platelet cyclic-3',5'-adenosine monophosphate (cAMP) levels. Via this mechanism, platelet aggregation is inhibited in response to various stimuli such as platelet activating factor (PAF), collagen and adenosine diphosphate (ADP).

DP inhibits phosphodiesterase (PDE) in various tissues. While the inhibition of cAMP-PDE is weak, therapeutic levels of DP inhibit cyclic-3',5'-guanosine monophosphate-PDE (cGMP-PDE), thereby augmenting the increase in cGMP produced by nitric oxide.

Pharmacokinetics:
Following an oral dose of DP tablets, the average time to peak concentration is about 75 minutes. The decline in plasma concentration following a dose of DP tablets fits a two-

compartment model. The alpha half-life (the initial decline following peak concentration) is approximately 40 minutes. The beta half-life (the terminal decline in plasma concentration) is approximately 10 hours. DP is highly bound to plasma proteins. It is metabolized in the liver where it is conjugated as a glucuronide and excreted with the bile. Drug interactions with HIV medications, including protease inhibitors, are not predicted.

Indications for DP:
DP (Persantine®) tablets are indicated as an adjunct to warfarin anticoagulants in the prevention of postoperative thromboembolic complications of cardiac valve replacement. The recommended oral dose is 75-100 mg four times daily as an adjunct to the usual warfarin therapy.

Precautions and Adverse Reactions:
DP has a vasodilatory effect and should be used with caution in patients with severe coronary artery disease (e.g., unstable angina or recently sustained myocardial infarction). Chest pain may be aggravated in patients with underlying coronary artery disease who are receiving DP. Elevations of hepatic enzymes and hepatic failure have been reported in association with DP administration. DP should be used with caution in patients with hypotension since it can produce peripheral vasodilation.

DP has been reported to increase the plasma levels and cardiovascular effects of adenosine and may counteract the anticholinesterase effect of cholinesterase inhibitors, thereby potentially aggravating myasthenia gravis.

Adverse reactions at therapeutic doses are usually minimal and transient. With long-term use of DP tablets, initial side effects usually disappear. The following reactions in Table 1 were reported in two heart valve replacement trials comparing DP tablets and warfarin therapy to either warfarin alone or warfarin and placebo:

Table 1 Adverse Reactions Reported in 2 Heart Valve Replacement Trials

Adverse Reaction	Dipyridamole Tablets/Warfarin	Placebo/ Warfarin
Number of Patients	147	170
Dizziness	13.6%	8.2%
Abdominal distress	6.1%	3.5%
Headache	2.3%	0.0%
Rash	2.3%	1.1%

Other reactions from uncontrolled studies include diarrhea, vomiting, flushing and pruritus. In addition, angina pectoris has been reported rarely and there have been rare reports of liver dysfunction. On those uncommon occasions when adverse reactions have been persistent or intolerable, they have ceased on withdrawal of the medication.

When DP tablets were administered concomitantly with warfarin, bleeding was no greater in frequency or severity than that observed when warfarin was administered alone. In rare cases, increased bleeding during or after surgery has been observed. The

guidelines from the American Society of Gastrointestinal Endoscopy state that low risk endoscopic procedures, including flexible sigmoidoscopy with biopsies, may be performed on patients using standard-dose DP or ASA-DP combinations.[43]

In post-marketing reporting experience, there have been rare reports of hypersensitivity reactions (such as rash, urticaria, severe bronchospasm, and angioedema), larynx edema, fatigue, malaise, myalgia, arthritis, nausea, dyspepsia, paresthesia, hepatitis, thrombocytopenia, alopecia, cholelithiasis, hypotension, palpitation, and tachycardia.

3.0 STUDY DESIGN

The proposed study is a randomized, placebo-controlled, two-arm, partial cross-over, single-site, pilot study comparing the effect of 12 weeks of DP or placebo on immune activation and inflammation. The study will enroll approximately 40 HIV-1-infected subjects, age 18 years or older, who have been on ART for at least 12 months prior to study entry and have HIV-1 RNA <50 copies/mL for at least 12 months prior to study entry. Subjects will be randomized equally to two regimens:

Arm A: Dipyridamole 100 mg four times daily for 24 weeks

or

Arm B: Dipyridamole placebo capsule four times daily for 12 weeks, then
Dipyridamole 100 mg four times daily for 12 weeks.

Each subject will be enrolled for 28 weeks. Accrual is expected to be completed in approximately 18 months.

In addition, this study will include Rectal Tissue evaluations in 20 subjects (10 per arm). Flexible sigmoidoscopy with biopsies will be done at Entry/Week 0 and Weeks 12, 24, and 28.

The primary endpoints will be change from baseline to week 12 in plasma levels of sCD163, sCD14, and IL-6. Secondary endpoints will include changes in immune activation as measured in blood (%HLA-DR+CD38+CD8+ and CD4+ T-cells and %CD69+CD25+ CD8+ and CD4+ T-cells) and rectal tissue (HLA-DR+CD45RO+CD4+ T-cells, gut-homing CD4+ T-cells), cell-associated HIV-1 RNA and total DNA, and safety and tolerability of DP in HIV-1-infected adults.

Assessment of the primary and secondary endpoints at Week 24 will be conducted to gather additional longitudinal data. Arm A subjects will provide data on the durability of the effect of DP on immune activation, as well as safety and tolerability. Arm B subjects will receive DP from week 12 to week 24 and will enhance the power of the study to detect an effect of DP as well provide additional safety and tolerability data.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

The inclusion and exclusion criteria in Sections 4.1 and 4.2 will be utilized to ensure the appropriate selection of study subjects. Potential subjects will primarily be recruited from the University of Pittsburgh HIV/AIDS primary care clinic. They will be identified by their primary care doctor or clinical team, including subjects who have previously expressed

interest in participating in a clinical research study by signing the IRB-approved HIPPA-compliant Research Registry consent document for sharing of health information. Interested persons will be provided with information by the research recruiter/educator who will include the potential subject's primary clinician in the discussion to participate. Subjects may also be referred to the study from other local HIV care providers, research projects, and health and social service providers. All recruitment materials will be approved by the University of Pittsburgh Institutional Review Board prior to use. Informed consent for participation will be obtained by a physician investigator. Eligible participants who agree to rectal tissue biopsies will be enrolled in the tissue subset.

4.1 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for inclusion in the study.

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

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

4.1.2 Men and women age 18 years or older.

4.1.3 On ART for at least 12 months prior to study entry with a regimen that includes three or more antiretroviral medications. Ritonavir used as a pharmacokinetic enhancer for another protease inhibitor will <u>not</u> be counted as an antiretroviral medication.

4.1.4 Plasma HIV-1 RNA <50 copies/mL by any standard clinical assay at screening and for a minimum of 12 months prior to entry, confirmed by by at least two measurements prior to study entry, one of which must be at least 48 weeks prior to study entry and one measurement that was obtained between 61 days and 48 weeks prior to study entry. All plasma HIV-1 RNA measurements in the 12 months prior to study entry must be <50 copies/mL (with the exception that a single detectable measurement of ≤ 200 copies/mL is permitted if the RNA levels immediately before and after are <50 copies/mL).

4.1.5 Stable ART regimen for at least 8 weeks prior to study entry and no plans to change ART regimen for at least 6 months following study entry.

4.1.6 Ability and willingness of subject to provide informed consent.

4.1.7 In the opinion of the investigator**,** no medical, mental health or other condition that precludes participation.

4.1.8 Laboratory values obtained within 60 days prior to entry:

- Hemoglobin ≥10.0 g/dL
- Platelet count ≥100,000/mm^3
- INR ≤ 1.5 (for rectal tissue subset only)
- PTT <2x ULN (for rectal tissue subset only)
- AST and ALT ≤ 2.5 x upper limit of normal (ULN)
- Total bilirubin ≤ 2.5 x ULN (except if hyperbilirubinemia is secondary to atazanavir).
- Creatinine ≤ 1.5 x ULN
- Hepatitis B surface antigen negative
- Hepatitis C antibody negative (note: subject with HCV Ab positive is eligible if Hepatitis C RNA PCR (viral load) is undetectable)

4.1.9 For women of reproductive potential, negative serum or urine pregnancy test, with a sensitivity of ≤ 50 mIU/mL, at screening and within 72 hours prior to study entry.

4.1.10 Female study volunteers of reproductive potential include women who have not been post-menopausal for at least 24 consecutive months, (i.e., who have had menses within the preceding 24 months, or women who have not undergone surgical sterilization, specifically hysterectomy and/or bilateral oophorectomy).

If participating in sexual activity that could lead to pregnancy, female subjects must agree to use one form of contraceptive as listed below while receiving protocol-specified treatment and for 4 weeks after stopping the treatment.

- Condoms (male or female) with or without a spermicidal agent. Condoms are recommended because their appropriate use is the only contraception method effective for preventing HIV transmission.
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive

NOTE: If the subject is taking a concomitant medication with stricter contraceptive requirements than what are listed in the protocol for the study drugs (such as in the case of efavirenz), then refer to the pregnancy category and product labeling for the concomitant medication with the strictest contraceptive requirements.

4.1.11 If the female subject is not of reproductive potential (girls who have not reached menarche, women who have been post-menopausal for at least 24 consecutive months, or women who have undergone surgical sterilization, e.g., hysterectomy, bilateral oophorectomy, or bilateral tubal ligation or salpingectomy), she is eligible without requiring the use of a contraceptive. Self- report is acceptable documentation of sterilization, other contraceptive methods, and menopause.

4.1.12 Rectal Tissue Subset only: Willing to abstain from receptive anal intercourse and practices involving insertion of anything in the rectum (drug, enema, penis, or sex

toy) for 72 hours prior to rectal biopsy and for 7 days post-biopsy to minimize risk of bleeding complications.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study:

4.2.1 Pregnancy or breast-feeding.

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

4.2.3 Known cardiovascular disease (history of MI, coronary artery bypass graft surgery, percutaneous coronary intervention, stroke, transient ischemic attack, peripheral arterial disease with ABI <0.9 or claudication).

4.2.4 Uncontrolled type II diabetes mellitus.

4.2.5 Known chronic inflammatory conditions such as, but not limited to, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, inflammatory bowel disease (i.e., Crohn's disease or ulcerative colitis), chronic pancreatitis, or autoimmune hepatitis, myositis, or myopathy.

4.2.6 History of asthma requiring medical treatment within 2 years prior to study entry with the exception of the use of albuterol inhaler for mild intermittent asthma.

4.2.7 Serious illness requiring systemic treatment and/or hospitalization within 14 days prior to entry.

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

- Immunosuppressives (e.g., azathioprine, , cyclosporine, mycophenolate, sirolimus, sulfasalazine, tacrolimus)
- Immune modulators (e.g., cytokines [e.g., IL-2], granulocyte colony stimulating factor, growth hormone, tumor necrosis factor antagonists, thalidomide)
- Antineoplastic agents
- Anticoagulants (e.g., warfarin and heparin)
- Anti-platelet drugs (e.g., clopidogrel)

4.2.9 Use of any of the following medications for more than 3 consecutive days within the 30 days prior to study entry:

- Corticosteroids [physiologic replacement doses are allowed]
- NSAIDs (nonsteroidal anti-inflammatory drugs)
- Aspirin

4.2.10 Vaccinations within 1 week prior to the pre-entry or study entry visits.

NOTE: Routine standard of care vaccinations including hepatitis A and/or B, influenza, pneumococcal, and tetanus are permitted if administered at least 7 days before pre-entry and entry evaluations.

4.2.11 Participation on any HIV immunotherapy or therapeutic vaccination trials within 6 months prior to study entry.

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

4.2.13 Use of investigational therapies within 30 days prior to study entry.

4.2.14 Rectal Tissue Subset only:

- Abnormalities of the colorectal mucosa or significant colorectal symptom(s), which in the opinion of the study investigator represent a contraindication to biopsy (including but not limited to presence of any unresolved injury, infectious or inflammatory condition of the local mucosa, and presence of symptomatic external hemorrhoids.

  NOTE: Abnormalities of the colorectal mucosa will be assessed at the time of the enrollment flexible sigmoidoscopy. If no significant colorectal abnormalities or symptoms are present then the participant will undergo the enrollment procedures. If abnormalities are present then no biopsies will be performed and the participant will not be enrolled into the rectal tissue subset but will continue participation in the main study.

- Active untreated gonorrhea, or chlamydia infection within 30 days prior to study entry (subjects diagnosed with rectal gonorrhea or chlamydia infection at screening may be treated during the screening period provided the treatment is at least 30 days prior to entry).

4.2.15 Exclusions for spirometry testing (for participants enrolled under Version 2.0): Participants will not undergo pre- and post-bronchodilator spirometry if they have any of the following:

- Abdominal or cataract surgery within 3 months.
- Myocardial infarction or stroke within the past 3 months.
- Acute onset of shortness of breath, cough, fever or heart condition such as tachycardia, angina or arrhythmias with 4 weeks prior to enrollment.
- Increasing respiratory symptoms or febrile (temperature >100.4°F [38°C]) within 4 weeks of study entry.
- Uncontrolled hypertension defined as systolic > 160 mm Hg or diastolic > 100 mm Hg from an average of two or more readings. Participant with controlled hypertension may undergo spirometry.
- Prior history of adverse reaction to albuterol

4.3 Study Enrollment Procedures

Prior to implementation of this protocol, and any subsequent full version amendments, the protocol and the protocol consent form(s) will be approved by the University of

Pittsburgh Institutional Review Board (IRB). Upon receiving final approval, all required protocol registration documents will be submitted to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC).The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

4.4 Coenrollment Guidelines

Coenrollment into observational studies is permitted provided maximum blood draw volumes are not exceeded.

5.0 STUDY TREATMENT

5.1 Regimens, Administration, and Duration

Study treatment is defined as DP 100 mg capsules and matching placebo, and will be provided by the study. Subjects will be asked to bring back their study product bottles for pill counts for all post-entry visits.

5.1.1 Regimens

At study entry, subjects will be randomized to one of the following arms:

Arm A: Dipyridamole 100 mg 4 times daily for 24 weeks

Arm B: Dipyridamole placebo 1 capsule 4 times daily for 12 weeks, then Dipyridamole 100 mg 4 times daily for 12 weeks.

5.1.2 Administration

DP capsules (and Placebo capsules) should be taken orally 4 times daily with or without food.

5.1.3 Study Treatment Duration

Subjects will remain on study treatment until Week 24.

5.2 Study Product Formulation and Storage

DP 100 mg and placebo for DP are provided as capsules.

Store at a controlled room temperature of 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F); protect from excessive moisture.

5.3 Pharmacy: Product Supply, Acquisition, and Accountability

5.3.1 Study Product Supply

DP and placebo for DP will be supplied by the Investigational Drug Service (IDS) of the University of Pittsburgh Medical Center.

5.3.2 Study Product Accountability

The IDS pharmacist will maintain complete records of all study products received and subsequently dispensed. All unused study products must be returned to IDS and will be destroyed after the study is completed or terminated.

5.4 Concomitant Medications

5.4.1 Required Medications

Subjects must be on combination ART (not provided by the study) as specified in the inclusion criteria (4.1.3).

5.5 Adherence Assessment

Pill counts and self-report adherence interviews for DP will be used to assess adherence during the treatment period. Subjects will be asked to bring in the bottles of study medication so that pill counts may be performed.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events (SOE)

Evaluation	Screening	Pre-Entry[1]	Entry	Week									Premature Treatment and/or Study Discontinuation
				2	4	8	12	14	16	20	24	28	
Documentation of HIV-1 infection	X												
Medical History/Medication History	X												
Complete Physical Exam	X												
Targeted Physical Exam		X	X		X	X	X		X	X	X	X	X
Clinical Assessments			X		X	X	X		X	X	X	X	X
Concomitant Medications		X	X		X	X	X		X	X	X	X	X
Hematology	X		X				X				X		X
Liver Function Tests/Blood Chemistries	X		X		X		X		X		X		X
Fasting Lipid Panel			X				X				X		X
Plasma HIV-1-1 RNA	X		X				X				X		X
CD4+/CD8+	X		X				X				X		X
Pregnancy Test	X		X		X	X	X		X	X	X	X	X
Prothrombin time/INR/PTT[2]		X											
Hepatitis B and C	X												
Stored Plasma for Immunology/Virology[5]		X	X		X		X		X		X	X	X
Stored PBMC for Immunology/Virology[5]		X	X		X		X		X		X	X	X
Urine adenosine level (stored) [5]			X		X		X		X		X		X
Serum for Dipyridamole concentration (stored)					X	X	X		X	X	X		X
Brachial Artery FMD			X		X		X				X		X[3]
Rectal swabs for GC/CT NAAT[2]	X		X				X				X	X	
HSV Serology[2]			X										
Flexible sigmoidoscopy (rectal biopsy)[2, 5]			X				X				X	X	
Adherence self-report					X		X		X		X		

Evaluation	Screening	Pre-Entry[1]	Entry	Week									Premature Treatment and/or Study Discontinuation
				2	4	8	12	14	16	20	24	28	
Medication Adherence Assessment [4]			X		X		X		X		X		
Telephone assessment of tolerability/adherence				X				X					
Pre- and post-bronchodilator spirometry [6]			X		X		X				X		

1 Pre-Entry evaluations must be completed within 14 days prior to study entry.
2 Rectal Tissue sub-group only.
3 Only if ≥ 4 weeks since the last FMD assessment and on study medication ≤72 hours prior to visit.
4 Capsule counts to take place at all post-entry visits.
5 See Section 6.3.9 for specific assays.
6 For participants enrolled under Version 2.0

6.2 Timing of Evaluations

6.2.1 Screening and Pre-Entry Evaluations

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

Screening
Screening evaluations to determine eligibility must be completed within 60 days prior to study entry unless otherwise specified.

Pre-Entry
Pre-entry evaluations must be completed at least 24 hours after screening evaluations have been completed and within 14 days prior to study entry.

6.2.2 Entry Evaluations

Entry evaluations must occur at least 24 hours after pre-entry evaluations unless otherwise specified. All entry evaluations must be completed before study drug is started. In order to facilitate scheduling of procedures, subjects may have the FMD test or the rectal biopsies performed on a separate day within 3 days after randomization (but prior to starting study medication).

6.2.3 Post-Entry Evaluations

On-Study Evaluations
All on-study evaluations must be scheduled as per Section 6.1 with a ±7 day window for all visits. In order to facilitate scheduling of procedures, subjects may have the FMD test or the rectal biopsies performed on a separate day within the visit window.

6.2.4 Discontinuation Evaluations

Evaluations for Randomized Subjects Who Do Not Start Study Treatment
Subjects who are randomized but do not start study treatment within 72 hours will be taken off study with no further evaluations required. All case report forms (CRFs) must be completed for the period up to and including week 0.

Premature Treatment Discontinuation Evaluations
Subjects who prematurely discontinue study treatment will have a study discontinuation visit as per Section 6.1 and then be taken off study.

6.2.5 Pregnancy

If a woman becomes pregnant while on study, study product will be discontinued immediately and no further study assessments will be performed except safety labs. See Section 7.2 regarding patient management. All pregnancies should be followed by monthly telephone contact until the final outcome can be determined.

In the event that the pregnancy has not been completed by the final study visit, then the subject will be contacted through monthly phone calls and review of medical records, if possible, until the pregnancy outcome can be ascertained. Intrapartum complications and/or pregnancy outcome will be recorded on the CRFs.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS Web site for information about what must be included in the source document: http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/SourceDocAppndx.pdf. All stated evaluations are to be recorded on the CRF and keyed into the database unless otherwise specified. This includes events that meet the International Conference on Harmonization (ICH) definitions for a serious adverse event.

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

To grade diagnoses, signs and symptoms, and laboratory results, sites must refer to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004 (Clarification, August 2009), which can be found on the DAIDS RSC Web site: http://rsc.tech-res.com/safetyandpharmacovigilance

6.3.1 Definition of Fasting

Subjects must be evaluated in a fasting state for those evaluations indicated as "fasting".

NOTE: Fasting is defined as nothing to eat or drink except water and required prescription medications for at least 8 hours. Drinking black decaffeinated coffee without sweetener or creamer is permissible, but is not advised. Subjects will be asked whether they have fasted, and if not, they should be scheduled to return in a fasting state within 7 days.

Although subjects must be fasting, they will be specifically instructed to take all medications according to their regular schedules and with the usual amounts of water, to the extent that they can tolerate them in a fasting condition. Drugs that need to be taken with food may be brought to the clinic and taken with food after the FMD evaluation and the fasting specimen collection.

In order to minimize diurnal variation, fasting samples for individual subjects should be obtained consistently in either the morning or the afternoon throughout

the study, if possible.

6.3.2 Documentation of HIV-1

Please refer to Section 4.1.1 regarding assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the CRF.

6.3.3 Medical History

The medical history must include all HIV-related and AIDS-defining diagnoses (Centers for Disease Control and Prevention [CDC] category B and C), and the following non-HIV diagnoses: diabetes mellitus, dyslipidemia, hypertension, hypotension, MI, coronary artery disease (not MI), congestive heart failure, stroke, malignancy, renal insufficiency, liver disease, pulmonary embolism, deep vein thrombosis, peripheral artery disease (including peripheral artery revascularization), chronic obstructive pulmonary disease (COPD), and asthma. Any allergies to any medications and their formulations must be documented in the source documentation.

HIV-1 RNA History
Pre-ART HIV-1 RNA: Record on the CRF the documented date and level of the subject's pre-ART viral load. If documentation is not available, then subject recollection will suffice.

Record on the CRF the documented date and viral load of the subject's first HIV-1 RNA level that was below the assay lower limit. Also, record the duration of time prior to study entry the subject has had continued HIV-1 RNA levels that were below assay lower limits. If documentation is unavailable, then subject recollection will suffice.

All known HIV-1 RNA levels obtained within 1 year prior to study entry should be documented and recorded on the CRF.

CD4+ T-cell History
Nadir CD4+ T-cell Count: The subject's nadir CD4+ T-cell count (absolute value and date) should be documented when possible with a copy of the nadir CD4+ T-cell count report. If this documentation is not available, then subject recollection will suffice. For subjects who do not know the exact nadir value and for whom there is no source documentation, then recall of the categorical nadir (e.g., < 50, < 100, < 200 cells/mm^3) will suffice.

All known CD4+ T-cell counts obtained within 1 year prior to study entry should be documented and recorded on the CRF.

6.3.4 Medication History

A medication history must be present, including start and stop dates, in the source documents and on the CRFs, including:

Complete HIV-1 treatment history of any ARV medication (estimated if the exact dates cannot be obtained), immune-based therapy, and HIV-related vaccines, including blinded study medications.

All prescription medications and non-prescription drugs, including herbal supplements or vitamin supplements taken within 30 days prior to study entry.

6.3.5 Clinical Assessments

Complete Physical Examination
A complete physical examination is performed at Screening and is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; examination of the extremities for edema. The complete physical exam will also include signs and symptoms, diagnoses, height, weight, and vital signs (temperature, pulse, respiration rate, and blood pressure).

Targeted Physical Exam
A targeted physical examination is performed at Entry and all other study visits to include weight and vital signs (temperature, pulse, respiration rate, and blood pressure) and is to be driven by any previously identified or new signs or symptoms, or any diagnoses that the subject has experienced within 30 days prior to entry/since the last visit.

Height
Height will be recorded on the CRF at the screening visit only.

Weight
Weight will be recorded on the CRF at each targeted physical exam.

Signs and Symptoms
At entry, all grades that occurred within 30 days before study entry must be recorded; post-entry, only new signs and symptoms Grade ≥ 2 must be recorded on CRFs. Record on CRFs all signs and symptoms that led to a change in treatment, regardless of grade. The source document must include date of onset and date of resolution.

Diagnoses
After entry, record all HIV-related and AIDS-defining diagnoses (Centers for Disease Control and Prevention [CDC] category B and C), and the following non-HIV diagnoses: diabetes mellitus, dyslipidemia, hypertension, hypotension, MI, coronary artery disease (not MI), congestive heart failure, stroke, malignancy, renal insufficiency, liver disease, pulmonary embolism, deep vein thrombosis, peripheral artery disease (including peripheral artery revascularization), chronic obstructive pulmonary disease (COPD), and asthma.

Each diagnosis must include:

a) Date of diagnosis, and date of resolution
b) Method of diagnosis

Concomitant Medications
Record new, or discontinued concomitant medications including immune-based therapy, blinded study treatment, prescription drugs, nonprescription drugs, alternative therapies, and dietary supplements since the last visit.

Study Drug Modifications
Record all study drug modifications, including initial doses, subject-initiated and/or protocol-mandated modifications, and inadvertent and deliberate interruptions. Record any permanent discontinuation of treatment on the CRF. More than three consecutive missed days will be considered an interruption and should be recorded on the CRF.

Antiretroviral Therapy
All modifications to ART medications including initial doses, subject-initiated modifications (more than three consecutive missed days), modifications, and permanent discontinuation will be recorded on the CRFs.

6.3.6 Laboratory Evaluations

At screening and entry all protocol required laboratory values must be recorded on CRFs. For post-entry assessments, record on CRFs all Grade ≥ 2 laboratory values. All laboratory toxicities that led to a change in treatment, regardless of grade, must be recorded on CRFs. Record on CRFs all values for serum creatinine, AST, ALT, and fasting lipid panel regardless of grade.

Hematology
Hemoglobin, hematocrit, white blood cell count (WBC), differential WBC, and platelet count.

Liver Function Tests/Chemistries
Total bilirubin (and indirect bilirubin if subject is receiving atazanavir), ALT (SGPT), AST (SGOT), and alkaline phosphatase, blood urea nitrogen (BUN), creatinine, glucose, sodium, potassium, chloride, and CO_2/bicarbonate.

Fasting Lipid Panel
Total cholesterol, HDL-c, calculated LDL-c (if triglycerides <400 mg/dL) and triglycerides.

Hepatitis Testing (at Screening)
Hepatitis B surface antigen, Hepatitis C antibody (if indicated, Hepatitis C RNA PCR).

Prothrombin time/INR/PTT
Prothrombin time/INR and PTT are required at pre-entry for rectal tissue subset only.

Rectal GC/CT NAAT
Rectal swab for GC/CT NAAT will be obtained for rectal tissue subset only

HSV Antibody
Serum for HSV antibody testing will be obtained at Entry visit for the rectal tissue subset only

Pregnancy Test
For women of reproductive potential as defined in section 4.1.11, serum or urine β-HCG (urine test must have a sensitivity of at least 50 mIU/mL) must be performed as indicated in section 6.1:

- At the screening visit.
- At the study entry visit.
- Every 4 weeks during study participation.

6.3.7 Immunologic Studies

CD4+
Obtain absolute CD4+ T-cell count and percentage within 60 days prior to entry from a laboratory that possesses a CLIA certification or equivalent.

Evaluations for CD4+/CD8+ T-cell counts and percentages will be performed in the same laboratory at the entry visit and throughout the course of the study.

Because of the diurnal variation in CD4+ and CD8+ T-cell counts, determinations for individual subjects should be obtained consistently in either the morning or the afternoon throughout the study, if possible.

6.3.8 Virologic Studies

Plasma HIV-1 RNA
All measurement of HIV-1 RNA will be performed by a laboratory that possesses a CLIA certification or equivalent. Screening HIV-1 RNA must be performed within 60 days prior to study entry. Eligibility will be determined based on the screening value.

6.3.9 Stored Samples

Blood and rectal tissue will be processed immediately after collection and stored for future analysis. Results of these tests do not have clinical relevance and will not be reported to participants.

Stored Specimens for Cellular Immune Activation

PBMC will be stored for batched analysis:

Immunology Lab:

Markers of cellular immune activation
Cell cycling (Ki67)
Immunoregulatory cell subsets (frequency and phenotype)
CD39 and CD73 expression in lymphocytes and monocytes

Stored Specimens for Markers of Inflammation, Coagulation and Activation

Plasma specimens for batch measurement (Immunology Lab):

Soluble markers of immune activation, inflammation, and coagulation

Stored Specimens for Adenosine and DP Pharmacokinetics

Plasma and Urine Specimens for HPLC-tandem mass spectrometry (Jackson Lab) of:

Adenosine, inosine, guanosine
5'-AMP and 3,5'-cAMP
Plasma creatinine
Dipyridamole assay

Stored Specimens for Virology:

PBMC and Plasma (Mellors Lab):

Cell-associated HIV-1 DNA
Cell-associated HIV-1 RNA
HIV-1 RNA Single copy assay

Rectal Mucosa:

Tissue mononuclear cells for immune activation (Mucosal Immunology Lab):

:

Markers of cellular immune activation
Immunoregulatory cell subsets
Expression of CD39 and CD73 in lymphocytes and monocytes

Tissue mononuclear cells for virology (Mellors Lab):

Cell-associated HIV-1 DNA
Cell-associated HIV-1 RNA

Refer to the protocol laboratory processing chart (LPC) for details of collection, processing, and storage.

6.3.10 Brachial Artery FMD

The brachial FMD assessment will be performed and interpreted at the University of Pittsburgh Department of Epidemiology Ultrasound Research Lab (URL) as per Section 6.1.

Subjects who are prematurely discontinuing the study should have a brachial FMD test only if it has been ≥4 weeks since the last FMD assessment.

Considerations for the Brachial Artery FMD Scanning:

1. Subjects should fast and abstain from exercise and smoking for at least 8 hours prior to the study visit and be afebrile (afebrile (oral T < 38 degrees C), at the visit; otherwise these assessments must be rescheduled.

    a) Subjects should be fasting (nothing but water or black decaffeinated coffee for 8 hours prior to evaluation; drinking black decaffeinated coffee is considered an exception but is not advised).

2. No blood pressure (BP) measurements or venipuncture are permitted in the right arm before the ultrasound scan.

3. FMD assessments should be done in the morning, approximately at the same time.

Brachial artery size in response to an endothelium-dependent stimulus will be evaluated by ultrasonographic measurement of the brachial artery. Baseline brachial artery diameter will be measured using 2-D ultrasonography. A blood pressure cuff will be placed proximal to the brachial artery and will be inflated to suprasystolic pressure (cuff inflation will be 40mmHg above the systolic blood pressure) for 5 minutes. After 5 minutes, the cuff will be deflated. Brachial artery diameter will be measured by 2-D ultrasonography at 1 minute following cuff deflation to measure brachial artery diameter response to reactive hyperemia, which is an endothelium-dependent vasomotor stimulus.

For quality control purposes, a subset of participants may be asked to have a second FMD at Weeks 4, 12, or 24.

6.3.11 Rectal Exam and Rectal Specimen Collection

Rectal tissues will be obtained from subjects at Enrollment and at Weeks 12, 24, and 28. These study visits will take place at the Magee Womens Hospital CTRC. Procedures will be performed by qualified physicians who perform rectal biopsies on a regular basis. All subjects in the Tissue Subset will be instructed to abstain from receptive anal intercourse and all rectal products for 72 hours prior to rectal biopsy, and for 7 days post-biopsy to minimize risk of bleeding complications. Subjects will be contacted by telephone 24-48 hours after sample collection to assess for any AEs.

The subject will be positioned in the left lateral decubitus position for the following procedures:

- Rectal swab for GC/CT
- Visual and digital rectal exam: The examiner will conduct a visual examination of the anus and surrounding area and note any abnormality. The examiner will then insert a lubricated gloved finger into the anal canal and sweep around the internal anal circumference.
- Enema: A 125 mL saline enema will be inserted through the anus and the contents gently squeezed into the rectum. The subject will hold the fluid in the rectum for 5 minutes then expel it.
- Flexible sigmoidoscopy and biopsy: A flexible sigmoidoscope will be inserted to approximately 10-20 cm, where approximately 17-21 biopsies will be taken using large-cup biopsy forceps.

6.3.12 Medication Adherence Assessment

A standardized assessment to monitor adherence and pill counts will be administered. Subjects will be instructed to return pill bottles for DP to the study site, and pill counts will be undertaken by the study nurse or designee and recorded on the CRFs.

6.3.13 Pre- and post-bronchodilator spirometry (for participants enrolled under Version 2.0)

Participants will be asked to withhold use of any short-acting bronchodilators for at least 4 hours prior to testing and long acting bronchodilators for at least 12 hours prior to testing. At least 3 acceptable forced vital capacity maneuvers should be performed according to ATS standards: a maximal inspiration followed by a maximal forced expiratory effort which lasts for at least seconds. The participant should be seated. Ensure that the 3 best FEV1 values are within 5% of each other. Participants will then be given 4puffs (360ug) of albuterol from a metered dose inhaler through a spacer. Three acceptable forced vital capacity maneuvers will again be performed according to ATS standards. Refer to the Manual of Procedures (MOPS) for detailed instructions.

7.0 CLINICAL MANAGEMENT ISSUES

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

The grading system is located in the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0 - December 2004 (Clarification dated August 2009), which can be found on the Division of AIDS Regulatory Support Center Web site: http://rsc.tech-res.com/safetyandpharmacovigilance/.

The management of toxicities suspected to be due to ART should be according to standard clinical practice.

7.1 Toxicity

7.1.1 General Reactions

- Grade 1 or 2

  Subjects who develop a Grade 1 or 2 AE or toxicity, except as stated in Section 7.2 may continue study treatment at the discretion of the investigator. If a subject chooses to discontinue study treatment, the subject will be followed until the toxicity resolves.

- Grade 3

  Subjects who develop a Grade 3 AE that is not specifically addressed below and is judged by the investigator to be study drug-related should have the study drug held. Subjects should be followed closely and if the AE does not return to Grade $\leq$ 2 within 4 weeks, the study drug must be permanently discontinued with subject evaluations as per Section 6.2.4.

  If the study drug is resumed and the same Grade 3 AE recurs within 4 weeks of reintroduction, and the investigator considers this AE related to the study drug, the drug must be permanently discontinued.

  With a Grade 3 AE that is judged not related to the study drug by the investigator, the study drugs may be continued at the discretion of the site investigator with close follow up.

  Subjects experiencing Grade 3 AEs requiring permanent discontinuation of study treatment should be followed closely for resolution of the AE to Grade ≤ 2.

  Subjects with Grade 3 asymptomatic laboratory abnormalities in cholesterol, creatine kinase (CK) or triglycerides may continue study treatment.

- Grade 4

Subjects who develop a Grade 4 AE that is not specifically addressed below and is judged by the site investigator to be study drug-related should have the specific study drug held. Clinical assessments and laboratory testing will be completed as described for Grade 3 toxicity. Subjects experiencing Grade 4 AEs should be followed closely with additional clinical assessments and laboratory testing as clinically indicated until resolution of the AE.

7.1.2 AST/ALT Elevations

- Grade 2 Asymptomatic

  For subjects who develop asymptomatic Grade 2 AST or ALT elevations, DP may continue and the AST and ALT should be repeated within 2 weeks.

- Grade 2 Symptomatic

  For symptomatic Grade 2 or greater elevations of AST or ALT for subjects on DP, the study drug should be held until symptoms have resolved and further evaluations, as indicated, have been conducted. If symptoms resolve and AST or ALT returns to < Grade 2, then DP may be resumed with close follow up.

- Grade 3

  Subjects who develop a Grade 3 AST or ALT that is judged by the investigator to be related to DP should have the DP held. The subject should be followed closely and if the AE does not return to Grade $\leq$ 2 within 4 weeks, the DP must be permanently discontinued with subject evaluations as per Section 6.2.4.

  If the study drug is resumed and the same Grade 3 AE recurs within 4 weeks of reintroduction, and the investigator again considers this AE related to the DP, the drug must be permanently discontinued.

- Grade 4

  Subjects who develop a Grade 4 AST or ALT AE that is judged to be related to DP by the investigator will have the drug permanently discontinued, with clinical assessments and laboratory testing as described for Grade 3 toxicity. Subjects experiencing Grade 4 AEs should be followed closely with additional clinical assessments and laboratory testing as clinically indicated.

7.1.3 Hypotension

- Grade 2

  Subjects who develop a symptomatic hypotension that resolves with oral fluids (Grade 2) may continue study medication and will be instructed to maintain adequate hydration. Recurrence of Grade 2 hypotension should prompt consideration of discontinuation of study treatment.

- Grade 3

  Subjects who develop Grade 3 or 4 hypotension that is judged by the investigator to be related to DP will have study treatment permanently discontinued.

7.1.4 Headache

- Grade 1

  Subjects who develop Grade 1 headache may continue study medications.

- Grade 2

  Subjects who develop Grade 2 headache may continue study medication and may be treated with over the counter analgesics (acetaminophen preferred, NSAIDS should be avoided and may not be taken for > 3 consecutive days).

- Grade 3

  Subjects who develop Grade 3 or 4 headache that is judged by the investigator to be related to DP will have study treatment permanently discontinued.

7.2 Pregnancy Management

If the pregnancy test is positive at entry, then the subject must not start study treatment. No further evaluations are necessary, provided that the subject did not initiate study drug.

Subjects who become pregnant after study entry must discontinue study treatment immediately. These subjects should be seen for a premature treatment discontinuation evaluation within 7 days. No further study evaluations except for safety labs will occur. These subjects will complete a study termination visit per Section 6.1. Study staff will request permission to contact pregnant participants by telephone monthly regarding pregnancy outcomes and/or obstetrical complications during and at the end of pregnancy.

Pregnancies that occur on study should be reported to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Phone: 800-258-4263; Fax: 800-800-1052. Intrapartum complications and/or pregnancy outcome will be recorded to The Antiretroviral Pregnancy Registry as well as on study case report forms, if possible.

7.3 Unblinding

It is not expected that subject unblinding will be necessary for management of toxicity. If deemed medically appropriate, unblinding will be requested from the Data Center.

8.0 CRITERIA FOR DISCONTINUATION

8.1 Permanent Treatment Discontinuation

- Requirement for prohibited concomitant medications except vaccinations (see the Manual of Procedures).
- Requirement for precautionary concomitant medications at the discretion of the investigator (see the Manual of Procedures)
- Drug-related toxicity as per Section 7.1
- Study drug is discontinued for 7 consecutive days for reasons other than toxicity at the discretion of the investigator. If study drug is discontinued for reasons other than toxicity, but the underlying reason for the discontinuation later resolves, study drug may be resumed at the discretion of the investigator.
- Pregnancy or breast-feeding
- Request by subject to terminate treatment.
- Clinical reasons believed to be life threatening by the physician, even if not addressed in the toxicity Section of the protocol.

8.2 Premature Study Discontinuation

- Subject repeatedly noncompliant with study treatment as prescribed.
- Request by the subject to withdraw or not able to attend study visits as required by study.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the institutional review board (IRB), Office for Human Research Protections (OHRP), NIAID, investigator, or IDSMB.

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This study is a randomized, placebo-controlled, two-arm, single-site, pilot study that will

evaluate whether 12 weeks of treatment with DP, decreases markers of immune activation and systemic inflammation in HIV-1 infected subjects virally suppressed on ART. DP will be administered to subjects for up to 24 weeks. Follow-up continues to week 28. The targeted sample size will be 40 subjects (20 of these subjects will be enrolled in the rectal tissue subset).

9.2 Endpoints

9.2.1 Primary Endpoints

- Change in Plasma level of sCD14 from baseline to week 12.
- Change in Plasma level of sCD163 and IL-6 from baseline to week 12.

9.2.2 Secondary Endpoints

- Changes from entry to week 12 and 24 (as applicable) in the following:
    - %HLA-DR+ CD38+ expression in CD8+ and CD4+ T-cells
    - %CD69+CD25+ expression in CD4+ and CD8+ T-cells
    - CD16 and tissue factor expression in monocytes
    - Plasma levels of biomarkers of systemic inflammation (sTNFαR, TNFα, hsCRP) and coagulation (D-dimer, tissue factor)
    - Percent brachial artery FMD
    - Proportion of cycling CD4+ and CD8+ T cells as measured by Ki-67 expression
- Grade 2 or higher AEs, treatment discontinuations
- Correlation of plasma DP level to urine adenosine, plasma IL-6, and soluble and cellular markers of immune activation

9.2.3 Exploratory Endpoints

- Changes from entry to week 12 and 24 (as applicable) in the following:
    - Rectal tissue: %CD38+/DR+ CD4+ and CD8+ T-cells by flow cytometry
    - Blood and rectal tissue CCR9+ β7high T-cells
    - Frequency and numbers of CD73 expressing T cells B cells and monocytes in peripheral blood and gut

- Purine and adenosine levels in blood
- Plasma HIV-1 RNA using single copy assay (SCA)
- Cell-associated HIV-1 RNA and DNA in blood and rectal tissue
- Pre- and post-bronchodilator FEV_1

9.3 Randomization and Stratification

At study entry, subjects will be allocated at a 1:1 ratio to the two arms using blocked randomization. In addition, randomization will be stratified by CD4 cell count at screening (<500 cells/mm^3 or ≥500 cells/mm^3), and enrollment into the rectal tissue subset.

9.4 Sample Size and Accrual

There will be 40 subjects (20 per arm) randomized to either DP 100 mg 4 times daily or matching placebo, and it is assumed that no more than 10% of participants will be excluded from the primary analysis due to any reason. sCD14 will be the main primary outcome, while sCD163 and IL-6 will be treated as other key outcomes of interest. As a result, the hypothesis for sCD14 will be tested at the 5% significance level and will be the basis for our power analysis. sCD163 and IL-6 will also be tested at the 5% significance level each. Based on the assumption that none of the 40 subjects are excluded from the primary analysis for any reason, we will have 80% power to detect standardized mean difference of 0.909 between treatment arms for the sCD14. In terms of clinically meaningful effects, this translates to a difference of 0.49 x 10^6 pg/mL in sCD14 between arms at 12 weeks assuming similar standard deviation seen in preliminary data (standard deviation is 0.54 x 10^6 pg/mL for sCD14). In the more realistic situation that 10% of subjects are excluded (18 per arm), we will have 80% power to detect standardized mean difference of 0.961 between treatment arms for the primary outcome. In terms of clinically meaningful effects, this translates to a difference of 0.52 x 10^6 pg/mL in sCD14 between arms at 12 weeks assuming similar standard deviations seen in preliminary data.

Because the ANCOVA model will adjust for baseline sCD14 as well as CD4 cell count (neither of which are expected to be related to treatment arm), it's likely that there will be sufficient power to detect even smaller effects than what is expected.

It is anticipated that subjects will accrue to the study at a rate of 2-3 subjects per month. At this rate, the study should fully accrue within 18 months after the first subject enrolls.

9.5 Primary Analyses

As this is a pilot study of biologic activity, our primary analysis of efficacy will be an as-treated analysis, limited to subjects who have data for baseline and week 12, and remain on study treatment through week 12 with no more than 7 consecutive days of medication

missed, and did not change ART or use prohibited medications or have virologic failure during this time period. To preserve the study power in the case of greater than 10% missing data due to discontinuation/non-adherence, additional subjects may enroll, at discretion of the protocol team, to replace subjects who are discontinued or non-adherent to study product or scheduled study visits prior to week 12. Therefore the total sample size may exceed 40 at the end of the study.

Changes from baseline to week 12 in sCD14, sCD163, and IL-6 (as well as all secondary and exploratory outcomes) will be summarized by means, medians, standard deviations, and 95% confidence intervals for each treatment arm. We will compare the two study arms at baseline with respect to clinical characteristics as well as subject demographics. Any baseline covariates that cause imbalance at the 5% significance level will be included in the primary analyses. Our primary analysis will utilize an Analysis of Covariance (ANCOVA) model of the primary outcomes, which compares the outcomes at 12 weeks between arms (placebo/DP) after adjusting for baseline activation (or plasma level) and CD4 cell count. If any of the primary outcomes violate assumptions of normality, suitable transformations will be investigated. If no transformation can be used, between-arm comparisons on 12 week change in sCD14, sCD163, and IL-6 will be assessed by the Wilcoxon rank sum test.

Statistical significance for the primary analysis of sCD14 will be tested using a two-sided alpha = 0.05. The other key outcomes of sCD163 and IL-6 will each be tested at 0.05 as well.

9.6 Secondary Analyses

Changes from baseline to week 12 and week 24 in other secondary and exploratory endpoints including measurements of immune activation, systemic inflammation, and residual virus in blood and rectal tissue, change in pre- and post-bronchodilator FEV_1, and percent FMD (see 9.2.2 and 9.2.3) will be summarized by means, medians, standard deviations, and 95% CIs for each of the treatment arms. As in the primary analysis, between-arm comparisons will be made using either an ANCOVA model or the Wilcoxon rank sum test. For secondary outcomes with more frequent measurements (i.e. baseline, 4, 12 week), longitudinal models such as Generalized Estimating Equations (GEE) will be used to compare the trajectories across time between treatment arms. Included in the model will be predictor variables for week, treatment group, and their interaction.

The study design allows for an additional 12 weeks of follow up in which the placebo arm crosses over to DP 100 mg 4 times daily for 12 weeks. As an exploratory measure, the trajectories of T cell activation and IL-6 expression for these subjects will be analyzed using a generalized linear mixed model with covariates for week (defined as time since initiating placebo/DP), drug (placebo/DP), and their interaction. The effects of long-term and short-term DP on T cell activation will be quantified using an ANCOVA model, similar to the primary analysis.

Safety will be evaluated by summarizing the number and frequency of Grade ≥ 2 adverse events within each study arm. Comparisons between study arms will be done

using Fisher's exact test at each study visit.

Statistical significance for each of the secondary analyses will be tested using the nominal 5% type I error rate.

9.7 Monitoring

Accrual and a summary of all Grade ≥ 2 signs and symptoms, all Grade ≥ 2 laboratory abnormalities and all reported AEs will be reviewed by the study team monthly. This summary will be pooled over the study arms. In addition, baseline characteristics as well as early study treatment and study discontinuations, pooled over study arms, will be reviewed regularly by the team. The Center for Research in Health Care Data Center (CRHC DC) will also prepare a quarterly report of all Grade ≥ 2 signs and symptoms, all Grade ≥ 2 laboratory abnormalities and reported AEs by study arm, to be reviewed quarterly [or as requested] by the DAIDS clinical representative or designee.

Approximately 6 months after enrollment of the first subject, (or, if earlier, when 50% of subjects have been enrolled), an interim review of the study will occur. An Institutional Data Safety and Monitoring Board (IDSMB) convened by the University of Pittsburgh will function as a Study Monitoring Committee (SMC) for the study and will review accrual, toxicity summaries, and off-treatment and off-study rates and reasons broken down by study arms. In addition, longitudinal changes in CD4+ T-cell count and HIV-1 RNA levels will be reviewed. Note that immune activation measurements, including the primary endpoints, will be run in batch after follow-up is concluded. The first IDSMB review will occur when 30% of the subjects are enrolled or at 1 years, whichever is earlier. IDSMB reviews will occur at least annually after the first review or at more frequent intervals as determined by the IDSMB. An interim IDSMB review may also be conducted if a reason is identified by the DAIDS clinical representative, study investigators, or study statistician in consultation with the team.

The specific responsibilities of the IDSMB are to:

1. Review the research protocol, informed consent documents and plans for data and safety monitoring;
2. Evaluate the progress of the study, including periodic assessments of data quality and timeliness, subject recruitment, accrual and retention, subject risk versus benefit, adverse events, unanticipated problems, performance of the trial site, and other factors that can affect study outcome;
3. Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the subjects or the ethics of the study;
4. Review clinical center performance, make recommendations and assist in the resolution of problems reported by the PIs;
5. Protect the safety of the study subjects;
6. Report on the safety and progress of the study;
7. Make recommendations to the PIs concerning continuation, termination or other modifications of the study based on the observed beneficial or adverse effects of the treatment under study;

8. Monitor the confidentiality of the study data and the results of monitoring;
9. Assist the PIs by commenting on any problems with study conduct, enrollment, sample size and/or data collection.

The IDSMB will include experts in infectious disease, immunology, and biostatistics. Members will consist of persons independent of the investigators who have no financial, scientific, or other conflict of interest with the study. Written documentation attesting to absence of conflict of interest will be required.

The University of Pittsburgh Office of Clinical Research, Health Sciences will provide the logistical management and support of the IDSMB. A safety officer (chairperson) will be identified at the first meeting. This person will be the contact person for serious adverse event reporting. Procedures for this will be discussed at the first meeting.

The first meeting will take place before initiation of the study to discuss the protocol, approve the commencement of the study, and to establish guidelines to monitor the study. The follow-up meeting frequency of the IDSMB will be determined during the first meeting. An emergency meeting of the IDSMB will be called at any time by the Chairperson should questions of patient safety arise.

10.0 PHARMACOLOGY PLAN

Not applicable

11.0 DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

11.1 Records to Be Kept

Case report forms (CRF) will be completed for each subject. Subjects must not be identified by name on any CRFs. Subjects will be identified by the patient identification number (PID).

11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the CRHC Data Center.

11.2.2 It is the responsibility of the CRHC Data Center to assure the quality of computerized data for this study. This role extends from protocol development to generation of the final study databases.

11.3 Clinical Site Monitoring and Record Availability

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

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

11.4 Expedited Adverse Event Reporting

11.4.1 Reporting Requirements for this Study

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

All serious adverse events which are unexpected, fatal or life-threatening, and related or possibly related to the research intervention will be reported to the University of Pittsburgh IRB within 24 hours of the investigator learning of the event. All serious adverse events, regardless of relationship to the research intervention, will be reported to the IDSMB within 24 hours. An emergency meeting may be called as determined by the IDSMB Chair. Other AEs that must be reported in an expedited manner are: congenital anomalies or abnormal pregnancy outcomes after week 20 of gestation, regardless of attribution to study agent(s).

All other adverse events that meet the IRB reporting requirements as per Section 11.5 will be reported to the IRB within 10 working days of the investigator learning of the event. All adverse events, regardless of relationship to the research intervention, will be reported to the IDSMB during regular reporting intervals (to be determined after the first initial meeting and depending upon risk).

The study agents for which expedited reporting are required are: dipyridamole and placebo for dipyridamole.

The DAIDS Medical Officer (MO) will receive a copy of all AEs submitted to the IRB in an expedited fashion. In addition to the events described above, the

DAIDS MO will receive all Grade 3 and 4 AEs that are attributed to study procedures graded as per Section 11.4.2.

11.4.2 Grading Severity of Events

The most current Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) is used and is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance

11.4.3 Expedited AE Reporting Period

The expedited AE reporting period for this study will be from the time the participant starts study agent until the end of the study follow-up for that participant.. After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the EAE Manual, will be reported to the IRB and IDSMB if the study staff become aware of the events on a passive basis (from publicly available information). A copy of these reports will also be sent to the DAIDS Medical Officer.

11.5 Unanticipated Problems

Unanticipated problems, as defined by the OHRP include any incident, experience, or outcome that meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, “possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Upon becoming aware of any AE, the investigator will assess whether the AE represents an unanticipated problem by applying the criteria described above. If the investigator determines that the AE represents an unanticipated problem, the PI will report it the University of Pittsburgh IRB within 10 working days as per the IRB Policies and Procedures Manual, Section XVII and to the IDSMB during regular reporting intervals. In addition, incidents, experiences and outcomes that occur during the conduct of this study that represent unanticipated problems but are not considered AEs may require reporting under the HHS regulations at 45 CFR 46.103(a) and 46.103(b)(5). Further details and examples of such scenarios are described in the OHRP policy (http://www.hhs.gov/ohrp/policy/advevntguid.html#Q1). Unanticipated problems involving risks to participants or others will be reported as detailed in the DAIDS Critical Event Policy

(http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Pages/Safety.aspx).

12.0 HUMAN SUBJECTS

12.1 Institutional Review Board (IRB) Review and Informed Consent

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

12.2 Protocol Registration

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

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

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

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual, available at http://rsc.tech-res.com/protocolregistration.

12.3 Risk-Benefit Statement

12.3.1 Risks

General

Phlebotomy may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection. Disclosure of STI status may cause sadness or depression in volunteers. Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions.

Brachial Artery Flow-Mediated Dilation (FMD)

Brachial artery FMD is a painless imaging test that has no known short or long-term risks. The test uses ultrasound and does not involve radiation exposure. The test may be mildly uncomfortable because a blood pressure cuff is applied tightly to the arm.

Dipyridamole

Adverse reactions at therapeutic doses are usually minimal and transient. On long-term use, initial side effects usually disappear. In two heart valve replacement trials, the following reactions were reported when DP tablets were given with warfarin: dizziness (13.6%), abdominal distress (6.1%), headache (2.3%), and rash (2.3%). Other reactions from uncontrolled studies include diarrhea, vomiting, flushing and pruritus. In addition, angina pectoris has been reported rarely and there have been rare reports of liver dysfunction. On those uncommon occasions when adverse reactions have been persistent or intolerable, they have ceased on withdrawal of the medication.

When DP tablets were administered concomitantly with warfarin, bleeding was no greater in frequency or severity than that observed when warfarin was administered alone. In rare cases, increased bleeding during or after surgery has been observed.

In post-marketing reporting experience, there have been rare reports of hypersensitivity reactions (such as rash, urticaria, severe bronchospasm, and angioedema), larynx edema, fatigue, malaise, myalgia, arthritis, nausea, dyspepsia, paresthesia, hepatitis, thrombocytopenia, alopecia, cholelithiasis, hypotension, palpitation, and tachycardia.

Enema

The main risk from having an enema is temporary discomfort, bloating and flatulence.

Flexible Sigmoidoscopy

Flexible sigmoidoscopy is a commonly practiced medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a “bloated stomach”. Mild rectal irritation, urgency to move bowels, or hypotension may also occur.

Endoscopic biopsies are painless, begin to heal within 2 hours, and are completely healed within 3-5 days. On extremely rare occasions, the endoscopic procedure or biopsies may lead to pain, infection (sepsis), bleeding or perforation of the gastrointestinal tract. Perforation secondary to mucosal biopsy occurs approximately once out of every 8,000 procedures. If this extremely rare complication occurs, antibiotics and surgery to repair the tear may be necessary.

Risk of bleeding due to DP: DP does not appear to increase the risk of bleeding and the Standards of Practice Committee of the American Society for Gastrointestinal Endoscopy does not recommend discontinuation of the drug prior to low risk endoscopic procedures, including flexible sigmoidoscopy with biopsy.

Pre- and post-bronchodilator spirometry and albuterol administration: Spirometry is a routine diagnostic procedure and involves a very low level of risk. A person taking a lung function test may feel a sensation of dyspnea and cough, faintness or feeling of light-headedness. These sensations subside rather quickly after completing the testing. All lung function tests will be performed sitting down so as to protect against injury from falling. Albuterol is a well-tolerated bronchodilator to be inhaled during the lung function testing. Common side effects of albuterol include heart palpitations, increased heart rate, chest pain, shakiness, nervousness, headache, dizziness, sore throat, and runny nose. Rarely, albuterol may cause an immediate hypersensitivity allergic reaction.

12.3.2 Benefits

There are no direct benefits for participation in this pilot study. Subjects and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to a better understanding of the causes of persistent immune activation and systemic inflammation in chronic HIV-1 infection. Subjects will be referred for treatment for any incidental findings detected during screening and other study-related examinations.

12.4 Informed Consent Process

It is the responsibility of the study site investigator to ensure that the Elements of Informed Consent (21 CFR 50.25, 45 CFR 46.116, and ICH GCP 4.8.10) and Health Insurance Portability Accountability Act (HIPAA) guidelines are followed and documented in the source document file. Written informed consent will be obtained from each study subject prior to screening. In obtaining and

documenting informed consent, the investigators and their sub-investigators will comply with applicable local and US regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with the Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials available at:

(http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/sourcedocpolicy.pdf).

Subjects will be provided with copies of the informed consent forms if they are willing to receive them. The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process will specifically address the following topics of importance to this study:

- The unknown safety and unproven efficacy of the study products
- The importance of adherence to the study visit and procedures schedule
- The potential medical risks of study participation (and what to do if such risks are experienced)
- The real yet limited benefits of study participation
- The distinction between research and clinical care
- The right to withdraw from the study at any time

The informed consent process will include an assessment of each potential subject's understanding prior to enrollment and randomization of concepts identified by the protocol team as essential to the informed consent decision. Subjects who are not able to demonstrate adequate understanding of key concepts after exhaustive educational efforts will not be enrolled in the study.

If during the trial a consent revision where new information that might affect the research subject's willingness to participate is presented, subjects will be informed of the revisions. If a research subject terminates the study and consent form revision occurs after their participation has ended, they do not need to sign the revised consent form.

12.5 Participant Confidentiality

All study procedures will be conducted in private and every effort will be made to protect subject privacy and confidentiality to the extent possible. All study-related information will be stored securely at the study site. All subject information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process and administrative forms, laboratory specimens and other reports will be identified by a coded number only to maintain subject confidentiality. Forms, lists, logbooks, appointment books and any other listings that link subject ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All local databases will be secured with password-protected access systems. Subjects' study information

will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by:

- Representatives of the US Federal Government, including the US OHRP, NIH, and/or contractors of the NIH
- University of Pittsburgh IRB

Authorized representatives of QUEST Diagnostics, Inc. will have access to limited identifiable information (including first and last name, date of birth, gender, and medical record number) for the purpose of analyzing biological samples obtained for this research study. Subjects who decline to allow this use of identifiers will have samples sent to QUEST using only a coded number.

12.6 Special Populations

This section outlines considerations made for the inclusion or exclusion of special populations in this study.

12.6.1 Pregnant Women

Women who test positive for pregnancy at screening or enrollment visits will not be eligible to participate in this study.

A urine pregnancy test will be performed on all women of childbearing potential at the Screening, Entry, and every 4 weeks during the study. Investigators will discontinue study product among subjects who test positive for pregnancy. All potential female subjects of childbearing potential will be required to be currently using a reliable method of contraception as outlined in the inclusion criteria.

12.6.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study meets Justifications for Exclusion criteria for younger children as set forth by the NIH. Specifically, “insufficient data are available in adults to judge potential risk in children” and “children should not be the initial group to be involved in research studies.” This study does not plan to enroll children or adolescents under 18 years of age.

12.7 Compensation

Pending IRB approval, subjects will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits and time away from work according to standard practice at the University of Pittsburgh. Reimbursement amounts will be specified in the informed consent form.

12.8 Communicable Disease Reporting

Study staff will comply with all applicable local requirements to report communicable diseases identified among study subjects to local health authorities. Subjects will be made aware of all reporting requirements during the study informed consent process.

12.9 Study Discontinuation

This study may be discontinued at any time by NIAID, the OHRP, the University of Pittsburgh IRB, the IDSMB or the Principal Investigators.

13.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by University of Pittsburgh policies.

14.0 BIOHAZARD CONTAINMENT

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

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

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