**A Randomized, Controlled Pilot Trial Assessing the Utility of Cognitive Behavioral Therapy to Improve Endothelial Function and Reduce Inflammation in Depressed, Virologically-Suppressed, Antiretroviral-Treated, HIV-infected Adults**

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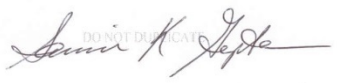

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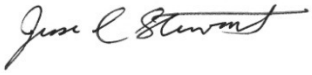

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SCHEMA

DESIGN

The objectives of this study will be met by performing a 24-week, randomized, controlled, single-blinded, two-arm, parallel group, pilot trial at a single center. A total of 200 subjects may be screened to identify 110 participants to be enrolled and randomized. These participants will be ≥ 18 years old, have been receiving antiretroviral therapy for at least one year with an HIV viral load < 75 copies/mL at screening, and have major depression using the PHQ-9 questionnaire. These participants will be randomized 1:1 to either depression treatment with the Beating-the-Blues cognitive behavioral therapy program (N=55) or usual care (N=55).

OBJECTIVES

The primary objective of this study is to compare 12-week changes in flow-mediated dilation (FMD) of the brachial artery in HIV-infected, depressed adults already receiving virologically suppressive antiretroviral therapy (ART) who are then randomized to either depression treatment with the Beating the Blues (BtB) cognitive behavioral therapy program or to Usual Care (UC). Key secondary objectives include comparing changes in FMD between the two groups at 24 weeks and comparing changes in circulating IL-6, hsCRP, D-dimer, EPCs/ECFCs, and markers of monocyte activation (CD14+CD16+ cell proportions and sCD14/sCD163) at both 12 and 24 weeks.

DURATION

Each individual participant will be followed for approximately 28 weeks (up to 4 weeks from screening to randomization and 24 weeks on study). We expect to enroll 3-4 participants per month. The total duration of this study will be approximately 38 months.

POPULATION

All participants will be HIV-infected, 18 years of age or older, have achieved HIV-1 viral loads less than 75 copies/mL while receiving stable ART for at least 12 months, and have a PHQ-9 score ≥ 10. Participants will be recruited from the infectious diseases outpatient clinics of Eskenazi Health Hospital and the Indiana University Health Hospitals.

SAMPLE SIZE

The total sample size for this study will consist of 110 enrolled participants. Up to 200 participants may be screened to identify the 110 eligible for enrollment. If a consented and otherwise eligible participant withdraws prior to the Baseline/Entry Visit, this participant will be replaced.

## 1.0 STUDY OBJECTIVES

### 1.1 Primary Objective

1.1.1 To compare 12-week changes in FMD between those undergoing depression treatment using the Beating the Blues (BtB) program vs those receiving Usual Care (UC).

### 1.2 Secondary Objectives

1.2.1 To compare 24-week changes in FMD between those undergoing depression treatment using BtB vs those receiving UC.

1.2.2 To compare 12 and 24-week changes in circulating levels of IL-6, hsCRP, D-dimer, fasting lipid profiles (total cholesterol, HDL-C, LDL-C, triglycerides), insulin resistance estimated using HOMA-IR, systolic and diastolic blood pressures, body mass indices (BMI), circulating EPC/ECFC levels, and circulating markers of monocyte activation (CD14+CD16+ cell proportions and sCD14/sCD163) between those undergoing depression treatment using BtB vs those receiving UC.

1.2.3 To compare 12 and 24-week changes in PHQ-9 scores and SCL-20 scores between those undergoing depression treatment using BtB vs those receiving UC.

1.2.4 To correlate FMD, IL-6, hsCRP, D-dimer, circulating EPCs/ECFCs, and markers of monocyte activation (CD14+CD16+ cell proportions and sCD14/sCD163) levels with PHQ-9 and SCL-20 scores at baseline.

1.2.5 To assess the safety, HIV-1 RNA levels, and CD4 cell counts of the BtB program in HIV-infected adults.

1.2.6 To explore the relationships between genetic markers with FMD, PHQ-9, SCL-20 scores at baseline and with response to depression treatment with BtB.

## 2.0 HYPOTHESES, BACKGROUND, AND INTRODUCTION

### 2.1 Hypotheses

2.1.1 Treatment with BtB will improve FMD, increase circulating EPC/ECFC levels, and reduce circulating levels of IL-6, hsCRP, D-dimer, and markers of monocyte activation (CD14+CD16+ cell proportions and sCD14/sCD163) at both 12 and 24 weeks when compared to UC.

2.1.2 Treatment with BtB will improve PHQ-9 and SCL-20 scores at both 12 and 24 weeks when compared to UC.

2.1.3 Treatment with BtB will be safely tolerated and have no effects on HIV-1 RNA suppression or CD4 cell counts.

2.1.4 Genetic variation is associated with endothelial function, depression severity, and response to depression treatment.

## 2.2 Background

### 2.2.1 HIV and cardiovascular events

Cardiovascular disease (CVD) has emerged as a leading cause of death in the HIV-infected population receiving ART (1). Given that lifespans of the HIV-infected population are nearing those of the uninfected population with the widespread use of ART (2-5), there is growing concern that the risk of HIV-CVD will be magnified several-fold worldwide, especially as the population ages (6). Although previously attributed to the dysmetabolic effects of ART, the greater risk of CVD in HIV remains, even when newer ART drugs are used that do not induce severe dyslipidemia, hypertension, or insulin resistance (7). Attention has thus turned to HIV-specific mechanisms as potential explanations for HIV-CVD. In fact, elevated markers of generalized inflammation (IL-6, hsCRP) and coagulation (D-dimer) have been found to predict HIV-CVD events (8-11). HIV may also be associated with impaired endothelial function (12, 13), perhaps due to this dysregulated inflammation and coagulation (14).

One possible etiology of the heightened inflammation, altered coagulation, and endothelial dysfunction in HIV, and consequently of the increased risk of HIV-CVD, may be depression. Depression is an independent predictor of incident CVD in the general population (15) with heightened inflammation (16), altered coagulation (17-19), and endothelial dysfunction (20-23) among the leading candidate mechanisms underlying this relationship. Thus, it stands to reason that depression may contribute to or exacerbate these pathways in HIV and thus increase the risk of HIV-CVD. Indeed, our preliminary data (section c1) indicate that depressive disorders independently increase the risk of incident acute myocardial infarction (MI) in HIV-infected veterans. However, it is unknown if depression leads to HIV-CVD through inflammatory, coagulatory, and/or endothelial dysfunction pathways. It is also unknown if depression treatments (antidepressants and/or psychotherapy) modulate these pathways and, therefore, may be useful as HIV-CVD primary prevention therapies.

It is important to note that there are as yet no definitive studies demonstrating that lowering systemic inflammation or coagulation reduces HIV-CVD events in those already on ART (12, 24, 25). Although pharmacological studies of low-dose methotrexate and ‘statins’ are underway to address this issue, it is not known if such interventions will be beneficial. Even if these interventions are found to be successful, they may be associated with adverse events, drug interactions, and increased cost when provided over the years to decades required to prevent CVD events in the aging HIV-infected population.

### 2.2.2 Depression, depression treatment, and CVD risk in the general population

Thirty years of evidence indicates that depression is an independent risk factor for CVD (26). Meta-analyses have shown that adults with depressive disorders or elevated symptoms have a 64% greater risk of future CVD (27, 28). The depression-CVD relationship is comparable in strength to conventional CVD risk factors and is independent of other emotional factors (29). In addition, CVD risk increases with depression severity (27). Finally, depression has been linked to several atherogenic factors (30, 31), which supports the biological plausibility of the depression-to-future-CVD connection. Because depression meets 8 of Hill’s 9 criteria for causation (32), it is reasonable to conclude that depression is an independent, and potentially causal, CVD risk factor. The criterion not yet met is experimental evidence; however, our recently published study (33) indicates that depression treatment may be a CVD primary prevention strategy.

To date, few clinical trials have evaluated if depression treatments reduce the likelihood of CVD events (34-38). In general, these trials have not observed the anticipated benefits. A key study was the Enhancing Recovery in Coronary Heart Disease (ENRICHD) Patients trial, in which 2,481 post-MI patients with depression and/or low social support were randomized to cognitive behavioral treatment or usual care. After 29 months, no difference in nonfatal MI or death was found (34). Similar negative results were observed in the Myocardial Infarction and Depression Intervention Trial (MIND-IT), in which depressed post-MI patients were randomized to a staged pharmacologic intervention or usual care (38).

We recently proposed a novel explanation: the depression interventions in past trials, all of which involved patients with pre-existing CVD, may have been delivered too late in the natural history of CVD (39). We hypothesized that treating depression before, versus after, the onset of clinical CVD could reduce risk of CVD events because: (1) earlier treatment of another CVD risk factor, hypercholesterolemia, yields more pronounced benefits (40-43), (2) depression exerts a cardiotoxic influence early in CVD pathogenesis (22, 29, 44, 45), (3) depression treatments may have greater antidepressive efficacy prior to clinical CVD onset (46-48), and (4) conventional prognostic factors may override depression's effect during the later stages of CVD (15, 49). To evaluate our hypothesis, we conducted an 8-year follow-up study (33) of the Improving Mood-Promoting Access to Collaborative Treatment (IMPACT) trial (48), in which older depressed patients were randomized to a collaborative stepped care intervention involving antidepressants and psychotherapy or usual care. Results supported our hypothesis. IMPACT patients (24/85=28%) without baseline CVD had a 48% lower risk of first MI or stroke (HR=0.52, 95% CI: 0.31-0.86) than Usual Care patients (39/83=47%). There was no treatment benefit among patients with baseline CVD. These findings strengthen the case for depression being a causal CVD risk factor and suggest that depression treatment is a potential CVD primary prevention strategy.

### 2.2.3 Associations between depression and depression therapy with inflammation, coagulation, and endothelial function in the general population

Systemic inflammation is one candidate mechanism that might explain how depression promotes CVD (50). A recent meta-analysis revealed that inflammatory markers, including IL-6 and hsCRP, are upregulated in depressed individuals (51). Depression also predicts increases in inflammatory marker levels up to 10 years later (51-53). Notably, the inflammatory markers elevated in depression are predictive of incident CVD (46). Depression-related dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system, two systems that normally exert anti-inflammatory effects, have been proposed to explain how depression may promote inflammation (50, 53). Selective serotonin reuptake inhibitors (SSRIs) have been found to reduce IL-6 levels in depressed patients (54, 55), and this potentially beneficial effect seems limited to those whose depression improved (56-60). In addition, two small trials in patients with cancer or recent CABG found that cognitive behavioral treatment led to reduced IL-6 and hsCRP levels (61, 62), results similar to our preliminary data (section c6).

Depression has been variably associated with altered coagulation (18, 19, 31, 63-71). For instance, Lukas et al. (18) found that depressive disorders were associated with higher D-dimer levels, and von Kanel et al. (72, 73) found that acute psychological stressors in depressed patients led to higher D-dimer levels even after the stressors resolved. However, other studies have not observed similar associations (70, 74, 75). Little is known about the effects of depression treatment on altered coagulation, although a small study recently found that SSRI treatment may counteract elevated D-dimer levels associated with depression (64). Given the known utility of D-dimer in predicting HIV-CVD and the availability of this marker in the large VACS Biomarker Cohort, it is reasonable to study this marker of coagulation in relation to depression in this application. Endothelial dysfunction is also a candidate mechanism through which depression may increase CVD risk. A recent meta-analysis of 12 studies revealed the depression is moderately and inversely associated with FMD in the general population (overall r=0.19, p=0.001) (22). Our pilot results (section c4) are in line with these findings. A few small studies have suggested improvements in FMD or nitric oxide availability with SSRI treatment (23, 76, 77). Of note, SSRIs could reduce inflammation, coagulation, and endothelial dysfunction either via depression reduction or potential direct medication effects on these systems (78-80). We are not aware of any studies, other than our pilot trial (section c6), that have examined the effect of cognitive behavioral treatment for depression on endothelial function.

### 2.2.4 HIV, depression, and CVD risk

Depression is highly prevalent in the HIV-infected population, including in those receiving ART, with rates ranging from to 20-40% (81-84). Several studies have suggested that the rate of depression in HIV is two-fold greater than in uninfected individuals (85). Given this high prevalence of depression, it stands to reason that this comorbidity may appreciably contribute to HIV complication. In fact, chronic depressive symptoms are associated with greater Framingham risk scores in HIV-infected persons (86), but our recent work is the first to show a relationship between depressive disorders and HIV-CVD (section c1). This current application seeks to identify the underlying mechanisms for this association and a new intervention to mitigate the risk of depression-related CVD in HIV.

### 2.2.5 Endothelial progenitor cells, soluble markers of monocyte activation, and cardiovascular disease

Endothelial Progenitor cells (EPCs) are defined as cells that are found circulating within the bloodstream and when cultured are capable of taking on the phenotypic and functional characteristics of mature endothelial cells. EPCs are thought to home in to sites of vascular damage and assist in angiogenesis by either direct integration into the endothelium or via paracrine stimulatory mechanisms. Multiple studies have now shown an inverse relationship between metabolic syndrome, CAD, and levels of circulating EPCs (87).

The published literature regarding EPCs in HIV are conflicting with some showing no difference between HIV-infected and uninfected groups and others suggesting a lower level in those with HIV and an inverse association with FMD (88-92). These studies use differing methodologies in assessing EPCs. However, the laboratory of Dr. Mervin Yoder and has established a 'gold standard' phenotype for differentiating EPCs from endothelial colony forming cell (ECFCs) (93). We will employ this method in the current study for assessing the relationships between depression treatment and ECFC levels.

In addition, $CD14^{+}CD16^{+}$ cell subsets and the soluble monocyte activation markers sCD14 and sCD163 have been linked to greater inflammatory atherosclerotic disease burden in those with HIV infection (94). Moreover, monocyte activation has been linked in some studies to more severe depression (95, 96). As such, these markers of monocyte activation will also be measured in this trial. Understanding the relationship between these cell subsets, monocyte activation, endothelial dysfunction in HIV during depression treatment is crucial to the continued efforts to explain the conferred increase in cardiovascular in those with HIV and possibly identify new biomarkers for risk assessment in this population.

### 2.2.6 Cognitive behavioral treatment to improve depression: the *Beating the Blues* program

*Beating the Blues* (U Squared Interactive; see www.beatingthebluesus.com for a video tutorial) is an empirically supported, stand-alone, cognitive behavioral treatment program appropriate for adults with little computer experience and at least a $5^{th}$-$6^{th}$ grade reading level (97). This program utilizes an interactive, multimedia format to deliver eight 50-minute, weekly sessions, the structure and content of which mirror face-to-face cognitive behavioral treatment. Although sessions are tailored to each patient's problems, general topics include challenging dysfunctional thoughts, activity scheduling, problem solving, graded exposure, task breakdown, sleep management, and relapse prevention. Patients are also assigned tailored homeworks. *Beating the Blues* is an appropriate selection for the proposed trial, as it is efficacious, acceptable, and an excellent fit with our goals. First, it has more empirical support than any other computerized psychotherapy, including a positive randomized trial of 274 depressed patients (98, 99). It is a potent intervention (98-106), with effect sizes comparable to face-to-face cognitive behavioral treatment (107). In our pilot trial, we also observed a large *Beating the Blues* effect size for depressive symptoms (d=1.33). Because of its strong evidence base, the National Institute for Health and Care Evidence recommended this program for managing depression in the English National Health Service in 2006 (108). In addition, since 2012, *Beating the Blues* has been listed in the Substance Abuse and Mental Health Services Administration's (SAMHSA) National Registry of

Evidence-based Programs and Practices. Second, *Beating the Blues* has been shown to be acceptable to patients (97, 105, 106, 109). Third, *Beating the Blues* is an excellent fit with our long-term goal of equipping HIV clinicians with a new tool to simultaneously manage depression and CVD risk in their patients, given that it is a safe, inexpensive, and easily implemented intervention due to the lack of dependence on trained therapists.

### 2.2.7 Flow-mediated dilation (FMD) of the brachial artery

Endothelial dysfunction is the impaired ability of the vascular lining to maintain normal homeostasis. Endothelial dysfunction is an early precursor to atherosclerosis and has been shown to predict future cardiovascular events in most population studies (110-112). Improvement in endothelial function, as measured by FMD of the brachial artery, is associated with a reduced risk of future cardiovascular events (113, 114). FMD is a non-invasive technique using high-resolution ultrasound which was developed to measure changes in brachial artery diameter due to a stimulus (115), with results correlating closely with coronary endothelial function (116, 117). Measurements are made before and after endothelium-dependent and endothelium-independent stimuli (118). The endothelium-dependent stimulus is hyperemic blood flow triggered after the release of an inflated blood pressure cuff around the forearm. The shear stress from the increased blood flow subsequently causes endogenous vasodilators, predominantly nitric oxide, to be released from the endothelium which then relaxes the vascular smooth muscle. This flow-mediated dilation (FMD) of the brachial artery is thus a measure of the endothelium's ability to respond normally to hyperemic shear stress. A second stimulus, nitroglycerin, which directly relaxes smooth muscle is then applied to determine the smooth muscle's functional capacity. This endothelium-independent measurement is called nitroglycerin-mediated dilation (NTGMD). If FMD is low, but NTGMD is normal, the endothelium, and not the smooth muscle, can be safely assumed to be dysfunctional. FMD and NTGMD are calculated as the percent increases, respectively, in diameter.

FMD is an ideal measure of cardiovascular risk in this current trial for several reasons. First, as a continuous measure, use of FMD as a CVD endpoint in the proposed trial provides greater statistical power and thus requires smaller sample sizes compared to a hard endpoint trial. Second, as opposed to anatomic measurements such as coronary calcium or carotid intima media thickness, FMD is a dynamic and mechanistic physiologic assessment of vascular function. Third, FMD responds within days to weeks of an intervention (119) and, thus, can be used to detect changes in cardiovascular risk quickly, which is vital when evaluating the potential efficacy of a new intervention such as cognitive behavioral treatment.

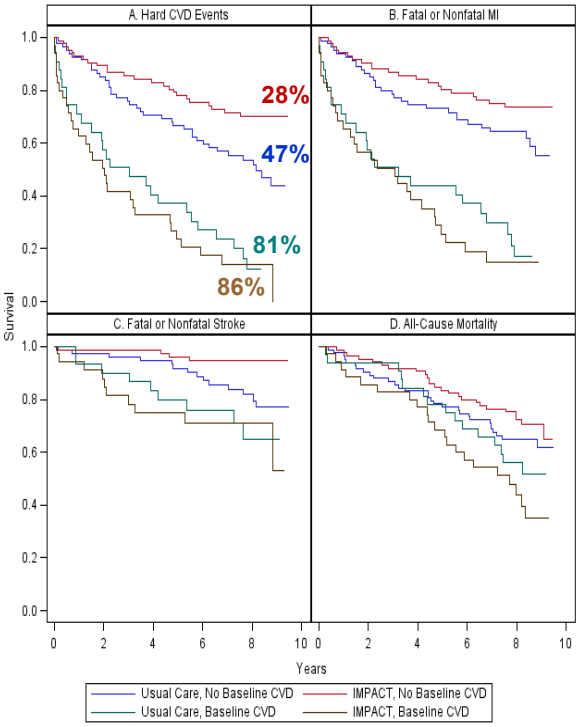
## 2.3 Preliminary Studies

### 2.3.1 Depression predicts incident CVD in HIV

We first sought to determine whether depressive disorders predict incident CVD in the HIV-infected population as they do in the general population. We previously found that HIV infection independently increases the risk of incident acute MI by 48% overall and by 39% in those with HIV-1 RNA levels <500 copies/ml in the VACS Virtual Cohort (120). Using this same cohort, we examined relationships between the presence of a depressive disorder at baseline and 6-year incidence of incident MI (CROI 2014). Of the 27,350 HIV-infected male veterans free of CVD at baseline (1998-2003), 6,219 (23%) had a baseline depressive disorder (ICD-9 codes 296.2, 296.3, or 300.4) and 367 (1.3%) suffered incident acute MI [defined using enzyme and EKG clinical data, inpatient ICD-9 code 410 in Medicare data, or ICD-10 code 121 as underlying cause of death on the death certificate] during the follow-up period (2003-2009). Cox models adjusted for demographic factors (age, race/ethnicity), CVD risk factors (hyperlipidemia, hypertension, smoking, diabetes, body mass index), and other potential confounders (hepatitis C infection, renal disease, alcohol and cocaine abuse, hemoglobin level, CD4 cell count, HIV-1 RNA level, antiretroviral medications) revealed that the presence of a baseline depressive disorder was an independent predictor of incident acute MI (HR=1.31, 95% CI: 1.03-1.67,

p=0.03). Our results (a) confirm that depressive disorders are highly prevalent in HIV-infected adults and (b) are the first to indicate that depression is a novel candidate risk factor for HIV-CVD events.

### 2.3.2 Depression treatment prevents first CVD events

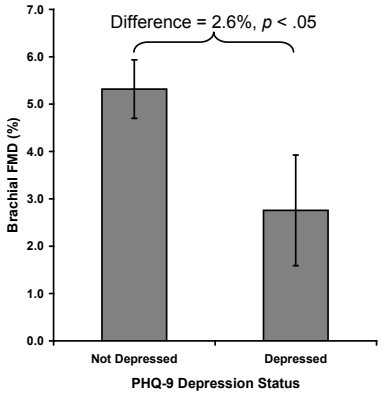



Depression treatment has been unsuccessful in preventing subsequent CVD events in HIV-uninfected patients with pre-existing CVD. Providing depression treatment as primary prevention, however, appears to hold promise. As reported in our 2014 paper in *Psychosomatic Medicine* (33), we tested our hypothesis that treating depression before clinical CVD onset reduces the risk of CVD events by conducting an 8-year follow-up study of the Indiana sites participating in the IMPACT trial (48, 121). In that multisite RCT, 1801 depressed primary care patients aged ≥ 60 years were randomized to the IMPACT depression intervention (a collaborative stepped care intervention involving primarily SSRIs and/or brief cognitive behavioral treatment) or usual care. The 235 patients (76% female, 47% Black) from the Indiana sites were assessed for hard CVD events during follow-up using electronic medical record data linked with Medicare/Medicaid claims.

During follow-up, 119 patients (51%) had a hard CVD event (MI or stroke). As hypothesized, we detected a Treatment x Baseline CVD interaction (p=0.021), indicating that time to CVD event varied across the groups (Figure). Among the 168 *without baseline CVD*, IMPACT patients had a 48% lower risk of a hard CVD event than Usual Care patients (28% vs. 47%, HR=0.52, 95% CI: 0.31-0.86; p=0.01). Highlighting clinical significance, the number needed to treat to prevent one hard CVD event over 5 years was just 6.1. In contrast, among the 67 patients with baseline CVD, the likelihood of a CVD event did not differ between arms (86% vs. 81%, HR=1.19, 95% CI: 0.70-2.03; p=0.52), consistent with prior trials (34, 38). A similar pattern of results was found for men and women, for MI and stroke, and after adjustment for potential confounders. The IMPACT intervention was efficacious for depression treatment among patients without (p<0.001) but not with baseline CVD (p=0.83). In sum, we found that depression treatment, delivered before clinical CVD onset, halved the excess risk of first CVD events among depressed, HIV-uninfected adults, suggesting that depression treatment could be used as CVD primary prevention strategy.

### 2.3.3 Depression is associated with lower FMD

In a pilot study at Indiana University, we examined the cross-sectional relationship between depression and FMD in 35 HIV-uninfected primary care patients. Participants were 41-72 years old (66% female, 49% non-white) with no history of CVD or other chronic conditions. A total of 29% screened positive for depression defined as Patient Health Questionnaire-9 (PHQ-9) score ≥10, which has 88% sensitivity and 88% specificity for major depressive disorder (122). Values [mean (SD)] for the PHQ-9 scores [6.0 (6.5)] and FMD [4.6% (3.1%)] fell within expected ranges. As is shown in the Figure, mean FMD of depressed adults was significantly lower than that of non-depressed adults (2.76% vs. 5.32%; p = 0.045), which is consistent with prior studies (22). Adjustment for brachial artery diameter, which is the strongest variable associated with FMD reactivity, increased the FMD difference to 3.5% (2.08% vs. 5.60%). This difference is likely clinically meaningful given that a 1.0% higher baseline FMD predicted a 6% lower risk of CVD events over five years in the Multi-Ethnic Study of Atherosclerosis (123, 124).




2.3.4 Depressive symptoms predict future increases in inflammation

We evaluated longitudinal associations between depressive symptoms and both IL-6 and hsCRP in 263 healthy, older adults (53). At baseline and follow-up visits, adults completed the Beck Depression Inventory-II (BDI-II) to assess depressive symptoms and underwent blood draws to quantify IL-6 and hsCRP. Path analyses revealed that baseline BDI-II score (β=0.18, p=0.005) predicted a 6-year increase in IL-6, even after adjustment for demographic, biomedical, and behavioral factors. Of all factors examined, BDI-II score was the strongest predictor of IL-6 change. In contrast, baseline IL-6 did not predict 6-year increases in BDI-II score (β=-0.04, p=0.48). These results suggest that depression promotes future increases in systemic inflammation over time as opposed to inflammation promoting depression severity. Thus, treatment of depression may consequently lead to reductions in inflammation.

2.3.5 Depression treatment with *Beating the Blues* improves FMD and decreases inflammation

This recently completed pilot RCT, funded by a grant from the American Heart Association to Dr. Stewart, provides additional proof-of-concept and feasibility data. We hypothesized that treating depression with *Beating the Blues* before clinical CVD onset would improve endothelial function, as measured by FMD in Dr. Gupta's laboratory, and reduce inflammation in HIV-uninfected adults. Primary care patients (41-64 years, 52% female, 38% Black) with depression (PHQ-9 score ≥10) and no known clinical CVD were randomized to *Beating the Blues* (*n*=13) or usual care (*n*=16). We assessed FMD, circulating inflammatory markers, and depressive symptoms at baseline and 12 weeks. The 8 *Beating the Blues* sessions occurred between these visits.

| | Beating the Blues Mean (SD) | Usual Care Mean (SD) | *p* | Cohen's *d* |
|---|---|---|---|---|
| **Pre-treatment Level** | | | | |
| FMD, % | 2.60 (4.18) | 2.51 (3.00) | 0.96 | --- |
| IL-6, pg/mL | 4.15 (2.33) | 4.90 (5.34) | 0.73 | --- |
| SCL-20 (range: 0-4) | 1.67 (0.78) | 1.59 (0.96) | 0.87 | --- |
| **Post-Treatment Level Adjusted for Pre-Treatment Level** | | | | |
| FMD, % | 4.18 (2.86) | 2.47 (2.72) | 0.21 | 0.61 |
| IL-6, pg/mL | 3.76 (1.65) | 4.75 (2.84) | 0.43 | 0.43 |
| SCL-20 (range: 0-4) | 0.83 (0.36) | 1.44 (0.54) | 0.02 | 1.33 |

As is shown in the Table, there were no meaningful differences between the groups in pre-treatment FMD, IL-6, or Symptom Checklist-20 (SCL-20) depression scores. As hypothesized, post-treatment FMD adjusted for pre-treatment FMD was higher in patients randomized to the intervention versus usual care (p=0.21, d=0.61). This difference was not statistically significant; however, the moderate effect size is clinically meaningful (123, 124). Similarly, post-treatment IL-6 levels adjusted for pre-treatment levels were lower in the

intervention versus usual care group (p=0.43, d=0.43 pg/mL). Although not statistically significant, this difference in IL-6 between groups are in line with levels associated with a greater risk of CVD events in the SMART trial (39% greater risk for each IL-6 increase of 0.34 pg/mL) (10). Finally, post-treatment SCL-20 score adjusted for pre-treatment score was significantly lower in the intervention versus usual care group (p=0.02, d=1.33), suggesting that *Beating the Blues* improved depression while improving FMD and lowering IL-6.

The lack of statistically significant group differences in FMD and IL-6 is likely due to insufficient power stemming from the small sample size. This pilot trial was designed to estimate effect sizes, not to detect significant group differences. Importantly, Beating the Blues alone (without antidepressant medication) led to the observed improvements in FMD and inflammatory markers, suggesting that cognitive behavioral treatment alone without pharmacologic therapy can reduce CVD risk.

### 2.3.6 ECFCs and monocyte activation in HIV

In a recently completed pilot trial, we compared ECFC, intermediate monocytes ($CD14^{+}CD16^{+}$), and non-classical monocytes ($CD14^{dim}CD16^{++}$) levels in HIV-infected participants virologically-suppressed on antiretroviral therapy, HIV-infected treatment-naïve participants, and HIV-uninfected healthy controls. ECFC levels were significantly higher in the HIV-infected, virologically-suppressed group compared to the uninfected controls. $CD14^{+}CD16^{+}$ percentages (but not $CD14^{dim}CD16^{++}$ cells) were significantly higher in both HIV-infected groups vs uninfected controls. In the HIV-infected groups, ECFCs and $CD14^{+}CD16^{+}$ intermediate monocytes were significantly and inversely correlated. Lower availability of ECFCs may partly explain the relationship between greater intermediate monocytes and atherosclerosis in HIV.

As such, this trial will provide an opportunity to confirm these associations in a depressed population. Furthermore, it will be of great interest to determine if depression treatment leads to reduction in monocyte activation, and in turn greater ECFC levels, in HIV-infected patients.

## 2.4 Study Rationale

To our knowledge, the current trial is the first ever to test the hypotheses that depressive disorders contribute to greater systemic inflammation, altered coagulation, and endothelial dysfunction in HIV-infected patients and that depression treatment reverses these potential mechanisms of HIV-CVD. Our preliminary data support these hypotheses by finding a strong relationship between depression and a higher risk of incident acute MI in HIV-infected veterans, thereby suggesting a new and, until now, completely unexamined approach (depression treatment) to reducing HIV-CVD events. However, we now need to determine the underlying mechanisms for this association to validate the link between depression and HIV-CVD risk and to justify future large-scale trials. These studies may also open a whole new area of investigation focused on the relationships between mood disorders in general and HIV complications. This application will also test the effects for the first time the *Beating the Blues* therapy program in an HIV-infected population.

We have chosen to use a cognitive behavioral treatment program as our intervention without the addition of pharmacologic antidepressant therapy based on the strength of our preliminary data in which the *Beating the Blues* program alone improved FMD and reduced inflammation. If *Beating the Blues* alone is found to be insufficient to improve depression and CVD risk in the proposed study, then we will investigate in future trials the effects of antidepressants (with and without CBT) on HIV-CVD. We cannot ethically restrict initiation of antidepressant therapies, especially in the Usual Care arm, by the participants' primary providers during the trial, but we will adjust the results for such antidepressant initiation.

We chose to IL-6, hsCRP, and D-dimer as our inflammation and coagulation biomarkers of interest due to their known associations both with depression and HIV-CVD. We will also measure markers of monocyte activation (sCD14, sCD163, CD14+CD16+ cells) and ECFCs. However, we will also archive plasma, serum, PBMCs, and urine at each visit for future investigation to measure other biomarkers of potential impact on HIV-

CVD if the primary markers are not affected by *Beating the Blues* or if newer and potentially better markers are identified in the literature.

If our hypotheses are supported, then the depression treatments studied in this application can be investigated in future trials to prevent HIV-CVD. In addition, these data may also justify trials of depression treatment to prevent other HIV complications linked with greater inflammation and coagulation, such as neurocognitive dysfunction, nephropathy, osteoporosis, and mortality (8).

## 3.0 STUDY DESIGN

### 3.1 Overview

The objectives of this study will be met by performing a 24-week, randomized, controlled, single-blinded, two-arm, parallel group, pilot trial at a single center. A total of 110 participants will be enrolled and randomized. These participants will be ≥ 18 years old, have been receiving antiretroviral therapy for at least one year with an HIV viral load < 75 copies/mL at screening, and have major depression using the PHQ-9 questionnaire. These participants will be randomized 1:1 to either depression treatment with the Beating-the-Blues cognitive behavioral therapy program (N=55) or usual care (N=55).

### 3.2 Screening Visit

If deemed eligible for screening, the participant will be approached (with permission/referral of the potential participant's primary care provider) by either a study investigator or by an Infectious Diseases Research Clinic study coordinator to enter the screening phase of the study. After written, informed consent is provided by the participant, a random study code number will be assigned to the participant to ensure confidentiality. This study code number will be used for laboratory transport and processing, result reporting, and data recording.

The study participant need not be fasting for this visit. At the Screening Visit, the participant's medical, psychiatric, and medication history will be reviewed. A brief physical examination will be performed. The PHQ-9 questionnaire will be administered. If the potential participant is eligible based on these initial assessments, then blood will be drawn to determine if the participant meets the laboratory eligibility criteria (see Section 4.0). Urine pregnancy testing for female study volunteers of reproductive potential will also be performed. All screening tests will be performed at the Indiana Clinical Research Center (ICRC). If the eligibility criteria are met, then the participants will enter the clinical trial. For those who score ≥ 10 on the PHQ-9 and are thus eligible for study participation, we will notify their primary HIV provider of these results so that the provider may institute any additional management as deemed best. A list of local mental health service providers and clinics will be given to the patients' primary HIV providers or social workers.

### 3.3 First Entry Visit

This visit will occur within 15 days of the Screening Visit and will occur at the ICRC. The primary purpose of this visit is to perform the first of two FMD studies and to obtain blood for the circulating markers of interest. Two Entry Visits are being conducted in order to establish more precise and stable estimates of endothelial function, inflammation, and coagulation prior to randomization. **The study participant must be fasting for this visit.** At this visit, FMD will be performed and blood will be obtained after vital signs are

measured and the PHQ-9 questionnaire administered. A urine pregnancy test will be repeated for women of reproductive potential. Blood and urine samples will be obtained for archiving.

## 3.4 Second Entry Visit

This visit will occur within 30 days of the Screening Visit and will occur at the ICRC. During this visit, updated psychiatric management (such as new medical or behavioral therapies initiated since screening) and medications will be reviewed. The PHQ-9, SCL-20, International Physical Activity, Moriskey, Tobacco Use, General Anxiety Disorder-7, Buss-Perry Aggression, PANAS (Positive Affect subscale of the Positive and Negative Affect Schedule), and Pittsburgh Sleep Quality Index questionnaires will be administered. **The study participant must be fasting for this visit.** Vital signs will be measured and a brief physical examination will be performed prior to performing the FMD procedure and obtaining blood for the circulating markers of interest. A urine pregnancy test will be repeated for women of reproductive potential. Urine will also be obtained for archiving.

Blood will also be drawn at this visit for archiving DNA for future studies of interest linking genetics with endothelial function and depression scores.

Randomization will occur at this visit. Randomization will be stratified by use of pharmacologic antidepressants at the time of the Second Entry Visit. The participants' primary providers will be notified of the randomization assignment.

## 3.4 Beating the Blues Treatment Sessions and Usual Care

### 3.4.1 Beating the Blues

To minimize treatment barriers, attrition, and subject burden, the BtB treatment sessions will occur in private at Dr. Stewart's laboratory, the ICRC, the Infectious Diseases Research Clinic at Eskenazi Health Hospital, or a location selected by the patient where s/he can access a computer with internet, such as the patient's home, the patient's work, a family member's/friend's home, or a public library. The location will be chosen by the participant. To help maintain privacy, patients will be provided with ear bud headphones if they plan to complete BtB sessions at remote locations and do not already own a pair of headphones that they would rather use. We will also tell patients that, if they choose to complete BtB session where others are present, these other people (e.g., family member or friend) may realize that they are completing a treatment for depression. It will be up to each patient to decide if the available remote locations provide sufficient privacy. Up to 8 sessions will be performed between the Second Entry Visit and the Week 12 Visit. The PHQ-9 questionnaire will be administered at each of the Weekly BtB Sessions. No physiologic or biologic assessments will be performed during these sessions. The participants need not be fasting for these sessions.

At each in-person BtB session, a research assistant trained in the use of this intervention will first collect homework and will start the appropriate session on a computer. The patient will then work through the session alone at his/her own pace. At the end of the session, the research assistant will give the patient the printed homework assignment and will schedule the next session.

Remote BtB sessions will scheduled, just like in-person sessions. Patients who select the remote treatment option will complete the first BtB session in person, at which time they will be given a BtB binder with printouts of all future homeworks. To begin each remote BtB session, an assistant will call the patient to instruct the patient to put last week's homework in the BtB binder, to address any technical issues, and to ensure that the patient has launched the correct BtB session for that day. The patient will then work alone through the BtB session at his/her own pace, with the assistant monitoring progress remotely on the secure BtB website set up for this trial. To end the session, the assistant will call the patient back to address any questions and ensure that the patient has identified the correct printed homework in the BtB binder.

### 3.4.2 Usual Care

This arm was modeled after the IMPACT trial's control group (48). Patients randomized to the Usual Care group will be informed of their depression diagnosis and will be encouraged to follow-up with their primary care or HIV provider. There will be no formal interaction with the participants between the Second Entry Visit and the Week 12 Visit. However, the participants will be encouraged to contact the study team for any changes in their condition.

Our team will also send their primary provider a letter indicating that their patient has a depressive disorder and was randomized to usual care and will include a list of local mental health services. Although feedback to patients and providers could result in treatment that would not have occurred naturally, studies have shown that informational support alone is unlikely to improve depression outcomes (125). In our pilot study which used this control group, only 14% of Usual Care patients had >50% reduction in symptoms versus 43% of *Beating the Blues* patients. There will be no restrictions on the care that can be received, although we will assess changes in care during the trial.

## 3.5 Week 12 and Week 24 Visits

The participant will come to the ICRC for both of these visits. The Week 12 visit will be scheduled between 84 and 105 days after the Second Entry Visit. The Week 24 Visit will be scheduled between 168 and 189 days after the Second Entry Visit. **The study participant must be fasting for these visits.**

During these visits, updated psychiatric management (such as new medical or behavioral therapies initiated since the Second Entry Visit) and medications will be reviewed. The PHQ-9, SCL-20, International Physical Activity, Moriskey, Tobacco Use, General Anxiety Disorder-7, Buss-Perry Aggression, PANAS (Positive Affect subscale of the Positive and Negative Affect Schedule), and Pittsburgh Sleep Quality Index questionnaires will be administered. Vital signs will be measured and a brief physical exam will be performed prior to performing the FMD procedure and obtaining blood for the circulating markers of interest. A urine pregnancy test will be repeated for women of reproductive potential. Urine will also be obtained for archiving.

Participants who continue to exhibit elevated depressive symptoms (PHQ-9 $\geq$ 10) at the final study visit will be urged to follow-up with their primary HIV provider and/or clinical social worker regarding their depression. In addition, the study team will notify the primary HIV provider that his/her patient has completed participation in our trial and still has clinically elevated depressive symptoms. A list of local mental health service providers and clinics will be given to the patients' primary HIV providers or social workers.

## 3.6 Brachial artery reactivity indices

FMD and NTGMD will be measured according to established guidelines (126) at both Entry Visits, the Week 12 Visit, and the Week 24 Visit. These measurements will be performed at the ICRC.

Brachial reactivity measurements have considerable day-to-day variability. Variables that will transiently affect vasomotor function include environmental conditions, tobacco and alcohol use, diet, and medications (127). Therefore, it is desirable to control these factors in order to obtain results that more accurately reflect the underlying condition being studied. Therefore, the room temperature will be controlled at 68-71°F. The participant will rest supine for 10 minutes prior to imaging. Participants will be told to not use tobacco-containing products or eat or drink anything other than water for 8 hours prior to the study. Female participants will first have urine pregnancy testing performed prior to the ultrasound tests; if the participant is found to be pregnant, the participant's participation will end.

A 10 mHZ linear array vascular probe for B-mode imaging will be used to visualize the brachial artery. Data will be stored on a compact disc for backup and also on the IU server with password protection. ECG leads will be placed on the participant's chest and automatic blood pressure cuff to left upper arm, set at q.15 minutes. A small forearm cuff is placed on the widest part of the participant's proximal right forearm

(approximately 1-2 cm distal to the antecubital fossa). The brachial artery will then be located with the image optimized for best resolution of all three layers of the anterior and posterior walls (with special emphasis on identifying the media-adventitia interface). Three consecutive cardiac cycles of B-mode images of the brachial artery to the disks will be acquired and stored.

After this baseline evaluation, the measurement of endothelium-dependent vasodilation, or FMD, due to increased blood flow will begin. The forearm pressure cuff will be inflated to 250 mmHg for 5 minutes. At that time, imaging will be switched to B-mode. At 60 and 90 seconds after cuff deflation, three consecutive cardiac cycles of B-mode images of the brachial artery to the disc will be acquired and stored. The greater of the two diameters measured at these time points will be used for FMD analysis.

The procedure for measuring brachial reactivity will require at least one hour of the participant's time. There will be no documentation on the ultrasound scans indicating the randomization assignment of the study participants. This will ensure that Dr. Gupta, who will assess all the ultrasound results, will be blinded when reading the ultrasound scans. Female participants of reproductive potential will have their main study visits during the same phase of their menstrual cycle to minimize confounding by hormonal changes that may affect FMD results (128, 129).

### 3.7 Study Questionnaires

We will monitor for changes in depression severity using the PHQ-9 questionnaire. As was done in our pilot trial and the IMPACT trial (48), we will also assess depressive symptoms using the SCL-20, a reliable and valid measure responsive to change (130-133). We will examine total score and % responded (≥ 50% reduction). We will also assess tobacco use (Tobacco Use Questionnaire from the Behavioral Risk Factor Surveillance System), exercise habits (International Physical Activity Questionnaire), and CVD medication adherence (Morisky Questionnaire). We will also assess anxiety symptoms using the Generalized Anxiety Disorder-7 (GAD-7),(134) hostility/anger using the Buss-Perry Aggression Questionnaire,(135) trait positive affect using the Positive Affect subscale of the Positive and Negative Affect Schedule (PANAS),(136, 137) and sleep quality using the Pittsburgh Sleep Quality Index,(137) all of which are widely used and validated instruments.

Each of these scales is widely used and possesses strong psychometric properties (138-140). Although our focus is on physiologic pathways, we are assessing changes in these factors as they are potential behavioral pathways through which depression and its treatment may influence the outcomes (141).

These questionnaires will be administered in private with availability of the study team to assist only when asked by the participant. The participant may refuse to complete any of the questionnaires.

### 3.7 Study Duration and Participant Retention

The maximum study period for each participant will be 31 weeks (screening phase of 30 days and on-study phase of at most 27 weeks if the participant requires extra time to schedule the Week 24 visit). In order to promote retention in the study, the participant participants will be financially compensated at each visit.

## 4.0 SELECTION AND ENROLLMENT CRITERIA

### 4.1 Inclusion Criteria

- 4.1.1 HIV-1 infection, documented by both: (1) any licensed rapid HIV test or HIV enzyme test kit at any time prior to study entry and (2) by at least one detectable HIV-1 antigen or at least one detectable plasma HIV-1 RNA viral load.
- 4.1.2 Age equal to or greater than 18 years.
- 4.1.3 Receipt of antiretroviral therapy of any kind for at least 360 days prior to screening.

Note: Interruptions in ART of up to 14 days total during the 360 days prior to screening are allowed.

4.1.4 HIV-1 RNA level < 75 copies/mL at screening.

NOTE: There are no CD4 cell count eligibility criteria for this trial.

4.1.5 For women who are still of reproductive potential, a negative urine pregnancy test.
4.1.6 Depression as defined by having a score ≥ 10 on the PHQ-9 questionnaire

4.2 Exclusion Criteria

4.2.1 Inability to complete written, informed consent.
4.2.2 Incarceration at the time of any study visit.
4.2.3 Active suicidality, as determined by the patient's HIV provider or social worker following a positive response (1, 2, or 3) to PHQ-9 Item #9 and a positive response (yes) to one or more of the three questions (for Question #3, the previous attempt must be within the past 10 years) on the Patient Suicidality Form (see Appendix).
4.2.4 Diagnosed vascular disease (documented history of angina pectoris, coronary disease, peripheral vascular disease, cerebrovascular disease, aortic aneurysm, or otherwise known atherosclerotic disease).
4.2.5 History of congestive heart failure, even if currently compensated.
4.2.6 Diagnosed disease or process, besides HIV infection, associated with increased systemic inflammation (including, but not limited to, systemic lupus erythematosis, inflammatory bowel diseases, other collagen vascular diseases).

Note: Hepatitis B or C co-infections are NOT exclusionary

4.2.7 Known or suspected malignancy requiring systemic treatment within 180 days of screening.

NOTE: Localized treatment for skin cancers is not exclusionary.

4.2.8 History of Raynaud's phenomenon.
4.2.9 History of cardiac arrhythmias or cardiomyopathy.
4.2.10 Uncontrolled hyperthyroidism or hypothyroidism, defined as TSH values outside of the local reference range on most recent clinical assessment.
4.2.11 History of carotid bruits.
4.2.12 Systolic blood pressure > 160 mmHg or diastolic blood pressure > 110 mmHg at screening.
4.2.13 Screening estimated glomerular filtration rate (eGFR) < 50 mL/min/1.73m$^2$ (calculated from the 2009 CKD-EPI equation) using a serum creatinine level measured at screening.
4.2.14 Screening glucose ≥ 140 mg/dL or hemoglobin A1c > 8.0%.
4.2.15 Screening total cholesterol > 240 mg/dL.
4.2.16 Therapy for serious medical illnesses within 14 days prior to screening.

Note: Therapy for serious medical illnesses that overlaps with a main study visit will result in postponement of that study visit until the course of therapy is completed; postponement outside of the allowed study visit timeframe will result in study discontinuation.

4.2.17 Pregnancy or breastfeeding during the course of the study.
4.2.18 Receipt of investigational agents, cytotoxic chemotherapy, systemic glucocorticoids (of any dose), or anabolic steroids at screening.

Note: Physiologic testosterone replacement therapy or topical steroids is not exclusionary. Inhaled/nasal steroids are not exclusionary as long as the participant is not also receiving HIV protease inhibitors.

4.2.19 Active drug use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.
4.2.20 History of schizophrenia or bipolar disorder.

If participants are excluded due to the above criteria, they may be approached again in the future or have their study visit rescheduled within the allowable timeframe if these criteria are no longer applicable. Co-enrollment into other studies will be permitted provided no drugs prohibited on this study will be given in the other study/ies and that the required blood draws do not exceed safe limits when combined with those for this study.

## 4.3 Co-enrollment Guidelines

Co-enrollment into other studies will be permitted provided no drugs prohibited on this study will be given in the other study/ies and that the required blood draws do not exceed safe limits when combined with those for this study.

# 5.0 STUDY TREATMENT

## 5.1 Beating the Blues

To minimize treatment barriers, attrition, and subject burden, the BtB treatment sessions will occur in private at Dr. Stewart's laboratory, the ICRC, the Infectious Diseases Research Clinic at Eskenazi Health Hospital, or a remote location selected by the patient where s/he can access a computer with internet, such as the patient's home, the patient's work, a family member's/friend's home, or a public library. The location will be chosen by the participant. Patients will be provided with ear bud headphones if they plan to use them at remote locations and do not already own a pair of headphones that they would rather use. Up to 8 sessions will be performed between the Second Entry Visit and the Week 12 Visit. The PHQ-9 questionnaire will be administered at each of the Weekly BtB Sessions. No physiologic or biologic assessments will be performed during these sessions. The participants need not be fasting for these sessions.

At each in-person BtB session, a psychology research assistant trained in the use of this intervention will first collect homework and will start the appropriate session on a computer. The patient will then work through the session alone at his/her own pace. At the end of the session, the research assistant will give the patient the printed homework assignment and will schedule the next session.

Remote BtB sessions will scheduled, just like in-person sessions. Patients who select the remote treatment option will be given a BtB binder with printouts of all future homeworks at the time of randomization (i.e., the end of the Second Entry Visit). To begin each remote BtB session, an assistant will call the patient to instruct the patient to put last week's homework in the BtB binder, to address any technical issues, and to ensure that the patient has launched the correct BtB session for that day. The patient will then work alone through the BtB session at his/her own pace, with the assistant monitoring progress remotely on the secure BtB website set

up for this trial. To end the session, the assistant will call the patient back to address any questions and ensure that the patient has identified the correct printed homework in the BtB binder.

## 5.2 Prohibited Medications

- Agents with significant anti-inflammatory activity (including, but not limited to, plaquenil, infliximab, etanercept, mycophenolate mofetil, sirolimus, tacrolimus, cyclosporine, pentoxifylline, theophylline, thalidomide)
- Investigational agents
- Cytotoxic chemotherapy
- Systemic glucocorticoids (topical steroids are allowed; inhaled/nasal steroids are allowed only if the participant is not also receiving HIV protease inhibitors)
- Anabolic steroids (physiologic testosterone replacement therapy is not exclusionary)

## 6.0 CLINICAL AND LABORATORY EVALUATIONS

### 6.1 Schedule of Events

| Evaluation | Screening Visit | First Entry Visit (within 15 days of Screening) | Second Entry Visit #2 (within 30 days after Screening) | Weekly BtB Sessions for the intervention group (8 in total) | Week 12 Visit (for both groups; 84-105 days after Second Entry Visit) | Week 24/Closeout Visit (for both groups; 168-189 days after Second Entry Visit) |
|---|---|---|---|---|---|---|
| **Informed Consent** | X | | | | | |
| **Documentation of HIV Status** | X | | | | | |
| **Medical/Psychiatric History** | X | | | | | |
| **Medication/Supplement Use History** | X | | | | | |
| **Updated Psychiatric Management** | | | X | | X | X |
| **Concomitant/Updated Medications** | | | X | | X | X |
| **PHQ-9 Assessment** | X | X | X | X | X | X |
| **Signs & Symptoms** | X | | X | | X | X |
| **Diagnoses/Updated Diagnoses** | X | | X | | X | X |
| **Height** | | X | | | | |
| **Weight** | | X | X | | X | X |
| **Vital Signs** | X | X | X | | X | X |
| **Brief Physical Examination** | X | X | X | | X | X |
| **Urine Pregnancy Test** | X | X | X | | X | X |
| **Randomization** | | | X | | | |
| **Serum chemistries/glucose, total cholesterol (non-fasting)** | X | | | | | |
| **Hematology, hemoglobin A1c** | X | | | | | |
| **HIV-1 RNA level** | X | | X | | X | X |
| **CD4 cell count** | | | X | | X | X |
| **Whole blood for ECFCs and monocyte subsets** | | X | X | | X | X |
| **DNA sampling** | | | X | | | |
| **Beating the Blues Behavioral Intervention** | | | | X | | |
| **Questionnaires** | | | X | | X | X |
| **FMD/NTGMD** | | X | X | | X | X |
| **Frozen Serum for Batched Testing of Inflammatory/Coagulation Markers, Metabolic Markers** | | X | X | | X | X |
| **Frozen Plasma, Serum, and Urine for Future Studies** | | X | X | | X | X |

## 6.2 Definitions for Schedule of Events – Special Instructions and Definitions of Evaluations

### 6.2.1 Documentation of HIV-1 Infection

HIV-1 infection documentation must be present in the source documentation at the Screening Visit. HIV-1 infection, documented by both: (1) any licensed rapid HIV test or HIV enzyme test kit at any time prior to study entry and (2) by at least one detectable HIV-1 antigen or at least one detectable plasma HIV-1 RNA viral load.

### 6.2.2 Medical/Psychiatric History

A medical/psychiatric history must be present in the source documents. Record the following on CRFs at either the Screening Visit:

- Birthdate
- Sex
- Patient's self-report of ethnicity and race
- Initial date of HIV infection documentation
- Route of HIV infection
- Most recent hepatitis B surface antigen result (including date of this value)
- Most recent hepatitis C antibody result (including date of this value)
- Diagnoses (all medical and psychiatric)

### 6.2.3 Medication and Psychiatric Treatment History

A medication history must be present in source documents. The following information will be recorded on the CRFs at the Screening Visit:

- Start dates of current antiretroviral treatments
- Listing of all previous antiretroviral treatments
- Start dates of current antidepressant treatments
- Any other prescription medications within 30 days of Screening
- Any vaccinations within 30 days of Screening
- Any supplements (non-prescription) used within 30 days of Screening
- Previous psychiatric cognitive behavioral therapy treatments within one year of screening

### 6.2.4 Diagnoses

All confirmed and probable new diagnoses made since the last visit will be recorded on the CRFs, including current status at the time of the study visit.

### 6.2.5 Concomitant Medications

All new and/or discontinued prescription medications (including antidepressant medications), taken since the last study visit will be recorded on CRFs with start and stop dates.

### 6.2.6 Clinical Assessments

#### 6.2.5.1 Height

Height will be recorded on CRFs at the First Entry Visit

#### 6.2.5.2 Weight

Weight will be recorded on CRFs at both Entry Visits, the Week 12 Visit, and the Week 24 Visit.

#### 6.2.5.3 Resting Blood Pressure

Blood pressure measurements will be recorded on the CRFs at each non-BtB study visit. Blood pressure measurements should be obtained prior to the brachial artery ultrasound measurements.

Blood pressure measurements should be performed on the same arm throughout the study. The participant should first sit quietly for five minutes. With the elbow and forearm resting comfortably on a flat table, the blood pressure should then be measured. After two minutes, repeat blood pressure measurement in the same arm. After another two minutes, repeat blood pressure measurements again. Therefore, three blood pressure measurements are to be documented in the CRFs.

#### 6.2.5.4 Resting Heart rate

Resting heart rate measurements will be recorded on the CRFs at each non-BtB study visit. Heart rate measurements should be obtained prior to the brachial artery ultrasound measurements. This may be done prior to the first blood pressure measurement. The participant should first sit quietly for five minutes prior to measurement of heart rate.

#### 6.2.5.5 Brief Physical Examination

The following assessments will be performed at each non-BtB study visit:

- Auscultation of heart for signs of cardiac arrhythmias and valvular murmurs
- Auscultation of carotid arteries for bruits

#### 6.2.5.6 Signs and Symptoms

All signs and symptoms must be documented in the source documentation. All signs and symptoms occurring within 30 days of Screening and/or since the last study visit, regardless of grade, must be recorded on CRFs.

#### 6.2.5.7 Laboratories

All study visit laboratory evaluations performed as part of this study must be present in source documentation. In addition, record on CRFs all values for hemoglobin A1c, white blood cell count, hemoglobin, creatinine and estimated GFR, glucose, urine pregnancy testing, total cholesterol, CD4 cell counts, and HIV-1 RNA levels.

All laboratories will be sent to the Indiana University Health clinical laboratory for immediate processing and analysis; results will be entered on CRFs and forwarded to the Biostatistical Division for electronic storage.

Brachial ultrasound results will be entered on CRFs and sent to the Biostatistical Division. Computer labels will be generated and affixed to the appropriate specimen containers or brachial ultrasound discs and will include the specimen number, participant number, specimen date, and primary specimen type. Labels must be affixed prior to freezing the vials.

NOTE: Either a single venipuncture or a peripheral IV heplock may be used for drawing blood samples; this venipuncture should be performed on an extremity other than the arm used for the brachial artery measurements.

6.2.5.8 Specimen Collection, Processing, Labeling, and Storage for Specimens for Future Analysis

DNA Sample

- Collection: Collect ONE (1) 7.0-mL K3 purple-top EDTA tube, FILL COMPLETELY. Place on ice immediately. Do not allow to clot. Do not spin.
- Processing: Prepare at least 4 aliquots of 1.5-mL each in 1.5-mL purple screw cap vials from the 7.0-mL whole blood EDTA tube and freeze at -80°C and store upright.
- Labeling: Label on each aliquot the protocol-subject number, date, specimen type "DNA".

Whole blood

- Collection:
  For the measurement of ECFCs and monocyte subsets, collect TWO (2) 6.0-mL, purple-top EDTA tubes, FILL COMPLETELY. After collection of blood, keep upright at room temperature.

- Processing:
  Do not spin after collection. Keep at room temperature pending transfer of these purple top EDTA tubes to Dr. Yoder's laboratory.

- Labeling:
  Label on each EDTA tube the protocol-subject number, date and time of collection, specimen type "WB ECFC", and tube number.

Plasma

- Collection:
  - For plasma #1, collect TWO (2) 6.0-mL, purple-top EDTA tubes, FILL COMPLETELY. After collection of blood, gently invert the tube 10 to 15 times and keep upright at room temperature until centrifugation. KEEP REFRIGERATED AT 4$^{O}$C AFTER DRAW IF UNABLE TO CENTRIFUGE IMMEDIATELY.
  - For plasma #2, collect an additional ONE (1) 2.7-ml, blue-top 3.2% buffered Sodium citrate (SCI) tubes, FILL COMPLETELY (for storage). Mix gently by inverting 8 times immediately after filling. DO NOT SHAKE the tube as this will break down fibrinogen in the sample. COMPLETE PROCESSING WITHIN 1 HR OF COLLECTION.
- Processing:
  - For plasma #1 tubes, spin 3000 x *g* for 10 minutes at 4ºC. Prepare at least 6 total aliquots containing at least 0.5-mL of plasma each and freeze at –80°C within 4 hours of collection.
  - For plasma #2 tube, spin at 1500 x *g* for 15 minutes at room temperature. Prepare at least 2 total aliquots containing at least 0.5-ml of plasma each and freeze at –80ºC within 60 minutes of collection.

- Labeling: Label on each aliquot the protocol-participant number, date, specimen type "E-PLA" and "S-PLA", and aliquot number as appropriate.

Serum

- Collection: Collect ONE (1) 10.0-mL red-top tube without additive at room temperature. Let blood clot 30 minutes at room temperature in vertical position.
- Processing: Spin tube for 10 minutes at 1300 x *g* to separate serum within 1 hour of collection. Prepare at least 6 total aliquots containing at least 0.5-mL of serum. Separated serum MUST be refrigerated until frozen. Freeze at -80ºC as soon as possible within 8 hours of collection. REFRIGERATE ALIQUOTS IF FREEZING CANNOT BE ACCOMPLISHED IMMEDIATELY AFTER PROCESSING.
- Labeling: Label on each aliquot the protocol-participant number, date, specimen type "SER", and aliquot number.

Urine

- Collection: Collect at least 5-mL of urine in a standard collection cup using clean catch technique.
- Processing: Prepare at least 4 total aliquots containing at least 0.5-mL in screw-top plastic vials and freeze at -80ºC.
- Labeling: Label on each aliquot the protocol-participant number, date, and specimen type "URI".

6.2.5.9 Brachial artery ultrasound measurements

All brachial artery ultrasound measurements must be recorded on CRFs.

Perform the assessment in the morning to avoid adrenergic stimulation after an overnight fast (approximately 8 hours).

Participants may not use tobacco-containing products or eat or drink anything other than water for 8 hours prior to the assessment and until it is completed.

Patients with a history of migraine headaches may participate in this trial but will not undergo the nitroglycerin-mediated dilation portion of the brachial artery ultrasound testing.

See section 3.6 for the ultrasound procedure.

## 7.0 ADVERSE EVENT MANAGEMENT

Although not an inherent risk of the study intervention or study procedures, participants for this study are eligible only if they have depression as determined through completion of the PHQ-9 questionnaire. As such, these participants may have suicidal ideation at screening or develop suicidal ideation during the course of the study. We have already put into place a protection protocol for just this event in our previous studies in HIV-uninfected depressed patients (please see Appendix at end of this protocol). If a participant reports having thoughts of being better off dead or of hurting him/herself (i.e., responds with a 1, 2, or 3 to PHQ-9 Item #9 or spontaneously reports suicidal ideation), the visit will be immediately stopped, and the research assistant will interview the participant to complete the Patient Suicidality Form (see Appendix). If the participant answers "no" to all three suicide questions or if the patient answers "yes" only to Question 3 (previous attempt) and the most recent attempt was ≥ 10 years ago, the visit will proceed as normal, and the completed Patient Suicidality Form will be given to the principal investigator.

If the participant answers "yes" to any of the three questions (for Question #3 the previous attempt must be within the past 10 years), the research assistant will immediately contact the principal investigators. Dr. Stewart (a clinical psychologist) and Dr. Gupta (a physician) will review the case as soon as possible, but no later than the same day, to determine the appropriate course of action (e.g., immediately contact the patient's primary care provider, primary HIV provider, clinical social worker, or care coordinator, consult with clinicians

at Midtown Community Mental Health Center, and/or escort the patient to the Crisis Intervention Unit at Eskenazi Health). Additional authorities, including the police, may be contacted if immediate harm is of concern. Participants may be withdrawn from the study.

A direct correspondence by phone call and letter (both an email and a hard copy version), will also be sent to the potential participant's primary provider notifying him/her of the situation (see below).

If an enrolled participant reports having thoughts of being better off dead or of hurting him/herself during any telephone calls (e.g., a scheduling call or a call to the study team initiated by the participant), the exact same procedures as outlined above will immediately be initiated. Please see the Appendix for the full Suicide Management Plan.

Since it is unknown if depression therapy (or lack thereof) results in somatic adverse events in antiretroviral-treated HIV-infected patients, we will also carefully document Grade 3 or 4 level toxicities defined using the Division of AIDS Table for Grading Adult Adverse Experiences. Clinical management decisions and decisions to discontinue participants from the trial will be made by the principal investigator(s) in conjunction with the participant's primary caregiver; care plans and outcomes must be included in the source documentation. All serious adverse events (SAEs) will be documented on CRFs with unexpected SAEs forwarded to the IUPUI IRB within 10 working days of the event and the remainder to be documented on the annual continuing review.

## 8.0 CRITERIA FOR STUDY DISCONTINUATION

- Request by the participant to withdraw
- Request of the primary care provider if s/he believes the study is no longer in the best interest of the participant
- If the participant is found to be pregnant or begins breastfeeding during the course of this study
- If the participant has develops fever, need for systemic therapy for acute or serious illness, or hypotension after screening that precludes completion of the study visits within the allowed timeframes
- Requirement for prohibited concomitant medication(s)
- Clinical reasons believed life threatening by the physician
- Participant, as judged by the investigators, to be at risk of failing to comply with the provisions of the study protocol as to cause harm to self or interfere with the validity of the study results

## 9.0 STATISTICAL CONSIDERATIONS AND DATA MANAGEMENT

### 9.1 General Considerations

Data management and statistical analyses will be the responsibility of the Department of Biostatistics at IUSM. Parameter estimates and relevant summary statistics will be reported for both efficacy and side effects. Continuous variables will be summarized by means, medians, minima, maxima, and standard deviations. Categorical variables will be summarized by frequencies and percentages. Additional exploratory analyses will be performed when appropriate. Normality of variables will be checked and, if violated, nonparametric methods will be adopted. Changes from the analysis plan will not require an amendment to the protocol unless it changes a significant feature of the study. All analyses will be performed using SAS 9.4 (SAS Inc., Cary, NC).

### 9.2 Study Design

This trial will be a randomized, controlled, single-blinded, two-arm, parallel group study. Eligible patients will be randomized 1:1 to either *Beating the Blues* (BtB) or Usual Care (UC). Stratifications for

randomization include usage of pharmacologic antidepressants at the Second Entry Visit and sex. Permuted-block randomization will be adopted to ensure the two arms are balanced. Randomization sequences will be prepared by the study statistician and forwarded to the study coordinator.

9.3 Sample Size Justification

We calculated the trial's required sample size to achieve sufficient power to detect treatment group differences in FMD at Week 12. Using the effect size of *Beating the Blues* on FMD in our pilot trial, and assuming a power level of 0.80 with a Type I error of 0.05, we will need 88 participants (44 per arm) to detect an absolute difference in FMD of 1.7% at Week 12. This would be a clinically meaningful difference, as a 1.0% higher baseline FMD predicted a 6% lower risk of CVD events over five years in MESA (123, 124). We will increase the sample size to 110 (55 per group) to account for a conservative attrition rate of 20%. For the secondary outcomes of FMD (Week 24) and circulating IL-6, hsCRP, and D-dimer levels (Weeks 12 and 24), we will be able to detect effect sizes as small as 0.61 standard deviations.

9.4 Criteria for Stopping the Study

No early stopping rule will be implemented.

9.5 Analysis Datasets

| Datasets | Definition |
|---|---|
| Intention-to-treat (ITT) | This will comprise all patients who meet the eligibility criteria and are randomized onto the study irrespective of their compliance to the planned course of treatment. |
| Per Protocol Set | This will comprise all UC patients and BtB participants who finish at least 6 out of 8 sessions. |
| Safety | This will comprise the BtB patients that attend at least one session of treatment. |

9.6 Patient Characteristics and Significant Protocol Violations

Demographic and other baseline data will be summarized descriptively for all patients in the ITT set. Comparisons between the two groups will be performed using Pearson's chi-square tests and Student's t-tests. Significant protocol violations will be documented.

9.7 Disposition

The number of enrolled subjects will be summarized in a flow chart with frequency of completion and discontinuation. The subjects discontinued from BtB and their corresponding information will be listed. Significant protocol violations will be tabulated and/or listed.

9.8 Compliance

Compliance status will be tabulated for the BtB arm.

## 9.10 Analysis Plan for the Primary Objective

To test our primary hypothesis, an analysis of covariance (ANCOVA) will be performed to test for treatment group differences in Week 12 FMD adjusted for baseline FMD using the intent-to-treat set. Statistical significance will be considered if two-sided p-value <0.05.This analysis will be also repeated using the per-protocol set.

## 9.11 Analysis Plan for Secondary Objectives

Both unadjusted comparisons and adjusted comparisons between UC and BtB will be performed for secondary outcomes defined in 1.2.1-1.2.3. For adjusted comparisons, multiple linear regression models will be constructed to include treatment group, baseline characteristics [demographics, SCL-20 scores, tobacco/exercise/adherence questionnaire results, ART drugs/regimens (including efavirenz (142-144)), antidepressant use, co-morbidities, CD4 cell counts, and HIV-1 RNA levels], and time-varying characteristics (changes in SCL-20 scores, questionnaire results, CD4 cell counts, ART drugs/regimens, HIV-1 RNA levels) to determine their effects on the outcomes. Pearson and Spearman correlation coefficients will be estimated for FMD, IL-6, hsCRP, D-dimer, circulating EPCs/ECFCs levels, and markers of monocyte activation (CD14+CD16+ cell proportions and sCD14/sCD163) with PHQ-9 and SCL-20 scores at baseline. These analyses will be performed for both the intent-to-treat set and the per-protocol set. Dose-response relationships between 12- and 24-week FMD and the number of BtB sessions, which have a range from 0 to 8, will be explored.

Safety assessments will be performed using the safety dataset. HIV-1 RNA levels and CD4 cell counts of the BtB arm during the trial will be summarized and compared to the UC arm.

## 9.12 Interim Analysis

No interim analysis will be performed.

## 9.13 Subgroup Analysis

Exploratory subgroup analysis will be performed based on sex, age (<50 vs. ≥50 years), and pharmaceutic antidepressants.

## 9.14 Missing Data

Multiple imputations will be adopted to account for missing data. If differential missing patterns are observed, sensitivity analyses will be performed to evaluate the consequences of potential missing mechanisms.

## 9.15 Data Management

A comprehensive web-based data management system will be developed for this study using REDCap by the IUCDCC which will allow controlled entry through the internet. REDCap provides a secure, web-based environment that provides an intuitive data entry interface and has real-time validation rules (with automated data type and range checks). The system offers easy data manipulation with logged auditing, functionality for reporting, monitoring and querying subject records. An experienced Database Administrator provides database creation, daily backup, and installation of security patches.

A unique identifier will be assigned to each study participant and their associated study specimens. Patient identifiers will be located only within the subject's study file in a separate, locked cabinet within the Infectious Diseases Research unit at the ICRC. Hardcopies of laboratory source records will also be stored in a locked file cabinet. All entryways to the IDRC are secured by padkey codes. Initial screening, consenting of potential subjects, and data abstraction and recording will be completed by either the primary investigators or by the research study coordinators from the IDRC.

The data management system will facilitate quality control efforts, prevent entry of erroneous values, provide warnings for possible outliers, generate missing data reports, track subjects and samples, and assist in the preparation of data for statistical analysis. All data will be reviewed and processed through multiple verification and edit checking programs post entry to ensure data quality.

## 10.0 HUMAN PARTICIPANTS RESEARCH AND PROTECTION

### 10.1 General Considerations/Investigator Training

The Human Participants Research outlined in this proposal meets the definition of a Phase II clinical trial for the purpose of identifying biological and physiological mechanisms of human disease (not for identifying the superiority of one agent over the other). Therefore, a formal Data and Safety Monitoring Board is not required, although appropriate monitoring through the Data and Safety Monitoring Plan with independent monitor as described below will be fully implemented. This trial will be posted on ClinicalTrials.gov and updated regularly as needed for protocol updates and results. All Indiana University personnel involved with this application have successfully completed the training and examination involved with the Collaborative Institutional Training Initiative Course.

### 10.2 Risks to the Participants

#### 10.2.1 Human Participants Involvement and Characteristics

- A total of 110 HIV-infected participants will be recruited to participate in this pilot trial investigating the efficacy of cognitive behavioral therapy using the Beating the Blues computer program to reduce inflammation and improve endothelial function.
    - Participants must be at least 18 years of age, have documented HIV infection, have a screening HIV-1 RNA level <75 copies/mL while on ART for at least one year, and have a screening PHQ-9 score ≥10.
    - The chief exclusion criteria include known CVD, congestive heart failure, treatment for malignancy (besides localized skin cancers) within 6 months of screening, uncontrolled diabetes (defined as screening Hgb A1c >8.0%) or newly diagnosed glucose intolerance (defined as screening glucose ≥140 mg/dL), uncontrolled hypertension (systolic BP >160 mmHg or diastolic BP >110 mmHg), screening eGFR <50mL/min/1.73$^2$ (using the 2009 CKD-EPI creatinine formula), screening total cholesterol >240 mg/dL, pro-inflammatory conditions besides HIV infection (e.g. autoimmune diseases, but allowing hepatitis B or C co-infection).
    - Potential participants will be recruited from the HIV outpatient clinics of Indiana University Health Hospitals and Eskenazi Health Hospital. All biologic/physiologic study procedures will occur in the Indiana Clinical Research Center at Indiana University Health University Hospital.

#### 10.2.2 Sources of Materials

- All data for this study will be obtained only after written, informed consent is provided by each participant. Existing medical records will be reviewed for demographics, medical diagnoses, and medications. Blood samples will be obtained for testing of chemistries, cell counts, HIV-1 RNA levels, CD4 cell counts, inflammatory/coagulation markers, and metabolic markers. Urine samples will be obtained for pregnancy testing. Urine, serum, plasma, and DNA will be obtained for future studies of interest. Questionnaires to assess depression, medication adherence, tobacco use, physical activity, anxiety, anger/aggression, positive/negative effects, and sleep quality will also be implemented. Ultrasound images from the brachial artery flow-mediated dilation testing will also be collected.
- Results from pertinent medical records and procedures performed for these studies, as outlined above, will be recorded on the human participants involved in the projects in this application.
- Data will be stored in a password-protected computerized database via REDCap that will include only the participants' study identification number (names and other identifiable information will not be included). Therefore, the SID# will be the only link to the participant. Only the principal investigators, co-investigators, and research personnel who will directly obtain the necessary data will have access to the participant identities. All data obtained for this study will be obtained only after written, informed consent is provided by each participant.
- Records will be reviewed manually. Urine specimens will be obtained via standard clean-catch technique. Blood specimens will be obtained via peripheral venipuncture. Flow-mediated dilation of the brachial artery will be measured using high frequency ultrasound with images downloaded to a secure, encrypted electronic database. Beating the Blues treatments and questionnaires will be completed in private settings. These data will be collected solely for the purpose of the proposed research projects.

10.2.3 Potential Risks

- There are minimal risks to the participants enrolled in the proposed research. The first is the potential loss of participant confidentiality. The second consists of the risks associated with blood drawing/needle sticks, which include pain, bruising, infection, and phlebitis. The amounts of blood to be drawn at screening and at each main study visit are 15 cc (one tablespoon) and 60 cc (four tablespoons), respectively. The total amount of blood to be obtained over the 24 week study period would be approximately 255 mL (17 tablespoons), which is well within the accepted standards for blood donation over a 6 month period. The primary risks associated with nitroglycerin administration as part of the brachial artery testing are headache and transient hypotension. NTG may also infrequently cause the sensation of flushing of the skin and rash. There may also be moderate pain associated with the inflation of forearm cuff as part of the brachial reactivity testing. There are no known risks related to the implementation of the Beating the Blues cognitive behavioral therapy program. Participants may also feel unease in completing the questionnaires.
- The principal alternative to these procedures would be not to participate in the research.

10.3 Adequacy of Protection Against Risks

10.3.1 Recruitment and Informed Consent

- Recruitment will only begin once the Indiana University Institutional Review Board has approved this study. All participants will be recruited from the outpatient HIV care clinics at Indiana University Health Hospitals and Eskenazi Health Hospital. Self-referrals from other venues will also be considered. If the primary caregiver for the patient believes he or she is eligible for the study and allows the patient to be

approached for screening, one of the study investigators or a study nurse will approach each potential participant during his or her regularly scheduled clinic visit. If eligibility is confirmed, then the purpose, procedures, and risks and benefits of the study will be discussed with the participant. Participants will have ample opportunity to ask questions and to have all concerns addressed. If the participant wishes to pursue screening, then written informed consent will be obtained (and a copy given to the participant). All consent forms will be stored in a locked file cabinet.

10.3.2 Protection Against Risk

10.3.2.1 Confidentiality. To minimize the risk to participant confidentiality, patient identifiers will be removed once his or her data is abstracted and recorded, and only the random study identification number (generated when consent is provided) will be used. All hardcopy study data will be kept in a secured and locked file cabinet. All electronic data will be kept in a password-protected computer database. The only link between patient identifiers and the randomized study identification number will be kept in separate files. Identifiers will never be used in the analysis or presentation of study results.

10.3.2.2 Blood draws. The risks of blood drawing will be minimized by having only experienced medical personnel perform this procedure. The amount of blood that will be drawn falls well within safety standards for blood donation.

10.3.2.3 Questionnaires. Participants may feel uneasy or discomfort in completing the depression, treatment adherence, and physical activity questionnaires. To minimize this risk, questionnaires will be completed in private settings with any questions regarding completion of the questionnaires addressed by trained study team psychology personnel.

10.3.2.4 Nitroglycerin. Brachial artery reactivity testing will be performed in a controlled setting in the Indiana University General Clinical Research Center. To protect against the risks of nitroglycerin administration, registered nurses in the ICRC will monitor the participant's blood pressure throughout brachial reactivity testing. Participants with inherent resting hypotension will be excluded from the NTG-mediated flow dilation portion of the brachial reactivity testing. If the participant becomes symptomatic (e.g. dizzy, perspiring, weak or nauseated, or if the systolic blood pressure falls below 80mmHg or more then 30mmHg below their baseline systolic blood pressure, the study will be stopped immediately). The patient will be placed in Trendelenburg position and be given fluids through the previously placed IV heplock if the blood pressure does not recover spontaneously. If the patient develops a headache, an analgesic will be recommended. In addition, participants with recent use of phosphodiesterase inhibitors (e.g. erectile dysfunction medications) prior to NTG administration or planned use after NTG administration will be excluded with the study visit to be rescheduled. Patients with a history of migraine headaches will also not receive NTG as part of their endothelial function assessments.

10.3.2.5 Suicidal ideation and management. Although not an inherent risk of the study intervention or study procedures, participants for this study are eligible only if they have depression as determined through completion of the PHQ-9 questionnaire. As such, these participants may have suicidal ideation at screening or develop suicidal ideation during the course of the study. We have already put into place a protection protocol for just this event in our previous studies in HIV-uninfected depressed patients (please see Appendix at end of this protocol). If a participant reports having thoughts of being better off dead or of hurting him/herself (i.e., responds with a 1, 2, or 3 to PHQ-9 Item #9 or spontaneously reports suicidal ideation), the visit will be immediately stopped, and the research assistant will interview the participant to complete the Patient Suicidality Form (see Appendix). If the participant answers "no" to all three suicide questions or if the patient answers

"yes" only to Question 3 (previous attempt) and the most recent attempt was ≥ 10 years ago, the visit will proceed as normal, and the completed Patient Suicidality Form will be given to the principal investigator.

If the participant answers "yes" to any of the three questions (for Question #3 the previous attempt must be within the past 10 years), the research assistant will immediately contact the principal investigators. Dr. Stewart (a clinical psychologist) and Dr. Gupta (a physician) will review the case as soon as possible, but no later than the same day, to determine the appropriate course of action (e.g., immediately contact the patient's primary care provider, primary HIV provider, clinical social worker, or care coordinator, consult with clinicians at Midtown Community Mental Health Center, and/or escort the patient to the Crisis Intervention Unit at Eskenazi Health). Additional authorities, including the police, may be contacted if immediate harm is of concern. Participants may be withdrawn from the study.

A letter, both an email and a hard copy version, will also be sent to the potential participant's primary provider notifying him/her of the situation (see below).

If an enrolled participant reports having thoughts of being better off dead or of hurting him/herself during any telephone calls (e.g., a scheduling call or a call to the study team initiated by the participant), the exact same process as outlined above will be initiated. Please see the Appendix for the full Suicide Management Plan.

10.3.2.6 Adverse event financial management, grading, and reporting. In the event of an adverse event, necessary medical and professional intervention will be provided immediately and billed to the participant's medical insurance (if available). If the participant does not have insurance, care will be provided via the indigent care program at Eskenazi Health Hospital. Standard procedures for reporting deviations from protocols will be followed; serious adverse events that meet the Indiana University IRB prompt reporting requirements will be reported within 10 business days. All adverse events will be graded using The Division of AIDS Table for Grading Adult Adverse Experiences is located at: http://roc.s-3.com/members/download/adulttox.pdf.

10.3.2.7 Data and Safety Monitoring. Dr. Diane Janowicz of the Division of Infectious Diseases, Indiana University will serve as the independent chair and monitor for this trial. She will receive reports at least every six months regarding the progress and participant safety during the trial.

10.3 Potential Benefits of the Proposed Research to the Participants and Others

- Potential benefits to the participants include an evaluation of their cardiovascular and immunologic status. They may also derive short-term benefits from the Beating the Blues depression treatment program, although this is not guaranteed.. Finally, the participants may also benefit from knowing that their participation will accrue knowledge that could benefit other HIV-infected patients.
- Although there are no guaranteed clinical benefits from those who are randomized to treatment with Beating the Blues, this program appears quite safe when used in HIV-uninfected participants. The standard of care will not be altered in the control participants. Therefore, the ancillary benefits to the participants in the proposed studies significantly outweigh the minimal risks in this study. Moreover, the proposed research may lead to other prevention and therapeutic studies that would demonstrate how to reduce inflammation and improve endothelial function, which consequently may reduce future cardiovascular events in the HIV-infected population. This would benefit society directly by impacting clinical practice.

10.4 Importance of the Knowledge to be Gained

- The knowledge that will be gained from this study will determine the relationships between HIV, depression, inflammation, and endothelial dysfunction. This would potentially impact the clinical care of HIV-infected patients at risk for cardiovascular disease. Furthermore, prevention and therapeutic strategies

for these highly prevalent diseases can then be formulated, thereby reducing morbidity, mortality, and cost to the patients and society in general.

- Again, the risks to the participants are considered minimal. Even if the results are negative, the results of these investigations will add substantially to our knowledge on the mechanisms underlying inflammation and cardiovascular risk in older, HIV-infected patients with depression. Therefore, the importance of the knowledge gained outweighs the risks to the participants.

10.6 Data and Safety Monitoring Plan

- Progress of these studies, including data monitoring, participant enrollment, protocol deviations, and all SAE, will be reviewed by a panel including the PIs (Drs. Gupta and Stewart), the study statistician (Dr. Liu), and an HIV expert investigator at Indiana University not directly connected with this study (Dr. Diane Janowicz of the Division of Infectious Diseases, Indiana University School of Medicine). Reports, which will include descriptions of all adverse events, will be prepared for review by this panel every 6 months. Any study participant prematurely discontinued due to an adverse event will be reviewed immediately. Standard procedures for reporting deviations from protocols to the Indiana University ICRC, IRB, and NHLBI will be implemented. Serious Adverse Events (SAEs) will also be reported to the IRB within 30 working days and subsequently forwarded to NHLBI as required.

10.7 Inclusion of Women and Minorities

There are no exclusion criteria based on gender, racial category, or ethnicity. Based on our previous cumulative experience and the general HIV-infected population cared for at the study sites at the Indiana University Health Medical Center (Eskenazi Hospital, Methodist Hospital, VA Roudebush Hospital, and Indiana University Health Hospital), it is anticipated that approximately 25% of the study participants will be women.

It is also anticipated that approximately 40% and 10% of the study participants will be black and Hispanic, respectively. American Indians, Alaskan Natives, Asians, Native Hawaiians or Other Pacific Islanders are not expected to be represented in the proposed study population due to extremely low representation of these groups within the Indiana University Health Hospitals HIV outpatient clinics and in Indianapolis in general.

10.8 Inclusion of Children

Subjects over the age of 18 will be eligible for enrollment into the proposed trial in Aim #2; as such, children of ages 18-21 will be included in this Aim. However, participants under age 18 will be excluded to minimize confounding from hormonal fluctuations that are encountered during adolescence on the proposal's endpoints of systemic inflammation, altered coagulation, and endothelial function.

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Appendix: Suicide Management Plan

Suicide management plans very similar to the one described below have previously been approved by the Indiana University IRB and were successfully implemented in the two other depression trials conducted by our team (IRB #'s 1105005448 and 1110007119). Those suicide management plans were constructed with input from Eskenazi Midtown Community Mental Health Center leadership (Dean Babcock, Associate Vice President, and Michael Hughes), who concluded that the plans provide a high level of protection while minimizing disruption to usual clinical activities. In the present trial, we will assess suicidal ideation at every study visit, and we are prepared to appropriately handle the situation should one of the enrolled patients exhibit suicidal ideation. Of note, zero enrolled participants in our two prior depression trials have exhibited suicidal ideation during their involvement in the studies, likely because patients exhibiting suicidality were not eligible to be enrolled, as is the case for the present trial.

(a) <u>In-Person Study Visits</u>
Suicidal ideation will be assessed using Item #9 of the PHQ-9 at the Screening Visit and all Main Study Visits (see Section 6.1). If a participant reports having thoughts of being better off dead or of hurting him/herself (i.e., responds with a 1, 2, or 3 to PHQ-9 Item #9 or spontaneously reports suicidal ideation) during the Screening, First Entry, Second Entry, Week 12, or Week 24 Visits, the visit will be immediately stopped, and the study coordinators (Patricia Anderson, Danielle Grounds) running these visits will interview the participant to complete the Patient Suicidality Form (see below). If this interview is required at one of the Weekly BtB Sessions, the graduate student research assistants will perform this interview, as they will conduct these treatment sessions.

The study coordinators have been trained by Dr. Stewart, a clinical psychologist, in conducting the interview to complete the Patient Suicidality Form and in following this protocol. Dr. Stewart will also serve as the primary supervisor to these study coordinators when it comes to their tasks related to this suicide management plan. In Dr. Stewart's completed (IRB #'s 1105005448 and 1110007119) and ongoing (IRB # 1411802537) clinical trials, he has trained ResNet research assistants to effectively conduct this interview and provide a high degree of protection to patients.

The graduate student research assistants are doctoral students enrolled full-time in IUPUI's clinical psychology Ph.D. program, which is accredited by the American Psychological Association and of which Dr. Stewart is a core member. The research assistants have completed graduate coursework in psychological assessment, psychological interventions, psychopathology, and ethics and have acquired supervised clinical experience in local healthcare settings. The graduate student research assistants have also been trained by Dr. Stewart in conducting the interview to complete the Patient Suicidality Form and in following this protocol. Dr. Stewart is also the primary supervisor of graduate student research assistants.

It is worth noting that neither the study coordinators nor the graduate student research assistants will be making any decisions regarding how to handle a situation. Instead, they will collect information by administering a highly structured, 3-question interview (Patient Suicidality Form, found below) and will follow the straightforward, step-by-step protocol described in the next paragraph. They will be instructed to call Dr. Stewart if they are unsure about a participant's response to any of the three questions. As he has done in his past depression trials and is described above, Dr. Stewart will train the study coordinators and the graduate student research assistants in administering the brief interview and in following the protocol. This training will be repeated periodically during the study period.

If the participant answers "no" to all three suicide questions or if the patient answers "yes" only to Question 3 (previous attempt) and the most recent attempt was ≥ 10 years ago, the visit will proceed as planned, and the completed Patient Suicidality Form will be given to Dr. Stewart.

If the participant answers "yes" to any of the three questions (for Question #3 the previous attempt must be within the past 10 years), the study coordinators or the graduate student research assistant will immediately stop the visit, will contact Drs. Stewart and/or Gupta, and will stay with the participant until a decision is made. If clinically indicated (e.g., the situation is an emergency), a graduate student research assistant and/or Dr. Stewart will go to the visit location (Infectious Diseases Research Clinic or ICRC) to assist the study coordinators.

Drs. Stewart and/or Gupta will review all cases screening positive for suicidal ideation immediately to determine the appropriate course of action – e.g., interview the patient to obtain further information, immediately contact the patient's HIV provider or clinical social worker to involve them in the decision-making process, consult with clinicians at the Eskenazi Health Midtown Community Mental Health Center to aid in the decision-making process, escort the patient to the Eskenazi Health Crisis Intervention Unit ('warm handoff'), and/or contact the police if the patient is at imminent danger of harm and is refusing all care. Either Dr. Stewart or Dr. Gupta will also call the participant's HIV provider notifying him/her of the situation if the provider was not involved in the decision-making process. Regardless of the exact course of action, the study team will ensure that the participant is connected to the appropriate existing clinical services. If the patient's HIV provider no longer believes that the patient is appropriate for this trial following this situation, the patient will be withdrawn from the trial.

Of note, because patients exhibiting active suicidal ideation are not eligible for this trial, we expect that it will be a rare occurrence that an enrolled patient will screen positive for suicidal ideation. Zero enrolled participants in our two prior depression trials have exhibited suicidal ideation during their involvement in the studies.

(b) Study Telephone Contacts

If a participant reports having thoughts of being better off dead or of hurting him/herself (i.e., responds with a 1, 2, or 3 to PHQ-9 Item #9 or spontaneously reports suicidal ideation) during a study call (e.g., the start or end of a remote BtB session, a scheduling call, or a call to the study team initiated by the participant), the study coordinator or a graduate student research assistant will interview the participant to complete the Patient Suicidality Form (found below).

If the potential participant answers "no" to all three suicide questions, the call will proceed as planned and the completed Patient Suicidality Form will be given to Dr. Stewart. If the participant answers "yes" to any of the three questions (for Question #3 the previous attempt must be within the past 10 years), the study coordinator or a graduate student research assistant will immediately contact Drs. Stewart and/or Gupta. Drs. Stewart and/or Gupta will review the case immediately to determine the appropriate course of action – e.g., interview the patient to obtain further information, immediately contact the patient's HIV provider or clinical social worker to involve them in the decision-making process, consult with clinicians at the Eskenazi Health Midtown Community Mental Health Center to aid in the decision-making process, escort the patient to the Eskenazi Health Crisis Intervention Unit, and/or contact the police if the patient is at imminent danger of harm and is refusing all care. If a participant prematurely terminates a call after reporting suicidal ideation, the study coordinator or a graduate student research assistant will immediately contact Drs. Stewart and/or Gupta. Once again, Drs. Stewart and/or Gupta will review the case immediately to determine the appropriate course of action. Either Dr. Stewart or Dr. Gupta will also call the participant's HIV provider notifying him/her of the situation if the provider was not involved in the decision-making process. Regardless of the exact course of action, the study team will ensure that the participant is connected to the appropriate existing clinical services.

If the patient's HIV provider no longer believes that the patient is appropriate for this trial following a situation, the patient will be withdrawn from the trial.

It should be noted that the informed consent form for this trial contains a section describing the steps that will be taken if an enrolled participant reports suicidal ideation on a questionnaire or spontaneously.

**Patient Suicidality Form (administered verbally by the research assistant)**

*****CONFIDENTIAL*****

Research Assistant: _____ Date: _____

Patient's Name: _____ Hospital ID: _____

Patient's Address: _____

Patient's Phone Number: _____ Patient's PCP: _____

**********

I'm going to ask you a few questions that are part of this study, because we have seen that in some patients with these symptoms, these are important concerns.

*1. Do you have a suicide plan?* Yes _____ No _____

Comments:

*2. Have you been struggling against thoughts about committing suicide?* Yes _____ No _____

Comments:

*3. Have you attempted suicide in the past?* Yes _____ No _____

If YES, in what year was the most recent attempt? _____

Comments:

**If the participant answers "yes" to any of the three questions (for Question #3 the previous attempt must be within the past 10 years), you must carefully follow the procedures described in the Suicidal Ideation Protection Protocol.**