Effects of Mindfulness Training on Chronic Inflammation in HIV-Infected Adults
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RESEARCH STRATEGY

A. Significance

The number of aging HIV-infected adults is rapidly and steadily increasing.[1,2] The implementation of antiretroviral therapy (ART) has dramatically changed the prognosis of HIV-infected individuals. HIV infection, once viewed as an almost-certain death sentence, has become a chronic and manageable viral condition.[3] By the end of 2009, the highest prevalence rate of HIV infection reported by the CDC was in adults aged 45–54 years. It is estimated that half of those living with HIV are 50 years old or older. This large population cohort of aging HIV-infected adults over 50 years old, a relatively new phenomenon in medicine, represents a critical and significant burden to the health care system.

HIV is associated with premature or accelerated immunosenescence.[4-6] Immunosenescence is defined as the natural aging of the immune system.[7] These age-associated alterations result in progressive deterioration and age-dependent defects, which includes what has been called *inflammaging*.[8-11] The clinical consequences of these alterations include a lower ability to respond to new infections and an increased frequency of diseases associated with older age. There is strong supportive evidence in both clinical and natural science, to indicate that HIV is associated with a premature and/or accelerated process of immunosenescence and inflammaging. In fact, the clinical aging of long-term HIV-infected adults mimics that of non-HIV peers 10-15 years older.[3,12,13]

Chronic inflammation is a crucial factor in the development of non-AIDS medical illnesses.[14-17] In patients with well-controlled plasma viremia, a low level of long-term and consistent chronic inflammation remains. Five plausible reasons have been described as underlying causes of this chronic inflammatory condition: 1) a persistent level of HIV replication below sensitivity of current plasma viremia assays, 2) direct toxicity resulting from ART, 3) microbial translocation caused by abnormal functioning of the gut-associated lymphoid tissue,[18-21] 4) co-infections commonly found in HIV-infected patients such as hepatitis C virus and cytomegalovirus, and, 5) irreversible damage to the immune system during the period of infection prior to beginning ART.[14,22-25] Chronic inflammation, likely a key factor in accelerated immunosenescence, results in detrimental clinical consequences for the aging long-term HIV-infected adult.

As HIV-infected adults live longer, diseases associated with aging are found at higher rates and are occurring earlier in this population in relation to non-infected individuals, an observation that has been described by some as *accelerated aging.* Although there are some conflicting reports on the concept of *accelerated aging*, most published data indicates that the clinical consequences of these underlying chronic inflammatory mechanisms are observed in outcomes of increased mortality, cardiovascular disease, lymphoma, venous thromboembolism, type II diabetes, cognitive dysfunction, and frailty.[1,3,12,13,22,23,26,27] The physical, emotional, social and financial implications of these clinical conditions manifesting 10-15 years earlier in the HIV-infected cohort as compared to an age-matched HIV-negative cohort, are not only of critical significance for patients, but are also a financial burden on the healthcare system.[1] More recently, this *accelerated aging* has been reported at both the *epigenetic* and *monocyte activation* levels in HIV-infected adults.[28,29] If the mind-body intervention proposed by this study proves of clinical value for these patients, it will represent a valuable complementary medicine tool that can be scaled up to improve health care outcomes.

Mindfulness-Based Stress Reduction (MBSR) is a holistic and complementary mind-body approach with well-documented efficacy and effectiveness on the treatment of a variety of medical conditions.[30-32] The MBSR program was founded by Jon Kabat-Zinn, Ph.D., in 1979 at the University of Massachusetts Medical School to help patients reduce stress and manage emotions through mindfulness meditation. MBSR is a group treatment that provides benefits in the management of a wide variety of medical conditions, including, but not limited to, chronic pain, cancer, lower back pain, fibromyalgia, rheumatoid arthritis, and cardiovascular disease.[33-38] MBSR has gained extensive public reputation in recent years, with dozens of mindfulness centers and hundreds of certified instructors available throughout the country, as shown in Appendix I.

There are 7 published studies on the use of MBSR in HIV-infected adults.[39-45] Robinson and colleagues showed increased numbers and functioning of natural killer cells for MBSR participants, but no significant differences in psychological, endocrine, or functional health variables. However, this study included only 24 MBSR participants with 10 matching controls in a quasi-experimental design.[39] Three additional studies examined the impact of MBSR on CD4 counts. Creswell and colleagues compared 33 MBSR participants to 15 controls, and CD4 counts were maintained in MBSR participants whereas a decline was observed among the controls who participated in a 1-day stress reduction education seminar. This study was limited by a small number of subjects, a 28% dropout rate, and measures of CD4 cells only pre- and post-intervention.[40] Two studies were conducted in Iran; the larger study included 173 HIV-infected adults, 87 of whom participated in MBSR, and 86 participated in a brief educational training. In both studies patients were not taking ART, and all subjects were followed for 12 months. Both CD4 count increases and psychological symptom improvements

were observed, but not sustained by the 12-month follow-up. Improvements in medical symptoms were also observed and sustained.[41,44] In 2011, a study was conducted in Toronto with 78 MBSR participants and 39 treatment-as-usual controls. No differences were observed in measures of depression or anxiety, but improvements in a mindfulness scale were found.[45] More recently, Gayner and colleagues studied 117 participants (78 MBSR, 39 treatment-as-usual) and observed that MBSR participants had significantly lower avoidance and higher positive affect compared to controls.[43] Johnson (consultant on this grant) and colleagues assessed the impact of MBSR on ART side effects. MBSR participants experienced a reduction in the frequency of symptoms associated with ART as well as a reduction in distress associated with those symptoms. Their study included 76 participants, 36 of which were in a wait-list control group with standard care.[42] Although most of the data collected in these studies relates to psychological outcomes, there is suggestive evidence that the treatment leads to significant biological outcomes.

The field of psychoneuroimmunology has demonstrated a strong negative correlation between stress and immune system functioning in the general population.[46,47] This correlation has been found in HIV disease with faster progression to AIDS associated with stressful life events.[48,49] The connection between stress and immunity is mediated through the central nervous system.[50-52] Specifically, activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic–adrenal–medullary (SAM) axis, results in production of hormones directly influencing the immune response. Cortisol, a glucocorticoid produced following HPA activation from stress, exerts immunosuppressive actions. Catecholamines, produced after SAM activation from stress, are also implicated in the immune response. The changes of the immune response caused by these hormones likely play a role in immune dysregulation and chronic inflammation observed in aging HIV-infected adults.[53,54] MBSR is hypothesized to ameliorate clinical outcomes observed in these patients by reducing stress-induced hormones from the HPA and SAM axes.

In this study, stress is hypothesized to exacerbate chronic inflammation in aging HIV-infected adults by stimulating production of hormones that directly impact the immune response. These changes directly impact biomarkers measured in the blood of these patients: IL-6, CRP, sCD14 and d-dimer. MBSR, by reducing the stress effect in these patients, could reduce levels of biomarkers associated with chronic inflammation. This conceptual model underlying the proposed study is graphically depicted in the Specific Aims.

B. Innovation

The proposed study will be the first to examine any form of alternative or complementary holistic therapy in older long-term HIV-infected persons with well-controlled plasma viremia.[55] It will also be the first MBSR study of HIV-infected adults to be conducted under the most rigorous RCT design with an active attention control condition that provides the same supportive climate of the MBSR group curriculum. From a translational medicine perspective, this study is innovative in that it examines a comprehensive set of biological markers specifically associated with chronic inflammation. No previous MBSR study has specifically targeted a set of biological markers that not only have been proven to be elevated in adults with HIV, but also been described as predictors of morbidity and overall mortality. This would be the first study to explore whether improvements in markers of psychosocial stress mediate the impact of MBSR on chronic inflammation.

This study is innovative in its effort to move HIV care beyond the current focus of virologic control, optimization of comorbid conditions, and encouragement of healthy lifestyle to include active interventions that can address the state of chronic inflammation and immune dysfunction underlying the early and accelerated aging process. Most bio-behavioral research in HIV to date has understandably focused on patients with poor adherence to HIV medication and treatment. This study is novel in its focus on highly adherent patients and the significant changes in profiles of biological markers associated with chronic inflammation documented in this cohort. The complementary medicine approach being studied has the potential to be integrated into the existing healthcare paradigm for HIV-positive persons.

C. Approach

General Study Design: The proposed study is a prospective, randomized, clinical trial with attention control under the direction of the dual PIs, a behavioral scientist and a virologist. The study team is also made up of investigators from infectious diseases, mind-body medicine, geriatrics, and biostatistics. Subjects will be randomized to participation in an 8-week group MBSR course as the study intervention or to the Health Enhancement Program (HEP) as the active control arm. The study population will consist of 120 individuals, 50 years old or older, long-term (5 years on ART or longer) HIV-infected adults with well-controlled plasma viremia. Randomization will be stratified to achieve balanced distribution between the groups based on gender and study site. The experimental group will participate in the 8-week MBSR course followed by six, once-per-month booster sessions, and the control group will participate in HEP, a validated group attention control condition for MBSR maintaining subjects inert to mindfulness, with additional monthly educational meetings for

six months. Both groups will be evaluated using two sets of measures: biological markers associated with chronic inflammation (IL-6, CRP, sCD14, d-dimer) and measures of psychosocial stress (anxiety, depression, fatigue, and mindfulness). Subjects will be assessed at three time points: baseline, 8 weeks (immediately after completion of MBSR intervention), and 6-months post MBSR course (immediately after completion of booster sessions). We will use mixed linear and structural equation model to test study hypotheses.

Intervention: Mindfulness-Based Stress Reduction (MBSR) Course: The MBSR course will follow the approved curriculum by the University of Massachusetts Medical School. Participants will attend 8 weekly sessions, 2.5 hours each, and a 5 hour silent retreat during the 6th week of the program. Two cohorts of MBSR sessions (in concurrence with HEP attentive control sessions) will be conducted in groups of 30 subjects each. Formal mindfulness meditation methods taught in the MBSR course include: Body Scan Meditation (a supine meditation), Gentle Hatha Yoga (practiced with mindful awareness of the body), Sitting Meditation (mindfulness of breath, body, feelings, thoughts, and emotions), and Walking Meditation. Informal Mindfulness Meditation Practices (mindfulness in everyday life) include: 1) awareness of pleasant and unpleasant events, and, 2) deliberate awareness of routine activities and events such as eating, weather, walking, and interpersonal communication. Daily home assignments include a minimum of 30 minutes daily formal mindfulness practices and 5-15 minutes informal practice, 6 days per week for the entire duration of the course. Course sessions will consist of discussions oriented around weekly homework assignments, including an exploration of obstacles, and development of self-regulatory skills and capacities. Monitoring for completion of course assignments and mindfulness practice will also be recorded on a weekly basis using a daily online log. Dr. Bloom will facilitate the MBSR course. She has taught MBSR to a wide range of patients and health professionals since 2006, and is a Certified MBSR Teacher, the highest level of teacher recognition by the University of Massachusetts Center for Mindfulness. After completion of the 8-week MBSR course, subjects will meet 6 times monthly for a 1-hour refresher session. A monthly online log will be used to record frequency of practice over this 6-month period. Subjects will receive biweekly email reminders to log their practice adherence.

Potential Challenges, Proactive Measures, Alternative Strategies Given that we are recruiting subjects with an undetectable HIV viral load and therefore likely to be highly adherent to ARV medications, we expect high rates of adherence to study sessions. However, several procedures will be implemented to maximize attendance to the two study conditions. Subjects will not have their study baseline evaluation conducted at the time of consent, but be asked to return on a separate date to complete the baseline study evaluation to demonstrate their ability to adhere to scheduled appointments. Participants will be offered a round-trip metro card at each intervention session for travel expenses. Facilitators (Dr. Bloom for MBSR and area experts for HEP) will be asked to provide information on subject attendance to the research coordinator (RC). Subjects will be asked to inform the facilitator if they plan to miss a session. For subjects that miss a session and do not contact the facilitator, the RC will reach out to the subject via email or phone.

Attention Control Arm: The Health Enhancement Program: Experts in mindfulness-based interventions argue for the use of an active control to eliminate the bias of the attention effect that can be encountered by the social interactions experienced by MBSR participants. MacCoon and colleagues have addressed this particular concern with the development of a validated attention control called the Health Enhancement Program (HEP).[56,57] HEP is an active control that matches MBSR in non-specific factors, such as the amount of time participants interact, but does not include mindfulness practice as an active component. HEP instructors will be selected from experts in the areas covered: music, nutrition education, and, functional movement. The study's RC will coordinate HEP sessions and monitor attendance. Following the 8-week HEP protocol, subjects will meet monthly in 1.5-hour educational sessions to match the booster sessions of MBSR participants.

Potential Challenges, Proactive Measures, Alternative Strategies: Although the HEP active control intervention provides the strongest RCT design, we are aware that it may also pose a challenge in observed outcomes if the effect size of our intervention is small. This is a plausible scenario for two reasons: (1) the attention effect of the active control intervention and (2) elements of the HEP intervention directly reducing psychosocial stress. To account for this possibility, statistical analysis will also include within subject differences. In addition, the sessions of HEP that could most influence stress reduction (i.e., physical activity)[58,59] will be replaced by educational sessions. Elements such as the weather, and community illnesses associated with weather (i.e., colds, flu, etc.) may impact outcomes of the proposed study. To account for this potential impact, both arms of the study will take place concurrently. Both weekly sessions (one MBSR and another HEP) will take place during the same week in the exact same location (a conference room at Mount Sinai Hospital).

Participants and Recruitment: Subjects will be recruited at the Mount Sinai Health System in Manhattan, New York. In 2013, an integrated health system was created through the merger of Mount Sinai Medical Center and Continuum Health Partners. It is expected that the study will be able to recruit enough subjects at

the Mount Sinai Medical Center, St Luke's and Roosevelt Hospital sites of HIV care which currently provide care to 9,031 HIV-positive patients. Based on quality improvement statistics from these sites, 45% of the patients are age 50 or older, 94% of patients are on ART, 73% of these patients have an HIV viral load less than 48 copies/mL, and 75% of these patients have a nadir CD4 count of below 250 cells/mm^3. Applying these percentages to the 9,031 patients, 2,091 meet the primary eligibility criteria. Taking into account additional inclusion/exclusion criteria, it is estimated that 1,800 patients would meet eligibility criteria and it is therefore only necessary to recruit less than 7% of the eligible patients to recruit the 120 subjects. Based on the demographics from subjects recruited in previous studies and number of patients at each site, it is expected that the recruited sample will be approximately 76% male, 40% black, 36% Hispanic, 19% non-Hispanic white, and 1% Asian. In order to ensure recruitment of adequate numbers of blacks, Hispanics, non-Hispanic whites and women, the PIs will continually monitor the demographics of recruited subjects and stop recruitment of blacks or Hispanics if either group reaches 45% or 40% respectively of the planned sample, stop recruitment of whites if it reaches 25% of the planned sample, and stop recruitment of males if it reaches 80% of the planned sample. To minimize attrition, incentive payments for study visits will be gradually increased over time.

Inclusion criteria: 1) HIV-infected for 5 or more years; 2) On ART for 5 or more years; 3) HIV viral load consistently <48 copies/mL for the last year; 4) no anticipated changes in ART by provider or patient; 5) CD4 nadir of 250 cells/mm^3 or less; 5) Fluency in the English language; 6) Age 50 years or older; 7) Willingness to complete the entire MBSR or HEP interventions; 8) baseline IL-6 level of 1.17 pg/mL or greater. Exclusion criteria: 1) Having participated in an MBSR course in the past; 2) Current meditation and/or yoga practice; 3) Began psychiatric medications in the past 2 months or plans to discontinue psychiatric medications; 4) Currently receiving statins, steroids, or immunosuppressant drugs; 5) Women who are pregnant or plan to become pregnant in the next year.

The list of eligible patients identified through the electronic medical record will be reviewed with each HIV Primary Care Provider (PCP) and permission requested from them for the RC to contact each of their eligible patients at the next clinic visit. If a PCP indicates not to contact a patient, the reason will be recorded. The patient's participation in this study will play no role in how the PCP manages his or her care. If permission is given, the RC will use a scripted explanation to explain the study to the patient including the inclusion and exclusion criteria. If the patient is interested in participating, he or she will undergo informed consent. The RC will place emphasis on the time demands of the intervention in order to screen out those that are unlikely to adhere to the demands of the intervention. After obtaining Informed Consent, the RC will assign a study identification number, collect demographic and contact information, and a blood sample will be collected to determine baseline IL-6 levels. Each consented subject that meets all inclusion criteria will be invited to join the study and complete three subsequent research visits (separate from the MBSR or HEP sessions). At these visits, the psychological assessments will be completed and blood will be collected. Separate blood draws will also occur within one week of each of these 3 study visits for the second measure of IL-6 values.

Measurement of Biological Markers: Inflammatory biomarkers to be measured belong to one or more of four broad-spectrum categories: i) general (IL-6, CRP), ii) innate immunity (IL-6, CRP, sCD14), iii) microbial translocation (sCD14), and, iv) system wide-associated (CRP, d-dimer). The selection of biological markers for the study is based on three primary considerations: i) markers which have been unequivocally demonstrated to be increased in HIV-infected adults when compared to uninfected counterparts, ii) markers with strong translational medicine correlates connecting basic immunological inflammation with clinical outcomes, and, iii) markers demonstrating scientific reliability with respect to laboratory assay measurements.[15-17,22,60,61]

The four markers selected using these criteria are:

(1) Interleukin 6 (IL-6): IL-6 is the primary outcome measure. Several large-scale studies of HIV-infected individuals have consistently shown IL-6 as a key predictor of mortality. In addition, other studies have shown IL-6 levels elevated in persons with HIV as compared to uninfected counterparts, and this fact remains true even after effective ART is implemented. IL-6 is the one marker that has been associated with all major domains of interest in this study: inflammation, HIV infection, aging and system-specific markers of disease. For example, IL-6 and TNF-alpha receptor-1 were shown as the best predictors of 10-year all-cause mortality in a study of aging adults in the Cardiovascular Health Study.[62] As a pleiotropic cytokine, with a major role in inflammation as well as a variety of systems, IL-6 is a key player connecting the basic mechanisms of the immune response to clinical outcomes.[17,18,22,23,62-64] From a translational medicine perspective, a vast array of recent reports have demonstrated IL-6's role in a variety of clinical conditions affecting HIV-infected individuals, including cardiovascular disease, non-AIDS-defining cancer, non-AIDS-defining serious bacterial infections, hepatic fibrosis, pulmonary function abnormalities, type 2 diabetes, functional impairment, anemia, sleep disorders and overall mortality.[65-75]

(2) C-reactive protein (CRP): CRP is a non-specific marker of inflammation produced by the liver. CRP levels have also been demonstrated to be elevated in HIV-infected adults, in particular in patients 45-76 years of age. In addition, study entry levels of CRP have been strongly associated with mortality, showing a key role of the protein in translational medicine of HIV disease progression.[22,23] Strong associations between CRP levels and cardiovascular disease, as well as metabolic syndrome, have recently been confirmed.[65,67] In combination with IL-6 levels, CRP have also been linked to pulmonary function abnormalities, type 2 diabetes, and sleep disorders in HIV-infected adults.[71,72,75]

(3) Soluble CD14 (sCD14): Evidence suggests that damage to the gastrointestinal immune system, and the consequential microbial translocation, are key elements in immune activation during chronic HIV infection. CD14+ macrophages secrete sCD14, which interacts with bacterial products from microbial translocation, such as lipopolysaccharides. sCD14 has been demonstrated to be at higher levels in HIV-infected individuals compared to uninfected counterparts.[17,19] Several studies of HIV-infected individuals have identified sCD14 as a biological marker linked to impaired cognitive function, increased risk of age-associated end-organ diseases, and mortality.[20,21,25,76-78] In more recent findings, sCD14 as a marker of gut epithelial barrier dysfunction (along with IL-6, CRP and D-dimer) was found to be a predictor of mortality in treated HIV infection.[66]

(4) D-dimer: D-dimer is a protein fragment produced from fibrin degradation, used as a marker of a hypercoagulable state and of endogenous fibrinolysis[79] Recent studies have identified D-dimer not only as a predictor of cardiovascular disease,[79,80] but also of mortality in HIV-infected individuals.[17,22,23,60] The role of D-dimer as a strong translational medicine predictor in HIV infection has recently been confirmed.[65,66 68,74,81,82]

The appropriateness of the biological markers selected for this study is not only evident from the significant number of clinical studies cited above, but also by numerous abstracts of preliminary studies presented at the 2015 Conference on Retroviruses and Opportunistic Infections investigating these molecules.[83-87]

Subjects will go to the Mount Sinai Clinical Research Unit (CRU) at screening time (after signing consent) plus two separate times within one week at each of the 3 study time points (baseline, after 8 week course, after 6 month booster sessions). Blood for serum analysis will be drawn into 4ml vacutainers and processed within 6 hrs. Tubes will be centrifuged and nonhemolyzed serum aliquoted and stored frozen at -80^{o}C until analysis. Assays for the four selected biological markers will be conducted at the Icahn School of Medicine at Mount Sinai in the laboratory of immunologist Dr. T. Moran. The core facility supervised by Dr. T. Kraus incorporates years of extensive experience in laboratory expertise with a wide variety of immunological approaches. Analysis of high sensitivity CRP, sCD14 and IL-6 will be performed using Quantikine ELISA kit (BIO RAD) and BioTek programmable plate reader and d-dimer will be analyzed using EMD Millipore bead based ELISA kit and a Luminex 200.

Potential Challenges, Proactive Measures, Alternative Strategies The key challenges when measuring the proposed 4 biological markers (IL-6, hsCRP, sCD14 and d-dimer) result from: 1) expertise and quality control of assay execution, 2) diurnal rhythm expression, and, 3) within subject variability.[88] To account for these potential challenges proactive measures have been established. First, and critically important, has been the establishment of collaboration with Drs. Moran and Kraus. The level of expertise, experience and quality control provided by this lab assures reliability on recorded data. All frozen samples will be processed for execution of biological assays at a single run after completion of all collections. This measure controls for any potential variability during assay execution. The potential challenge of diurnal rhythm expression for all of the 4 biological markers will be addressed by strict serum collection in the three hour period between 3 pm and 6 pm. All blood samples for the entirety of the study will be collected in this three-hour time period and processed immediately, followed by immediate storage at a minus 80-degree freezer, assuring preservation of each marker. The concern of within subject variability is a key issue for IL-6 in particular.[89] For that reason, a critical approach measure has been established: two separate collections of blood for IL-6 measure at each time point, averaging the value for the two. Although it is recognized that this poses an additional level of demand on subjects, as they have to report to the study site twice for each of the three study time points, given the crucial role of IL-6 as the key biological marker, collecting two separate measures at each study time point provides the strongest level of reliability.

Measures of Psychosocial Stress and Mindfulness: Psychosocial measures will be assessed at 3 time points: baseline, after the 8 week course, and after the subsequent 6 month booster sessions.

The Patient Reported Outcomes Measurement Information System (PROMIS) is a system of highly reliable self-report measures, with substantial qualitative and quantitative evidence supporting validity, developed with funding from NIH.[90,91] Two instruments have been selected from the PROMIS Neuro-QOL Item Bank v1.0 Short Forms: Anxiety and Fatigue (8 validated items each, for a combined instrument of 16 items).

The Beck Depression Inventory (BDI-II)[92] is a reliable and valid self-report measure of depression.[93] The

Total Score of the BDI-II will be used to measure depression. This 21-item measure asks about how the subject has been feeling in the "past two weeks including today" and requires 5-10 minutes to complete.

The Five Facet Mindfulness Questionnaire (FFMQ) will be used in the study to measure mindfulness. This is a validated scale is commonly used in MBSR scientific studies. FFMQ will also provide an indication of rates of adoption of MBSR activities by participants, and permit analysis within 5 separate subscales: observing, describing, acting with awareness, nonjudging of inner experience, and nonreactivity of inner experience.[94-97]

We will also evaluate the feasibility and acceptability of each intervention condition. Attendance records will provide completion and retention rates. In addition, the RC will conduct a structured interview with each subject at the visit following the 8 week course to assess acceptability of the intervention.

Data Management and Statistical Analysis: We will use SPSS version 22 (IBM), and TrueCrypt 7.1a software (TrueCrypt Foundation) to enter, manage, and encrypt all electronic data. We will perform random checks and double entry of data to minimize coding errors. Data will be stored in a secured network that is backed up daily by Mount Sinai's Information Technology Department. The distributional properties of study outcomes will be evaluated and appropriate transformations will be applied, if necessary. The equivalence of baseline characteristics of study groups will be assessed with the t-test, Wilcoxon test, or chi-square test, as appropriate. We will also assess patterns of missing data and use multiple imputation or full information maximum likelihood methods, if missing at random.

Aim 1: *To assess the effect of MBSR on biomarkers of chronic inflammation in HIV-infected adults with well-controlled plasma viremia*. The primary outcome for the analyses of treatment effectiveness will be IL-6 levels. We will use mixed linear models to assess the effect of the intervention on markers of inflammation. The model will include fixed effect terms for treatment group, time, and time-treatment interactions. In secondary analyses, we will similarly assess the effect of the intervention on secondary markers of inflammation (CRP, sCD14, d-dimer) using appropriate adjustments for multiple comparisons. If there are imbalances in patients' characteristics across groups, statistical adjustment including these factors as covariates will be performed. All analysis will use an intent-to-treat principle. Power Calculation: Sample size calculations were based on the following assumptions: 1) mean baseline IL-6 of 2.0 pg/ml (SD:0.5)[17,98]; 2) stable IL-6 levels in the control arm; 3) clinical significant effect of MBSR will be a net reduction of IL-6 of >0.5 pg/ml.[98,99] Under these assumptions, a sample size of 100 patients (50 per group) will provide a power >80% using 2-sided test and a 0.05 significance level. One hundred and twenty study participants will be enrolled. Dropout rates reported in the literature for MBSR studies range significantly (range 0 to 48%, mean 10%). In order to lean on a conservative side a higher dropout rate of 15% is estimated for this study. With a 120 enrolled, a 15% dropout rate provides 102 completed participants. It is quite feasible to recruit 120 patients from this estimated eligible sample of 1,800 subjects at the three recruitment sites (requiring participation of ~7% of the eligible sample).

Aim 2: *To explore whether changes in markers of psychosocial stress mediate the impact of MBSR on chronic inflammation*. We will compare depression, anxiety, fatigue, and mindfulness scores among subjects in the active vs. control arm using a t-test or Wilcoxon test, as appropriate. The association between depression, anxiety, fatigue, and mindfulness scores with IL-6 (and other inflammatory markers) will be tested using the Pearson correlation coefficient. SEM analyses (using Mplus) will be based on the preliminary model depicted in Figure 1. We hypothesize that MBSR will impact inflammatory markers indirectly via effect on markers of psychosocial stress and mindfulness. Latent variables will be measured using a combination of factor scores (i.e., parceling) and items. Psychosocial stress will be measured with four factors (e.g., depression, anxiety, fatigue, mindfulness) allowing for the testing of specific pathways. This model will also include control variables. We will estimate parameters by using Weighted Least Squares Mean-Variance adjusted (WLSMV) estimator. The WLSMV method provides a robust estimation when sample size is moderate. Model fit will be evaluated based on various indices of exact and close fit: χ^2 (>0.05), the comparative fit index (CFI>0.90), the Tucker-Lewis fit index (TLI>0.90), the root mean squared error of approximation (RMSEA<0.05), and the weighted root mean square residual when using WLSMV (WRMR<0.90). To ensure consistent parameter estimates, we will estimate models including a latent class variable identifying the patterns of missingness. These analyses will be conducted using a latent class pattern mixture model. Sample Size Calculations were performed using the method presented by MacCallum et al.[100] Effect size in this approach is defined in terms of a null hypothesis and alternative hypothesis value of the root-mean-square error of approximation (RMSEA) index, which we hypothesized would be 0.05 (adequate fit) and 0.03 (good exact fit), respectively. We estimated that we would have, at minimum, 80 degrees of freedom, having a sample size of 120 subjects, and an error rate (alpha) of 0.05. Under these specified assumptions we will have >80% power.

Data Safety Monitoring Board (DSMB) Dr. H. Sacks and Dr. S. Morgello will serve on the DSMB for the study. The DSMB will meet twice yearly and Dr. Weiss and Dr. Barbosa will attend the DSMB meetings.