Reductions in Biopsychosocial Risks for Pregnant Minority Women and Their Infants:

The Mastery Lifestyle Intervention

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812).

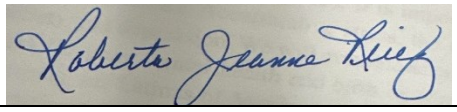
National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form(s) must be obtained before any participant is consented. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form(s) will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

INVESTIGATOR'S SIGNATURE

The signature below constitutes the approval of this protocol and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines, as described in the *Statement of Compliance* above.

Principal Investigator or Clinical Site Investigator:

Signed:  Date: 9-16-2024

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1.1 SYNOPSIS

Title: Reductions in Biopsychosocial Risks for Pregnant Latinas and Their Infants: The Mastery Lifestyle Intervention

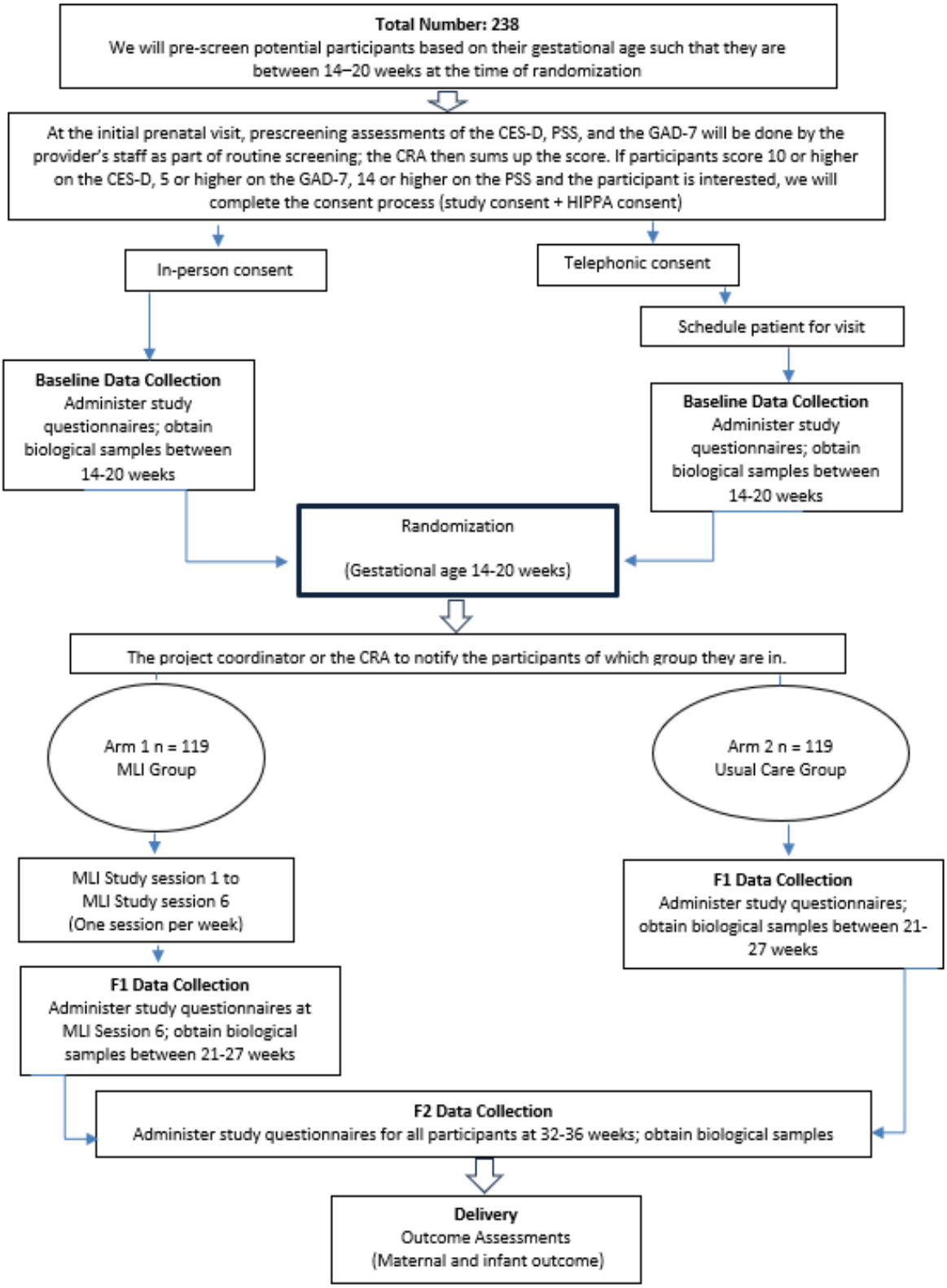
Grant Number: ***R01 HD101535-01A1-02***

Study Description: To address the gaps related to interventions for minority (Hispanic and African American) pregnant women, we have developed and successfully pilot tested the Mastery Lifestyle Intervention **(MLI**): a culturally-relevant, manualized psychosocial group intervention that integrates evidence-based behavioral therapies – Acceptance and Commitment Therapy (ACT), Problem-Solving Therapy (PST) and Mindfulness. The MLI is a 6-week program designed to be integrated into regular prenatal care to facilitate more comprehensive care delivered by a nurse practitioner (NP) or certified nurse midwife (CNM). We propose the following hypotheses for a randomized controlled trial: **Hypothesis 1a:** Participants in the 6-week MLI will have decreased depressive symptoms, anxiety, stress, disengaged coping, and increased active coping compared to UC (our control group receiving only standard prenatal care) at end-of-treatment and after 6 weeks. **Hypothesis 1b:** The effects of MLI versus UC on depression, anxiety, stress, acculturative stress, and coping will be mediated via psychological flexibility and moderated by acculturation. **Hypothesis 2a:** Compared to UC, MLI participants will have significantly lower mean levels of CRH (Corticotropin Releasing Hormone) over time from baseline to end-of-treatment. **Hypothesis 2b**: Compared to UC, MLI participants will have significantly higher progesterone levels and lower estriol levels (higher progesterone/estriol ratios) over time from baseline to end-of-treatment. **Hypothesis 3:** As compared to UC, infants from mothers in the MLI group will have longer gestational age, greater birth weight, and fewer NICU admissions.

Objectives:	**Primary Aim 1:** Determine the efficacy of the MLI in pregnant minority women to decrease depressive symptoms, anxiety, perceived and acculturative stress, and to improve coping, versus usual care **(UC),** from baseline (14-20 weeks' gestation) to end-of-treatment (20-26 weeks' gestation) and at a 6-week follow-up (26-32 weeks' gestation), with acculturation as a moderator and psychological flexibility as a mediator. **Exploratory Aim 2:** Explore the effect of the MLI on neuroendocrine risk factors of preterm birth (PTB) (CRH, progesterone, and estriol) versus UC from baseline to end-of treatment. **Exploratory Aim 3**: Explore the effect of the MLI on infant birth outcomes (gestational age, birthweight, NICU admission in comparison to birth outcomes in the UC.
Endpoints:	The primary endpoints are depressive symptoms, anxiety, perceived and acculturative stress, and coping at 20-26 weeks and 32-36 weeks' gestation of pregnancy, and the secondary endpoints are blood levels of CRH, progesterone and estriol as well as infant outcomes at delivery.
Study Population:	The populations of interest are Latinas and African American pregnant women living in the Houston metropolitan area. Our target sample is 238 pregnant women; allowing for a 20% attrition rate. Ages of pregnant women will be between 18 and 45 years; participants will need to identify as Hispanic or as African American. They will need to be in good health without systemic infections. The geographic area is the greater Houston area including outlying areas the Texas Medical Center (TMC) serves.
Phase or Stage:	This is a phase 2 study
Description of Sites/Facilities Enrolling Participants:	Participants will be enrolled from various obstetricians throughout the greater Houston area.

Description of Study Intervention/Experimental Manipulation:	We will hold six weekly sessions of the study intervention (the Mastery Lifestyle Intervention, MLI) starting at 14-20 weeks' gestation to 20-26 weeks' gestation with each session lasting ~1-1½ hours. We will give participants in the MLI group a participant handbook in either English or Spanish that has space for reflection and activities to complete at home. We have refined our facilitator handbooks extensively. Conducting the MLI with groups is feasible, acceptable, and advantageous as it is a) cost-effective, b) allows for group support and building a social network and c) encourages good role modeling. A recent meta-analysis found no significant differences in effectiveness in individual versus group formats for Cognitive Behavioral Therapy (CBT). We also have been using a web-based format over Zoom for groups.
Study Duration:	The study should take 52 months from first enrollment to completion of data collection.
Participant Duration:	Each individual participant will take 12 weeks for complete maternal data collection. Data about infants born to participants will be collected via chart review from delivery hospitals.

1.2 SCHEMA (SEE NEXT PAGE)


Total Number: 238
We will pre-screen potential participants based on their gestational age such that they are between 14–20 weeks at the time of randomization
At the initial prenatal visit, prescreening assessments of the CES-D, PSS, and the GAD-7 will be done by the provider's staff as part of routine screening; the CRA then sums up the score. If participants score 10 or higher on the CES-D, 5 or higher on the GAD-7, 14 or higher on the PSS and the participant is interested, we will complete the consent process (study consent + HIPPA consent)
In-person consent
Telephonic consent
Schedule patient for visit
Baseline Data Collection
Administer study questionnaires; obtain biological samples between 14-20 weeks
Baseline Data Collection
Administer study questionnaires; obtain biological samples between 14-20 weeks
Randomization
(Gestational age 14-20 weeks)
The project coordinator or the CRA to notify the participants of which group they are in.
Arm 1 n = 119
MLI Group
Arm 2 n = 119
Usual Care Group
MLI Study session 1 to
MLI Study session 6
(One session per week)
F1 Data Collection
Administer study questionnaires; obtain biological samples between 21-27 weeks
F1 Data Collection
Administer study questionnaires at MLI Session 6; obtain biological samples between 21-27 weeks
F2 Data Collection
Administer study questionnaires for all participants at 32-36 weeks; obtain biological samples
Delivery
Outcome Assessments
(Maternal and infant outcome)

1.3 SCHEDULE OF ACTIVITIES

	Pre-screening	Consenting	Visit 1: Baseline (14-20 weeks)	Randomization	Visit 2: MLI Session 1	Visit 3: MLI Session 2	Visit 4: MLI Session 3	Visit 5: MLI Session 4	Visit 6: MLI Session 5	Visit 7: MLI Session 6	Visit 8: F1 (21-27 weeks)	Visit 9: F2 (32-36 weeks)	Delivery
EMR Review for medical eligibility	X												
Score Review of CES-D, PSS, GAD-7	X												
Clinical and prenatal history	X												
Informed Consent & HIPPA		X											
Demographics			X										
Questionnaires			X								X	X	
Obtain biological samples			X								X	X	
Randomization of groups after 4-8 women are enrolled				X									
Experimental Intervention (MLI) Group			X		X	X	X	X	X	X	X	X	
Usual Care Group (UCG)			X								X	X	
Collection of infant outcomes data-both groups													X
Adverse Events Reporting			X		X	X	X	X	X	X	X	X	X

2 INTRODUCTION

Background and Significance for Clinical Trial

Psychosocial distress (depression, anxiety, chronic stress, acculturative stress) is prevalent in pregnant Hispanics (hereafter referred to as Latinas) and is associated with preterm birth (PTB) and low birth weight (LBW). PTB in 2017 for all births rose (9.93%), rising in Latinas to 9.61% (1**).** Depressive symptoms, anxiety, perceived, and acculturative stress, and deleterious coping are important risk factors related to these results. About 10-25% of pregnant women in the U.S. have depressive symptoms, with resulting negative sequelae for women, children, and families (2). Antenatal depression, a strong risk factor for postpartum depression, highlights the need to intervene early during pregnancy (3). Although postpartum depression is outside the scope of study, we will examine it in future studies, and we will examine the effect of untreated antenatal depression on infant outcomes at birth (LBW, PTB). A meta-analysis found that pregnant women with untreated depression had an odds ratio of 1.56 (1.25-1.94) for PTB and 1.96 (1.24-3.10) for LBW (2). Most evidence indicates that there are ethnic disparities in antenatal depression; Latinas have higher risk factors for depression as compared to non-Hispanic white women (2), with rates of depression as high as 35-40%. In our previous study with Latinas (Ruiz-R01NR07891), 30% had antenatal depression. The prevalence of depression and accompanying psychosocial distress, particularly among impoverished women, is a significant public health problem. Anxiety commonly co-occurs with depression. As many as 60% of women diagnosed with depression also met diagnostic criteria for anxiety (5). Similarly, perceived stress is another well-established risk factor for antenatal depression (6) that affects PTB (7-10). Of concern, studies of antenatal depression in Latinas failed to account for acculturation, the multidimensional process of psychological and cultural change that occurs when a person interacts with two or more cultures (11). Previous results for ours and other's studies indicate that greater acculturation results in worsened mental health (12, 13); it is important to consider acculturation as a moderating risk factor for Latinas. Acculturative stress, the stress associated with acculturation, also relates to anxiety and depression. We will examine acculturation and acculturative stress as part of the social risks for poor mental health and birth outcomes. We will avoid methodological weaknesses by focusing on Latinas of Mexican origin, avoiding heterogeneity (14). We will not ascertain if a participant has a social security number, as doing so was noted as a human subject concern by reviewers.

Psychosocial distress is also very prevalent in pregnant African American (Black) women. A diagnosis of depression, anxiety and stress during pregnancy worsens the risk for PTB and LBW, particularly in Black women. Racial and ethnic differences in preterm birth rates continue. In 2020, the rate of preterm birth among African American women (14.4%) was about 50 percent higher than the rate of preterm birth among white women (9.1%)) (CDC website, 2021). These stark numbers nationally indicate a need for better preventive interventions. Data also indicates

disparities in the use of mental health services for both Black and Latina women (Ponting et al., 2020). In a systematic review (Ponting), the rates for both Black and Latina women to use mental health services during pregnancy are between 4 and 5%, and they are less likely to receive follow up for treatment (Goodman & Tyer-Viola, 2010). Barriers are infrequent screening, particularly in clinics attended by a lot of Latinas and Blacks, as well as healthcare mistrust (Levy & O'Hara 2010). The most common treatment for psychosocial distress has been cognitive behavioral therapy (CBT) (Ponting, 2020). Most randomized controlled trials (RCTs) do not measure anxiety as an outcome, that is vitally needed. Additionally, cultural adaptations with CBT programs can improve clinical outcomes in ethnic minorities (such as coping with discrimination).

Psychosocial factors increase the risk of adverse birth outcomes.

Depressive Symptoms and Anxiety. Evidence from several meta-analyses (16-18) indicates that the use of antidepressant medications (particularly Selective Serotonin Reuptake Inhibitors [SSRIs]) is possibly related to increased risk of PTB as well as LBW. These findings underscore the need to weigh the effect of untreated depression against the potential effects of antidepressant exposure, (18) and to identify and implement effective nonpharmacological treatments. Treating depression during pregnancy for low-income women can be difficult due to limited insurance coverage for mental health and out of pocket costs, (19) as well as the stigma associated with mental health visits. Depression is often comorbid with anxiety; together, they increase the risk for PTB (20). Several researchers have found strong links between anxiety and PTB (21). There is also evidence that maternal prenatal anxiety programs the fetus resulting in emotional and behavioral problems for the child (21-23). *Based on the evidence related to risk, we will target depression and anxiety to reduce psychosocial risk, ultimately reducing the risk of adverse birth outcomes.*

Chronic Stress. Perceived stress has been extensively studied in relationship to PTB, with strong empirical evidence indicating links between prenatal stress and PTB (7,8). However, acculturative stress and perceived stress together have the greatest impact on elevated depressive symptoms in Latinas (24).

Acculturative Stress refers to stress responses by immigrants culturally adjusting to a new society as they are challenged by an unknown culture and differing social norms. Acculturation involves embracing new behaviors and customs while either preserving or losing those of the traditional culture. Often acculturation and acculturation stress result in intergenerational family conflict, maladjustment, and marginalization (25, 26). Most recently, acculturative stress for Latinas may be exacerbated given the recent increase in sociopolitical stressors (e.g., anti-immigrant, anti-Hispanic policies, hate crimes). Although evidence on the health effects of anti-immigration rhetoric and policies is still being collected currently, studies have related sociopolitical stress (i.e., anxiety about deportation, awareness of anti-immigration policies) with poorer mental health (27) as well as higher systolic blood pressure and pulse pressure among Latina adults, known risk factors for PTB (28). Documented increases in heightened profiling and deportation may not only

result in increased acculturative stress but also decreased healthcare use (27). For instance, in a study using birth certificate data, severe sociopolitical stressors among Latinas indicated a detrimental impact on PTB (29). This study illustrated the implications of racial stressors for increased PTB in both the health of Latina immigrants as well as U.S. born Latinas. Rates of LBW and PTB also increased in Latina mothers in Iowa after a surprise immigration raid (30). Further, LBW risks in this study were worse for mothers with low education, who had fewer coping resources. Investigators speculated that the pregnant Latinas' neuroendocrine balance and coping resources were affected after the raid, leaving their infants vulnerable to a dysregulated endocrine environment. Previous scientists found acculturative stress to be a major contributor to poor health outcomes (31,32), but more work is needed to understand the role of maternal mental health. Our last major study with Latinas (Ruiz-R01NR07891) found acculturative stress (as measured by the Acculturative Stress Scale) (33) to be significantly associated with depression ($r = .21$, $p < .000$) and chronic stress ($r = .24$, $p < .000$), as measured by the Perceived Stress Scale (34). *Both types of stress are proposed targets to reduce psychosocial risk*.

Coping. Poor coping skills, such as disengaged coping, may also be associated with compromised mental health and risk of poor birth outcomes. Disengaged coping may be conceptually defined as avoidance, behavioral disengagement, or self-blame. Relations between coping and antenatal depression among Latinas remain minimally explored; however, among Latinos in general, Torres found among 148 adults, poor coping was associated with worsened depression (35). Of further concern, Borders, studying 294 welfare recipients, found poor coping skills (avoidant or disengaged coping) were associated with LBW babies (36). In general, pregnant women with coping behaviors of avoidance or distancing (i.e., behavioral disengagement) have had more PTB and postpartum depression (36-39). Alternatively, active coping may be conceptually defined as the use of emotional and instrumental support, use of humor, and/or acceptance (vs. avoidance) of internal distress (40). Ours and other's evidence indicate that first-generation Latinas used less active coping skills than acculturated Latinas (41,42). *We will focus on increasing acceptance of difficult internal states (i.e., depression, anxiety) and the use of more action-oriented (vs. avoidant coping) to reduce psychosocial risks both of Latinas and Black women.*

Neuroendocrine factors are important for the maternal-fetal response to psychological distress. Research has demonstrated that neuroendocrine pathways mediate the effects of psychosocial factors on health outcomes, i.e., particularly fetal development and infant birth outcomes (43). In seminal work, Chrousos (44) linked the neuroendocrine system to the stress response and health outcomes. The hypothalamic-pituitary-adrenal **(HPA)** axis (in pregnancy, the placental-pituitary-adrenal-axis) is one of the major systems involved with the stress response. The main agent of this axis is Corticotropin Releasing Hormone **(CRH);** placental CRH is identical to hypothalamic CRH both biologically and in its response to stress (45). Wadhwa (46,47) states that the placenta has the ability to receive, process, and respond to certain stimuli, and thus may take

on functions similar to the central nervous system in the stress response. Placental CRH facilitates the placenta getting information to the fetus. Increases of CRH early in pregnancy have predicted the onset of preterm labor in ours and other's results (48-52). The chronicity of stress is particularly important in relation to the initiation of early labor (53). Estrogen and progesterone are other important endocrine factors linked to CRH and related to labor. Increases in estrogen (estriol, **E3)** and decreases in progesterone shift the endocrine balance. Placental CRH stimulates E3 induced changes in the cervix and uterus, part of the initiation of labor (54). It is important to explore the effects of an intervention to reduce psychosocial distress on some of the key pathways, such as CRH, progesterone, and estriol. In our previous study with Latinas (55), the combination of acculturation, depressive symptoms, progesterone, and E3 predicted PTB, indicating that the combined impact of these factors produced the greatest risk. *We propose to a) test a behavioral intervention to reduce psychosocial risks, and b) explore the impact on neuroendocrine factors and infant birth outcomes.*

Cognitive behavioral therapy (CBT) is recommended for reducing antenatal depression, yet effects are small, and few studies have targeted PTB or Latinas. In recent evidence, a report of interventions to prevent perinatal depression by the U.S. Preventive Task Force (USPSTF) (56), counseling interventions, primarily CBT, were recommended to prevent perinatal depression. Fifteen of the 20 trials reviewed had counseling interventions that were focused on women who were known to be at risk for perinatal depression, particularly depression history or symptoms. Analysis of counseling interventions revealed a pooled standardized effect size of 0.2, considered a small effect (57), which *suggests new therapies are needed to obtain larger effects.*

Moreover, the only study to examine the effect of therapy on PTB was an RCT conducted in Spain and France to evaluate the Tourne approach versus usual care to reduce depressive symptoms (58). The Tourne approach used humanistic and cognitive techniques during 10 antenatal group sessions for both the woman and her partner. Nurse midwives were facilitators. Depressive symptoms were not significantly reduced; however, there were major differences in the infant outcomes. In the intervention group there were only 3 PTBs (4.4%) with higher birthweights (p =.01), compared to 13 PTBs in the control group (22.4%) (p = 0.003). *Infant outcomes were much improved in women who received behavioral therapy, suggesting the need for trials evaluating both proximal psychosocial risk factors and infant outcomes, such as we are proposing.*

CBT has primarily been used with women who are Caucasian, married and middle class, and not well tested in Latinas; we found only a few RCTs using CBT targeting depression in pregnancy that included Latina women (59-61). These studies did not specifically address the unique situations associated with Latinas and their distinctive risk factors. Jesse (59) et al. focused on perinatal depression and used traditional CBT integrated into prenatal care, for rural low-income minority women, both Latinas and Black women. They could not recruit enough Latinas, so the feasibility of this intervention with Spanish speaking Latinas is still unknown. This RCT was more

effective for low to moderate risk depressive symptoms on the Edinburgh Postnatal Depression Scale (EPDS) and was particularly effective for Black women. This study provides evidence of the effectiveness of CBT for especially Black pregnant women. Munoz (60) focused on Latinas and limited the sample to women with major depression. The 12-week program used social learning concepts, attachment theory, and reality management. It was ineffective in several areas: a) the women completed only 7 of the 12 sessions on average, and importantly, b) the depression scores were not changed after the intervention. Le (61) recruited Central American Hispanics, who, as new immigrants (4 years or less in America), had a low risk for depression. A study limitation was the lack of consideration for psychosocial risks related to acculturation. Another study focused on using CBT with pregnant Black women in Washington, DC. focusing on several risk factors, not just psychosocial ones. This study reported results indicating it was possible to combine such an intervention for Black women integrated into prenatal care. In sum, studies applying CBT with Latinas did highlight that behavioral therapy can be integrated into the prenatal setting to serve this population. *Our proposed intervention is based on the third wave (newest) generation of CBTs not tested in any studies reviewed in the USPSTF analysis, thus filling a gap and advancing science by testing the newest CBT methods. The three studies using traditional CBT with Latinas focused exclusively on depression, indicating a need for a broader transdiagnostic intervention for other psychosocial distress.*

Acceptance and Commitment Therapy (ACT) (62), a third-wave Cognitive Behavioral Therapy, has a strong evidence-base for effects on depression and anxiety, with burgeoning support for women in the perinatal period. Although it is a CBT, ACT is an innovative behavioral treatment that represents a significant shift in philosophy and perspective. A primary tenet of ACT involves the notion that *avoidance* or attempts to exert strong control of negative emotions, thoughts, or bodily sensations results in and perpetuates psychopathology (e.g., depression, anxiety) (63-65). ACT methods endorse an acceptance rather than a control-based model to promote more flexible and adaptive behavior consistent with client-identified, personal values. ACT has been applied to a myriad of problem areas (e.g., substance abuse, chronic pain) and has a particularly strong evidence base in depression with moderate evidence for anxiety (66, 67). Most studies with pregnant women tend to focus narrowly on perinatal depression, yet ours and other's work (9, 46, 68, 69) suggest a high prevalence of anxiety and stress as well. *Interventions are sorely needed to address co-occurring mental health needs among perinatal women (e.g., stress, depression, anxiety, coping), and therefore a "transdiagnostic" approach, such as ACT, is ideal (67).*

ACT has benefit over other similar treatments in that it links to a theoretical framework with a corresponding comprehensive set of behavioral change principles. Psychological processes involved in psychopathology and target mechanisms for change are identified. Primary processes include acceptance (as opposed to avoidance), values identification, committed action, mindfulness, and cognitive defusion, only a few of which may be targeted with currently

recommended CBTs, including interpersonal therapy (IPT). Psychological flexibility is the overarching process comprising the six primary, individual processes, and has been associated with most forms of psychopathology and related problems (70). Stotts (co-I), Villarreal (co-I), Suchting (co-I), and Northrup (co-I) (71) conducted a longitudinal secondary analysis that found psychological flexibility to be a mediator of early (1-2 weeks postpartum) and later (6 months postpartum) depressive symptoms among new mothers of NICU infants. Depressive symptoms in women low in psychological flexibility persisted or increased by 6 months postpartum relative to women who were more psychologically flexible. Many studies support ACT processes as mechanisms of change and potential targets of treatment. In fact, among ACT studies investigating mediators of treatment, about 50% of the between-group differences in follow-up outcomes can be accounted for by differential levels of psychological flexibility (72). *Due to its emphasis on psychological flexibility, we believe ACT is well suited to reduce stress among Latinas as they adapt to the American culture, as well as African American women with the challenges they face related to discrimination. Other CBT treatments have identified very few mechanisms of change for psychosocial distress in perinatal women. The proposed study will fill this gap.*

Problem Solving Therapy (PST) (73) may target stressful external problems experienced by both pregnant Latinas and Black women as it teaches tangible problem-solving skills. PST, a behavioral intervention developed from traditional CBT, has been found effective in treating depression (73-76) and anxiety (77,78) Unlike IPT (79) and significant to the proposed study, PST has been better adapted for use within primary and other health care settings and can be administered by physicians and NPs (80). Further, PST has demonstrated effects on mental health outcomes when delivered either individually or in group settings. PST treatment focuses on helping individuals solve multilevel stressors and concrete problems. ***We contend that a combined treatment of ACT and PST is a much-needed intervention to reduce maternal psychosocial risks for vulnerable Latinas and African Americans, thus improving clinical practice.***

2.1 STUDY RATIONALE AND BACKGROUND

Pregnant Hispanic women hereafter referred to as Latinas, are at increasing risk for psychological distress which leads to adverse birth outcomes such as preterm birth (PTB, gestational age < 37 weeks) and low birthweight (LBW, <2500 grams) (81,82). African Americans are also at considerable risk for poor pregnancy outcomes, of even greater concern than Latinas are. The cause of PTB is multifactorial (83, 84), with many of the risk factors difficult to modify (85). Extensive research has been focused on preventing PTB in the United States (US) (86), predominantly testing medical interventions such as drugs (17-alpha hydroxyprogesterone caproate) or cervical cerclage; with minimal focus on decreasing psychosocial risks. Women reporting "high" levels of psychological stress are at 25-60% increased risk for PTB compared to those with "low" stress (46). The American College of Obstetrics and Gynecology has endorsed psychosocial screening at least once each trimester in pregnancy (87). Acculturative stress and

perceived stress together are known to elevate depressive symptoms in Latinas (88, 89), particularly Latinas who have assimilated into U.S. culture, and are associated with PTB (68, 90-93). Despite this, many care providers do not recognize or diagnose depression during pregnancy; and even if depressed women are identified, few are treated (94). This is a significant unmet need for both Latinas and Blacks (95), leaving them at increased risk of both PTB and postpartum depression (92) which has devastating and long-term effects for mother and child (93). Our prior research, using a psychoneuroimmunology (PNI) framework, has identified psychological risk factors (depressive symptoms, anxiety, stress, acculturative stress, coping) and neuroendocrine risk factors (high Corticotropin Releasing Hormone [CRH], lower progesterone, higher estriol) at 22-24 weeks gestation as strong predictors of PTB in Latina women. The rate of prematurity was unacceptably high in this understudied population of Latina women (as high as ~16% in our previous study) (97). **New interventions targeting additional risk factors need to be identified and rigorously tested for two of the at-risk minorities** (85).

To address the gaps related to interventions for Latinas and Black women, we have developed, and pilot tested the novel Mastery Lifestyle Intervention **(MLI**): a culturally-relevant, manualized psychosocial group intervention that integrates two evidence-based cognitive behavioral therapies **(CBT)** – Acceptance and Commitment Therapy **(ACT)** and Problem-Solving Therapy **(PST**). Our study is unique in that we propose to target psychological risk factors with the MLI and also assess the impact on associated neuroendocrine risk factors, based on our evidence (97,98) as well as extensive empirical evidence in other populations (7, -9, 99). This innovative combination of two interventions during pregnancy is expected to have an effect on clinical outcomes as well. The MLI is a 6-week program designed to be integrated into regular prenatal care to facilitate more comprehensive care delivered by a nurse practitioner **(NP)** or certified nurse midwife **(CNM)**. We have specifically targeted the timing of the MLI prior to maternal biological changes seen at 22-24 weeks gestation in our previous work. *Our pilot study of the MLI demonstrated feasibility, acceptability and a moderate to large pre-post effect size for reducing anxiety, and a moderate effect size in reducing depressive symptoms.* We are now poised to test the MLI in a rigorous RCT conducted in early pregnancy and integrated into a prenatal care setting for Latinas and African Americans. To our knowledge, this will be the first study to a) explore both the psychosocial and biological effects of ACT and PST and, b) explore the effects of ACT and PST related to improving gestational age, birthweight and NICU admission. We propose the following aims:

Primary Aim 1: Determine the efficacy of the MLI in pregnant Latina and Black women to decrease depressive symptoms, anxiety, perceived and acculturative stress, and to improve coping, with psychological flexibility as a mediating factor and acculturation as a moderator, versus usual care **(UC),** from baseline (14-20 weeks' gestation) to end-of-treatment (20-26 weeks' gestation) and at a 6-week follow-up (32-26 weeks' gestation). **Hypothesis 1:** Participants in the 6-week MLI will have decreased depressive symptoms, anxiety, stress, disengaged coping and increased active coping compared to UC at end-of-treatment and at a 6-week follow-up. **Exploratory Aim 2:**

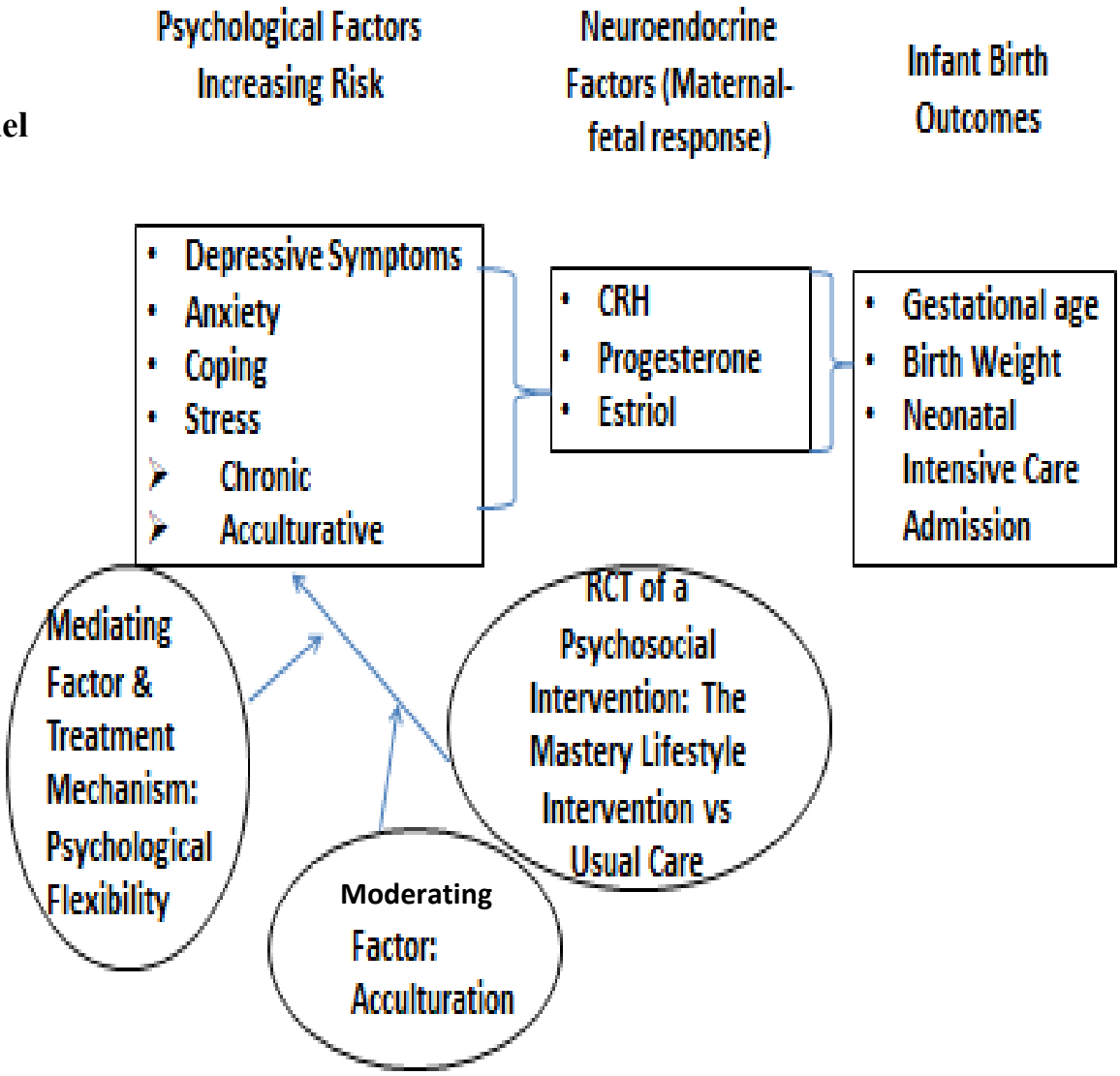
Explore the effect of the MLI, versus UC, on neuroendocrine risk factors of PTB (CRH, progesterone, and estriol) from baseline to end-of treatment. **Hypothesis 2a:** Compared to UC, MLI participants will have significantly lower mean levels of CRH over time from baseline to end-of treatment. **Hypothesis 2b**: Compared to UC, MLI participants will have significantly higher progesterone levels and lower estriol levels (higher progesterone/estriol ratios) over time from baseline to end-of treatment. **Exploratory Aim 3**: Explore the effect of the MLI on infant birth outcomes (gestational age, birthweight, NICU admission). **Hypothesis 3:** As compared to UC, infants from mothers in the MLI group will have longer gestational age, greater birthweight, and less NICU admissions.

Impact: We expect the MLI will provide a greatly needed, novel, feasible, and effective nonpharmacological program added to the toolbox of treatments assisting providers to improve health during pregnancy. This work is consistent with the mission of NICHD to improve prenatal health, particularly in relationship to health disparities. It is an important step in identifying new pregnancy interventions to possibly reduce the risk of PTB.

Background

Conceptual Framework. We have based our previous work for the last 20 years on the psychoneuroimmunology (PNI) model. This model (Figure 1) of "psycho," or psychological factors (such as depressive symptoms, anxiety, etc.), with neuroendocrine factors (such as hormones and corticosteroids), with immunological factors (inflammatory markers, infections) predicts health outcomes. From this framework, we have tested depressive symptoms, anxiety, stress, and coping as psychological factors impacting birth outcomes in Latina pregnant women. Our evidence, as well as extensive evidence from the literature, indicates the importance of testing a model to reduce psychobiological risk and, subsequently, poor infant outcomes (100-104).

Figure 1
PNI model



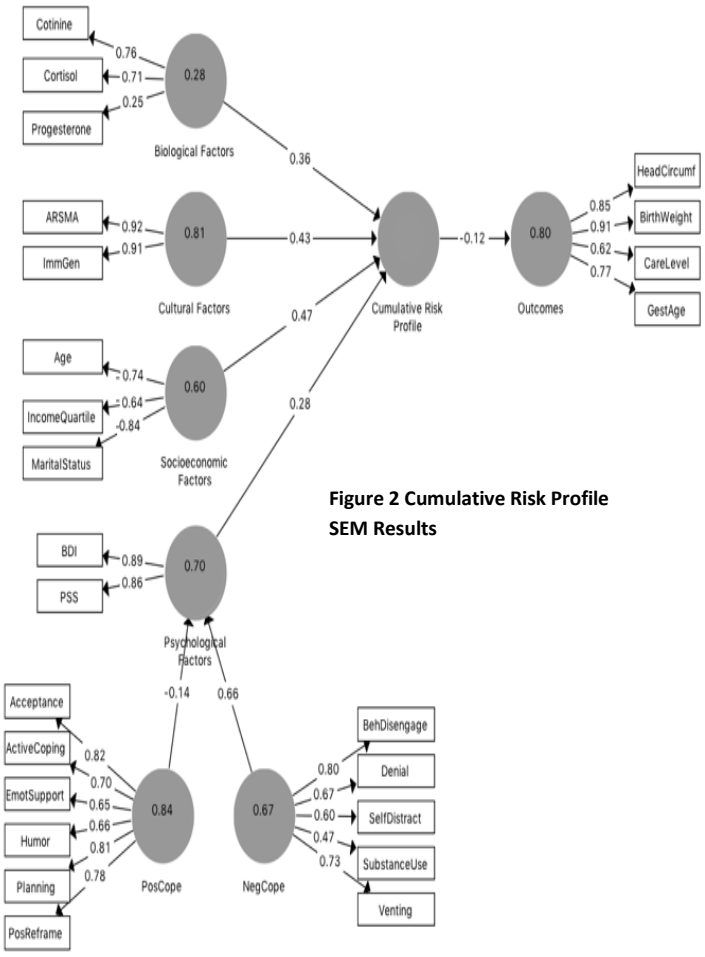


Figure 2 Cumulative Risk Profile SEM Results

Observational Results. In our last study, PI-Ruiz-R01NR07891, we had a sample of 515 pregnant Latinas. We conducted an observational study at 22-24 weeks gestation and measured acculturation, progesterone, cortisol, cotinine, age, marital status, income, stress, depressive symptoms, and coping. These risk factors were tested as predictors of a higher-order latent factor, that we named the *Cumulative Risk Profile* (98). We hypothesized that the cumulative effect of biological, cultural, socioeconomic, and psychological risk factors would predict neonatal health, represented through a latent outcomes construct. We sought to evaluate the strength of the factors that put these women at risk for poor outcomes by a predictive structural equation model. Consistent with our hypotheses, poor birth outcomes were predicted by risk factors at multiple levels of analysis, including biological, cultural, socioeconomic, and psychological. The tested empirical model of a *Cumulative Risk Profile* predicting poor birth outcomes (lower birth weight, decreased gestational age, more NICU admissions) had a good model fit and was scientifically rigorous (See Figure 2). Young, low income, single (socioeconomic factor), and greater acculturation (cultural factor) have the highest regression coefficients predicting the risk of having a poor pregnancy outcome. Stress and depressive symptoms were also important psychological factors predicting risk and are modifiable, unlike most other risk factors. Coping, particularly negative coping as noted from the different subscales in this figure, predicted an increase in depressive symptoms and stress, suggesting another treatment target. *These results not only demonstrate our ability to conduct large studies but also provide the rationale to target modifiable psychological risk factors to prevent PTB. We*

posit that many of the same risks may be modifiable also in the Black population. The MLI is a targeted intervention to reduce stress and depressive symptoms and to increase adaptive coping. We also have results using latent profile analysis to identify characteristics of low-, moderate-, and high-risk groups for PTB (97). The low-risk group (*n* = 157) had 7.7% PTBs (*n* = 12). Characteristics of women in the low-risk group: slightly older (mean = 25), married (56%), the least acculturated (more Spanish speakers, fewer years in the U.S., more identification with Mexican cultural identity) as compared to the other two groups. The low-risk group had very low depression scores on the BDI (mean = 6). The moderate-risk group for PTB (*n* = 272) had 12% (*n* = 32) PTBs. Characteristics of the moderate-risk group: average childbearing age (mean = 24), slightly fewer married women than the low-risk group (48%), more acculturated (greater English speakers, more years in the U.S., mean between second and third generation) as compared to the low-risk group. Depression scores were low (mean = 9 on the BDI), and coping was more active vs. avoidant. The high-risk group had the least married women; *n* = 21), the most acculturated (more English proficiency, the least Spanish proficiency, and the longest time in the U.S.) as compared to the moderate- or low-risk group. They had high scores on the BDI (mean of 22 or moderate depression). They also had the highest scores on disengaged coping and lowest scores on active coping. *These results indicate an association between psychological symptoms and PTB and the need to target* coping and lowest scores on active coping. *These results indicate an association between psychological symptoms and PTB and the need to target psychological factors, particularly disengaged, avoidant coping, with pregnant Latinas.* (86)

Development of the Mastery Lifestyle Intervention (the MLI): Rationale and Justification. The MLI is a manualized psychosocial group treatment that integrates two evidence-based interventions – Acceptance and Commitment Therapy (ACT) and Problem-Solving Therapy (PST). ACT is a contextual behavioral treatment systematically applied and investigated in diverse settings, with diverse populations and cultural backgrounds, and for a variety of behavioral health problems (62,105,106-108). ACT can easily be done in group formats and has reduced depression in various populations. For example, in a recent study (108), stroke survivors who received group-based ACT for 4 weekly 2-hour didactic sessions, as compared to a treatment-as-usual group, had significantly reduced depression and increased hopefulness with medium effect sizes. ACT helps increase psychological flexibility, identify personal values, and implement behavioral changes that are personally meaningful to the individual (i.e., committed action). Kashdan and Rotterburg (109) define psychological flexibility as "*the measure of how a person: (1) adapts to fluctuating situational demands, (2) reconfigures mental resources, (3) shifts perspective, and (4)* ***balances competing desires, needs, and life domains."*** This concept addresses much of what Latina women are required to do in pregnancy and as they acculturate. This concept that also pertains to many challenges Black women face in daily life. PST is a behavioral treatment that teaches recognition of how one orients to a problem and focuses on the development of specific problem-solving

skills. It is flexible in its application and works well as part of a larger, integrated treatment package for different populations. PST provides training about how to better resolve and cope with stressful life problems (75) has been used as a stand-alone treatment, and has been effective in pregnancy (110, 111). PST promises to also provide an effective treatment for Black women to cope with some of their difficult problems. We therefore believe that the MLI may easily work well with both Latinas as well as African Americans, as the participants identify their own values and problems and determine the way best way to problem solve considering their own situation.

We have integrated ACT and PST in the MLI to complement and build on each other. ACT enhances flexibility in responses to internal stimuli (thoughts, feelings, physical sensations), while PST teaches more concrete problem-solving skills in the external environment. Both assist women in pursuing valued goals. PST is a natural extension of the ACT process of committed action. ACT and PST both teach effective coping strategies related to changes and roles Latinas may face due to pregnancy as well as acculturation, and difficulties related to discrimination in Black women. The MLI facilitator manual and the participant handbook have been translated into Spanish.

D.2.c. Pilot Study Results of the Mastery Lifestyle Intervention (MLI) (112) with Latina women.

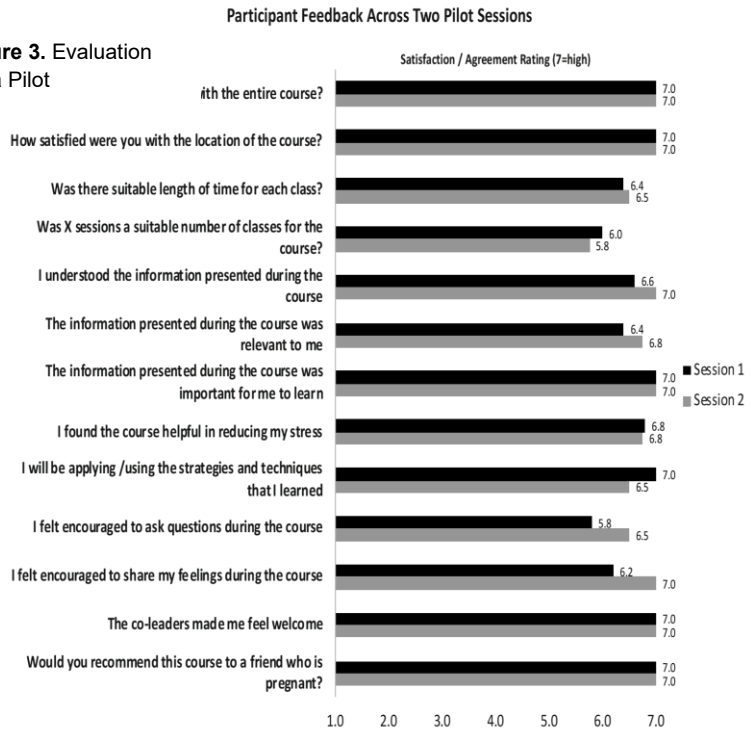


Figure 3. Evaluation Data Pilot

Feasibility. We conducted a one arm, pre-post test pilot study that included 3 cohorts with 15 participants. All but one of 15 participants attended 100% of the sessions. Several attempts at recruitment were initiated (e.g., recruitment with a research assistant not part of the obstetrics practice), however most efficacious was contracting a medical assistant from the obstetrics practice to recruit pregnant women at the care site and holding the sessions in the lobby of their provider, resulting in better recruitment and attendance rate for the sessions. The pilot trial elucidated that the recruitment window needed to be 14-20 weeks gestation to maximize the number of participants. Six sessions were well tolerated and attended, and we will continue that number for the proposed RCT. The use of electronic tablets was highly feasible; the use of the tablets by

participants worked well.

Acceptability. Figure 3 above provides evaluation data from the MLI pilot participants. Women ranked the answers on a survey on a scale of 1-7, with 7 being excellent and 1 being very poor. Participants reported that 6 sessions from 1.5 to 2 hours appeared to be ideal. From these results, we have added more group engagement.

Pretest and Posttest Results. From pre- to post-assessment, the intervention reduced anxiety and depression and negative coping (effect sizes ranged from -.30 to -1.5 for anxiety and -0.45 to -0.58 for depression). Positive coping (e.g., use of emotional or instrumental support, use of humor; acceptance) was increased as well, with subscale effect sizes ranging from 0.2 to 0.39. Negative coping (e.g., avoidance and distraction) was decreased with subscale effect sizes ranging from -0.28 to -0.61. **Our pilot results were positive and support proceeding to a larger RCT to further establish efficacy for the MLI**.

2.2 RISK/BENEFIT ASSESSMENT

2.2.1 KNOWN POTENTIAL RISKS

What are the risks of taking part in this study?

For <u>the MLI (or intervention) group</u>:

- There is a risk that the MLI may not be as good as traditional therapy for anxiety, depression or stress.
- There is also a risk that a participant can have some emotional discomfort from taking part in the MLI group. Due to the nature of the MLI group sessions, there may be sensitive emotions or problems revealed. To decrease this risk, if needed, the study team will have a list of resources we can give to the participants should they experience discomfort.
- At the first group session, we will discuss the rules of the group session in order to minimize the risk of participation and maintain confidentiality. **

**Exceptions to the confidentiality rule include: 1) if child or elder abuse is revealed, the session leaders are required by law to report that to child or adult protective services; and, 2) if a participant indicates suicidal or homicidal thoughts (group leaders may disclose this information to protect the participants or others [in accordance with Texas law]).

Discomforts **<u>for either group</u>** associated with this research include:

- There is a minimal risk of mild pain or discomfort, bruising and swelling from the blood draw at the puncture site, or there may be dizziness or fainting. There is a small risk of infection. We will apply pressure with a sterile gauze dressing for several minutes to avoid bruising and infection and assist anyone if they are faint.
- A participant may get tired or bored when they are filling out the questionnaires. The study

team will make sure the participant does not have answer any question they do not want to answer.

- A participant may have mild emotional discomfort from answering the surveys.
- With research participation, there is a small risk of a loss of confidentiality. Research personnel will work hard to maintain participant confidentiality (privacy). We will do our best to maintain participant privacy throughout the time in the study.

2.2.2 KNOWN POTENTIAL BENEFITS

What are the potential benefits to taking part in this study?

- The MLI group will be facilitated by a nurse practitioner/licensed advanced practice nurse, who will be able to address anxieties or problems related to pregnancy and mental health
- Improved emotional health
- Improved coping
- Decreased stress
- Decreased depression and anxiety symptoms
- Improved relationships with the partner of the participant and/or people close to participant
- Improved sleep
- Benefit to other pregnant women in the future
- Lowered risk for preterm births as a result of improved emotional health in the MLI group

2.2.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Protections against Risk: There should be minimal risks from participating in the research study. The risks to the participants related to the effects of untreated depression and anxiety on the fetus are much greater than any of the risks delineated in participating in the study. A physical risk is that of bruising and /or pain from the venipuncture site. Pressure will be applied with a sterile gauze dressing for several minutes to avoid bruising. Study personnel will also make sure they use aseptic techniques to clean the skin before venipuncture, as well as ensuring the use of sterile needles. Women may have some emotional distress from answering the questionnaires. There is some risk for the participants to travel to the sites either by bus or by car, but this risk is no greater than they have for everyday activities that they travel to or risk to come to their prenatal appointment. In addition, the fact the sessions are facilitated by a certified NP/CNM gives an added benefit as she should be able to handle medical concerns that may arise. The group sessions will be conducted either on-site in the MLI office or via Zoom. The group sessions will be planned for maximum privacy if conducted in person.

To minimize any potential risks, we will use the following protocols:

Handling of Depression or Suicidality: Depression questionnaires will be completed at 14-20 weeks' gestation, at 21-27 weeks' gestation, and at 32-36 weeks' gestation for participants in either group. The Clinical Team Member (CTMB) will review the baseline score for the CES-D immediately on-site after the participant completes it and is received by CTMB. We will consider:

- If the score is $\geq$ 36 i.e., 36 or above, we will administer the Columbia- Suicide Severity Rating Scale (C-SSRS) and follow protocol for scoring (see attached protocol)
- The CTMB will also advise the prenatal care provider if the score on the CES-D is $\geq$ 36 so that they may make an immediate referral for treatment of severe depression.
- The CTMB will also complete an "Adverse Score Follow-up Care Report" to indicate the score, action taken, and any additional notes that might become important should reporting to the IRB become necessary. In the MLI group, the NP/CNM facilitator will monitor depression and suicidality while conducting the group therapy. In the UC group, the prenatal care provider will monitor women as is customary in their practice and follow the previously established standard of care.

Handling of Disclosure of Domestic Violence: Although not a focus of this study, it is possible that a woman would disclose interpersonal violence during or after a group session. Our initial curriculum packets contain resources and supports in the Houston community for women experiencing interpersonal violence. If domestic violence is revealed, the CTMB or NP/CNM will individually and discretely talk to the woman to see if she would like help developing a safety plan and will offer her a list of resources for domestic violence. The NP/CNM should have training from her academic classes and experience assessing for domestic violence from her professional practice. We will also include a refresher training during our team trainings for practitioners so that all team members know the current, active available resources for women experiencing interpersonal violence. We will encourage women to share their experiences with their prenatal care providers, and, if needed – will inform her care provider on her behalf and with her permission as noted on the Adverse Score Report Form.

Emotional Upset During or After Completing Questionnaires: Some of the items on the questionnaires may cause emotional upset, particularly as items may cause a participant to consider how she is feeling. If she is not feeling well and is more aware of her feelings during or after completing the questionnaire, the NP/CNM will discreetly take her to a private area to reassure her and support her. The group sessions should also provide an opportunity for others to offer support. The woman will be reminded that she can check in with her prenatal care provider, too. Although the risk for more severe emotional upset is possible, it is very unlikely and will be noted on our Adverse Score Report Form if it does occur. In our prior R01s using these questionnaires with 1000+ women this situation did not arise.

Biohazard Safety: Handling of Body Fluids and Universal Precautions: Only sterilized, disposable butterfly needles will be used for drawing blood. Participants will be instructed to let the CTMB know of any problems during the blood draw; however, procedures are such that the risks of problems occurring during blood draws are rare. Pressure will be applied at the venipuncture site to avoid bruising. The CTMB will monitor the participant for any problems after the blood draw. All persons handling blood or body fluids will employ strict universal precautions to avoid the spread of blood borne pathogens to themselves or others and use personal protective equipment. Blood will be transported in coolers by the CTMB that has had training in blood borne pathogens and universal precautions. There should be no legal risks in obtaining urine for cotinine checks since smoking is legal.

Loss of Confidentiality: Due to the nature of group sessions there may be sensitive emotions or problems revealed by the participants. At the first group session, we will review the rules for the sessions. The first rule is that what is said in the group stays in the group so that people are not afraid to share and help each other.

The only exception to this rule is if child abuse, elderly abuse, suicidal indications or homicidal thoughts are revealed. For child and elderly abuse, mandatory reporting will be done to child and elderly protective services by session leaders as required by law. For suicidal indications or homicidal thoughts session leaders may disclose information to prenatal providers in accordance with Texas law.

Confidentiality of participants will be assiduously protected. No names of individuals will be shared or published at any time. Each participant will be assigned a unique code number. Only the NP/CNM, and CTMBs will have access to the master list of participant names and code numbers with contact information. However, the PI and other investigators will make every effort to not examine this list as they need to be blinded as to groups for analysis purposes. The master list will remain under a protected password for the CTMB or NP/CNM to use for follow-up. The CTMB who will be drawing the blood will know the participant names and numbers of the blood samples and urine samples to ensure proper labeling of the biological samples and the questionnaires. Study personnel will remain blinded as to results until the study is completed. Staff at the physician office will not have access to any individual's questionnaire results, laboratory specimens, or data analysis results.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS	PUTATIVE MECHANISMS OF ACTION
Primary			
To determine the efficacy of the MLI in pregnant minority women to decrease depressive symptoms, anxiety, perceived and acculturative stress, and to improve coping, with psychological flexibility as a mediating factor and acculturation as a moderating factor, versus usual care **(UC),** from baseline (14-20 weeks' gestation) to end-of-treatment (20-26 weeks' gestation) and at a 6-week follow-up (32-36 weeks' gestation). This objective is to demonstrate efficacy.	The primary endpoints are depressive symptoms, anxiety, perceived and acculturative stress, and coping at the end of a six-week treatment at 20-26 weeks' gestation of pregnancy and at 32-36 weeks' gestation to see if the MLI group has improved scores as compared to usual care.	Primary antecedents are: depressive symptoms, anxiety, perceived and acculturative stress and coping. Psychological flexibility is a mediator and acculturation is a moderator for the antecedents.	We propose that the primary endpoints affect the neuroendocrine response of Corticotropin Releasing Hormone (CRH), progesterone and estriol in the maternal/fetal response.
Secondary			
To explore the effect of the MLI, versus a usual care group, on neuroendocrine risk factors of preterm birth (CRH, progesterone, and estriol) from baseline to end-of treatment.	Neuroendocrine responses (CRH, progesterone, and estriol) previously demonstrated as causally related to the antecedents	The neuroendocrine measures are potential causal mechanisms linking the changes in the antecedents to the improved infant health outcomes.	CRH, progesterone and estriol are all part of the maternal fetal responses to stress, anxiety, and depressive symptoms.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS	PUTATIVE MECHANISMS OF ACTION
	and to the infant health outcomes.		
Tertiary/Exploratory			
To explore the effect of the MLI on infant birth outcomes (gestational age, birthweight, NICU admission).	These are clinically important events for the infants in the study to show a possible treatment effect.	Health outcomes of the infants after usual care or after treatment with the intervention to further determine efficacy.	

4 STUDY DESIGN

4.1 OVERALL DESIGN

Hypothesis 1a: Participants in the 6-week MLI will have decreased depressive symptoms, anxiety, stress, and disengaged coping, and increased active coping compared to UC at the end of treatment and after 6 weeks.
Hypothesis 1b: The effects of MLI versus UC on depression, anxiety, stress, acculturative stress, and coping will be mediated by psychological flexibility and moderated by acculturation.
Hypothesis 2a: Compared to UC, MLI participants will have significantly lower mean levels of CRH over time, from baseline to the end of treatment.
Hypothesis 2b: Compared to UC, MLI participants will have significantly higher progesterone levels and lower estriol levels (resulting in higher progesterone/estriol ratios) over time, from baseline to the end of treatment.
Hypothesis 3: Compared to UC, infants of mothers in the MLI group will have longer gestational ages, greater birth weights, and fewer NICU admissions.

Design of Trial. Building on our data and findings from previous studies, we believe that testing the MLI in this phase 2 trial is both feasible and justified. The intervention is expected to lead to significant reductions in distressing psychological symptoms, with the potential to decrease the incidence of preterm births (PTBs). We propose conducting a parallel group, randomized

controlled trial (RCT) comparing the efficacy of the MLI to Usual Care (UC). This phase 2 trial will include psychological assessments at pre-intervention, post-intervention, and 6 weeks after the completion of the sessions to evaluate the effectiveness of the MLI compared to routine prenatal care. Our clinical team member (CTMB) will recruit eligible pregnant Latina and African American women, who are between 14- and 20-weeks' gestation, from the Greater Houston area. We chose this gestational age because it is late enough to minimize the risk of miscarriages, yet early enough to potentially mitigate biological responses associated with preterm birth. The MLI group sessions will be held weekly for 1-1/2 hours over a 6-week period, in person at the MLI office (Suite 303, 7580 Fannin Drive, Houston). Participants will have the option to attend makeup sessions if they are unable to attend the regularly scheduled ones. For those who cannot meet in person, sessions can be conducted via Zoom. Alternatively, we offer the option to meet via telehealth using an encrypted electronic platform to ensure the safety and privacy of the group. The MLI group sessions will be provided in both English and Spanish to meet participants' language needs. To minimize the risk of cross-group contamination, we will ensure that UC participants are scheduled at times when MLI group sessions are not in progress.

Random Assignment. The random assignment of participants to the groups was conducted using GraphPad software, which utilizes its built-in randomization tool. This process ensures that participants are randomly assigned to different groups, minimizing bias and providing a fair distribution across the study groups. If necessary, the randomization can be repeated using the same settings to maintain consistency. The assignment of all groups is securely stored in a database protected by the statistician, with groups numbered 1 to 42/45. This information will be shared with the CTMB and the research team on the day of randomization, once the process is complete. We considered two methods for random assignment: 1) randomly assigning individuals to either the MLI or UC groups, and 2) randomly assigning pre-formed groups to either the MLI or UC. We chose to randomize participants individually, ensuring that each person is independently assigned to either the MLI or UC group. This approach minimizes potential contamination across treatments by allowing the study to be conducted in a controlled manner, facilitating effective follow-up throughout the post-treatment period. We recognize that in order to form groups with sufficient participants, some individuals may need to wait briefly before beginning their group treatment, based on the date of randomization, which is scheduled to occur once a month. The CTMB, who is blinded to group assignments to avoid bias, conducts baseline intake assessments. After participants have been consented, completed their intake/baseline assessments, and had their biological measures, the CTMB will inform them of their group assignment and schedule them for either MLI or UC. Our first group began in September 2021, and we have adjusted session formats to accommodate participants, offering varying types of sessions based on their availability and needs. The randomized group assignments are securely stored in a restricted database, accessible only by the database manager or statistician, and remain confidential until the day of

randomization.

Number of study groups and duration of intervention. Considering the possibility of refusals and exclusions, we initially anticipated recruiting approximately 6 women every 6 weeks. In the first year, recruitment began in the fourth month after funding, with the first group randomized to the intervention or UC after 6 women were recruited. We successfully recruited 3 groups in the first year. For years 2-5, we propose forming multiple groups per year, with 6 to 8 women per group, aiming to recruit a total of 238 women over 5 years across both the MLI and UC groups. This estimate accounts for a ~20% attrition rate. To reach our enrollment target of 238 women, we estimate screening 378 women. Data collection for infant outcomes will occur 4-8 weeks after the final data collection at 32-36 weeks of gestation for the participants.

Rationale and Justification for Recruiting African American Women in the Study in Addition to Latinas. Since September 2021, we have been prescreening African American women at their first obstetrical visit using the same criteria applied to Latina women for inclusion in the study. It is critically important to include African American women in this intervention, as they exhibit higher levels of emotional distress compared to Latina women. By incorporating African American women into the study, we aim to ensure that each group retains at least 6 participants by initially recruiting 9-10 women. Given that 3-4 women typically drop out of the group sessions, expanding our recruitment pool is essential to meet the study's milestones. The consistently higher emotional distress scores among African American women underscore the need for MLI as a crucial component of their prenatal care.

The Intervention. Table 2 gives module content, processes, and goals for the MLI.

Table 2. MLI Content, Processes & Goals

#	Session Content	ACT/PST Processes	Goal of Topics
1	A. Impact of stress on physical and emotional wellbeing B. Strategies to deal with stress: Externalize, Visualize, Simplify, Acceptance C. Introduce values - Identify values in various life domains	Identify and manage stress Identify values	Defuse from stress to avoid biological responses Identification of valued life areas
2	A. Identify and describe barriers that impact problem-solving skills: negative feelings, negative thinking, feelings of hopelessness B. Defusion exercises: learn to look AT thoughts rather than FROM them. C. Introduce and practice two problem solving skills to live according to identified values D. Introduce "Stop, slow down, think and act" and rational problem solving	Identify barriers to solving problems Defusion	Understand barriers to achieving values Problem solving ways to get around the barriers

3	A. Introduce & practice generating alternative solutions to overcome barriers to reach value-related goals B. Strategies: a. Quality principle; b. Deferment principle; c. Getting unstuck	Committing to Action	Identification of obstacles to problem solving Define alternatives to solve problems
4	A. Predict Consequences of Alternatives; B. Generate Pros and Cons	Evaluation of solutions Psychological flexibility Acceptance	Determine the best solution; Possible reevaluation if no best solution
5	Develop an Action Plan, carry plan out; Monitor, evaluate, reward self	Commitment	Development of an action plan
6	Summary of content and skills & how to apply in one's life Note: Mindfulness exercises are at the end of each session		Review of content and plan to apply in everyday life

The techniques from Acceptance and Commitment Therapy (ACT) encourage individuals to experience difficult thoughts and feelings rather than avoid them. Paired with mindfulness, cognitive defusion helps women acknowledge troubling thoughts and feelings and observe them from a distance rather than accepting them as literal truths (e.g., "I can't handle having another baby."). The goal is to change the impact of distressing thoughts and feelings, rather than eliminating them, which may in turn alter biological responses in the body. Indeed, mindfulness alone has been associated with reduced distress (109) and changes in the brain (110). Problem-Solving Therapy (PST) techniques include making effective decisions, generating creative solutions, and identifying barriers to reaching one's goals. We made cultural modifications to the MLI, such as considering family roles and hierarchies, incorporating culturally relevant metaphors, and including mindfulness exercises, to make it more beneficial for pregnant Latinas. The intervention consists of six weekly sessions, starting between 14-20 weeks' gestation and continuing to 21-27 weeks' gestation, each lasting approximately 1 ½ hours. This session length aligns with other intervention studies using Cognitive Behavioral Therapy (CBT) during pregnancy and was supported by feedback from our pilot sessions. Participants in the MLI group will receive a handbook in either English or Spanish, containing space for reflection and activities to complete at home. We have also extensively refined our facilitator handbooks. Conducting the MLI in group settings is feasible, acceptable, and advantageous because it is cost-effective, fosters group support and social network building (113), and encourages positive role modeling (113). A recent meta-analysis (114) found no significant differences in effectiveness between individual and group formats for CBT.

Rationale for the use of NP or CNMs. Authors of systematic reviews (115,116) recommend the use of CNMs/NPs to improve maternal mental health. They argue that there is a great need for innovative ways to provide effective mental health interventions for pregnant women. NPs scope of practice includes the treatment of mental health (117). Interventions such as Creating Opportunities for Personal Empowerment (COPE) (118-121) (a 2nd generation CBT) have successfully used registered nurses as well as NPs as facilitators with good outcomes. NPs were

also effective in our pilot studies. *NPs/CNMs are ideal facilitators because of their knowledge of both physical and mental health aspects of pregnancy; a focus on both may reduce prematurity risks.* NPs or CNMs will facilitate the groups with participants.

Control or Usual Care (UC) Group. Participants in the UC group will receive standard, traditional individual prenatal care following the schedule of visits recommended by the American College of Obstetricians and Gynecologists. This group allows us to determine whether the MLI is more effective in reducing psychosocial risks compared to the usual information and advice provided as part of current prenatal care practices. If women in the UC group require mental health treatment, they will be provided with a list of resources, and their obstetrician will be notified.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The selection of usual care for the comparison group is based on the current baseline for prenatal care, i.e., no intervention for mental health, unless pathology is diagnosed and then treatment is referred. The emotional well-being of the participants is not routinely assessed in care, unless it is a simple screen for depression. Inclusion of a usual care group allows for evaluation of treatment outside of prenatal care (i.e., psychologist or psychiatrist), including use of medications. The purpose of the intervention is to ensure emotional wellbeing as part of the patient's prenatal care. As noted, we will avoid MLI group sessions when the usual care group is being seen to avoid contamination from one group to the other.

4.3 JUSTIFICATION FOR INTERVENTION

Please see section on study design for the justification for data collection related to infant outcomes and length and number and frequency of intervention contacts. The intervention was originally designed for the Hispanic culture, as described earlier, but has since been expanded to include African American women as well. To meet the minimum acceptable participation criteria, participants must attend at least 3 out of the 6 sessions. In our data analysis, we will account for the number of sessions attended.

4.4 END-OF-STUDY DEFINITION

A participant is considered to have completed the study if she has fulfilled the following criteria: completed the baseline (first) assessment, attended at least 3 intervention sessions (for those in the experimental group), and completed both the F1 (second) assessment and the F2 (third and final) 6-week follow-up assessment for either group.

The end of the study for a participant is defined as the completion of all required assessments for both the UC and MLI groups.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Inclusion criteria are: a) providing informed consent; b) ability to read and speak English or Spanish; c) pregnant at 14-20 weeks' gestation with one fetus, intrauterine pregnancy, gestational dating will be reviewed before enrollment via ultrasound administered per standard of care; d) self-identification as African American or Hispanic; e) age 18 to 45 years; and f) currently living in the U.S.; g) Medicaid or other government supported insurance or low income (less than $35,000 per year); h) women who score 10 or greater on the CES-D (possibility of mild depression) OR 5 or greater on the Generalized Anxiety Disorder-7 scale (GAD-7) (mild anxiety), OR greater than or = to 14 on the PSS (mild stress); i) willingness to adhere to the MLI regimen or usual care regimen. **Justification for prescreening scores of the participants:** The aim of this project is to both prevent and treat problems that Latina and Black pregnant women may have with their emotional health.

5.2 EXCLUSION CRITERIA

All individuals meeting any of the exclusion criteria at baseline will be excluded from study participation as noted below:

Exclusions after initial review of the electronic health record (EHR) are: a) major systemic infections such as HIV, hepatitis; b) <18 years of age or >45 years of age; c) enrollment in a prenatal program such as the Nurse Family Partnership; d) severe cognitive or psychiatric impairment per judgment of providers, that precludes cooperation with study protocol; e) inability to read English or Spanish. Women who develop GDM after enrollment in the study will remain in the study. Development of hypertension or preeclampsia, pyelonephritis, or GDM will be considered an effect modifier in analysis of infant outcomes. Current antidepressant use will not be exclusionary and will also be used as an effect modifier. Further rationale for the exclusion criteria is:

- Only singleton pregnancies are included as the mechanisms of PTB are thought to differ with multiples.
- We do not exclude participants based on language (English or Spanish) spoken. We expect heterogeneity in language spoken and expect that there may be women who use both languages. Our prior study findings indicate that the risk of PTB is greatest among English-speaking and bilingual women, although we believe that all women will receive benefit from the MLI.
- Girls <18 are excluded as the intervention is designed to improve emotional wellbeing and quality of life in mature adults; girls <18 may not be cognitively ready for the adult

intervention and may need an intervention tailored to their stage of development. Girls under 18 also have a different set of risks than women over 18 years of age.

- We will control the use of progesterone treatment statistically.
- Women who develop gestational diabetes after enrolling in the study will remain in the study.
- At enrollment or during the study, anyone who is prescribed antidepressant or anti-anxiety medications will be eligible for the study. We will make note of it and control it in analysis. If a participant is referred for individual therapy, we will obtain that data from the prenatal record and control for that in the analysis.

5.3 LIFESTYLE CONSIDERATIONS

N/A

5.4 SCREEN FAILURES

Our prescreening of the prenatal chart and the use of three different instruments to include participants that at least have mild anxiety, depression or stress should be sufficient to avoid the need for screening failures.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Sample and Setting: Dr. Chavez, based in the Texas Medical Center in Houston, sees approximately 40 new pregnant women each month. In our previous study conducted within this practice, 61% of the patients had scores of 10 or higher on the CES-D, indicating mild depression, while 30% had scores of 14 or higher, indicating moderate to severe depression. For this study, we will use the CES-D to prescreen participants for scores of 10 or up to 35, the GAD-7 for scores of 5 or greater (mild anxiety), and the Perceived Stress Scale (PSS) for scores of 14 or greater. Given the potential for refusals and exclusions, we conservatively estimate recruiting 6 women every 6 weeks. In the first year, recruitment began in the fourth month after funding, and we successfully recruited 3 groups of 6-9 women each. For years 2-5, we propose forming 8 groups per year, with 6-8 women per group, aiming to recruit a total of 238 women across both the MLI and UC groups over the 5-year period. This target accounts for an estimated 20% attrition rate, and we anticipate screening at least 378 women to achieve our enrollment goal. In the same obstetrical practice from our last study, 66% of the women spoke English, 24% were bilingual, and 10% spoke only Spanish. Participation in our previous study was high, with 80-85% of those approached gave a consent to participate in the study. We plan to offer the MLI in separate sessions to accommodate English speakers, bilingual English/Spanish speakers, and Spanish-only speakers.

Procedures for Recruitment, Consent, and Data Collection. Our clinical team member (CTMB) will coordinate with the staff of participating obstetricians to identify Latina and African American

pregnant women prior to, or withing the timeframe of 14-20 weeks at their first prenatal visit (as long as the women are 14-20 weeks at the time of randomization). This will be done by the staff giving out pre-screening questionnaires (the CES-D, GAD-7 and PSS) and an informative brochure to pregnant women within the appropriate gestational range. We will place flyers and posters as informational materials in the office and the restrooms. The CTMB will either be present at the practice site making the feasibility of ongoing screening easier, or they will be monitoring pre-screening scores remotely and be available by phone for any issues that arise. If the participant is interested in the study, the CTMB will review the exclusion/inclusion criteria for the participant and the scores for the CES-D, GAD-7 and PSS. The CTMB will plan to obtain informed consent as soon as possible, either in-person or via telephone consent. The CTMB will then schedule a time for the participant to come to the office and complete the baseline assessments and corresponding biological samples. To ensure blindness of the data, the CTMB will transfer the electronic data directly from the preprogrammed tablets to the REDCAP database, that is Internet based. Identification of which group the participants are in will be after 4-8 participants have consented. The CTMB will then let the participants know what group they are in.

Post-treatment data collection will occur immediately after the last MLI session or 21-27 weeks for the UC group, and again at the final 6-week follow-up between 32-36 weeks. If the participant does not come for a data collection visit, the CTMB will make every effort to contact them via approved contact methods to reschedule the appointment.

Rationale for the six-week follow-up data collection after treatment: We believe it is important to assess potential ongoing effects of the MLI to decrease psychological risks in the third trimester prior to delivery.

Processes for Each Group: The MLI office will serve as a place to host group sessions. Groups will run every week for 6 weeks with MLI sessions lasting about 90 minutes. The last session (Session 6) will require an additional 1.25 hours of time to accommodate completion of study questionnaires and biologic data collection (blood, urine). If the participant is attending the final session via Zoom, the CTMB will schedule a separate time, within the appropriate time frame, for them to come in and complete their study questionnaires and biological data collection. For the UC, the third data collection will require about 1.25 hours for data collection. The final data collection for both groups will require about the same amount of time.

The MLI office has comfortable chairs and available paid parking. Sessions will start at 14-20 weeks gestation and continue through 21-27 weeks gestation. A 14-20-week window will avoid women who may miscarry yet will be early enough in the 2nd trimester to enhance the probability of expected outcomes. Each session will be led by a NP facilitator or CNM (certified nurse midwife) trained to deliver the intervention.

Either a trained phlebotomist or nurse will data collect after session 6, performing venipuncture, testing urine specimens, and ensuring samples are in the -40-degree freezer as soon as possible. The same process for biologic samples and questionnaire data collection will be followed for both

groups. As much as possible, we will hold all sessions at the same time of day throughout the study to control diurnal variability. The phlebotomist and CTMB RN have been trained by the PI in the collection and safe storage/transport of biological samples. Samples will be stored in a -40-degree freezer at the MLI office. The serum samples designated for estriol and progesterone will either be kept at the MLI office to be ran by a trained CTMB or sent to the University of Texas Medical Branch (UTMB) to be ran by the staff there. The serum samples designated for CRH will be stored at the MLI office until it is appropriate to send them to Australia to be ran in the lab of Laureate Professor Roger Smith by him and his team.To avoid biased results, the CTMB will administer questionnaires using an electronic tablet device but will not participate directly with the groups. We will clarify questions only, not discuss answers. For infant health data, a CTMB, who also works for the participating obstetrician, will enter delivery data from the delivery records directly into an Excel database on an encrypted shared drive.

Retention and Missed Sessions: We will take inspiration from a protocol that resulted in 87% retention in prior studies (122): 1) prior to obtaining informed consent, the CTMB will read with the women a Commitment Pledge to complete all 6 MLI sessions and all 3 data collection appointments (122); 2) we will collect alternative contact information for each participant and conduct multiple reminder phone calls if needed (123); 3) the NP or CNM will report group attendance to the CTMB and data manager who will track attendance across the duration of the study and create a weekly and summative attendance/attrition report; 4) the PI will review attendance and attrition rates weekly; 5) the NP or CTMB will phone or text any participant who misses a session to encourage continuation and assist with issues related to attending the sessions; 6) incentives will be provided for the completion of data collection instruments at baseline, during treatment, and follow-up; and also 7) maintain positive working relationships with family members. To evaluate participants' satisfaction with the content and delivery of each session, Likert-type measures will be used unofficially after sessions 1 and 3 (to allow for real-time improvements to the intervention) and officially at the end of the session 6. We will use these evaluations to examine factors that may affect attrition, such as timing, place, and day of the week, as well as overall satisfaction with the program. The CTMB will coordinate with the NP or CNM to call participants that miss a session. The NP or CNM will review with them individually, or in a group format (depending on the number of participants who missed the session), the session over Zoom will use the content of the facilitator's manual. Although the make-up session will not have the benefit of the in-person group, it will at least allow the participant to keep up with the knowledge from the module. We will use data about missed sessions to assess dose response.
Although we have had a successful experience recruiting out of Dr Chavez's private practice, if our recruitment is delayed or we are not able to retain the participants as hoped, we are recruiting new physicians to have their patients participate in the study. This will allow a backup plan should we not meet milestones at the end of any year.

Once challenging participants are identified, the research team will engage in considerable brainstorming/problem-solving to ensure the completion of the intervention and any follow-up visits in a large diverse city such as Houston. We have a designated cellphone for participants to text or call to reschedule/schedule/confirm upcoming visits. The project cellphone will be covered by the CTMB during office hours. Project staff send messages or make calls in each participant's preferred medium (i.e., e-mail, text, or voice call). At baseline, via the IRB-approved consent form, we will obtain permission to contact participants via multiple methods (e.g., email, text, or voice call). These communication methods will begin within hours of a missed visit.
If the CTMB is not successful with engaging/reengaging a participant, then a senior member of the team, such as Dr Ruiz, will reach out to participants to problem-solve missed visits. Further, we may contact other family members or friends (i.e., "locators" identified during the consent process). To increase adherence, appropriate incentives (e.g., monetary) commensurate with participants' time and effort are planned for distribution. Research staff will make every effort to let participants know they are valued and appreciated. Staff will use non-judgmental language and try to help participants enjoy their time in the study as other methods to improve retention. Feedback from the pilot study has been incredibly positive related to acceptability and retention.

Participant Incentives: The CTMB will ensure that each participant receives payment via a reloadable Clincard after each data collection visit, even if a participant does not complete the entire study, and regardless of which group they are in. The participants will receive a reloadable Clincard that is refilled at these intervals: $60 after completing the baseline data collection visit (14-20 weeks of pregnancy), $70 at the follow-up 1 data collection visit (21-27 weeks of pregnancy) and $50 at the final follow-up 2 data collection visit (32-36 weeks of pregnancy). If a participant misses a data collection appointment, the CTMB will contact them as to when they can come in for make-up appointment. Therefore, for either usual care or MLI group, a participant may potentially receive a total of $180 for participating. The participants may also be eligible for parking reimbursement for MLI group sessions due to the cost of parking at the medical center.

6 STUDY INTERVENTION(S) OR EXPERIMENTAL MANIPULATION(S)

6.1 STUDY INTERVENTION(S) OR EXPERIMENTAL MANIPULATION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION OR EXPERIMENTAL MANIPULATION DESCRIPTION

Please see Table 2 under Study Design for session content and goals of the intervention **for the MLI.**

6.1.2 ADMINISTRATION AND/OR DOSING

We will hold six weekly group sessions starting at 14-20 weeks' gestation and ending at 21-27 weeks gestation. They will last ~1 ½ hours and will be at the MLI office lobby or delivered via Zoom. Our primary plan is that the participants will interact with each other face to face and the NP or CNM interventionist in the MLI group. We may have to use Zoom or another electronic delivery method as needed if they cannot attend in person. We consider 3 sessions to be the minimum for a full dose intervention. Six is the average number of sessions delivered in other intervention studies using CBT in pregnancy and was supported in feedback from our pilot sessions. Transportation will be provided if the participant requires assistance. We will give participants in the MLI group a participant handbook in either English or Spanish that has space for reflection and activities to complete at home. We have refined our facilitator handbooks extensively. Conducting the MLI with groups is feasible, acceptable, and advantageous as it is a) cost-effective, b) allows for group support and building a social network (113), and c) encourages good role modeling (114). A recent meta-analysis (114) found no significant differences in effectiveness in individual versus group formats for CBT.

6.2 FIDELITY

6.2.1 INTERVENTIONIST TRAINING AND TRACKING

Training and Intervention Fidelity. The NP or CNM training for the MLI was initially facilitated by Dr Villarreal (expert in ACT processes) and PI Ruiz. The sequential training elements were the following: a) The NP or CNM completed knowledge assessments pre-independent study and post-in-person training. b) 5-hours of independent study, including reading the MLI manual and relevant empirical literature, plus listening to audio recordings about different components of ACT or PST, c) experiential training on how to facilitate an MLI including ACT and PST strategies. A brief didactic review of the MLI model, a description of important teaching strategies, and an explanation of logistical considerations for an MLI group encompassed part of the training. The significant and lengthy portion of the training focused on the practice facilitation of MLI group sessions through role-plays, plus troubleshooting common problems that occur during groups with challenging group members, and d) 6-weeks of consultation in which the group facilitator implements the MLI for the first time, conducts group sessions, and does audio/video recordings of their group sessions. Dr Villarreal had initially reviewed the videotaped sessions and given feedback. The DNP that has been trained and conducted the sessions for the last three years is now helping the PI with the training of any new NPs or CNMs.

The PI has conducted education and training sessions for the CTMB and personnel at the clinical practice site about the study, eligibility, data collection, questionnaire administration, protocol for venipuncture and blood specimen management, and testing and storage of urine samples. Universal precautions were taught for safe sample handling. The PI has trained the CTMB to

collect data from the prenatal chart and hospital record and to evaluate the effectiveness and accuracy of data collection while ensuring blinding.
We will monitor and evaluate the fidelity of content delivery for MLI groups, conducted in person, or via Zoom. We currently have a psychiatric nurse practitioner that is assigned to evaluate the content delivery two times for every 6 sessions for fidelity. She will give feedback to the NP after the session. Thus, audiovisual recordings are important for quality control and are mandatory for the participants. Compliance with the protocol will be monitored using the MLI Fidelity Checklist (adherence scale).

Measures to Minimize Bias: Randomization and Blinding

Random Assignment. The random assignment of participants to groups was conducted using GraphPad software, which utilizes its built-in randomization tool to ensure that participants are randomly assigned, minimizing bias and ensuring a fair distribution across the study groups. All group assignments are securely stored in a database protected by the statistician, with groups numbered 1 to 42/45. This information will be shared with the CTMB and the research team on the day of randomization, once the process is complete. We considered two methods for random assignment: 1) assigning individuals randomly to either the MLI or UC groups, and 2) assigning pre-formed groups to either the MLI or UC. We chose to randomize participants individually, ensuring that each person is independently assigned to either the MLI or UC group. This approach minimizes potential contamination across treatments, allowing the study to proceed in a controlled manner and facilitating effective follow-up throughout the post-treatment period. To ensure adequate group sizes, some participants may need to wait briefly before beginning their group treatment, based on the date of randomization, which is scheduled to occur once a month. Our first group began in September 2021, following a 3-month period of training and startup, with session formats adjusted to accommodate both in-person and electronic delivery as needed. In this setup, the CTMB and the NP involved in the intervention, data collection, and follow-up for participants who miss sessions are aware of the group assignments. However, these personnel are strictly instructed not to share participant ID numbers with the PI or Co-Investigators unless explicitly approved by the PI. The statistician, who manages the randomization process, remains blinded to the specific participant numbers associated with each group and has no direct exposure to the individual participants. Laboratory samples are labeled with participant ID numbers and are blinded to the laboratory technician. The Principal Investigator (PI), Dr. Ruiz, will make every effort to remain blinded to group assignments to ensure unbiased data analysis. While the psychiatric NP (consultant) reviews videotapes of the intervention and is thus aware of who is in the intervention group, they are not informed of which participant numbers are associated with the data.

6.3 STUDY INTERVENTION/EXPERIMENTAL MANIPULATION ADHERENCE

The CTMB will initiate and maintain an Excel spreadsheet of all participants in the MLI group as well as the UC group. After each group session, the CTMB will check with the NP as to who the participants were and log them on the participation log. Mandatory is completion of the two times the questionnaires are to be given and the laboratory assessments for two different times. An active participant will need to have completed 3 MLI sessions if in the experimental group, both sets of questionnaires for two different data collections, and two sets of laboratory assessments.

6.4 CONCOMITANT THERAPY

N/A

6.4.1 RESCUE THERAPY

N/A

7. STUDY INTERVENTION/EXPERIMENTAL MANIPULATION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION/EXPERIMENTAL MANIPULATION

When a subject discontinues from the MLI group but not from the study, the participants will complete the remaining study procedures (i.e., completion of second round of questionnaires and laboratory assessment).

If a deleterious clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the PI, Dr Ruiz, will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected by the CTMB at the time of study intervention discontinuation will include the following:

- The reason(s) for discontinuing the participant from the intervention, and methods for determining the need to discontinue.
- If the participant is due to complete assessments within 2 weeks of being discontinued from the study intervention, those assessments will be administered at the time of discontinuation; if the next scheduled assessments are more than 2 weeks from the discontinuation date, the discontinued

participant will wait for the next scheduled assessment. Thereafter, the participant will be included in all future scheduled assessments, even though not participating in the intervention.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants have the right to withdraw from the study at any time upon request.
An investigator may discontinue a participant from the study for the following reasons:

- **Significant non-compliance** with the study intervention, such as attending fewer than 3 out of 6 sessions in the MLI group, or unwillingness to complete required questionnaires and laboratory assessments.
- **Lost to follow-up:** If the study staff is unable to contact the participant after multiple attempts (see Section 14.3, Lost to Follow-Up).
- **Medical events or conditions** that arise during the study, where continued participation may not be in the best interest of the participant or may require additional treatment that could interfere with the interpretation of the study results (e.g., severe depression requiring hospitalization).
- **Newly identified exclusion criteria** or conditions not previously recognized that disqualify the participant from further study participation.

The reason for any participant's discontinuation or withdrawal will be documented in the Case Report Form (CRF). Participants who sign the informed consent form and are randomized into either group will continue to be monitored in the "intent to treat" group unless they formally request in writing to be removed from the study.

7.3 LOST TO FOLLOW-UP

A participant is considered lost to follow-up if they fail to attend 3 sessions of the MLI, and study staff is unable to re-establish contact after at least three attempts.
The following steps will be taken if a participant misses a required study visit:

- The CTMB or Licensed Advanced Practice Nurse/NP will attempt to contact the participant and reschedule the missed visit within the following week, possibly offering the session via electronic means. They will also counsel the participant on the importance of adhering to the assigned visit schedule and assess whether the participant wishes to continue in the study.
- Before declaring a participant lost to follow-up, the CTMB will make every reasonable effort to re-establish contact, including making three telephone calls and, if necessary, sending a certified letter to the participant's last known address or using other locally appropriate methods. All contact attempts will be recorded in the participant's study file.
- If the participant remains unreachable, they will be considered withdrawn from the study, with "lost to follow-up" as the primary reason.

Attrition, it refers to the loss of participants during the course of a study, which can occur due to dropouts, withdrawal, loss to follow-up, or other reasons. The impact of attrition is significant because it can lead to reduced statistical power and potentially biased results if the attrition is not random.

Please see also Retention and Missed Visits

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 ENDPOINT AND OTHER NON-SAFETY ASSESSMENTS

See also Procedures for Recruitment, Consent and Data Collection

Table 3. Self-Report Questionnaires for Data Analysis

Measure	Purpose; Variable type	Times Measured Baseline =16-20 wks. Time 2 = 20-26 wks. Time 3 = 26-32 wks.	# Items	α	Spanish
MASII Multidimensional Acculturation Scale (127)	Cultural identity; Language proficiency: **Moderator**	Baseline	22	.78-.93	**Yes**
CESD (129)	Depressive symptoms **Primary outcome**	Prescreen, Baseline; Midtreatment (19-23 weeks) for MLI group only. Time 2 and 3	20	.85-.94	**Yes**
GAD-7 (130)	Anxiety; **Primary outcome**	Prescreen, Baseline, Time 2 and 3	7	.89	**Yes**
PSS Perceived Stress Scale (34)	Global measure of stress **Primary outcome**	Prescreen, Baseline, Time 2 and 3	10	.79	**Yes**
MASI Multidimensional Acculturative Stress Inventory (33)	Measure of acculturative stress **Primary outcome**	Baseline, Time 2 and 3	36	.93	**Yes**
The Brief Cope (131)	Positive versus negative coping; **Primary outcome**	Baseline, Time 2 and 3	28	.86-.89	**Yes**
Multidimensional Psychological Flexibility Inventory (MPFI) 132)	Psychological Flexibility **Mediator**	Baseline, Mid-treatment (19-23 weeks), Time 2 and 3	22	.97	**Yes**
Group Climate Questionnaire (133)	Measures if the group is engaged, conflictual, or avoiding interaction; **Covariate**	Time 2 taken by NP and participants	12	.9	N/A

D.10. Study variables. **Demographic variables** include age, marital status, education, insurance provider or self-pay, gravida, para, number of abortions, country of birth, years living in the US, primary language, residence in public housing, and number of times the participant has moved within the last year. Data on income, housing, mobility, and type of insurance will be used to assess socioeconomic status. This data will be collected by the CTMB after training by the PI.

We will not ask the participants if they have a social security number, to satisfy the concerns of the scientific review related to Human Subjects. **Obstetric variables**. A CTMB who has been professionally trained in obtaining obstetrical histories by working in an obstetrical setting, will collect obstetrical history, last menstrual period and ultrasound determination of expected date of confinement, height/weight for BMI, infections (vaginal or systemic), medications (particularly SSRI use or progesterone) and other prescription or illegal drug use, and any other medical risk factors for PTB (GDM, preeclampsia, history of PTB, etc.) from the prenatal chart. This data will be obtained only after a HIPPA consent has been signed seeking permission of review of the entire prenatal chart. The

HIPPA consent will be obtained with informed consent as it is completed at the initiation of the participation in the study.

Psychological and acculturation measures, listed in Table 3, will be scored as recommended by scales' authors. The team has used these questionnaires extensively. These measures have been tested in Spanish without problems or reliability issues. We will also ask women if they have been receiving any mental health therapy as well. The NP or CNM facilitator will assess group climate themselves, and for the participants, using the Group Climate Questionnaire (134) at follow-up 1. This questionnaire will be used as a covariate in analysis. **Hormones, cotinine**. We will collect all biological samples at baseline (14-20 weeks), follow-up 1 (21-27 weeks), and follow-up 2 (32-36 weeks). After drawing the blood, we will centrifuge the blood and separate into aliquots of plasma as done in our previous studies. The blood is stored in the laboratory at the MLI office in a -40-degree centigrade freezer. The serum samples designated for estriol and progesterone will either be kept at the MLI office to be ran by a trained CTMB or sent to the University of Texas Medical Branch (UTMB) to be ran by the staff there. The serum samples designated for CRH will be stored at the MLI office until it is appropriate to send them to Australia to be ran in the lab of Laureate Professor Roger Smith by him and his team. Progesterone and estriol will be run in batches with ELISAs. We will prepare plasma samples for CRH with Aprotinin 500 IU/ml added. We plan to analyze CRH by radioimmunoassay. Urine samples will be pre-screened for cotinine (with 1-Step Rapid Nicotine test) and THC (with 1-Step Rapid THC Marijuana test) for both groups at all data collection points. We have focused on testing the hormones at the timepoints mentioned as our previous results, as well as others, justify these critical time periods for the neuroendocrine response. We will follow the World Health Organization's Good Laboratory Practices (136).

Infant outcomes. Pregnancy duration will be recorded in completed weeks and days, and birthweight will be recorded in grams. We will collect data on any NICU admissions. In Ruiz-R01-NR07891, we collected data on ~200 babies without difficulty from Dr Chavez's delivery records. This data will include diagnoses for the infant, especially those linked to pregnancy complications. We will collect data on medically indicated PTB versus spontaneous PTB, as well as early term births (37-39 weeks). We will use gestational age as a continuous variable as longer gestation, even among term infants, benefits both cognitive and motor development (137). **Infant sex as a covariate of biological and infant outcomes.** Evidence indicates that male infant outcomes may be more affected by prenatal adversities than female infant outcomes (138), including a link between maternal prenatal depression and impaired neonatal motor behavior in male infants (139) and a link between prenatal maternal depression and increased anxiety seen in females (140).

8.2 SAFETY ASSESSMENTS

Please see section 9.9 for detailed discussion of safety assessments and protections.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS

Adverse Event: Any unfavorable and unintended sign associated with the MLI, regardless of whether it is considered related to the intervention. These include such things as revelations of child abuse, domestic violence. Adverse events also include "unanticipated problems" of any nature (e.g., psychological or social harm) and will be designated as unrelated, related, probably related, or possible related (see below).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS

Serious adverse event: Any adverse event that is fatal or life threatening, is permanently disabling, requires inpatient hospitalization, or results in a congenital anomaly or birth defect. Examples include suicidal attempts, homicidal attempts, drug overdoses.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

SEVERITY OF EVENT

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Life-threatening event: Any adverse event in which the subject is at immediate risk of death from the reaction as it occurs. This does not include a reaction that, if it were to occur in a more serious form, might cause death. Although unlikely for this behavioral intervention, an example of this might be a severe allergic reaction during data collection or while participating in the group sessions.

Unexpected event: Any adverse event that is not identified in nature, severity, or frequency in the investigator brochure, study protocol, consent form; or the event was more serious than anticipated. An example of an unexpected event would be a participant falling.

RELATIONSHIP TO STUDY INTERVENTION/EXPERIMENTAL MANIPULATION

Definitely Related: An adverse event that has a timely relationship to the administration of the investigational study procedure and follows a known pattern of response for which no alternative cause is present.

Probably Related: An adverse event that has a timely relationship to the administration of the investigational study procedure and follows a known pattern of response, but for which a potential alternative cause may be present.

Possibly Related: An adverse event that has a timely relationship to the administration of the investigational study procedure and follows no known pattern of response, but a potential alternative cause does not exist.

Unrelated: An adverse event for which there is evidence that it is related to a cause other than the study procedure; in general, no timely relationship to the administration of the procedure exists, or if so, the event does not follow a pattern of response and an alternative cause is present.

EXPECTEDNESS

An AE or suspected adverse reaction is considered "unexpected" if it is unlikely to occur in the study population, or it is unlikely to occur at the severity that has been observed. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study procedures.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during data collection visits, during a group session for participants of the MLI group, or during a prenatal care visit with the physician.

All AEs, not otherwise precluded per the protocol, will be captured on the Adverse Event Reporting Form as delineated in this section. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study procedures (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on the study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Documentation of onset and duration of each episode will be maintained for AEs characterized as intermittent.

The CTMB will record events with start dates occurring any time after informed consent is obtained until 7 days (for non-serious AEs) or 42 days (length of group sessions) (for SAEs) after the last day of study participation. At each data collection visit for the UC group, the CTMB will inquire about the occurrence of AE/SAEs. For the UC Group, events will be followed for outcome information by the prenatal care provider until resolution or stabilization. The NP/CNM will follow up with a MLI group member privately after a group session weekly, or as needed, for AEs. The following are the Adverse Event Reporting Forms for the study:

Participant ID # _____

Name: _____
Date: _____

Phone: _____

1. Reason for follow up on site after a session:

Verbal admission of <u>child abuse</u> during group session. (unsolicited response if present)

Time period: _____

NP discussed with participant. (enter summary of discussion)

Follow up:

____ Participant was notified of need for further follow up with child protective services
____ Documentation of referral (include who made the call, date and time of the call, who was contacted, and brief summary of information relayed):

3. Reason for follow up on-site after a session:

2. Verbal admission of <u>domestic violence</u> during group session. (unsolicited)

Time period: _____

Discussed with participant: (enter summary)

Follow up:
_____Participant was given resource list
_____Safety plan was generated
_____Participant refused assistance at this time

3.. Reason for followup on site either after a group session or after a data collection session:

Unsolicited comments that indicate an actual or potential adverse event (generally).

Discussed with participant (enter summary)

Follow up:

8.3.5 ADVERSE EVENT REPORTING

ADVERSE EVENT REPORTING:
Serious adverse events will be reported to the IRB and DSMB within 48 hours of learning of the event. Unexpected adverse events that are not serious, but may be associated with the intervention, will be reported to the IRB and NIH no later than 30 days after the event on an annual basis. After considering the symptoms, a decision will be made by the PI in conjunction with the DSMB, if indicated, whether to recommend that the participant stop the intervention and/or refer the participant for further evaluation. The PI is responsible for reporting adverse events to the DSMB within 48 hours of the occurrence. The DSMB will determine whether said events are related to the study. All adverse events, serious and non-serious, will be fully documented on the appropriate Adverse Event (AE) Reporting Form. For each adverse event, the investigator will provide the onset, duration, intensity, treatment, required, outcome and action taken. Serious adverse events are not expected to occur during this study.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

In consultation with the PI, the CTMB will be responsible for conducting an evaluation of a serious adverse event and shall report the results of such evaluation to the NIH and the Institutional Review Board (IRB) as soon as possible. This reporting should be no later than 10 working days after the investigator first learns of the event.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

N/A

8.3.8 EVENTS OF SPECIAL INTEREST

N/A

8.3.9 REPORTING OF PREGNANCY

N/A all participants are pregnant.

8.4UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS

Unanticipated Problems include problem situations that arise during the course of a study but are not directly related to study procedures.

This protocol uses the definition of Unanticipated Problems as defined by the Office for Human Research Protections (OHRP). OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents and (b) the characteristics of the participant population being studied.
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

These UPs may include loss of data or data collection documents and psychological distress related to the intervention or control condition (as described above).

8.4.2 UNANTICIPATED PROBLEMS REPORTING

A research team member will report any unanticipated problems (UPs) to the Data Coordinating Center (DCC) and to the principal investigator (PI) Dr Ruiz, who will report to the reviewing Western-Copernicus Group Institutional Review Board (WCG IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number
- A detailed description of the event, incident, experience, or outcome

- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP.
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DSMB/study sponsor/funding agency within 48 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DSMB/study sponsor/funding agency within 14 days of the investigator becoming aware of the problem
- All UPs will be reported to appropriate institutional officials, the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 30 days of the IRB's receipt of the report of the problem from the investigator]

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

N/A

9. STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary Endpoints to Test Efficacy:

- **Hypothesis 1a:** Participants in the 6-week MLI will have decreased depressive symptoms, anxiety, and stress, disengaged coping, and increased active coping compared to UC at end-of-treatment and after 6 weeks.
- **Hypothesis 1b:** The effects of MLI versus UC on depression, anxiety, stress, acculturative stress, and coping will be mediated via psychological flexibility and acculturation.

Secondary Endpoints to Test Biological Mechanisms of Efficacy and Health Outcome of Infants:

- **Hypothesis 2a:** Compared to UC, MLI participants will have significantly lower mean levels of CRH over time from baseline to end-of-treatment.
- **Hypothesis 2b**: Compared to UC, MLI participants will have significantly higher progesterone levels and lower estriol levels (higher progesterone/estriol ratios) over time from baseline to end-of-treatment.
- **Hypothesis 3:** As compared to UC, infants from mothers in the MLI group will have longer gestational age, greater birth weight, and fewer NICU admissions.

9.2 SAMPLE SIZE DETERMINATION

Table 1. **Power to detect given effect sizes assuming different rates of attrition.**

Sample Size	15% Attrition *N* = 200 (100/group)			20% Attrition *N* = 190 (95/group)			25% Attrition *N* = 180 (90/group)		
Power	81.1%	86.3%	91.6%	78.8%	84.5%	90.6%	75.4%	82.6%	88.6%
Effect Size	0.300	0.325	0.350	0.300	0.325	0.350	0.300	0.325	0.350

This study is designed to have sufficient power to test the primary hypothesis in Aim 1, with calculations conducted using k = 1000 Monte Carlo simulations under various conditions in SAS 9.4. Specifically, the power analysis focuses on detecting a statistically significant interaction between time and treatment group using generalized linear mixed models (GLMM). Table 1 outlines the power to detect effect sizes under the following conditions: (1) a range of plausible attrition rates (~15%, ~20%, and ~25%); (2) a total recruitment target of 238 women; (3) a correlation of r = .50 between consecutive observations (e.g., baseline to week 6, week 6 to week 12); (4) a correlation of r = .05 for all observations collected at the same time point; and (5) a significance level of $\alpha = .01$ (after applying the Bonferroni correction across five outcomes as described in Hypothesis 1). In practice, statistical significance will be assessed using the false discovery rate (FDR) to adjust for multiple comparisons, based on the observed p-values. The expected effect sizes align with those observed in previous research and pilot studies. For instance, our pilot study (n=15) showed effect sizes for anxiety ranging from d = -.37 to d = -1.5, and for depression from d = -.45 to d = -.76, which are consistent with findings in other studies utilizing ACT. For example, McCracken (108) found a Cohen's d = 0.58 for depression using the PHQ-9 in an RCT (n=73) following ACT for chronic pain, and Majumdar (102) reported a medium effect size for group ACT ($\eta 2 = .07$, or d = 0.55).

9.3 POPULATIONS FOR ANALYSES

Intention-to-treat analyses will be conducted using SPSS software. We will examine any missing data to determine whether it is missing at random. Since the proposed analyses can fully incorporate unbalanced data, all collected data will be utilized, even if some repeated measures are missing. Missing data will be managed through maximum likelihood estimation, explicit modeling of missingness, and/or multiple imputations, each of which is robust to data missing at random. We will also assess the sensitivity of the analyses to different patterns of missing data. To address multiple comparisons within families of tests, we will apply the false discovery rate (FDR) approach.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

All variables will be summarized via descriptive statistics. Categorical data will be summarized by frequency and percent, and continuous data will be summarized in terms of central tendency (i.e., mean/median) and dispersion (i.e., standard deviation). Inferential tests will rely on the statistical significance criterion $\alpha = .05$ (two-tailed). Hypothesis tests that find values $p \leq .05$ will be considered statistically significant. Covariates may potentially be included in statistical models; details of covariate inclusion are described below in Section 9.4.2. Modeling assumptions will be evaluated via inspection of residual plots and formal statistical tests. Violations of assumptions will be addressed as needed via by transformation, robust estimation coefficient scaling, stratification, or model respecification. Any hypothesis tests that are unable to satisfy statistical modeling assumptions will be reevaluated via equivalent non-parametric tests if possible.

9.4.2 ANALYSIS OF THE PRIMARY ENDPOINTS

Analyses will primarily rely on generalized linear mixed modeling (GLMM) to evaluate the relationships between predictors and outcomes. This flexible approach allows for modeling of normally or non-normally distributed outcomes, specified by distribution family and link function, as well as longitudinal or multilevel data through the inclusion of level-2 terms for participant ID. Nonlinear effects can also be modeled using spline or polynomial terms. The measurement scales for all study outcomes are described in Section 8.1.

For longitudinal analyses, each outcome will be modeled as a function of the treatment group, time, and the interaction between treatment group and time. If the interaction term is not statistically significant, the model will be simplified to include only the main effects. Follow-up tests of simple effects will be conducted to evaluate changes in each outcome over time within treatment groups (141, 142). Cross-sectional analyses at each time point will also use GLMM to model each outcome as a function of the treatment group. In all models, random intercepts will be included for participant ID and other multilevel data structures, such as participants within groups. Interaction effects will be described as significant or non-significant. If significant, the focus will be on the simple effects of time within each group; if non-significant, the focus will shift to models of main effects without the interaction. Results will be presented as parameter estimates with 95% confidence intervals for models that assess either main effects or within-group models of simple effects in the presence of a significant interaction. Graphical presentations will include plots of estimated marginal means of outcomes over time by group.

Mediation analyses will investigate the indirect effects of treatment on 6-week follow-up measures of depression, anxiety, stress, and coping through intervening (end-of-treatment) measures of psychological flexibility and acculturation. These analyses will utilize Structural Equation

Modeling/Path Analysis with MPlus (v. 8.4) to evaluate both direct and indirect effects of treatment on outcomes. Indirect effects will be estimated using the product coefficient method, with 95% confidence intervals constructed via bootstrap resampling.

Prior to hypothesis testing, relationships between baseline and demographic variables, treatment groups, and outcomes will be assessed using GLMM. Covariates that demonstrate relationships with both predictors and outcomes will be considered potential confounders, leading to the development of two models: one with and one without covariate adjustment. If covariate adjustment impacts inferences, both models will be reported; otherwise, the simpler model will be presented.

Specific covariates to be examined as potential confounders include age, pre-pregnancy body mass index, gravidity, positive cotinine, positive drug screens, fetal sex, group climate scores for MLI sessions, concurrent behavioral therapy, acculturation scores, country of birth, SSRI or progesterone use, obstetrical variables such as preeclampsia, gestational diabetes, history of preterm birth, and infections during the periods between and during data collection and delivery, as well as the frequency of intervention attendance. For the MLI group, the number and type of sessions, whether phone-based for missed sessions or in-person, will be examined as moderating dose-response effects of treatment.

Assumptions of statistical modeling will be evaluated via inspection of residual plots and formal statistical tests. Missing data will be handled through methods such as multiple imputation or maximum likelihood estimation, ensuring that all available data are utilized. Sensitivity analyses will be conducted to evaluate the robustness of results under different assumptions or data handling methods.

Finally, for a priori defined outcomes, a significance level of $\alpha = 0.05$ (two-tailed) will be applied. The false discovery rate (FDR) correction will be used to control for Type I error in any follow-up or post hoc tests.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The analytic details provided in Section 9.4.2. also apply to any endpoints that may be established *post hoc*.

9.4.4 SAFETY ANALYSES

N/A

9.4.5 BASELINE DESCRIPTIVE STATISTICS

N/A

9.4.6 PLANNED INTERIM ANALYSES

N/A

9.4.7 SUB-GROUP ANALYSES

If a given baseline characteristic is determined to influence the relationship between predictors and outcomes via the criteria for confounding described above, that characteristic will also be evaluated as a potential moderator of the time x treatment interaction; in essence providing a three-way interaction. Subgroup analyses will then evaluate the primary statistical models within each group, sample size permitting (model convergence may be an issue if group sizes end up being too small).

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

We do not plan to tabulate individual participant data.

9.4.9 EXPLORATORY ANALYSES

N/A

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks will be given to the participant and written documentation of informed consent will be completed prior to starting the study intervention. The following consent materials are submitted with this protocol:

a. Consent form to participate in the study
b. HIPPA form to release prenatal record and birth record

a. CONSENT FORM TO TAKE PART IN RESEARCH

RESEARCH SUBJECT CONSENT FORM

TITLE: Reductions in Biopsychosocial Risks for Pregnant Minority Women and Their Infants: The Mastery Lifestyle Intervention

PROTOCOL NO.: R01 HD101535-01A1
WCG IRB Protocol #20235111

SPONSOR: Microgen Laboratories LLC

INVESTIGATOR: Roberta Jeanne Ruiz, Ph.D., WHCNP-BC, FAAN
7580 Fannin, Suite 303
Houston, TX 77054
United States

STUDY-RELATED PHONE NUMBER(S): 713-383-7020

RESEARCH CONSENT SUMMARY

You are being asked for your consent to take part in a research study. This document provides a concise summary of this research. It describes the key information that we believe most people need to decide whether to take part in this research. Later sections of this document will provide all relevant details.

What should I know about this research?

Someone will explain this research to you.
Taking part in this research is voluntary. Whether you take part is up to you.
If you don't take part, it won't be held against you.
You can take part now and later drop out, and it won't be held against you
If you don't understand, ask questions.
Ask all the questions you want before you decide.

How long will I be in this research?

We expect that your taking part in this research will last until the birth of your baby.

Why is this research being done?

The purpose of this research is to determine the helpfulness of group counseling to reduce risks for you and your baby. These risks are related to stress, anxiety, and depression (how you feel).

The group counseling is called, "the Mastery Lifestyle Intervention" (MLI).

What happens to me if I agree to take part in this research?

If you decide to take part in this research study, the general procedures include:

- Getting assigned to the investigational MLI treatment group (the study treatment PLUS usual prenatal care) or the Usual Care (UC) group
- Filling out questionnaires about how you feel and how you handle change, 3 different times. You may skip any questions on the questionnaires that you do not want to answer.
- Having your blood drawn and urine collected, 3 separate times (baseline, follow-up 1, follow-up 2)

If you are assigned to the MLI treatment group, you will still receive usual prenatal care, but additional procedures include:

- Attending 6 group MLI sessions with a Nurse Practitioner (NP) or Certified Nurse Midwife (CNM) and other group participants. Group sessions will be videorecorded

Could being in this research hurt me?

Discomforts for the MLI group:

- There is a risk that the MLI may not be as good as current therapy for anxiety, depression, or stress.
- There is a risk that you could have some embarrassment, guilt, fear, sadness, or other psychological feelings from taking part in the MLI group because sensitive emotions or problems may be revealed. To decrease this risk, we have a list of resources we can give you should you have excessive discomfort.
- Your privacy is important and your participation in this study will be kept confidential. However, absolute confidentiality cannot be guaranteed. You will be assigned a study number with your name protected. At the first MLI session, we will talk about the rules to lower the risk of your being in the study in order to help keep your privacy. **

**Exceptions to the confidentiality rule include: 1) if you reveal child or elder abuse, the session leaders are required by law to report that to child or adult protective services; and 2) if you indicate suicidal or homicidal thoughts, we may disclose this information to your physician to protect you or others in accordance with Texas law.

Discomforts for either group (MLI or UC) include:

- There is a small risk of mild pain or discomfort, bruising and swelling from your blood drawn at the puncture site. It is possible that you might become dizzy or faint.

- There is a small risk of infection from the blood draw sites. We will use disinfectant to clean the area where we will draw blood and will use a sterile needle to reduce the risk of infection. We will apply pressure with sterile gauze to avoid bruising and infection and help you to avoid fainting.
- You may get tired or bored when we are asking you questions, or you are completing questionnaires. You do not have to answer any question you do not want to answer.
- You may have mild emotional discomfort from answering the surveys. We have a list of resources that we can provide if this discomfort continues.
- There is a small risk of a loss of confidentiality. We will work hard to keep your confidentiality. We will do our best to maintain your privacy throughout your time in the study.

This research is unlikely to harm your pregnancy or fetus in any way.

Will being in this research benefit me?
The most important benefits that you may expect from taking part in this research, particularly when in the MLI group, include:

- MLI group will be led by a Nurse Practitioner or Certified Nurse Midwife, who will be able to help address anxieties or problems related to pregnancy
- Improved emotional health
- Improved coping to stressors
- Decrease in stress, depression, and/or anxiety
- Decreased risk for preterm birth (birth before 37 completed weeks of pregnancy)
- Improved relationships between yourself and people close to you
- Improved sleep quality

Possible benefits to others include additional evidence-based information that may contribute to reducing perinatal depression, preterm births, and number of admissions to the neonatal intensive care unit (NICU).

What other choices do I have besides taking part in this research?
Instead of being in this research, you may choose to consult with your physician and ask for a referral to a licensed therapist. Another option is to decline participating (which you can do at any point) and continue receiving usual prenatal care.

What else should I know about this research?
Other information that may be important for you to consider so you can decide whether to take

part in this research is that the MLI group requires attendance to 6 weekly meetings, so barriers to your attendance (work conflicts, school conflicts, childcare conflicts, vacation, transportation, etc.) will need to be considered. The clinical research team members will work with you to accommodate the attendance conflicts, but early notice helps ensure accommodation.

MLI sessions will be videotaped to ensure quality and to confirm that the NP or CNM is running the session effectively. Recordings will be reviewed by members of the research team who are trained to review recordings and may include experts located at other research facilities also involved in this research. All study recordings will be kept in locked file cabinets and/or password protected computers with only study staff having access to them. Information from the recordings may be published or shared in study reports as a group to help describe the sessions and how they were conducted, but your name or other data that might reveal who you are will not be revealed in any reports or writings that may result from this study.

DETAILED RESEARCH CONSENT

You are being invited to take part in a research study. A person who takes part in a research study is called a research subject, or research participant.

What should I know about this research?

Someone will explain this research to you.
This form sums up that explanation.
Taking part in this research is voluntary. Whether you take part is up to you.
You can choose not to take part. There will be no penalty or loss of benefits to which you are otherwise entitled.
You can agree to take part and later change your mind. There will be no penalty or loss of benefits to which you are otherwise entitled.
If you don't understand, ask questions.
Ask all the questions you want before you decide.

Why is this research being done?

The purpose of this research is to determine the helpfulness of group counseling to reduce risks for you and your baby. These risks are related to stress, anxiety, and depression (how you feel). The group counseling is called, "the Mastery Lifestyle Intervention" (MLI). The potential for improved emotional health by being in the MLI group may lower the risk for preterm births, a known risk for your baby to be sick at birth.

About 238 subjects will take part in this research.

How long will I be in this research?
We expect that your taking part in this research will last until the birth of your baby.

For the UC group, there are three (3) visits for data collection, which may take about an 1–1 ½ hours each, for a total of 4 ½ hours.

For the MLI treatment group, there are an extra six (6) group visits that may take about 1 – 1 ½ hours each. This totals to about 6–9 hours for group sessions and 4 ½ hours for data collection until 32–36 weeks of your pregnancy.

What happens to me if I agree to take part in this research?
If you decide to take part in this research study, the general procedures include:

- Getting assigned to the MLI treatment group (the study treatment PLUS usual prenatal care) or the Usual Care (UC) group
- Filling out questionnaires about how you feel and how you handle change, 3 different times. You will be requested to fill out all the questionnaires three different times during your pregnancy (baseline, follow-up 1 and follow-up 2) and it will take about 90 minutes or less each time. You may skip any questions on the questionnaires that you do not want to answer.
- Having your blood drawn and urine collected, 3 separate times (baseline, follow-up 1, follow-up 2)

If you are assigned to the MLI treatment group, you will still receive usual prenatal care, but additional procedures include:

- Attending 6 group MLI sessions with a Nurse Practitioner (NP) or Certified Nurse Midwife (CNM) and other group participants. Group sessions will be videorecorded. All of your group sessions will be held at the same location. If you change OB/GYN providers, you will no longer be able to participate in the study and will not need to attend group sessions anymore. Your group session will be held at the following site:

  Microgen Laboratories LLC, Mastery Lifestyle Intervention
  7580 Fannin Street, Suite 303
  Houston, Texas 77054

You will be put into a study group by chance (like a coin toss). You have an equal (50 out of 100) chance of being placed in each group. You cannot choose your study group. You will be notified

of which group you are assigned to by a member of the research team.

The Usual Care (UC) group will receive standard prenatal care without attending the MLI sessions. The study will still collect biological data (blood, urine), psychological data (questionnaires), and birth and newborn outcome data from the UC group.

Activities of the study will occur from the time of consent until the birth of your baby. The timeline is as follows:

- Signing recruitment documents, including informed consent and patient rights to privacy and confidentiality (HIPAA)
- **Baseline data collection (14–20 weeks of pregnancy)** – Includes blood draw, urine sample and electronic questionnaires on a tablet. This step is completed for both groups.
- **MLI Treatment Group Start** – **This is only for the participants randomized to the MLI group.** MLI group sessions include participating in the MLI therapy content with an NP or CNM and other group participants. Groups will take place in a conference area at the Microgen Laboratories MLI location mentioned above.
- **1st Follow up data collection (21–27 weeks of pregnancy) –** Includes blood draw, urine sample, and electronic questionnaires on a tablet. This step is completed for both groups.
- **2nd Follow up data collection (32–36 weeks of pregnancy) –** Includes blood draw, urine sample and electronic questionnaires on a tablet. This step is completed for both groups.

What are my responsibilities if I take part in this research?

If you decide to take part in this research study, the general procedures include:

- Getting assigned to the MLI treatment group (the study treatment PLUS usual prenatal care) or the Usual Care (UC) group
- Filling out questionnaires about how you feel and how you handle change, 3 different times. You may skip any questions on the questionnaires that you do not want to answer.
- Having your blood drawn and urine collected, 3 separate times (baseline, follow-up 1, follow-up 2)

If you are assigned to the MLI treatment group, you will still receive usual prenatal care, but additional procedures include:

- Attending 6 group MLI sessions with a Nurse Practitioner (NP) or Certified Nurse Midwife (CNM) and other group participants. Group sessions will be videorecorded

If you take part in this research, you will be responsible to:

- Make sure members of the research team have current contact information to be able to

contact you (they will reach out to you when data collection times are coming up)

- Maintain appointment times for data collection and MLI sessions
- Disclose to members of the research team if you are currently receiving any other psychological therapy or psychiatric care
- Disclose to members of the research team any medications, if any, you are using or begin using
- Disclose to members of the research team any medical conditions you currently have or may develop throughout the pregnancy
- Describe any problems or concerns regarding the study to the investigator promptly
- Notify study team members if you cannot attend an appointment time or need to reschedule
- Immediately contact the investigator or immediately seek medical attention should you begin experiencing thoughts of hurting yourself, hurting others or killing yourself or others

Could being in this research hurt me?

Discomforts for the MLI group:

- There is a risk that the MLI may not be as good as current therapy for anxiety, depression, or stress.
- There is a risk that you could have some embarrassment, guilt, fear, sadness, or other psychological feelings from taking part in the MLI group because sensitive emotions or problems may be revealed. To decrease this risk, we have a list of resources we can give you should you have excessive discomfort.
- Your privacy is important and your participation in this study will be kept confidential. However, absolute confidentiality cannot be guaranteed. You will be assigned a study number with your name protected. At the first MLI session, we will talk about the rules to lower the risk of your being in the study in order to keep your privacy. **

**Exceptions to the confidentiality rule include: 1) if you reveal child or elder abuse, the session leaders are required by law to report that to child or adult protective services; and 2) if you indicate suicidal or homicidal thoughts, we may disclose this information to your physician to protect you or others in accordance with Texas law.

Discomforts for either group (MLI or UC) include:

- There is a small risk of mild pain or discomfort, bruising and swelling from your blood drawn at the puncture site. It is possible that you might become dizzy or faint.
- There is a small risk of infection from the blood draw sites. We will use disinfectant to clean the area where we will draw blood, and will use a sterile needle to reduce the risk of infection. We will apply pressure with sterile gauze to avoid bruising and infection and

help you to avoid fainting.

- You may get tired or bored when we are asking you questions, or you are completing questionnaires. You do not have to answer any question you do not want to answer.
- You may have mild emotional discomfort from answering the surveys. We have a list of resources that we can provided if this discomfort continues.
- There is a small risk of a loss of confidentiality. We will work hard to keep your confidentiality. We will do our best to maintain your privacy throughout your time in the study.

This research is unlikely to harm your pregnancy or fetus in any way.

Will it cost me money to take part in this research?
Taking part in this research requires no additional cost, only your time.

Parking fees accrued during data collection or MLI session visits will be reimbursed by the study.

Will being in this research benefit me?
We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits to you (particularly when in the MLI treatment group) include improved emotional health, improved coping to stressors, improved relationships with people close to you, improved sleep quality and a decrease in stress, depression, and/or anxiety. These benefits may continue even after the study ends. You may also experience a decreased risk for preterm birth (birth before 36 completed weeks of pregnancy), which may reduce risk of your baby being admitted to the neonatal intensive care unit (NICU).

Possible benefits to others include additional evidence-based information that may contribute to reducing perinatal depression, preterm births, and number of admissions to the NICU.

What other choices do I have besides taking part in this research?
Instead of being in this research, you may choose to consult with your physician and ask for a referral to a licensed therapist. Another option is to decline participating (which you can do at any point) and continue receiving usual prenatal care.

What happens to the information collected for this research?
Your private information and your medical record will be shared with individuals and organizations that conduct or watch over this research, including:

The research sponsor, Microgen Laboratories, LLC
People who work with the research sponsor

Government agencies, such as the National Institutes of Health (NIH)
WCG IRB, the Institutional Review Board (IRB) that reviewed this research
Companies engaged with Microgen Laboratories, LLC to commercialize the MLI

We may publish the results of this research. However, we will keep your name and other identifying information confidential.

We protect your information from disclosure to others to the extent required by law. We cannot promise complete secrecy.

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

Data or specimens collected in this research might be de-identified and used for future research or distributed to another investigator for future research without your consent.

Who can answer my questions about this research?
If you have questions, concerns, or complaints, or think this research has hurt you or made you sick, talk to the research team at the phone number listed on the first page.

This research is being overseen by WCG IRB. An IRB is a group of people who perform independent reviews of research studies. You may talk to them at 855-818-2289 or clientcare@wcgclinical.com if:

You have questions, concerns, or complaints that are not being answered by the research team.
You are not getting answers from the research team.
You cannot reach the research team.
You want to talk to someone else about the research.
You have questions about your rights as a research subject.

What if I am injured because of taking part in this research?
If you are injured or get sick because of being in this research study, call the study doctor immediately. Free treatment has not been arranged, and no other payment for injury is routinely available from the study doctor or sponsor. However, emergency treatment and professional services are available to you. You should call Dr. Roberta J. Ruiz at the study phone number, 713-383-7020, to tell her of your injury. You will not give up any of your legal rights by signing this consent form.

Can I be removed from this research without my approval?
The person in charge of this research can remove you from this research without your approval. Possible reasons for removal include:

- It is in your best interest
- You have a side effect that requires stopping the research
- You need a treatment not allowed in this research
- You have a miscarriage or induced abortion
- The research is canceled by the DHHS or the sponsor
- You are found to have an infection in your blood
- You are unable to keep your scheduled appointments
- You transfer your prenatal care to a different OB provider

We will tell you about any new information that may affect your health, welfare, or choice to stay in this research.

What happens if I agree to be in this research, but I change my mind later?
If you decide to leave this research, contact the research team so that the investigator can understand the purpose of your decision to stop participating. The investigator will also make sure you have received all the incentives that you have provided data for and update your OB physician that you have removed yourself from the study.

If you decide to leave the research early, there may be risks with this decision. This may include reduced ability to cope with stressors as well as you did while in the study, and you will not be able to receive incentive payments for data that was not provided for the study's use.

Will I be paid for taking part in this research?
For taking part in this research, you may be paid up to a total of $180.00. Your compensation will be broken down as follows:

- $60.00 after collection of the baseline data (collected at 14 – 20 weeks of pregnancy)
- $70.00 after collection of 1st follow-up data (collected at 21- 27 weeks of pregnancy)
- $50.00 after collection of 2nd follow-up data (collected at 32 – 36 weeks of pregnancy)

You will receive payments after each data collection visit, even if you do not complete the entire study. Participants in both groups, intervention and usual care, will receive payment.

If you drop out of the study before the next data collection, you will not receive incentives for the remainder of data collection visits

We will need your name and current address to receive your incentive payment. The incentive payments will be added to a reloadable *ClinCard* gift card and given to you. Every time you have complete data visits, your *ClinCard* gift card will be reloaded with the incentive amount for that visit.

Your specimens (even if identifiers are removed) will not be used for commercial profit.

Statement of Consent:

Your signature documents your consent to take part in this research.

Signature of adult subject capable of consent	Date
Signature of person obtaining consent	Date

My signature below documents that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject, and that consent was freely given by the subject.

Signature of witness to consent process	Date

b. HIPAA Privacy Authorization Form

**Authorization for Use or Disclosure of Protected Health Information (Required by the Health Insurance Portability and Accountability Act, 45 C.F.R. Parts 160 and 164) **

Participant Name: _____

Participant Date of Birth: _____/_____/_____

I give permission for my obstetrical healthcare provider to use and disclose (share) the protected health information described below to Roberta Jeanne Ruiz, PhD, WHCNP-BC, FAAN, Primary Investigator of this research project.

The study staff will get the medical information of you and your baby. For example: past and present medical records, research records, records of correspondence between you and your provider's office (as it pertains to this research), records of your prenatal and study visits, records of your outcome/birth and records of you baby's outcome/birth.

The study staff will share the records generated from this research, regulatory agencies such as DHHS (Department of Health and Human Services), and the IRB (Institutional Review Board).

This information is shared so the research can be conducted and properly monitored. The people receiving this information may be re-disclosed without your permission.

If you do not provide permission to use your information, you cannot be in the study.

The permission will not end unless you cancel it.

You may cancel your permission by sending written notice to the study staff on the first page of the main consent document.

Any information collected before you withdraw your permission may still be used.

This authorization for release of information covers the period of healthcare from: June 2021 to June 2026.

I authorize the release of my complete prenatal health record (including records relating to mental healthcare, communicable diseases, HIV or AIDS, and treatment of alcohol or drug abuse).

This authorization shall be in force and effect until the end of the research study (June 2026), at which time this authorization expires.

I understand that I have the right to revoke (cancel) this authorization, in writing, at any time. I understand that a revocation (cancellation) is not effective if any person or entity has already acted, relying upon this authorization.

I understand that my treatment, payment, enrollment, or eligibility for benefits will not be controlled by whether or not I sign this authorization.

I understand that the health information used in this research study as a result of this authorization may no longer be protected by federal or state law.

I understand that when giving my contact information (phone number and email address) I am consenting to the research personnel contacting me via these methods, including leaving voicemails on the provided phone number.

_____ _____ ____/____/____

Participant Name (Print)	Participant Name (Signature)	Date
_____	_____	____/____/____
Witness Name (Print)	Witness Name (Signature)	Date

CONSENT PROCEDURES AND DOCUMENTATION

Our plans for recruitment and consent were informed by results of our pilot work.

- Our CTMB will coordinate with the staff of participating obstetricians to have pre-screening questionnaires, and an informative brochure, handed to potential participants upon signing-in for their appointment. The brochure contains a phone number for our CTMB, who can be contacted during office hours to answer any questions the potential participant may have.
- The CTMB will either be in-person at the office of the participating obstetrician, or will be available remotely to answer questions and review eligibility information for potential participants. This will allow us to reach as many participants as possible.
- We will place flyers and posters as informational materials in the office and the restrooms.
- The CTMB will ensure the discussion of the study, determination of eligibility, and obtaining informed consent are done in a manner respectful of the potential participant's privacy. These conversations may happen in-person or over the phone.
- We will also ask participants to sign a Personal Health Information (HIPPA) release to examine their medical records, as well as those of their future infant. The participating obstetrician will also allow a CTMB (from either the MLI office or their office) to collect data from the enrolled participants' prenatal records (after participants have consented) that are relevant to the medical confounders to be controlled for in the study. They will collect data from the prenatal records and the birth records, and will be able to link the participant's release to their study identification number for data entry purposes.

Processes for Each Group.

One group will run at a time, either MLI or UC. The MLI groups are given at the MLI office. Groups will run for 6 weeks with MLI sessions lasting about 90 minutes. A separate Zoom session will be conducted in Spanish with a fluent Spanish speaking NP or CNM throughout all sessions. This is because there is a much smaller group of Spanish speakers and it would be difficult to integrate them with the English speakers. The last session (Session 6) will require an additional 1.25 hours of time to accommodate completion of study questionnaires and biologic data collection (blood, urine). For the UC, the second data collection will require about 1.25 hours for data collection. The final data collection for both groups will require about an hour or so of time.

The office for the MLI has comfortable chairs and couches and available paid parking. Sessions will start at 14-20 weeks' gestation and continue through 21-27 weeks' gestation. A 14-20-week window will avoid women who may miscarry yet will be early enough in the 2nd trimester to enhance the probability of expected outcomes. Each session will be led by an NP or CNM facilitator trained to deliver the intervention.

Either a trained phlebotomist or nurse will data collect at Session 6 and will perform venipuncture and test urine specimens. As much as possible, we will hold all sessions at the same time of day throughout the study to control for diurnal variability. The CTMB has been trained by the PI in the collection and safe storage/transport of biological samples. All specimens will be kept in a -40-degree freezer at the MLI office laboratory. To avoid biased results, the CTMB will administer questionnaires using an electronic tablet device but will not participate directly with the groups. We will clarify questions only, not discuss answers. For infant health data, the CTMB will enter delivery data from the hospital charts directly into an encrypted Excel database that will be then used for the statistical database. The same process for biological samples and questionnaire data collection will be followed for both groups.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, NICHD, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and NICHD and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance of study staff to the protocol (i.e., significant protocol violations)
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met.
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the funding agency, sponsor, IRB, or other relevant regulatory or oversight bodies (OHRP, DSMB).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, the safety and oversight monitor(s), and the sponsor(s) and funding agency. This confidentiality is extended to the data being collected as part of this study. Data that could be used to identify a specific study participant will be held in strict confidence within the research team. No personally identifiable information from the study will be released to any unauthorized third party without prior written approval of the sponsor/funding agency.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor or funding agency, representatives of the Institutional Review Board (IRB), regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor/funding agency requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored on an encrypted shared drive. The PI and Co-Is will be able to log and access the data. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

Measures Taken to Ensure Confidentiality of Data Shared per the NIH Data Sharing Policies
It is NIH policy that the results and accomplishments of the activities that it funds should be made available to the public (see *https://grants.nih.gov/policy/sharing.htm*). The PI will ensure all mechanisms used to share data will include proper plans and safeguards for the protection of privacy, confidentiality, and security for data dissemination and reuse (e.g., all data will be

thoroughly de-identified and will not be traceable to a specific study participant). Plans for archiving and long-term preservation of the data will be implemented, as appropriate.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored on a shared drive. After the study is completed, the de-identified, archived data will be transmitted to and stored at Microgen Laboratories, for use by other researchers including those outside of the study.

With the participant's approval, and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at Microgen Laboratories. These samples could be used to research the causes of premature birth, its complications and other conditions for which individuals with reactivated viruses such as Epstein Barr Virus and Cytomegalovirus are at potential increased risk, and to improve treatment. Microgen Laboratories will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio sample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through Microgen Laboratories.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Institutional Monitor**
Roberta Jeanne Ruiz, PhD, Senior Clinical Health Research Scientist	*Raymond Stowe, PhD, CEO of the Microgen Laboratories*
Microgen Laboratories, LLC	*Microgen Laboratories, LLC*
7580 Fannin Street Suite 303 Houston, TX	*903 Texas Avenue La Marque Tx*
281-300-5265	*409-935-6700*
jruiz@microgenlabs.com	*rpstowe@microgenlabs.com*

Direct Report	Team Member	Responsibilities
NIH	PI Ruiz	Oversight of all team members and training for human subjects including but not limited to rigor with data collection; fidelity of the intervention; ensure use of decision algorithm for referral of women reporting violence or depression; Ensure attainment of milestones; and overall quality of investigation. Prepares IRB application and monitors for any adverse events. Prepares progress report for NICHD.
PI Ruiz	Consultant: Dr. Misty Richmond PMHNP	Oversee MLI groups for effectiveness and any problems with group sessions; Interventionist initial training for ACT and PST and group dynamics
PI Ruiz	Interventionist training: Dr. Liza Rivera, PI Ruiz	Serves as a role model for group sessions
PI Ruiz	Co-I Dr. Darpan Patel	Oversee analysis of laboratory tests for blood and urine-ensuring quality results, Available as back up for running ELISA samples as needed and ensures safe laboratory practices.
PI Ruiz	CTMB/ Clinical Research Nurse Coordinator: Amanda Thomas (RN, BSN) Ester Godbold (RN, BSN)	Assists Dr Ruiz in operationalization of the aims by overseeing immediate data and sample collection with the CRA, data collection for questionnaires in absence of the CRA; venipuncture and collection of urine samples as needed; assuring planning of groups with CRA after randomization, assists NPs with supplies and needed preparation for groups sessions; monitors a weekly report of

		recruitment and retention of participants. Assists with progress reports and IRB updates. Laboratory: Organize and store biological samples, prepares samples and sets up samples needing transport to lab; Conduct ELISA analysis of progesterone, estriol and cotinine. Ester serves as a consultant for ensuring research integrity and protection of human subjects.
PI Ruiz & PC	CTMB/Clinical Research Associate: Irma Pecina	Recruitment; Informed Consent; Assign each patient with unique de-identified ID number; Tracks incentives; monies used for mileage, parking, and taxis; Assists in setting up group sessions; Assists with solving transportation problems and attendance problems for data collection if needed
PI Ruiz	Data Manager/ Statistician onsite analysis Devanshi Majeethia, MS, BDS	Data: Set-up database; ensures randomization is blinded during recruitment and allocation of groups after all participants are in a group; monitor missing data; Review data with PI for accuracy, integrity, and completeness on a weekly basis.
PI Ruiz	Co-I Dr. Rita Pickler	Setup data collection for nuances related to infant outcomes; Review infant health data monthly after participants deliver; Consultant regarding expert advice surrounding randomized clinical trials
PI Ruiz	NPs Dr. Liza Rivera	Conduct group sessions for the intervention either electronically or in person.
PI Ruiz	CTMB	Trained phlebotomist responsible for obtaining biological samples (blood,

	Laura Guzman	urine) from participants. Aids in preparation and appropriate storage of samples post collection.
PI Ruiz	CTMB Chelsea Rodriguez	Staff member of a participating obstetrician, accessing participant's medical records to obtain relevant information for study use.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB)/Safety Monitoring Committee (SMC). Members of the DSMB will be independent from the study conduct and free of conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data from each arm of the study. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to NICHD.

10.1.7 CLINICAL MONITORING

The PI will monitor data accuracy and quality on the shared drive at a minimum every other week, as well as monitoring the training for all new staff. The PI will verify data with the data manager, comprehensively.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Our Co-Investigators will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. All sites will follow a common quality management plan.
Quality control (QC) procedures will be implemented as follows:

Informed consent --- Study staff will review both the documentation of the consenting process as well as a percentage of the completed consent documents. This review will evaluate compliance with GCP, accuracy, and completeness. Feedback will be provided to the study team to ensure proper consenting procedures are followed.

Source documents and the electronic data --- Data will be initially captured on source documents via electronic tablets (see **Section 10.1.9, Data Handling and Record Keeping**) that

will ultimately be entered into the study database. To ensure accuracy site staff will compare a representative sample of source data against the database, targeting key data points in that review.

Intervention Fidelity — Consistent delivery of the study interventions will be monitored throughout the intervention phase of the study. Procedures for ensuring fidelity of intervention delivery are described in **Section 0, Interventionist Training and Tracking**.

Protocol Deviations – The study team will review protocol deviations on an ongoing basis and will implement corrective actions when the quantity or nature of deviations is deemed to be at a level of concern.

Should independent monitoring become necessary, the PI will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor/funding agency, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection will be the responsibility of the CTMB, and the NP Interventionist at the clinical obstetrical site. The PI will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents will be completed in a neat, legible manner (i.e., consent forms, HIPPA forms) to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant consented/enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents will be consistent with the data recorded on the source documents.
Clinical data (including adverse events (AEs) and expected adverse reactions data) and clinical laboratory data will be entered into an Excel database and then exported into SPSS. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

STUDY RECORDS RETENTION

Study documents will be retained for a minimum of 7 years after the last approval of a marketing application in an International Council on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These

documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor/funding agency, if applicable. It is the responsibility of the sponsor/funding agency to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS AND PUBLICATION AND DATA SHARING POLICY

This protocol defines a protocol deviation as any noncompliance with the clinical trial protocol, International Council on Harmonization Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions will be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- Section 4.5 Compliance with Protocol, subsections 4.5.1, 4.5.2, and 4.5.3
- Section 5.1 Quality Assurance and Quality Control, subsection 5.1.1
- Section 5.20 Noncompliance, subsections 5.20.1, and 5.20.2.

It will be the responsibility of the PI to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation. All deviations will be addressed in study source documents, reported to NICHD Program Official. Protocol deviations will be sent to the reviewing Institutional Review Board (IRB) per their policies. The PI will be responsible for knowing and adhering to the reviewing IRB requirements.

Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other

researchers 10 years after the completion of the primary endpoint by contacting Dr Ruiz, PI. Considerations for ensuring confidentiality of these shared data are described in Section 10.1.3.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NICHD has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS AND SPECIAL TERMS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list). Special terms are those terms used in a specific way in the protocol. For instance, if the protocol has therapist-participants and patient-participants, those terms could be included here for purposes of consistency and specificity.

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form

CTMB	Clinical Team Member
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Council on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee

SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2	8-11-21	Updated Consent and minor edits	Most current consent is needed for the protocol
3	2-22-22	Minor updates and updated consent	Current edits are made to reflect changes needed as the study grew larger.
4	10-29-2023	Updated changes in procedures and data management Addition of African American women to the study Lowered target sample from 234 to 221	Organizational changes have been necessary High pre-screen scoring on questionnaires, need for larger recruitment
5	8-27-2024	Formatting changes Grammar corrections Clarified date ranges for milestones Changed reimbursement schedule, amounts and method Changes to recruitment process Changes to schema Changes in wording Revisions to suicide protocol Added debarment process Updated involved personnel Increased total number of participants Added definition of attrition Added more methods for analyzing data Corrected randomization process	Corrected table of contents, section numbering, added page numbers Removed midpoint, redistributed milestone payments (total amount unchanged) To reflect current process Updated to better care for participants Per instructions from Microgen Laboratories, LLC Updated current CTMBs and removed Dr. Fagundes To account for an attrition rate of 20% To increase the scope of the study

5.1	09-16-2024	Removal of debarment language throughout protocol	Removed as required per WCG IRB SOP 21
5.1	09-16-2021	Removal of research cell phone number 713-330-9311	Cell phone number no longer in use
5.1	09-16-2024	Formatting correction	Corrected field control error to table of contents

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