Protocol Title: The effects of an estrogen receptor (ER) beta agonist (Lilly Compound LY500307) on estradiol-withdrawal-induced mood symptoms in women with past perimenopausal depression.
Abbreviated Title: Estrogen receptor beta and mood
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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff: None

Total requested accrual:
80 Women reporting a past depression during the perimenopause to be screened

Project Uses Ionizing Radiation: ☒ No ☐ Yes (attach *RSC/RDSC documentation)*
☐ Medically-indicated only
☐ Research-related only
☐ Both

IND/IDE ☐ No ☒ Yes *(attach FDA documentation)*
Drug/Device/# IND 128256
Sponsor: NIMH (Dr. Maryland Pao is the official representing the Institute.)

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes
Institution_____FWA #_____

Date of IRB approval_____*(attach IRB documentation)*

Data and Safety Monitoring Board ☐ No ☒ Yes

Technology Transfer Agreement ☐ No ☒ Yes
Agreement type and number _Clinical Trial Agreement 2015-0279
Expiration Date_None_

Confidential Disclosure Agreement ☐ No ☒ Yes

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form: 9.7

Précis

A. Objective

During the perimenopause, the incidence of depression increases [1-5] and predicts increased all-cause and cardiovascular mortality [6]. A role of estradiol withdrawal in the onset of mood disorders in some perimenopausal women has been suggested indirectly by estradiol's antidepressant efficacy and safety in perimenopausal depression [7;8]. Moreover, observational studies report the emergence of depressive symptoms after the discontinuation of menopausal hormone therapy (HT) in 5-10% of women [9-11]. The coincidence of declining ovarian function with the onset of depression led to the inference that "withdrawal" from physiologic estradiol levels underpinned depression during the perimenopause. To test this inference, we undertook a study to examine the role of estradiol withdrawal in perimenopausal depression. We evaluated the effects of the acute withdrawal of estradiol therapy in postmenopausal women with and those without a past perimenopausal depression. Results demonstrated that estradiol withdrawal induces depressive symptoms in women with a past perimenopausal depression, but not in those without such a history [12]. This study was the first to provide direct evidence that estradiol withdrawal is the relevant physiologic trigger for depressive symptoms in women with this condition. In women with past perimenopausal depression, the recurrence of depressive symptoms during blinded hormone withdrawal suggests that normal changes in ovarian estradiol secretion can trigger an abnormal behavioral state in these susceptible women. These data also suggest that the effects of estradiol withdrawal are processed differently in some women, presumably by altering the brain network composition or activity that underlies affective state. In this next protocol, we will examine a possible mechanism mediating the effects of estradiol-withdrawal on mood symptoms in asymptomatic postmenopausal women with a past

perimenopausal depression. We propose to evaluate the efficacy and safety of a selective estrogen receptor (ER) beta agonist (Lilly Compound LY500307) to prevent estradiol withdrawal-induced mood symptoms. The effects of estradiol primarily occur through activation of two receptor subtypes, often with opposing outcomes: estrogen receptor (ER) alpha, and ER beta. We focus on ER beta because the beta estrogen receptor is reported to mediate the effects of estradiol on the serotonergic system and mediate the antidepressant-like effects of estradiol in the forced-swim test [13-16]. Moreover, selective agonists of estrogen receptor beta have been demonstrated to attenuate the behavioral and hypothalamic-pituitary- adrenal (HPA) axis response to stress [17-19]in animal studies. We propose to employ the selective estrogen receptor agonist LY500307 under double-blind, placebo controlled conditions to examine the specific role of estrogen receptor beta in the effects of estrogen withdrawal in women with a past perimenopause-related depression. Depressive symptoms will be measured with standardized ratings scales (i.e., Center for Epidemiologic Studies Depression scale (CES-D) and 17-item Hamilton Rating Scale of Depression (HRSD)). We will also generate patient-derived LCLs and IPSCs from the women participating in this protocol to investigate both intrinsic cellular differences between women with PMD and controls as well as examining the effects of estradiol withdrawal with and without invitro exposures to an ER beta agonist. Additionally, we will perform whole exome (WES) and whole transcriptome (WTS) sequencing (and possibly whole genome sequencing (WGS) under this protocol in the future). Results of this study will determine the role of ER beta in estradiol withdrawal-induced mood symptoms and can provide preliminary data to support the efficacy and safety of this compound as a treatment for depression during the perimenopausal transition.

B. Study Population

We propose to employ the selective estrogen receptor agonist LY500307 under double-blind, placebo-controlled conditions to examine the specific role of estrogen receptor beta in the effects of estrogen withdrawal in women with a past perimenopause-related depression. Depressive symptoms will be measured with standardized ratings scales (i.e., Center for Epidemiologic Studies Depression scale (CES-D) and 17-item Hamilton Rating Scale of Depression (HRSD)). Results of this study will determine the role of ER beta in estradiol withdrawal-induced mood symptoms and can provide preliminary data to support the efficacy and safety of this compound as a treatment for depression during the perimenopausal transition.

C. Design

We propose to employ the selective estrogen receptor agonist LY500307 under double-blind, placebo-controlled conditions to examine the specific role of estrogen receptor beta in the effects of estrogen withdrawal in women with a past perimenopause-related depression. Depressive symptoms will be measured with standardized ratings scales (i.e., Center for Epidemiologic Studies Depression scale (CES-D) and 17-item Hamilton Rating Scale of Depression (HRSD)). Results of this study will determine the role of ER beta in estradiol withdrawal-induced mood symptoms and can provide preliminary data to support the efficacy and safety of this compound as a treatment for depression during the perimenopausal transition.

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List of Abbreviations:
ET – estradiol therapy
E2 – estradiol
HT – menopausal hormone therapy
ER – estrogen receptor
CES-D – Center for Epidemiologic Studies Depression scale
HRSD – Hamilton Rating Scale of Depression

1. Introduction

Background:

Depression risk increases during the perimenopause, and depression is cited as a primary reason for resuming menopausal hormone therapy (HT) [20-22]. Community-based epidemiologic studies document a 1.5-3 fold greater risk of first onset and recurrent depressions in women during the perimenopause compared with those who are premenopausal (or who are several years postmenopausal) [1-4;23]. Observational studies report the emergence of depressive symptoms after the discontinuation of HT in 5-10% of women [9-11].

The role of estradiol (E2) - either declining or low levels - in the precipitation of perimenopausal depression (PMD) is unknown, largely due to the associational and indirect nature of the evidence linking ovarian function and depression. Correlative studies with plasma FSH or response to hormonal therapy [4;7;8;24;25] do provide indirect evidence of the relevance of changes in reproductive hormones to mood disturbances. However, even prospective epidemiological studies cannot test the estradiol withdrawal hypothesis since perimenopausal changes in the secretion of several hormonal and metabolic factors could confound the effects of estradiol withdrawal. In other studies of the role of ovarian steroids in affective disturbance, changes in ovarian steroids (in the context of otherwise normal levels) have been shown to directly trigger depression, but only in a subset of women with histories of mood disorders linked to reproductive function [26;27]. In subsequent work (protocol 03-M-0175), we directly examined the hypothesis that declining ovarian function – estradiol "withdrawal" - underpins depression occurring during the perimenopause. In this recently completed study, asymptomatic, postmenopausal women who experienced a depression during the menopause transition and asymptomatic postmenopausal women with no past depression received a standard dose of estradiol and then were withdrawn from estradiol under double-blind, placebo-controlled conditions.

Results demonstrated that estradiol withdrawal induces depressive symptoms in women with a past perimenopausal depression (n = 26), but not in those without such a history (n = 30). In women with a past depression during the perimenopause, estradiol withdrawal was associated with a significant increase in depressive symptoms (as measured with standardized depression rating scales including the Hamilton Rating Scale of Depression (HRSD) and the Center for Epidemiologic Studies Depression scale (CES-D) ($p < 0.05$, $p < 0.001$, respectively) compared with those women who were maintained on estradiol therapy under double-blind conditions. Additionally, no significant depressive symptoms emerged in the women lacking a history of depression who were either withdrawn or maintained on estradiol therapy. The severity of vasomotor symptoms did not differ significantly between estradiol and placebo conditions in either group of women [12]. This study is the first to provide direct evidence that estradiol withdrawal is the relevant physiologic trigger for depressive symptoms in women with perimenopausal depression. These data also suggest that the effects of estradiol withdrawal are processed differently in some women, presumably by altering the brain network composition or activity that underlies regulation of affective state.

Identification of the mechanisms underlying the affect-modulating effects of estradiol withdrawal will potentially uncover novel treatments with either superior therapeutic benefits or more acceptable long-term safety profiles than those of estradiol therapy. Of note, the effects of estradiol primarily occur through activation of two receptor subtypes, often with opposing outcomes: estrogen receptor (ER) alpha, and ER beta. In this current protocol, therefore, we will examine the ability of a selective ER beta agonist (LY500307) to prevent estradiol withdrawal-induced mood symptoms in women with past perimenopausal depression. We focus on ER beta because the beta estrogen receptor is reported to mediate the effects of estradiol on the serotonergic

system and mediate the antidepressant-like effects of estradiol in the forced-swim test [13-16]. Moreover, selective agonists of estrogen receptor beta have been demonstrated to attenuate the behavioral and HPA axis response to stress [17-19]. Results of this study will determine the role of ER beta in estradiol withdrawal-induced mood symptoms and, possibly, suggest the efficacy and safety of this compound as a treatment for depression during the perimenopause.

Given the markedly different adverse effect profiles of ER alpha and beta agonism, the results of this study may substantially impact current treatment recommendations for women with perimenopausal depression.

Justification for study sample: The sample of women who will be recruited for this protocol consists of women who have a history of a Structured Clinical Interview for DSM V (SCID) confirmed major or minor depression in the context of the perimenopause and who now are asymptomatic and postmenopausal. Our prior findings and those of others [7;8] have demonstrated the short-term antidepressant effects of estradiol in this condition. Longitudinal studies also have demonstrated that the onset of depression in these women typically occurs during a stage of the perimenopause (stages of reproductive aging workshop [STRAW] stage -1) characterized by estradiol withdrawal. The results of our most recent study now provide, for the first time, direct evidence that estradiol withdrawal triggers a recurrence of depressive symptoms in these women (but not in matched controls). It is unclear whether the relevant signal in perimenopausal depression is mediated through estrogen receptor alpha or estrogen receptor beta. Consequently, we want to use the same design described above in euthymic women with a past history of perimenopausal depression to investigate whether the physiologically-relevant signal is mediated by estrogen receptor beta. Drawing our sample from this population ensures the inclusion of women who have been shown to be differentially sensitive to declining estradiol levels and who will likely develop

mood symptoms in our withdrawal paradigm, critical for demonstrating the role of ER beta in perimenopausal depression.

Estradiol has many biological effects, some of them undesirable in peri and postmenopausal women as observed in the Women's Health Initiative (WHI) [28;29]. Estradiol acts through at least three receptor systems [30], with very different physiologic profiles associated with each. In fact, in many tissues, ER alpha and ER beta have antagonistic actions [31]. Evidence suggests that ER beta is involved in regulation of anxiety and mood [13-16]. Thus, demonstration that ER beta mediated the beneficial effects on depression would greatly advance both our understanding of relevant mechanisms underlying affective state regulation, and offer therapeutic options that may have a greater safety profile, avoid undesirable side effects, and address the needs of women with depression who are reluctant to take psychotropic medication or who experience intolerable antidepressant-related side effects.

Our study hypothesis is that in women with a history of perimenopausal depression, mood symptoms consequent to experimentally induced estrogen withdrawal will be prevented by the administration of an ER beta agonist but not by placebo. Failure to confirm this hypothesis could suggest that either ER alpha alone or both ER alpha and ER beta are involved in the recurrence of mood symptoms. However, if the hypothesis is confirmed, then ER beta agonists could serve as effective alternatives for women receiving estrogen therapy (ET) for mood disturbances during the perimenopause since these compounds would be expected to have a more acceptable long-term safety profile than estradiol.

The ER beta compound to be employed in this protocol demonstrates a dose-dependent loss of ER beta selectivity and overlapping effects on ER alpha activity potentially could be observed at higher doses of LY500307. Thus, we plan to employ two doses of LY500307 (i.e., 25 mg and 75

mg), as this will, in effect, enable us to compare selective ER beta agonism with mixed (ER alpha and beta) agonism, since the higher 75 mg dose could mitigate the recurrence of depressive symptoms by acting through ER alpha. We also will monitor other possible targets of ER alpha activation in these postmenopausal women including plasma luteinizing hormone (LH) levels, plasma prolactin secretion, and endometrial thickness (by vaginal ultrasound). These measures were selected for the following reasons: First, plasma LH levels are increased in postmenopausal women, and studies in mice demonstrate that ER alpha is responsible for ongoing negative feedback effects of estradiol on pituitary LH secretion [32-34]; consequently, LY500307-related decreases in plasma LH levels would suggest an ER alpha action. Second, prolactin secretion also has been identified in preclinical studies to be mediated by ER alpha [35;36]; consequently, an LY500307-related increase in plasma prolactin secretion would suggest ER alpha activity. Finally, increased endometrial tissue, as measured by vaginal ultrasound, also would suggest the presence of ER alpha activity. These additional outcomes will assist in our efforts to distinguish ER beta activity from ER alpha activity in this protocol.

Medication Administered in this Protocol

Selective Estrogen Receptor beta agonist LY500307: LY500307 is an orally active, selective estrogen receptor beta (ER beta) agonist designed to maximize binding to the ER beta receptor and minimize binding to the estrogen receptor alpha (ER alpha). LY500307 was initially developed for the treatment of benign prostate hyperplasia (BPH). However, due to inadequate efficacy in prostate volume reduction or improvement in International Prostate Symptom Score (IPSS), the benefit-to-risk balance failed to support further development of LY500307 for the BPH indication. LY500307 was selected for use in this protocol since it is the only ER beta selective agonist that both has been

approved by the FDA for use under an IND (held by Lilly) in humans and has evidence to support its ability to penetrate the central nervous system.

In competitive binding assays, LY500307 affinity for ER alpha is 2.04 nM, while its affinity for ER beta is 0.16 nM, representing an approximately 12-fold selectivity towards ER beta (data available from Lilly upon request). There have been no studies of LY500307 in women. In men, the terminal half-life of LY500307 is approximately 15 hours. Single dose studies in men have been conducted involving oral doses between 0.5 and 500 mg. In addition to phase 1 and 2 treatment trials of LY500307 (at doses of 25-150 mg per day) in men for the treatment of BPH, there is one ongoing trial of LY500307 in men with schizophrenia targeting negative symptoms which employs three doses of LY500307 of 25 mg, 75mg, and 150 mg per day (conducted by PI Alan Brier at the University of Indiana). As of 04-13-18, 95 men with schizophrenia have participated in this trial. The study remains blinded; however, all three doses have been well-tolerated, and only three serious adverse events considered by the investigators to be probably related to study drug have occurred, including two events (dyspepsia and facial flushing) that occurred within 48 hours of initially starting the trial, and one case of urticaria resulting in this patient being withdrawn from the study (Alan Brier, personal communications, 07-27-15 and 05-21-18). Few adverse effects have been reported in men at the proposed dose to be administered in this study.

The ability of LY500307 to inhibit the metabolism of marker catalytic activities for cytochromes P450 (CYP) CYP3A4, CYP2D6, CYP2C19, CYP2C9, CYP2C8, CYP2B6, and CYP1A2 was examined using isozyme-selective probes. CYP enzyme inhibition constants (Ki) ranged from Ki = 2.5 ± 0.3 μM for CYP2C8 to Ki = 16 ± 2 μM for CYP1A2. Following 14 daily 100-mg doses of LY500307 in healthy volunteers, the highest clinical plasma concentration observed was 27.3 ng/mL. Using this plasma concentration and the Ki that was calculated for

CYP2C8, the I/Ki result is 0.04, or <0.1. Thus, LY500307 is unlikely to produce significant inhibition in vivo. Additionally, preliminary screens suggested no mechanism-based inactivation of any of these CYPs by LY500307 (LY500307 Investigator's Brochure).

Tissue distribution studies in rodents demonstrate that radiolabeled [^{14}C] LY500307 penetrates the central nervous system and crosses the blood-brain barrier (LY500307 Investigator's Brochure).

According to data provided by Lilly (LY500307 Investigator's Brochure) a 25 mg daily dose will provide optimal selectivity for estrogen receptor beta, as the impact on estrogen receptor alpha increases with increasing doses. Nonetheless, the optimal dose for ER beta selectivity and CNS penetrance has not been established in humans. We plan to employ two doses of LY500307 (i.e., 25 mg and 75 mg), as this will in effect enable us to compare selective ER beta agonism with mixed (ER alpha and beta) agonism, since the higher 75 mg dose could mitigate the recurrence of depressive symptoms by acting through ER alpha. The dose of 75 mg was selected for the following reasons: (1) the higher dose should enhance the CNS penetrance of LY500307; (2) the 75 mg dose remains within the safe range for toxicity based on animal studies (LY500307 Investigator's Brochure); and (3) the 75 mg dose has been safely employed without the occurrence of any serious adverse events in over 15 men with schizophrenia in a randomized controlled trial of LY500307 of 8 weeks duration (conducted by PI Alan Brier at the University of Indiana; [personal communication, 07-27-15]).

Pre-Clinical Studies of LY500307:

Summary (see LY500307 Investigator Brochure) – The results of nonclinical safety, pharmacology and toxicology studies demonstrate acceptable safety profile. No CNS respiratory or cardiovascular risks were identified at doses as high as 30 mg per kilograms in rats and mice, and 1500 mg per

kilograms in monkeys. In rat and monkey, one, six and nine month toxicity studies, the no-observed-adverse-affect level (NOAEL)-defining toxicity was consistent with the pharmacology of an estrogen agonist and it was considered to be nonlife threatening, monitorable, and generally reversible. Few sex-differences were observed in these studies and the majority related to sex-differences in gonadal function.

Plasma pharmacokinetics of LY500307 have been examined in CD-1 mice, Sprague-Dawley rats, and Cynomolgus monkeys after administration of [^{14}C] LY500307 (both male and female). Peak plasma concentrations (C_{max}) of parent compound and radioactivity were observed from 0.5 to 8 hours after oral dosing. Following the $C_{max,}$ plasma concentrations declined with an elimination half-life ranging between three and eight hours for the parent compound and between six and 22 hours for radioactivity after oral administration. Of the absorbed dose only 2.7%, 1.2%, and 0.3% circulated as parent compound in mice, rats and monkeys, respectively. The toxicokinetics of LY500307 were determined after daily oral administration in Sprague-Dawley rats for up to six months, and in Cynomolgus monkeys for up to nine months. In general exposure increased with increasing dose. Exposure was higher in female rats but there were no sex-related differences observed in monkeys. LY500307 is highly bound to plasma proteins from mice, rats, and monkeys. The degree of plasma protein binding was independent of concentration over the ranges tested for all species. Tissue distribution studies indicated that after an oral 5 mg per kilogram dose of [^{14}C] labeled LY500307 to Sprague-Dawley and Long-Evans rats, distribution of radioactivity was extensive, and there was no apparent melanin-binding observed (a potential marker of ocular toxicity in humans but with unclear predictive power). Quantifiable levels of radioactivity were observed in testes, cerebellum, cerebrum, medulla, olfactory lobe, and spinal

cord of Long-Evans rats indicating that [^{14}C] labeled LY500307-derived radioactivity crosses the blood:testes and the blood:brain barriers.

In multiple-dose pharmacokinetics studies, plasma toxicokinetics of LY500307 have been determined in Sprague-Dawley rats and Cynomolgus monkeys following daily oral exposure for up to six months for rats and nine months for monkeys. In general, greater exposure was observed in female rats compared to male rats especially at doses of .1 and 1 mg per kilogram per day. In male rats, the increase in exposure was greater than proportional to the increasing dose, whereas in females, the increase in exposure was generally proportional with dose. Following daily nasogastric administration of LY500307 in Cynomolgus monkeys, no significant sex-differences in exposure were observed. There was some evidence of accumulation of LY500307 over the duration of the study in the mid-dose group in females (15 mg per kilogram per day) and at the highest dose group (375 mg per kilogram per day) in both sexes on day 270 of the study.

Non-clinical safety pharmacology and toxicology: The toxicity profile of LY500307 has been characterized in rat, mouse, and monkey through a package of acute, repeat dose, immunotoxicity and reproductive toxicity, safety pharmacology and genetic toxicology studies. The primary treatment-related findings in repeat dose toxicity studies up to six months in duration in the rat and nine months in duration in monkey were consistent with the estrogenic effects of LY500307 and included decreased feed consumption and growth, changes in the endocrine, reproductive, hematopoietic, immune, and hepatic systems. All of these findings were considered to be nonlife threatening monitorable and reversible following cessation of exposure. The principal concern arose with continued exposure to high doses could which resulted in dose limiting toxicity due to body weight loss and poor physical condition. Other potentially non-estrogen related findings in organ systems were considered minor in nature secondary to stress or marked changes in body weight and

food consumption or limited to higher doses that met or exceeded the maximum tolerated dose. LY500307 has a low order of acute oral toxicity and is classified as a non-irritant. Results of the in vivo safety pharmacology studies indicate that the oral administration of LY500307 does not produce CNS or respiratory effects at doses up to 30 mg per kilogram in rats or mice. In addition, the nonclinical data has not revealed any substantial risk of QT/corrected QT (QTc) prolongation or hemodynamic changes at doses up to 1500 mg per kilogram in monkeys. The 50% inhibitory concentration (IC_{50}) for human ether-a-go-go-related gene (hERG) (the ion channel produced by this gene mediates the potentially fatal long QT syndrome) inhibition was greater than 2.82 µg per ml, the highest concentration tested which was at least 100 times higher than the highest maximum LY500307 clinical plasma concentration at a clinical dose of 25 mg.

Repeat dose toxicity findings in female rats were consistent with exaggerated estrogen pharmacology with similar systems affected as described in males (i.e. growth, hepatic, hematopoietic, endocrine, reproductive systems) after six months of treatment. There was complete reversal of all adverse treatment-related changes with the exception of partial recovery in body weight and female mammary gland findings and limited to no recovery of male follicle-stimulating hormone (FSH) and Inhibin B levels. In one and six month repeat dose toxicity studies in rats, at daily doses greater than or equal to 3 mg per kilogram, clinical signs were consistent with prolonged estrogen exposure and included signs of thin appearance, decreased body weight, food consumption, FSH, cholesterol and testosterone, alterations in levels and inhibin B; increased prolactin, degeneration the seminiferous tubules of the testes, atrophy of Leydig cells and secondary sex glands; oligospermia/aspermia of epididymis, mammary gland feminization; centrilobular hepatocellular hypertrophy, and bone marrow hypocellularity. Additional important findings in males given greater than 10 mg per kilogram dose of LY500307 included decreased erythrocyte

parameters (red blood cells), hematocrit, hemoglobin, reticulocytes, leukocyte count, spleen and thymus weights, increased gamma glutamyl transferase, alkaline phosphatase, total bilirubin and incidence of hepatic eosinophilic cell foci.

Treatment-related findings in monkeys given LY500307 for one, six, or nine months were generally consistent with estrogen agonist pharmacology and paralleled the effects observed in rats. Findings in female monkeys specifically, were consistent with the organ systems affected as described in males with the additional observations of a decrease in white pulp lymphocytes in the spleen, menstrual cycle prolongation, decreased corpus luteum and squamous metaplasia of the cervix epithelium and greater than 15 mg per kilogram. In general the findings recovered by the end of the third month of non-treatment.

In reproductive toxicity studies in female rats altered estrous cycles increased moribundity/mortality, abortion, post-implantation loss and fetal skeletal variations were observed at a dose of 3 mg per kilogram. In a rat pre-and postnatal study, dystocia, decreased postnatal growth and survival, delayed postnatal sexual maturation, estrous cycle prolongation, decreased fertility and a decrease in startle response were observed at 3 mg per kilograms in the F1 generation.

In conclusion, the results of nonclinical safety, pharmacology and toxicology studies demonstrate acceptable safety profile. No CNS respiratory or cardiovascular risks were identified at doses as high as 30 mg per kilograms in rats and mice, and 1500 mg per kilograms in monkeys. In rat and monkey, one, six and nine-month toxicity studies, the no-observed-adverse-affect level (NOAEL)-defining toxicity was consistent with the pharmacology of an estrogen agonist and it was considered to be nonlife threatening, monitorable, and generally reversible. Few sex-differences were observed in these studies and the majority related to sex-differences in gonadal function. The

NOAEL level was determined to be 1 mg per kilogram per day in the male rat and 1.5 mg per kilograms per day in the male monkey following nine months of daily dosing.

Estradiol

The administration of 17ß-estradiol via a transdermal system was chosen for this study because it has several relevant advantages over oral preparations [37;38]. First, it delivers the primary ovarian estrogen, estradiol, into the circulation at a constant rate and results in sustained and easily measurable plasma levels of estradiol and in estrone/estradiol ratios less than one (as seen in the pre-climacteric period of life) [39]. Second, it delivers sufficient estradiol into the circulation to raise estradiol plasma concentrations to levels similar to those of women in the early follicular to mid-follicular phases of the menstrual cycle [37;40-42], levels reported by some investigators as the minimum necessary for the relief of menopausal symptoms, particularly hot flushes [40], without increasing synthesis of renin substrate. An increase in renin substrate has been suggested to accentuate or initiate the development of high blood pressure in susceptible women with other predisposing factors [43] and has been implicated as a factor in the association of hypertension with the administration of oral conjugated estrogen [44]. In our experience with the transdermal estradiol patch in over 100 women in several protocols, it has been well tolerated, and, with the exception of an occasional skin rash, we have observed no adverse effects. With the application of the estrogen patch every three days, patient compliance has been reported to be excellent, and only occasional local irritation has been observed [40].

Provera

Provera and synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty [45].

Provera is widely prescribed to induce menses and shedding of the endometrial lining in women receiving ET. In our experience and that of others [46], a daily dose of 5 mg for seven days is sufficient to successfully induce menses in women receiving 3-8 weeks of ET.

2. Study Objectives

a. Primary objectives

To determine whether the selective ER beta agonist LY500307 prevents mood symptoms (as measured by the CES-D and HRSD) from developing after the acute withdrawal of ET under double-blind, placebo-controlled conditions in asymptomatic postmenopausal women who have previously experienced a carefully confirmed perimenopause-related depression. Additionally, we will assess the safety of LY500307 in the context of this study.

b. Secondary objectives

Secondary objectives will be to evaluate the effects of LY500307 on measures of estradiol-withdrawal-induced individual affective and behavioral symptoms (daily rating form [DRF] and VAS ratings), vasomotor symptoms, plasma hormone levels (estradiol, estrone), and menstrual bleeding. Exploratory objectives will include an examination of the effects of LY500307 on gene expression profiles in lymphoblastoid cells (LCLs) or induced pluripotent cells (IPCs) from study participants. We plan to generate patient-derived LCLs and IPCs from the women participating in this protocol to investigate both intrinsic cellular differences between women with PMD and controls as well as examining the effects of estradiol withdrawal with and without invitro exposures to an ER beta agonist. Potential differences in gene expression profiles between women with and those without past perimenopausal depression, and the corresponding effects of estradiol withdrawal on these same systems are currently being examined in collaboration with the Laboratory of Neurogenetics, NIAAA. Analyses of gene expression changes in lymphoblastoid cells will be performed by RNAseq, employing gene set enrichment analysis (GSEA), weighted

gene co-expression network analysis (WGCNA) and database for annotation, visualization, and integrative discovery (DAVID) analyses, quantitative PCR to confirm the changes identified in RNAseq, and subsequent confirmation of changes in protein expression corresponding to results of gene expression data. Additionally, we will perform whole exome and whole transcriptome sequencing (and possibly whole genome sequencing) at a later date. Finally, we will monitor the effects of LY500307 on presumed estrogen receptor alpha-related tissue effects including endometrial proliferation (by vaginal ultrasound), lipids, selected plasma proteins (e.g., sex hormone binding globulin [SHBG]), plasma LH, and plasma prolactin levels.

3. Participants

a. Description of study populations:

The screening for this protocol was initially conducted as part of protocol 88-M-0131; however, this protocol (18-M-0144) was amended to include the screening phase. Due to attrition during screening (i.e., screen failures), we will need to screen approximately 80 women in this protocol to ensure that our target enrollment is met.

The study population will be forty-five non-depressed postmenopausal women with well documented past perimenopausal depression (i.e., 15 women in each of three study arms). Dropouts will only be replaced if they withdraw before randomization. All randomized subjects will be included in analyses.

b. Inclusion Criteria:

1. Women with a past perimenopause-related depression (within 12 years). The diagnosis of perimenopause-related depression will be based on a history of a past depressive episode (major or minor depression confirmed by Structured Clinical Interview for DSM-V (SCID)) at midlife in association with menstrual cycle irregularity (and possibly hot flushes and/or vaginal dryness) and in whom menopausal hormone therapy was reported to improve their depression at

any time within the prior twelve years. All women participating in this protocol will be screened with psychiatric, medical, and reproductive evaluations to confirm they are in good medical health.

2. Age 45 to 65;

Medication free (including no mood stabilizers, no sleep medication) except for the following: women on menopausal hormone therapy who will discontinue these medications at the start of this study and have their hormone therapy replaced with estradiol 100mcg per day (as described below), women who are on stable doses of thyroid replacement for at least six months prior to study enrollment, or women who occasionally take non-steroidal anti- inflammatory drugs [NSAIDs] or allergy medications (although we will ask women to minimize the use of these medications during the study).

3. Subjects must have consent capacity.

c. Exclusion criteria:

The following conditions will constitute contraindications to participate in this protocol:

1. Any current Axis 1 psychiatric illness or any clinically significant sleep disorder;

2. Women with histories of hormone replacement therapy-induced dysphoria due to either the estrogen or the progesterone components of their hormone replacement;

3. Past history of major depression with suicidal ideation;

4. History of ischemic cardiac disease, pulmonary embolism, or thrombophlebitis;

5. Renal disease; hepatic dysfunction; history of cholecystitis; hypertension;

6. Women with a history of carcinoma of the breast or any undiagnosed breast nodule/mass;

7. Women with a history of uterine cancer, ill-defined pelvic lesions, particularly undiagnosed ovarian enlargement, undiagnosed vaginal bleeding;

8. Pregnant women; sexually active women will be required to employ barrier contraceptive

methods;

9. Cerebrovascular disease (stroke);

10. Recurrent migraine headaches;

11. Women who have had a hysterectomy before one year after their last menstrual period;

12. NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.

4. Study Design and Methods

a. Study overview

Participants will be screened over 3-4 clinic visits to ensure inclusion criteria is met. Following screening, the medication phase of this study is a seven-week randomized, double blind, placebo controlled study and there is a four week follow-up evaluation phase to monitor all women for the emergence of adverse effects post-medication exposure. Participants will have weekly outpatient visits, weekly blood draws and will also complete daily symptom rating scales. The study involves a three week baseline phase in which all women receive open label (OL) estradiol therapy (ET) at a dose of 100 micrograms per day by transdermal skin patch, after which all women receive three weeks of double blind (DB) medication (i.e., LY500307 [at a daily dose of either 25 mg or 75 mg] or placebo), and then in non-menstruating women (i.e., the absence of reported menstrual bleeding of greater than 1-2 days during the double blind phase of this study), one week of Provera to precipitate a progestin-induced menses. The week of Provera is not a research-related intervention but is clinically-indicated to induce endometrial shedding that will eliminate potentially abnormal endometrial tissue consequent to the three weeks of unopposed estradiol exposure. The duration of the OL and DB phases of this study were selected to match those employed in our previous protocol (03-M-0175) [12] in which we demonstrated that three weeks of estradiol followed by a three week withdrawal of estradiol were sufficient for the majority of

women (who met identical selection criteria as in this study) randomized to estrogen withdrawal to develop a recurrence of their mood symptoms after a three week asymptomatic period while on OL estradiol.

All participants will be instructed to ingest study medications on an empty stomach after an overnight fast, since in all clinical pharmacology studies, LY500307 was administered orally after overnight fast, followed by breakfast approximately 30 minutes after the dosing. The effect of food on LY500307 has not been formally assessed. All participants will receive three capsules of LY500307 or placebo each morning consisting of the following formulations: 1) women randomized to 75 mg LY500307 will receive three capsules each containing 25 mg LY500307; 2) women randomized to 25 mg LY500307 will receive one capsule containing 25 mg LY500307 and two capsules of placebo; and 3) women randomized to placebo will receive three capsules each containing placebo. There will be a 1-1-1 distribution of women in each study arm (i.e., each arm will contain 15 women). A table of random numbers will be used for randomization in this trial. A set of numbers, equivalent to the proposed "N" will be selected and an appropriate block size within that set will be chosen. Within that block, the highest third of the numbers will be assigned to one treatment arm, the middle third will be assigned to the second treatment arm, and the lowest third will be assigned to a third treatment arm. The block size is not revealed to the investigator.

Genetics Studies: Subjects will be invited to provide a 30ml blood sample for genetic characterization studies, including investigation of specific gene polymorphisms (e.g., estrogen, progesterone and androgen receptors) and establishment of lymphoblastoid cell lines (LCLs) and induced pluripotent cell (IPCs) for epigenomic (WES), and transcriptomic (WTS) sequencing. We also plan to perform whole genomic sequencing (WGS) in this protocol in the future. The potential relevance of genetic polymorphisms for women with menstrual cycle-related mood disorders is evidenced in our recent studies (Dubey et. al, 2017;Rudzinskas et. al, 2020), where we have

demonstrated that cell lines derived from these women show intrinsic differences in their gene expression, both prior to and after in vitro hormone manipulation, as well as in analogous studies, such as those demonstrating that decreasing CAG trinucleotide repeats in Exon 1 of the androgen receptor lead to greater the transcriptional efficiency of this receptor resulting in the increased likelihood of developing prostate cancer , and a greater likelihood that the cancer will be of high stage and grade (124). Menstrual-related mood disorders may, like prostate cancer, be seen as a different phenotypic response to a gonadal steroid signal, where it is the response, and not the signal, that is aberrant. Wherever possible, internal control samples will also be acquired.

b. Recruitment

All subjects for this protocol will be either self-referred in response to newspaper advertisements or referred by their personal physician. Pre-screening telephone interviews will be performed prior to scheduling any woman for a screening clinic visit.

IRB approved recruitment material will be to initiate a first contact with women who may be interested in participating in research or know someone who is. When a woman calls the Branch for more information she is given an overview of the study, told the study is taking place on an outpatient basis at the NIH Clinical Research Center in Bethesda, Maryland. She is informed there is no cost to her if she participates and informed of time commitments and compensation. The following recruitment material has been uploaded into iRIS:

1. Menopause and Mood Ad 2c x 5
2. Menopause and Mood Flyer with Tear offs
3. Menopause and Mood Poster
4. Perimenopausal Depression Postcard
5. Menopause and Mood Direct Mail Card (2 pages)
6. Menopause and Mood Dear Neighbor Letter

7. Menopause and Mood 2-sided note card 5c x 3 (2 pages)
8. Menopause and Mood Online Button ad (300 x 250, standard sidebar)

Ad link to Study-specific page on the NIMH-IRP Join A Study Website. URL (placeholder) http://www.nimh.nih.gov/labs-at-nimh/join-a-study/adults/adults-perimenopause-related-mood-disorders.shtml

9. Menopause and Mood Mobile ad (300 x 50, mobile or 728 x 90 banner)

Ad link to Study-specific page on the NIMH-IRP Join A Study Website. URL (placeholder) http://www.nimh.nih.gov/labs-at-nimh/join-a-study/adults/adults-perimenopause-related-mood-disorders.shtml

10. Menopause and Mood text for NIMH "Join a study" website
11. Menopause and Mood Long text
12. Menopause and Mood Medium text
13. Menopause and Mood Short text

Menopause and Mood Twitter text (2)The material will be distributed, as appropriate, by the following methods:

2c x 5 advertisements may be used in local newspapers (e.g., The Washington Post, Express, Gazette, Washington Jewish Week, Military papers) and/or in local and national magazines with targeted local advertising (e.g., Bethesda Magazine, Washingtonian, More, Washington Parent, Washington Woman, Oprah, Women's Health), on websites of radio stations, newspaper and magazines, in advocacy group newsletters of local women's groups, churches, community based organizations, professional groups (APA, MHA). Flyers (with tear offs) and posters (without tear offs) may be posted on NIH bulletin boards, community bulletin boards (with permission and according to the guidelines of the facility), emailed as PDFs to local providers or callers who

request a copy, at conferences at which NIMH has a table with permission to distribute material (e.g., Montgomery County Mental Health Association sponsored CEU events for social workers). The postcard and the 4.5 inch x 6 inch note card may be sent using commercially-available mailing lists via direct mail. The postcard and the note card contain the IRB required disclaimer "Names and addresses of women ages 45-65 living in the greater Washington DC area were obtain from a consumer information database." The Dear Neighbor Letter may be sent using commercially-available mailing lists via direct mail and contains the IRB required disclaimer "Names and addresses of women ages 45-65 living in the greater Washington DC area were obtain from a consumer information database." It will be mailed along with the 2-sided notecard (5c x 3).

The 2-sided notecard and postcard may be posted on NIH bulletin boards, community bulletin boards (with permission and according to the guidelines of the facility), emailed as PDFs to local providers or callers who request a copy, distributed at conferences at which NIMH has a table with permission to distribute material (e.g., Montgomery County Mental Health Association sponsored CEU events for social workers), mailed to women in the greater Washington DC area and distributed, as appropriate, to community organization (e.g., Montgomery County Mental Health Association sponsored CEU events for social workers).

The online electronic ad and mobile ad may be placed on websites of community groups (e.g. Wednesday Morning group), advocacy groups (e.g. Consortium for Women's Mental Health), e-newsletters, local newspapers (Gazette, Express), local magazines (Washington Woman), women's health or community blogs (Just up The Pike, Bethesda Actually), radio stations (WBIG 100, WASH FM), and Facebook pages (e.g. NIH and NIMH or of women who call that may request a link to share).

Long Text ads may be used for Listservs, Craig's List, Advocacy e-news, or print news postings with the permission of the moderator and the IRB-required statement on how the receiver was

identified. Examples of Listservs that may be contacted include professional groups, parenting/ family, women's health and fitness. SBE will retain copies of all correspondence with the administrator of each Listserv.

Medium Text ads may be used for e-news or print news postings with limited space requirements such as Washington Parent or professional Newsletters.

Short Text ads may be used for e-news or print news postings with limited space requirements such as Washington Parent or professional Newsletters such as the Greater Washington Society of Clinical Social Workers.

Twitter text may be used by the NIH and NIMH extramural Communications Office as well as by community or advocacy groups such as Consortium for Women's Mental Health. The Branch will not post directly to Twitter.

c. Screening

An initial pre-screening phone call is done prior to obtaining consent via a waiver of consent for screening activities. Initial pre-screening will be conducted by phone interviews to determine if the subject appears to meet inclusion criteria.

Pre-screening activities performed prior to obtaining informed consent:

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

Please see request for waiver of consent for pre-screening activities in Section 13 b: Consent Process Section.

After the pre-screening phone call, all potential participants will come to the Clinical Center, consent will be obtained and further screening will commence. All women will be personally interviewed to confirm the inclusion criteria by a psychiatrist, psychiatric nurse, or social worker to obtain a description of the following (if any): 1) the nature of the relationships between mood disorders and the perimenopause, with emphasis on the relationship of symptom appearance to onset of perimenopause, vasomotor symptoms, and development and evolution of the disorder; 2) type, severity and duration of symptoms, including non-mood related menopausal symptoms; 3) premorbid psychiatric history; 4) medical and medication history, including medication taken for mood problems; 5) psychiatric history.

Consent is obtained before any study procedures, including screening procedures, are done.

Women will be screened to have the presence or absence of menopausal status evaluated and documented. Postmenopausal reproductive status will be defined by a history of at least twelve months of amenorrhea and biological evidence of ovarian dysfunction, specifically at least three of four plasma FSH values > 14 IU/L on consecutive occasions drawn at 10-14 day intervals over a period of six to eight weeks.

The following screening procedures will be done in this protocol during the screening phase:

- A complete medical history, physical exam and EKG
- Participants will have a psychiatric history taken which will include diagnostic interviews such as the Structured Clinical Interview for Axis I DSM-V Disorders (SCID)
- Symptom ratings:

  Women may be asked to return for follow-up interviews on approximately 10-14 day

intervals for up to six weeks in this screening phase. During these interviews, phenomenological assessments may be performed by instruments including the following: Beck Depression Inventory (BDI), 17-item Hamilton Depression Scale (HRSD); Center for Epidemiologic Studies Depression Scale (CESD); and questionnaires focusing on early life traumatic events (i.e. Adverse Childhood Experience (ACE) Questionnaire and Childhood Trauma Questionnaire [CTQ]).

- Blood tests:
    - complete blood count (CBC)
    - electrolytes;
    - glucose,
    - BUN and creatinine;
    - Liver function tests;
    - thyroid function tests,
    - plasma prolactin,
    - plasma FSH levels (to confirm reproductive status)
    - lipid profile.
    - plasma βHCG pregnancy test (if indicated)
  - Urine –
    - urinalysis; urine pregnancy test

Any woman with significant physical, EKG, or laboratory abnormalities, or who meets any of the exclusion criteria will not participate in this protocol. Additionally, prior to participation all subjects will be examined for any contraindications to ET.

All women will have had a normal PAP smear, negative HPV test results and normal gynecologic

exam within the 5 years prior to study enrollment as recommended for women between the ages of 30 and 65 years by the USPSTF, the American Society for Colposcopy and Cervical Pathology and the American College of Obstetricians and Gynecologists, unless the woman has a previous positive test for HPV in which case the exam and testing will have occurred within 3 years of starting this study. The gynecologic exam done is a standard gynecological exam. Exams done by an outside provider will require the release of a copy of the report from the provider with permission of the subject. If the inclusion criteria is met, participants may move to the medication phase of the study.

d. Study Procedures

During the seven-week medication phase the following will be conducted:

- The effects of LY500307 or placebo on mood symptoms will be monitored with daily symptom rating scales.
- During the 8 weekly clinic visits all women will also have blood pressure, pulse, and weight measured and will be assessed for any side effects to the prescribed medications.
- Blood samples will be drawn via venipuncture at each of the first 7 clinic visits; approximately 50 ml of blood will be collected at each draw (total = 350 ml).
- One time blood draw of 30ml for genetics which includes the establishment of LCLs and IPCs, and analysis including WES, WTS and possibly WGS.
- Transvaginal ultrasound will be performed on two occasions: before randomization (i.e., during week 3 of open label estradiol), and then after 3 weeks of treatment with LY500307 or placebo.

Baseline period (Open Label estradiol treatment [ET]):

During a three-week baseline phase, all women will receive open label (OL) estradiol (100 micrograms/day) by skin patch and will complete mood and behavioral

symptom ratings to confirm the absence of clinically significant mood symptoms prior to entry into the study.

Double-blinded Randomization phase:

After the three-week baseline period and the determination that ET does not precipitate symptoms, women will be randomized to one of three arms as follows: LY500307 at a dose of 25 mg per day, LY500307 at a dose of 75 mg per day, or matched placebo for three weeks. Once the three weeks of LY500307 or placebo treatment is completed, non-menstruating women will receive one week of Provera (5 mg/day) at the end of the trial to induce a progestin withdrawal bleed. The justification for employing two separate doses of LY500307 is that the lower dose (i.e., 25 mg) may be insufficient to effectively produce agonist effects at ER beta. Prevention of the emergence of estradiol withdrawal-induced mood symptoms at the 75 mg dose (but not the 25 mg dose) would suggest either a dose-dependent agonism at ER beta or the requirement for activation of ER alpha. The latter possibility will be examined by monitoring ERα activity at the levels of the endometrium, the pituitary (plasma LH and prolactin), and possibly hypothalamus (plasma LH [33]), which will help us evaluate potential ER alpha actions accompanying the higher dose of LY500307. Of note, the 75 mg daily dose is within the safety margins established by Lilly.

After the trial, those women who were taking ET prior to the study will be re-started on their previous HT regimen unless they request to continue the withdrawal from HT under medical supervision. Outcome measures will include standardized mood and behavioral rating scales. The main comparisons in this study will be between LY500307 and placebo in women with a past history of perimenopause-related depression. Results will be analyzed by analysis of variance for mixed effects and post-hoc Bonferroni testing.

Weekly visits and daily mood ratings:

During the seven-week study, the effects of LY500307 or placebo on mood symptoms will be monitored with daily symptom rating scales and weekly clinic visits. Outcome measures include the following: Primary outcome measures: self ratings - the Center for Epidemiologic Studies-Depression Scale (CES-D) [47], observer ratings - the 17-item Hamilton Rating Scale of Depression (HRSD)[48]. Secondary outcome measures: the daily symptom rating form (DRF)[49] consisting of a 14 item six point likert-type scale measuring the severity of several mood and behavior symptoms including hot flushes (completed on a daily basis at home by each participant); the Beck Depression Inventory (BDI)[50]; a visual analogue scale (VAS) measuring the reported severity of 13 mood and behavioral symptoms (completed at each weekly clinic visit); and whole genome expression/exome sequencing.

During eight weekly clinic visits all women will also have blood pressure, pulse, and weight measured and will be assessed for any side effects to the prescribed medications (including specific evaluations of any complaints of calf pain or other symptoms and signs of blood clots in the legs). In addition, blood samples will be drawn via venipuncture at each of the first seven clinic visits; approximately 50 ml of blood will be collected at each draw (total = 350 ml). This blood will be analyzed for several measures such as the following: estradiol, estrone, FSH, LH, prolactin, testosterone, dihydrotestosterone, SHBG, electrolytes; glucose, CBC, renal, hepatic, thyroid and lipid functions. These measures will be employed to monitor safety parameters of LY500307 (i.e., CBC, renal, hepatic, thyroid and lipid functions), and the potential effects of LY500307 on measures of ER alpha function (see above). Blood samples for estradiol will be processed within 24 hours in the NIH Clinical Center Pathology Service and sent to a staff member not involved with patient

care in order to monitor compliance with estradiol therapy.

Timing of clinic visits: It is anticipated that participants in this study will occasionally miss or fail to complete an assessment or procedure, such as a study visit, completion of a rating scale or a blood draw. Omissions such as these will be considered expected events and not protocol deviations provided they are infrequent and do not include data needed to assess safety or the primary study outcome. Cumulative proportions of these missed events in the study population will be presented to the IRB annually. In addition, the rate of omissions will be monitored by the Investigators. If an individual misses more than 15% of the required assessments/procedures or if more than 15% of the participants miss completion of the same assessment or procedure, it will be considered a deviation and a deviation report will be sent to the IRB within 14 days.

Transvaginal Ultrasound:

To monitor potential effects of LY500307 on the endometrium (ER alpha-mediated), we will perform transvaginal ultrasound on two occasions as follows: before randomization (i.e., during week 3 of open label estradiol), and then after 3 weeks of treatment with LY500307 or placebo. The transvaginal ultrasound will be performed either in the Department of Radiology, NIH, or in the ACRF-9 under the consultation of Lynnette Nieman, M.D or Alan DeCherney, M.D. Any abnormality identified by transvaginal ultrasound will be further investigated by the consultant gynecologist. If any woman experiences marked discomfort during the insertion of the ultrasound probe the procedure will be discontinued, and we will perform instead a trans-abdominal ultrasound. Otherwise, there are no additional associated risks or discomforts with the transvaginal ultrasound.

All women will receive an information hand-out describing the transvaginal ultrasound

procedure and possible associated discomforts that will be reviewed with each woman by the research team prior to the first scheduled procedure.

e. End of Participation:

After the completion of this trial, all participants will be evaluated for the presence of clinically significant mood symptoms. Additionally, the results (including symptom ratings and plasma hormone levels) of the study will be reviewed with each participant. If negative mood symptoms or distressing hot flushes are present, we will discuss therapeutic options prior to referral of each participant back to her community health care provider. Additionally, all women will be requested to return for a follow-up clinic visit four weeks after completion of the seven week medication phase (i.e., three week open estradiol treatment, three week double blind placebo/ LY500307 treatment, and one week progestin treatment in non-menstruating women) to evaluate possible adverse effects emerging post-treatment – although no late-onset adverse effects are anticipated. At the time of this follow-up visit an EKG will be performed on all subjects to assess QTc. The options for follow-up in those women who report persisting negative mood symptoms include no treatment with follow-up by community health care provider, initiation of standard antidepressant therapy such as selective serotonin uptake inhibitors with follow-up in community, or resumption of previous menopausal hormone therapy by her community health care provider. Our experience in protocol 03-M-0175, which employed a similar study design with a symptom-provocation paradigm, suggests that in the majority of women depressive symptoms that emerged during estradiol withdrawal were of mild to moderate severity and time-limited. In those women in whom depressive symptoms persisted and were accompanied by distress, either resuming their previous ET or commencing standard antidepressant therapy led to a remission of symptoms. However, in

the fourteen women with past perimenopausal depression who participated in 03-M-0175 and were randomized to estradiol withdrawal, only three women elected to resume ET or use antidepressant therapy because of distressing mood symptoms.

5. Management of Data and Samples

a. Storage

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Data from structured diagnostic interviews and symptom ratings are kept in secure research files and electronically on the Branch server space or within the CTDB database. Access to these research files is only available to study investigators. Symptom ratings, diagnostic interviews, and other questionnaires will be collected through an online system using a subject-specific log in and password to protect confidentiality (see Appendix 2). Samples will be kept in pass-card protected locked storage. Only study investigators will have access to the samples and data. DNA and cell cultures for lymphoblastoid cells and/or induced-pluripotent cells (IPCs) will be coded and stored under locked conditions at Dr. David Goldman's laboratory (NIAAA) at Fisher's Lane, Rockville. Dr. Goldman and other associate investigators in his lab will have access to the code key for specimens and data. Any loss or destruction of samples will be reported to the IRB.

b. Data (including genomic data) and sample sharing plan

This study requires submission of genomic data under the NIH GDS policy. Genomic data will be submitted to the following NIH-designated repositories: dbGAP.

Samples and data including genomic data will be shared with NIAAA for the purposes of collaborative research.

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was

obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators but will remain at NIH.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

c. Future use

Samples and data are stored with the intent of future use by the Principal Investigator and other investigators. Participants can opt out of having their samples and data used in future studies by initialing the consent form.

Access by other researchers to the study data is an important component of this study. Most commonly, researchers seek access to de-identified clinical data and biomaterials via the NIMH Center for Genetics (NCG), where data requests are handled by the Genomics Research Branch in the Office of the Director, NIMH, in accordance with a Distribution Agreement (https://www.nimhgenetics.org/documents/Distribution%20Agreement.pdf).

Access to clinical data and biomaterials not available through the NCG is handled by the PI, who works with the requestor and the NIMH Office of Technology Transfer to provide anonymized or coded data or biomaterials in accordance with an appropriate Material Transfer Agreement. Personally identifiable information is never shared outside the NIMH IRP.

d. Disposition

Samples and data will be kept indefinitely.

6. Additional considerations

a. Research with investigational drugs or devices:

LY500307 drug substance will be provided as active pharmaceutical ingredient (API) from Eli Lilly and Company allowing reference to the existing regulatory information in IND 100,916. The drug product will be prepared under a new IND for LY500307 (ER beta agonist) with the NIMH as the sponsor. Dr. Maryland Pao is the official representing the Institute.

Role of a commercial company: Eli Lilly will provide suitable amounts of active pharmaceutical ingredient (API) of LY500307 to the Contract Manufacturing Organization (CMO) where quality assurance tests will be performed and appropriately dosed capsules will be formulated.

In addition to drug substance, Eli Lilly and Company will provide the CMO with access to the information in the existing IND 100,916, including information on formulation development and drug product stability. The CMO we propose to employ is Pine Pharmaceuticals and is responsible for the manufacturing and release of the medications to be employed in this protocol. The facility at the Pine Pharmaceuticals, (100 Colvin Woods Pkwy, Tonawanda, NY 14150; website: www.PinePharmaceuticals.com, Ph 716.248.1025) is an FDA certified facility for good manufacturing practice (c-GMP) and will produce the appropriate capsules containing LY500307 and matched placebos. Similar contracts are established with Pine Pharmaceuticals and the University of Iowa for the manufacturing and release of medications currently employed within other NIH protocols including several within the NIAID.

b. Gene therapy

Not applicable.

7. Risks and Discomforts

Rating Scales and Interviews: The risks and discomforts of the evaluations are minimal. Mood ratings and interviews in the clinic pose little risk. Daily ratings require approximately 10 - 15 minutes each evening to complete, which may be inconvenient and potentially tiring but the majority of our participants are very dedicated to collecting this information while participating in our studies.

No discomfort is expected to be associated with or the clinical interview other than potential stress of answering personal questions and completing a questionnaire about early life trauma. All clinical assessors have extensive experience in clinical psychiatric assessment and will make every effort to implement protocol procedures in a sensitive and supportive manner. Research interviews will be interrupted if subjects become distressed or object to answering questions. Other measures to minimize risks include the careful assessment of each subject before the study, and close clinical scrutiny during all aspects of the study.

History and Physical Exam and Laboratory Tests: It is possible that during the evaluation a subject may be found to have a medical problem of which they were previously unaware. In that event, the participant will be given the results and an appropriate referral made as indicated by the condition identified.

Genetic Testing and Return of Incidental Findings: These risks are only relevant if testing is conducted in CLIA-certified labs so that incidental findings can be returned and the results are placed in the subjects' medical record. Under some circumstances, it can be a risk for genetic information about an individual to be known. Variation in some genes is known to be directly related to risk for certain illnesses. Other genes may be shown at some point in the future to be related to illness. Since the results of these genetic tests may allow prediction of risk of illness in some cases, it is possible this information could be used against a subject participating in the study. Although there are no physical risks associated with participation in genetic studies, apart from those routinely associated with phlebotomy, the psychological and social/economic risks associated with genetic studies of the kind described here are difficult to define and remain the subject of heated controversy in the ethics community. The major risk, to the degree that any exists, is that a breach of confidentiality regarding genetic studies that resulted in third parties finding out genetic information about a person could theoretically place a person at risk for loss of insurance, loss of employment, etc. because of

genotype-based discrimination. To our knowledge, no person has ever suffered harm for the reasons just described as a result of participating in a genetics research study. Regardless, our written informed consent process will go over these risks carefully. The Genetic Information Nondiscrimination Act (GINA) is a federal law that prohibits plans and health insurers from requesting genetic information or using genetic information. It also prohibits employment discrimination based on health information. However, GINA does not address discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect against discrimination based on an already-diagnosed condition or disease that has a genetic component.

Learning about the changes in genes could mean something about the subject's family members and might cause them distress. In addition, subjects could receive a result for an unexpected gene change that turns out not to cause that health condition. This could potentially cause unnecessary distress or lead to unnecessary medical testing risks and costs.

Subjects will have the right to "withdraw" from the research and have their DNA sample destroyed, if not already used. Finally, all research records containing any subject-identifying information will be stored under lock and key, or in secured computing environments by the CTDB. Personal identifiers are never associated directly with genotypes in the same data file - all genotype information is indexed only to de-identified subject codes. With these safeguards in place, we are confident the research is virtually without psycho-social-economic risks to subjects; however, subjects will be informed that risks of sharing genomic data may include possibility of reidentification.

Plan for Return of Incidental Findings: Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations

for the return of incidental findings that is current at the time of primary analysis. Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Study medications: LY500307 has not previously been administered to women, and, therefore, existing safety data in humans is based on experience with LY500307 in men only. This protocol will be the first to evaluate LY500307 in women.

1. Selective Estrogen Receptor beta agonist LY500307: LY500307 is a selective ER beta agonist. At doses of 25 – 75 mg per day, LY500307 has been found to be well tolerated, with both low (i.e., 25mg) and high doses (75 mg) having only modest effects on circulating testosterone concentrations in healthy middle-aged men (an effect presumed to reflect the actions of ER alpha not ER beta on negative feedback regulation of testosterone in men). No changes in vital signs, blood counts, liver and renal functions, and electrocardiograms (ECGs) have been observed in the Phase 1 studies.

Preclinical pharmacology and toxicology studies suggested that at higher doses, LY500307 loses its selectivity towards ER beta; as such, classic estrogenic effects have been observed in toxicology and Phase 1 clinical trials. These effects include the following:

Weight loss: Reduced food intake and weight loss was observed consistently in the toxicology studies in animals. Clinically, no statistically significant weight loss was observed in phase 1 studies.

Gynecomastia: Mammary gland hyperplasia (also considered a consequence of ER alpha activity) has been consistently observed in toxicology studies in animals receiving high doses of LY500307, therefore, gynecomastia is a potential adverse effect of LY500307 in clinical dosing. Gynecomastia has been observed in men receiving hormone therapy for prostate cancer, and also in men receiving 5-α-reductase inhibitors for BPH.

There are several estrogenic adverse effects that cannot be reliably predicted with available toxicology studies. These include: the worsening of atherosclerosis and increased risk for strokes and thromboembolic events (e.g., DVT).

These less common, but potentially life-threatening, AEs have been reported in association with estradiol therapy but are unlikely to occur with the duration of time that LY500307 is administered in this study and in this relatively young age range of women who are otherwise healthy. Estrogens have been known to alter hepatic lipid metabolism and reduce low-density lipoprotein (LDL) in women (see below). In one study (study Lilly Study BPAD, Lilly Investigator Brochure), after two weeks of LY500307 administration, there was an increase in calculated LDL concentration without a change in the LDL particle number or size. However, in a longer study lasting 24 weeks (Lilly Study BPAE, Lilly Investigator Brochure) no changes in plasma lipid profiles were observed. In contrast to effects seen with estrogens, no increase in serum triglyceride was observed. Changes in liver function tests and renal function have not been observed during short-term clinical dosing, but will be monitored in this study. No changes in complete blood count and lymphocyte subtype were observed in the Phase 1 studies.

In all clinical pharmacology studies, LY500307 was administered orally after overnight fast, followed by breakfast approximately 30 minutes after the dosing. The effect of food on LY500307 has not been formally assessed.

Following a single 25-mg oral dose of LY500307 containing [14C]LY500307 (approximately 100

μCi or 3.7-MBq) to 6 healthy male subjects, approximately 98% of the administered radioactivity was recovered over a duration of 216 hours post-dose. The principal routes of elimination of LY500307 are in the urine, and to a lesser extent in the feces (see Lilly Investigator Brochure). In plasma and urine, direct sulfate and glucuronide conjugates of LY500307 were identified as the predominant metabolites.

Four clinical pharmacology studies have been conducted in healthy males. As of November 2010, a total of 109 males have received LY500307 in clinical pharmacology studies. Doses of LY500307 were generally well tolerated. No clinically significant safety concerns have been encountered after administration of LY500307 in studies to date. Few serious adverse events (SAEs) and treatment emergent adverse events (TEAE) were observed at any dose level in these studies (see Lilly Investigator Brochure, page 36, tables 6.3 and 6.4). The incidence rates of SAEs and TEAEs were similar between placebo and active treatment groups. All AEs reported in these studies were mild to moderate in severity, and all were self-limiting without discontinuation of LY500307 dosing. No clinically significant changes in vital signs or EKGs were observed during these clinical pharmacology studies. No clinically significant changes in complete blood count, lymphocyte subtype, serum electrolytes, glucose, blood lipids, and liver and renal function tests were observed in these studies. Sporadic statistically significant QTc prolongation was noted in one study (Lilly BPAC study, see Lilly Investigator's brochure); however, no dose/concentration-dependent prolongation of QTc interval was observed in the other clinical pharmacology studies. In men with BPH, a phase 2 dose-ranging trial of 24 weeks duration designed to test the safety and efficacy of LY500307 was completed (Lilly BPAE, see Lilly Investigator's brochure pp 36-39). At the end of this phase 2 study, approximately 177 men experienced at least 1 treatment emergent adverse event and 54 men were considered to experience events possibly related to study drug including the following (in order of frequency): gastrointestinal disorders (such as constipation, diarrhea, nausea,

dry mouth, and flatulence), cough, nasopharyngitis, back pain, headache, increased prostatic specific antigen, dizziness and fatigue.

Of note, the incidence rates of SAEs, including AEs leading to discontinuation were similar between active and placebo treatment groups.

In this study, we will evaluate at each clinic visit signs and symptoms of any potential adverse events including those associated with deep venous thrombosis, pulmonary embolism, stroke, or severe migraine headaches. Additionally, laboratory tests will monitor renal, hepatic function as well as blood counts and blood lipid levels. Finally, during the last clinic visit an EKG will be repeated to rule out emergent cardiac conduction abnormalities.

2. Estradiol Replacement

Nausea is the most common side effect of estrogen administration. At conventional replacement doses, higher than those employed in this protocol, this complaint seldom interferes with eating, and no weight loss has been reported. Breast engorgement, endometrial hyperplasia and bleeding are also common side effects of estrogen administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of estrogen; however, at the dosage and for the duration of estrogen administration in this protocol this risk is small.

The relationship between estrogen, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation [51].

Numerous retrospective case control studies published since 1975 have indicated that postmenopausal exposure to unopposed estrogens for more than one year results in a two to 12 fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen therapy appears to decrease the risk of endometrial hyperplasia and

endometrial cancer to equal or below that of women receiving no hormonal treatment. Recent studies suggest that the optimal regimen to prevent hyperplasia and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progestin treatment each month when estrogens are administered [52]. There is an increase in thromboembolism and stroke in women receiving estrogen therapy [53-59]; however, this complication is unlikely at the dose and duration of estrogen therapy employed in this protocol and in the younger age of the women participating in this trial [60]. One study [40] reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use [52;61;62]. Additionally, case-control studies [63;64] observed that an increased risk of venous thromboembolism was associated with oral but not transdermal estrogen compared with nonusers (odds ratios = .42 [95% CI, 1.5 to 11.6] and 0.9 [95% CI, 0.4 to 2.1] respectively). Blood pressure, on average, appears to be unaffected by estrogen replacement therapy, although both increases and decreases have been reported. Post-menopausal estrogen therapy has been observed to increase the relative risk of cardiovascular disease in some [57;65;66] but not all studies [67-69]. Indeed recent analyses of the Women's Health Initiative demonstrate that the adverse effects of estrogen therapy on cardiovascular outcomes were largely confined to older women compared with younger perimenopausal women [70-78]. High doses of oral estrogens have been reported to elevate hepatocellular enzyme levels and, less commonly, cause cholestatic jaundice. The risk for gall stones and hepatocellular adenomas has been reported to be increased in association with oral contraceptive use, and although uncommon these complications may also occur with the use of replacement doses of estrogen [79-81]. Further, most studies have suggested an increased relative risk of breast cancer after four or five years of use[70;82-94], similar to the risk expected if the onset of menopause was delayed for a comparable length of time.

Estrogens may precipitate migraine headaches, and depression has also been reported to occur with the use of estrogens. In general, considering the dose and duration of treatment that we

propose to use in this protocol, the risk of developing such side effects is negligible.

Due to the publicity surrounding the cancellation of the treatment arm of the Women's Health Initiative study [28;29] that involved the administration of combined conjugated estrogens and medroxyprogesterone acetate (Prempro), we will include the following statement in the consent documents:

Adverse Events Related to Combined Hormone Replacement and the Results of the Women's Health Initiative (WHI):

The WHI study demonstrated that continuous administration of one form of estrogen (conjugated estrogens) in combination with one form of progesterone (medroxyprogesterone acetate) is associated with an increased risk of dementia, heart attacks, stroke, blood clots, and breast cancer. Estradiol, the form of estrogen that we use in the first three weeks of this study, is administered for a short time (three weeks) and as a sole agent (followed by one week's administration of medroxyprogesterone acetate) and, consequently, should not pose the increased risks observed with the chronic combination of the conjugated estrogens and medroxyprogesterone administered in the WHI study.

Estrogens may precipitate migraine headaches, and depression has also been reported to occur with the use of estrogens. In general, considering the dose and duration of treatment that we propose to use in this protocol, the risk of developing such side effects is very small. Also, we have excluded any subjects with a history of mood worsening in association with ET.

A skin rash at the site of patch placement is not uncommon and resolves usually with re-positioning of the skin patch. Thus a skin rash from the estradiol patch is an expected adverse event that will be tracked and reported at the time of CR.

Possible Risks related to Estradiol withdrawal:

Menstrual bleeding, hot flushes, vaginal dryness, and changes in mood may occur after

estradiol withdrawal; otherwise there are no known medical risks associated with estradiol withdrawal.

3. Progesterone and Synthetic Progestins (Provera)

a. Side Effects

Side effects reported in women taking progestins are uncommon but may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression of mood, easy fatigue, lack of initiative, and chloasma. Since progestins are often used in women with antecedent menstrual irregularity, it is not clear whether the breakthrough bleeding represents an effect of the medication or refractoriness to treatment. In the large majority of patients, menstruation occurs predictably following withdrawal of progestins. Finally, one investigation observed no differences in reports of physical or emotional symptoms between medroxyprogesterone acetate and placebo when they were added to ET under double blind crossover conditions [95]. Similarly, in protocol #90-M-0077 we did not observe significant mood changes during the addition of Provera to ET [7]. Side effects observed in patients receiving combined oral contraceptives include nausea, breast soreness, vaginal discharge, fluid retention, hypertension, and clotting abnormalities, which have been associated with the estrogen component of the oral contraceptive. Thromboembolic disorders including thrombophlebitis, pulmonary embolism, and cerebral and coronary thrombosis appear to occur with greater frequency in women taking oral contraceptives. While the increased incidence of these disorders has been associated with the estrogen component of the oral contraceptives, it is now believed that the progestin component may, to a lesser extent, contribute to the increased risk. There are relatively few reports associating oral contraceptives with the development of carcinomas (vaginal, uterine, hepatic, and mammary) despite the vast use of these agents, although this may reflect the latent period needed for cellular transformation. Finally, several reports suggest an association between intrauterine

exposure to female sex hormones and congenital anomalies.

Summary of potential adverse effects of the medications to be administered in this study in order of severity and timeliness of reporting to clinic staff:

- Cough with or without blood, sudden chest pain, shortness of breath consistent with a pulmonary embolism
- Calf pain, consistent with venous thromboembolism (e.g., DVT)
- Increased risk for stroke, worsening of atherosclerosis
- Headache
- Nausea, weight loss, or vomiting
- High blood pressure
- EKG abnormalities including QTc prolongation
- Breast tenderness or Gynecomastia
- Chloasma, spotty darkening of the skin (mostly of the face)
- Fluid retention
- An increase in calculated LDL concentration without a change in the LDL particle number or size
- Gastrointestinal disorders (such as constipation, diarrhea, nausea, dry mouth, and flatulence)
- Depression
- Nasopharyngitis
- Vaginal bleeding
- Back pain
- Dizziness
- Fatigue

b. Symptom Provocation

Some women may experience a recurrence of their previous depressive symptoms during the estradiol withdrawal phase. These symptoms are likely to be similar in severity to those experienced prior to study entry. However, if symptoms are intolerable we will discontinue their participation in the study and provide guidance for symptom remediation as described above.

c. Blood Drawing

Total research blood withdrawal during this seven-week study is 380 mls and falls within NIH guidelines (550 ml. per eight week period). Fifty ml's of research blood will be drawn at each of seven clinic visits in this protocol (total = 350ml). One potential discomfort of this study may result from the venipuncture and multiple blood sampling. A one-time blood draw of 30ml will be drawn for genetics.

d. Rating scales

e. Vaginal Ultrasound

Any abnormality identified by transvaginal ultrasound will be further investigated by the consultant gynecologist. If any woman experiences marked discomfort during the insertion of the ultrasound probe the procedure will be discontinued. Otherwise, there are no additional associated risks or discomforts with the transvaginal ultrasound.

f. Data Sharing

Under some circumstances, it can be a risk for information about an individual to be known. However, all research records containing any subject- identifying information will be stored under lock and key, or in secured computing environments by the CTDB. With these safeguards in place, we are confident data sharing is virtually without risk to subjects; however, subjects will be informed that risks of sharing data may include possibility of reidentification.

8. Subject Safety Monitoring

Subjects are evaluated by one of the Associate Investigators or the PI. Overall participant safety will be monitored by the PI. During weekly clinic visits all women will have blood pressure, pulse, and weight measured and will be assessed for any side effects to the prescribed medications (including specific evaluations of any complaints of calf pain or other symptoms and signs of blood clots in the legs). After completing the study (including the one month follow-up evaluation in every woman), participants will either be discharged from this protocol with a referral to the community, or will be referred to other NIMH studies.

Patients will be instructed not to become pregnant during the study and sexually active women will be required to employ barrier contraceptive methods.

The Investigators will consult with the Independent Medical Monitor, the NIMH Office of the Clinical Director and the Human Subjects Protection Unit (HSPU) team throughout study participation around issues of participant safety. Participants will be assessed for adverse experiences by the investigator. The reasons for study withdrawal include:

a. Clinically significant abnormal lab values or adverse events inconsistent with continuation in the protocol.

b. Clinical judgment of the investigator, the multidisciplinary team, or Independent Medical Monitor. The multidisciplinary team will meet throughout the week to coordinate treatment efforts, monitor the progress/safety of the patient and determine whether further participation would put the patient's safety at risk. The multidisciplinary team will make regular (i.e., every 3 weeks) on the clinical status of the participant to the Independent Medical Monitor. If the participant demonstrates clinical worsening (whether through clinical observation or scores on the CGI), the Independent Medical Monitor may recommend that the participant be withdrawn from protocol. Other members of the multidisciplinary team also reserve the right to discontinue a participant from

further participation for any reason.

c. Withdrawal of consent and/or patient decision to terminate.

d. Unable to tolerate the study medication, e.g., severe breast pain, severe headaches or nausea, disabling mood symptoms. Any patient experiencing clinically significant side-effects such as severe breast pain, severe headaches, nausea, hypertension, vomiting, extreme fluid retention, disabling mood symptoms, thrombotic conditions or extensive chloasma will discontinue taking the study drug. If the study drug is discontinued, patients continued involvement with our clinic will not be jeopardized; they can still receive care to ensure a safe discontinuation from the study.

While it is not anticipated, any subject who becomes acutely suicidal or whose depression becomes incapacitating (inability to perform previously managed daily vocational or home-related activities; self report of increased and overwhelming distress) during the course of this study will, after careful evaluation, have their customary schedule of hormone therapy resumed (if they were taking hormone therapy prior to the study), their participation in the protocol terminated, and if necessary, their care transferred to an appropriate outpatient or inpatient psychiatric facility. Any adverse events will be reported as per NIH policy (See Appendix A). NIH will not be able to pay for outside hospitalization.

9. Outcome Measures

a. Primary outcome measures

Outcome measures will include standardized mood and behavioral rating scales. The main comparisons in this study will be between the selective estrogen receptor beta agonist LY500307 and placebo in women with a past history of perimenopause-related depression. Outcome measures will include the following: Primary outcome measures: self ratings - the Center for Epidemiologic Studies-Depression Scale (CES-D) [47]; observer ratings - the 17-item Hamilton Rating Scale of Depression (HRSD) [48].

b. Secondary outcome measures: the daily symptom rating form (DRF) consisting of a 14 item six point likert-type scale [49] measuring the severity of several mood and behavior symptoms including hot flushes; the Beck Depression Inventory (BDI) [50]; and a visual analogue scale (VAS) measuring the reported severity of 13 mood and behavioral symptoms during the week prior to completion. Additionally, the relative impacts of LY500307 on ER alpha versus ER beta activity will be assessed by plasma LH, FSH, prolactin, and lipid levels, and endometrial thickness as measured by vaginal ultrasound. Finally, we plan to generate patient-derived LCLs and IPSCs from the women participating in this protocol to investigate both intrinsic cellular differences between women with PMD and controls as well as examining the effects of estradiol withdrawal with and without invitro exposures to an ER beta agonist.

10. Statistical Analysis

a. Analysis of data/study outcomes

The double blind will be broken and the randomization status of each participant will be revealed only after the completion of this study in all 45 women. Interim **blinded** analyses will be performed episodically (for example prior to the PI's Board of Scientific Counsellors) to inform ongoing scientific efforts and productivity of the Branch; however, these interim analyses will not serve as the basis for decisions to change study procedures or to terminate the study. Both study outcome measures and safety assessments will be reviewed by the DSMB.

We will first test for significant differences in mood symptoms arising during the double blind (DB) conditions compared with the open label (OL) ET (baseline) conditions in each of the three groups (i.e., women with past PMD randomized to DB LY500307 [25mg or 75mg] or placebo). Second, we will compare symptom ratings during DB treatment conditions across randomization groups (i.e., women with past PMD who were randomized to either DB LY500307 or placebo).

We will obtain six repeated measures on the same woman during two phases: baseline OL

ET (three weekly measures) and DB treatment (three weekly measures). Analyses will be performed with SAS Version 9.2 software (SAS Institute, Inc, Cary, NC), using PROC MIXED (for mixed models). For each symptom rating and blood hormone level, the predictor variables of interest, DB treatment (placebo or LY500307), will be modeled as fixed effects. The covariance pattern across time will be structured as first-order autoregressive. We will use the Kenward-Roger method for computing the degrees of freedom for tests of fixed effects.

For each of the symptom outcomes, we will perform analyses to explore the effect of DB medication in the two treatment groups. We will first examine if symptoms changed from baseline (i.e., OL ET [weeks 1-3 of the trial]) to DB treatment phase (i.e., weeks 4-6 of the trial) in any of the three randomization groups (as described above). For the second analysis, if necessary, we will include the mean of the baseline (OL ET) symptom measure (for each symptom) as a covariate and compare differences in symptom ratings across each of the three randomization groups to test for effects of DB treatment. Plasma levels of estradiol, estrone, prolactin, LH and FSH during DB treatment also will be analyzed across randomization groups. For all analyses the value of the estimator ("least square means" in SAS terminology) and associated standard error of the estimator and p values will be reported. The p values will be adjusted for multiple comparisons (k=3-6) by the Bonferroni method, and two-sided $P < 0.05$ will be considered statistically significant.

Clinical characteristics (tables 1-3) in women with past PMD will be compared to ensure similarity across groups and include the following measures: age, BMI, menstrual bleeding, weekly average hot-flush severity scores > 2 (minimal severity), CES-D scores > 16 (consistent with clinically significant depressive symptoms of at least moderate severity [1;96]), and CES-D scores >8 and <16 (consistent with subsyndromal depression [97-99]). We will employ Fisher's exact test for categorical variables and Student's t-tests for continuous variables.

b. Power analysis

Based on the results of protocol 03-M-0175 [12] and with standardized differences of .6 to .7 (and consistent with a prior meta-analysis of estrogen's effects on depressed mood [100]), 10 -15 women will be required in each of the three treatment cells to achieve a power of 80% with an $\alpha = 0.05$ to detect a significant difference between LY500307 and placebo with analyses of variance with repeated measures and Bonferroni t-tests in the principal outcome measures of HRSD and CES-D scales [101]. We intend to recruit up to 80 women for this trial through this protocol. Dropouts will be replaced. Since LY500307 has never been administered to women, there is no data available to estimate the sample size needed to evaluate safety measures. Safety measures (unblinded) including symptom inventories, clinical evaluations, laboratory results, and results of EKG of all women participating in this study will be reviewed by the DSMB.

11. Human Subjects Protection

Subject selection:

a. Subject selection will be equitable. All subjects must meet the inclusion and exclusion criteria listed in Section 4. We will select physically healthy adult female individuals. The proportion of ethnic minorities (vs. Caucasians) in the total sample will be approximately consistent with the overall U.S. population proportions. The specified age range for participants in this study (i.e., ages 45 – 65 years) was selected to match the sample selection criteria we previously employed in protocol 03-M-0175. The lower age limit was to avoid enrolling women with early menopause prior to age 45 and the upper age limit would restrict participants to those who had menopause by age 55 (and therefore not women who had menopause after age 55 years). Thus we wish to study women who experienced menopause within the more normal range between ages 45 and 55 years.

b. Justification for exclusion of children:

We will exclude children or minors because the study population is women with past

perimenopausal depression.

c. Justification for exclusion of vulnerable subjects:

Those without consent capacity are excluded because the research question can be answered by enrolling only adults who can provide their own consent and there is no direct benefit for these participants. Therefore, the risks outweigh the benefits for this population.

Since this study is more than minimal risk, and there is no safety data from non-pregnant women, pregnant women will be excluded from this study. Men will also be excluded because the study population is women with past perimenopausal depression.

Justification of sensitive procedures: Depressive symptoms will be provoked to see if the study drug is effective. Any subject who becomes acutely suicidal or whose depression becomes incapacitating (inability to perform previously managed daily vocational or home-related activities; self report of increased and overwhelming distress) during the course of this study will, after careful evaluation, have their customary schedule of hormone therapy resumed (if they were taking hormone therapy prior to the study), their participation in the protocol terminated, and if necessary, their care transferred to an appropriate outpatient or inpatient psychiatric facility. Any adverse events will be reported as per NIH policy (See Appendix A).

d. Safeguards for vulnerable populations:

Pregnancy testing will be required prior to study entry and in sexually active women barrier contraceptive use will be required during this study.

The enrollment of NIH employees will be in accordance with NIH policy 404 titled “Research Involving NIH Staff as Subjects”. Employees wishing to participate will be given the “NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research”. Every effort will be made to protect participant information but such information may be available in medical records and may be available to authorized users outside of the study team

in both an identifiable and unidentifiable manner.

12. Anticipated Benefits

There are no direct benefits of participating in this study. We hope that the results of the study will help us better understand perimenopause-related depression, which may help others in the future.

Overall risk and benefit consideration:

The overall risks for this study are more than minimal; the risks are reasonable in relation to anticipated benefit.

13. Consent Documentation and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

b. Consent procedures

The informed consent document will be provided as a physical or electronic document to the participant or consent designee as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort, and benefits. To minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

The initial consent process as well as re-consent, when required, may take place in person or

remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on either on paper or on screens at their respective locations; the same screen or paper document may be used when both the investigator and the participant are co-located but this is not required. When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. The process for documenting signatures on an electronic document is described below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the iMedConsent platform (which is 21 CFR Part 11 compliant) or the Adobe Acrobat platform (which is not 21 CFR Part 11 compliant).

Both the investigator and the participant will physically sign the paper document, or sign the electronic document using a finger, stylus, or mouse. Electronic signatures (i.e., the “signature” and a timestamp are digitally generated) will not be used. A record of the communication of this

information and of the consent to participate in this study will be placed in the medical record. The right of the subjects to withdraw from the study or to refuse any procedure will be made clear. Any patient whose symptoms become excessive during this study will be offered another medication without completing the seven-week trial. Confidentiality of patients will be assured according to the laws of the federal Privacy Act. In case of published data resulting from the study, care will be taken to protect the anonymity of patients.

c. Consent documents

The Patient consent and the Genetics consent forms contain all required elements. All women enrolled in this study will first sign the Patient consent. Additionally, blood samples for lymphoblastoid cell lines and induced-pluripotent cell lines will be obtained with a separate Genetics consent form from this protocol.

d. Considerations for Consent of NIH staff or family members of study team members:

Consent for NIH staff will be obtained as detailed above with following additional protections: Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

e. Request for Waiver of Consent for Pre-Screening Activities:

Prior to the subject signing the consent for this study, pre-screening activities listed in the screening section may be performed. We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the waiver as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening

activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

14. Data and Safety Monitoring

a. Data and safety monitor:

The protocol will be monitored by the NIMH-IRP Data and Safety Monitoring Board (DSMB). The NIMH-IRP DSMB SOP and membership is on file with the IRB.

b. Data and safety monitoring plan:

This study will be reviewed by the NIMH DSMB prior to implementation and at least twice annually. Additional reviews may be scheduled at the discretion of the DSMB. The DSMB will review study accrual and progress, adverse events related to the study and safety and outcome data as well as other study specific data elements at their request. The medically responsible investigator has the authority to break the blind in the event of an emergency. Breaking of the blind, life threatening injury or death all require immediate reporting to the DSMB and the NIMH Clinical Director. A written notification to the DSMB chair must follow within 7 days. Results of the DSMB review are provided to the PI, the Clinical Director and the IRB.

The PI is responsible for participants in this protocol and will report all serious adverse events to the NIMH Clinical Director and the IRB within the guidelines set by the standards for clinical research within the NIH intramural research program.

As part of the Clinical Operations Committee, the Principal Investigator (PI) and Associate Investigators will discuss subjects' participation during weekly rounds and will review all data and procedures. During weekly clinic visits all women will also have blood pressure, pulse, and weight measured and will be assessed for any side effects to the prescribed medications (including specific

evaluations of any complaints of calf pain or other symptoms and signs of blood clots in the legs). The PI will be ultimately responsible for monitoring the data and safety and will provide continuous, close monitoring of adverse events. A yearly summary of adverse event data will be reported to the NIH IRB and the external Data and Safety Monitoring Board (DSMB).

c. Criteria for stopping the study or suspending enrollment or procedures:

In the event of unanticipated problems or serious side effects, the PI will consider whether the study should continue.

The DSMB will have the authority to require changes in the study design, or to stop all or part of any study based on accumulating safety data. Any changes required as conditions for resuming the research must be submitted as an amendment and IRB-approved before the changes can be implemented.

15. Quality Assurance

a. Quality assurance monitor

Quality assurance will be monitored by the PI, the research team and the NIMH Office of Regulatory Oversight (ORO).

b. Quality assurance plan

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the ORO SOP based on the study level of risk. Results of ORO audits are provided to the PI, The Clinical Director and the CIRB. As an IND study, this protocol will be subject to GCP audits at study initiation and after the first enrolled subject. Timing of subsequent review will be established by the ORO but no less frequent than every year.

16. Reporting of Unanticipated problems, adverse events and protocol deviations

Reportable events for this protocol will be tracked and reported in compliance with Policy

801.

a. For Drugs and Biologics

The PI and the IND Sponsor for this study, will report SAEs according to the requirements of 21 CFR 312.64(b). The PI and Sponsor will record nonserious AEs.

17. Alternatives to Participation

Subjects either do not receive any treatment in this study or forego treatment in order to participate in this study. The alternative, therefore, is not to participate.

18. Privacy

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

19. Confidentiality

a. For research data and investigator medical records

Data will be stored using codes that we assign. Data will be kept in password-protected computers. Only study investigators will have access to the data.

No sensitive information is collected, so employee information will be treated the same as all other participants.

b. For stored samples

Samples will be stored using codes that we assign. Samples will be kept in locked storage. Only study investigators will have access to the samples.

c. Special precautions

Not applicable.

20. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

There are no conflicts-of-interest to report. Non-NIH investigators will abide by the conflict-of-interest policies of their own Institutions.

b. Conflict of interest: There are no conflicts to report.

c. Role of a commercial company: Eli Lilly will provide suitable amounts of LY500307 to the NIH Pharmacy which will be provided to the contract manufacturing organization (CMO) (i.e., Pine Pharmaceuticals) where quality assurance tests will be performed and appropriately dosed capsules will be formulated. A contract for manufacturing and a quality agreement are being initiated between NIMH and the CMO.

21. Technology Transfer approval from Lilly

In addition to drug substance, Eli Lilly and Company provided the NIH with access to the information in the existing IND (IND - 100,916 for LY500307 study I1A-MC-E001 (ER beta agonist) including information on formulation development and drug product stability. The Clinical Trial Agreement number is 2015-0279. There is no expiration date.

21. Reimbursement and Travel Compensation

Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.
Each volunteer will be compensated according to the following schedule:

Screening Procedures:

Initial evaluation, physical exam (2 hours)	30.00
Venipuncture $20 X 3	60.00
Completion of symptom ratings $10 X 3	30.00

ER beta trial:

Venipuncture $20 X 8	160.00
Genetics Bloods	60.00
Clinic visits (weekly) $20 X 8	160.00
Completion of symptom ratings $10 X 8	80.00
Transvaginal ultrasound $100 X 2	200.00

Investigational drugs:

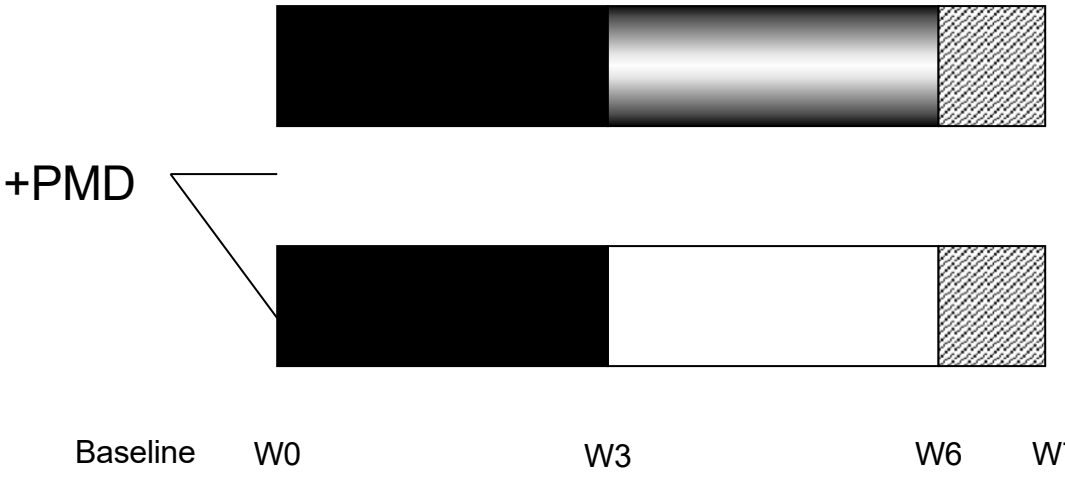
Estradiol Patch $30 X 2	60.00
LY500307 (or placebo) 21 doses	150.00
Provera	30.00
Total	**$1020.00**

Compensation will be prorated for parts completed if subjects do not complete the study. No escort fee will be provided.

Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Flow Sheet

Protocol Schematic



+PMD = Asymptomatic Postmenopausal Women with past perimenopause-related depression

- -Estradiol 100 μg/day (skin patch)
- -Placebo (PO)
- -LY500307 25 mg or 75 mg daily (PO)
- -Provera 5 mg daily (PO)

22. References

1. Freeman EW, Sammel MD, Liu L, et al. Hormones and menopausal status as predictors of depression in women in transition to menopause. Arch Gen Psychiatry 2004;61: 62-70

2. Cohen LS, Soares CN, Vitonis AF, et al. Risk for new onset of depression during the menopausal transition. The Harvard study of moods and cycles. Arch Gen Psychiatry 2006;63: 385-390

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23. Appendix 1

Adverse Experiences/Concurrent Illnesses

Any event of an adverse nature that is reported or observed to occur during the study after initiation of treatment, whether or not believed to be related to the use of the test article will be documented in the patient record and on the Adverse Event Case Report Form.

Definition of Terms

An **adverse event** means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

Exacerbation of pre-existing conditions means any condition or disease state that was present prior to first treatment with test article that changes adversely in nature, severity, or frequency during the study.

An adverse event (AE) or suspected adverse reaction is considered "**serious**" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Life threatening means the subject is at immediate risk of death as a result of the event as it occurred. It does not mean that a reaction, had it occurred in a more serious form, might have

caused death.

Classification of an Adverse Event:

Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **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".

Relationship to Study Intervention:

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Expectedness

The PI will be responsible for determining whether an adverse event (AE) is expected or unexpected. 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 intervention.

Eliciting Reports of Adverse Events

In addition to documenting observed changes in a subject's medical or physical condition, information regarding the occurrence of adverse events during the study will be solicited through non-directed questioning of the subject by the investigator at each clinic visit. Subjects will also be asked if they have been hospitalized for any reason, had any accidents, used any new medications, or changed dose or regimen of any concomitant medication (prescription or over-the-counter), and the reasons for such.

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 study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (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 study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

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. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Adverse Event Reporting:

Serious Adverse Event Reporting

The study investigator will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information

qualifies for reporting.

In consultation with the PI, a trained member of the study team will be responsible for conducting an evaluation of all adverse events and shall report the results of such evaluation to the NIH Institutional Review Board (IRB) as per Policy 801.

Definition of Unanticipated Problems (UP)

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, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- 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 (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

Appendix 2

Clinical Trials Database - Security Overview

There are multiple aspects to the security framework for the Clinical Trials Database (CTDB) and Clinical Trials Survey System. The following features allow for the safe and secure collection of research variables:

- Application Firewall- The NICHD has recently upgraded their application firewall which protects both the front end web server and back end database server for the CTDB. Strict policies are in place which control exactly who has pre-defined, limited access to the application. This firewall is a state-of-the-art hardware solution which blocks access to everyone but authorized users for the CTDB system.

- Data Encryption and SSL Certificates - The CTDB system makes use of military grade encryption both for the session and the data storage. The CTSS collects de-identified self-reported data. Both systems protect information from interception by encrypting the data flow using SSL with a 1024 bit signed certificate. The entire communication session from the time a client requests a connection to the system to the time a user logs out is encrypted using a certificate from an industry recognized vendor. The result is a secure communications channel for our partners, providing data confidentiality and integrity. In further, the CTDB program encrypts data stored within the system, thereby providing an additional layer or security for the sensitive CTDB clinical data.

- CTSS HIPAA Requirements - Below is the list of identifiers to be removed for the de-identification of health information under HIPAA. This is found in 45 C.F.R. 164.514(a),(b)&(c)

(A) Names;

(B) All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of the Census:

(1) The geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and

(2) The initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.

(C) All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older;

(D) Telephone numbers;

(E) Fax numbers;

(F) Electronic mail addresses;

(G) Social security numbers;

(H) Medical record numbers;

(I) Health plan beneficiary numbers;

(J) Account numbers;

(K) Certificate/license numbers;

(L) Vehicle identifiers and serial numbers, including license plate numbers;

(M) Device identifiers and serial numbers;

(N) Web Universal Resource Locators (URLs);

(O) Internet Protocol (IP) address numbers;

(P) Biometric identifiers, including finger and voice prints;

(Q) Full face photographic images and any comparable images; and

(R) Any other unique identifying number, characteristic, or code, except as permitted by paragraph (c) of this section

- Logical Access Controls (Role/Privileges) – Logical access controls are in placed using role based security for database access and application account access. Security controls are in place to detect unauthorized access attempts. The application is further protected by the NICHD firewall and NIH firewall. Inactive user accounts are monitored and removed when not needed, and users are disconnected after a specific period of inactivity. Encryption is used (1024bit SSL key) and data is HIPPA compliant. Access is monitored and apparent security violations are investigated when identified. Insecure protocols are disabled on all application servers. Guest and anonymous accounts/access is disabled.

- Audit Trails – Activity involving access to and modification of sensitive or critical files is logged and monitored for possible security violations. Access to these audit trails is strictly controlled and can be used to support after-the-fact investigations of how, when and why normal operations ceased should this occur. Off-line storage of audit logs is retained for a period of at least 1 year. Suspicious activity is investigated and appropriate action is taken when warranted.

- Physical and Environmental Protection – The servers are physically located in a secured NIH data center with controlled limited access. All work products from the system including Data backup tapes are rotated to off-site storage with must be authorize and are recorded. All visitors to sensitive areas are escorted with entry codes changed periodically. Fire prevention and suppression devices are installed and in working condition. All heating and air-cooling systems are periodically checked to ensure proper working condition.

- Production Input and Output Control – Audit trails are in place to record data changes. Only authorized system administrators are allowed access to this data, as well as any data backup tapes. Damaged media is sanitized or destroyed, and any hardcopy media is shredded when no longer needed.
- Contingency Planning – All critical data files, database files and web server files have been identified. A regularly scheduled data backup solution is in place with identified resources supporting critical operations. A comprehensive contingency plan has been developed and documented. This plan has been approved by key affected parties. The Contingency plan/Disaster recovery plan is regularly tested and adjusted as appropriate.
- Hardware and Software System Maintenance – Access is limited to the hardware and software infrastructure. Restrictions are in place as to who performs maintenance activities. Procedures are in place to monitor the use of system resources. All new and revised hardware and software are tested and approved before implementation. All system and application components are tested, documented and approved prior to promotion to production environment. Detailed system specification as prepared and reviewed by management. A version control system is in place for all key application and operating system files. The systems are actively managed to monitor and reduce vulnerabilities with unnecessary services eliminated.

Strategic framework – The tactical security framework provides a mechanism whereby the Clinical Trials support personnel provide day-to-day operational support activities for the regular maintenance of the CTDB system. These initiatives incorporate application and software security. The CTDB application was designed using the latest Java technology. This allows the adaptation of the application to ever-changing business rules within the application. An Oracle 10g relational database provides the repository for the clinical data. The use of a robust, industry standard

relational database provides a modular architecture design of CTDB which allows for the CTDB program to assign role based security to the participants in the system. This allows roles to be defined and implemented for different users- such as investigators, study participants, report writes, etc- in order to secure database access and the application data stored within the system. This implies that the NICHD CTDB partners can implement custom roles and maintain their own clinical data with a high level of confidence that the data will not be compromised nor shared with non-participants. As technology evolves, this attention to the strategic framework allows us to address individual software components and target them for enhancements or upgrades all while maintaining the integrity and confidentiality of the CTDB system. Another example of this strategic framework is the reporting interface. The modular design of CTDB allows provides the ability to upgrade to Cognos reporting with the result being more detailed comprehensive reporting abilities. The net result is a feature-enhanced system while maintain the strict security framework of the system.

(1) Transvaginal Ultrasound

What Is a Transvaginal Ultrasound?

An ultrasound test uses high-frequency sound waves to create images of your internal organs. The sound waves bounce off your organs, creating images of their structures. Imaging tests can identify abnormalities and help doctors diagnose conditions. A transvaginal ultrasound is a type of pelvic ultrasound used by doctors to examine female reproductive organs. This includes the uterus, fallopian tubes, ovaries, cervix, and vagina.

"Transvaginal" means "through the vagina." This is an internal examination. Unlike a regular pelvic ultrasound, where the ultrasound wand rests on the outside of the pelvis, this procedure involves your doctor or a technician inserting an ultrasound probe about two or three inches into your vaginal canal.

When Is a Transvaginal Ultrasound Performed?

There are many reasons a transvaginal ultrasound might be necessary, including:

•an abnormal pelvic or abdominal exam
•unexplained vaginal bleeding
•pelvic pain
•an ectopic pregnancy (which occurs when the fetus implants outside of the uterus, usually in the fallopian tubes)
•infertility
•checking for cysts or uterine fibroids
•checking for proper placement of an IUD

How Should I Prepare for a Transvaginal Ultrasound?

In most cases, a transvaginal ultrasound requires little preparation on your part. Once you've arrived at your doctor's office or the hospital and you're in the examination room, you will have to remove your clothes from the waist down and put on a gown.

Depending on your doctor's instructions and the reasons for the ultrasound, your bladder might need to be empty or partially full. A full bladder helps lift the intestines and allows for a clearer picture of your pelvic organs. If your bladder needs to be full, you'll have to drink 32 ounces of water or any other liquid about 30 minutes to one hour before the procedure begins.

If you're on your menstrual cycle or if you're spotting, you'll have to remove any tampon you're using before the ultrasound.

What Happens During a Transvaginal Ultrasound?

When it's time to begin the procedure, you'll lie down on an examination table and place both of your feet in stirrups. Your doctor will cover the ultrasound wand with a condom and lubricating gel, and then insert the wand into your vagina.

You might feel some pressure as your doctor inserts the wand. This feeling is similar to the pressure felt during a pap smear when your doctor inserts the speculum into your vagina. Once the wand is inside of you, sound waves bounce off your internal organs and transmit pictures of the inside of your pelvis onto a monitor. The technician or doctor will slowly move the wand around while it's still inside of your body. This provides a comprehensive picture of your organs.

What Do the Results Show?
You might get your results immediately if your doctor performs the ultrasound. If a technician performs the procedure, the images are saved and then analyzed by a radiologist. The radiologist will send the results to your doctor.

A transvaginal ultrasound helps diagnose multiple conditions, including:

- cancer of the reproductive organs
- cysts
- fibroids
- pelvic infection
- the thickness of the uterine lining

Outlook
There are virtually no risks associated with a transvaginal ultrasound, although you might experience some discomfort. The entire test takes about 30 to 60 minutes, and the results are typically ready in about 24 hours. If your doctor is unable to get a clear picture, you might be called back to repeat the test.

(2) Deep Vein Thrombosis (DVT)

What Is Deep Vein Thrombosis?

Deep vein thrombosis (DVT) is a serious condition that occurs when a blood clot forms in a vein located deep inside your body. A blood clot is a clump of blood that is in a gelatinous, solid state. Deep vein blood clots typically form in your thigh or lower leg, but they can also develop in other areas of your body. Other names for this condition include thromboembolism, post-thrombotic syndrome, and post-phlebitic syndrome.

Who Is at Risk for Deep Vein Thrombosis?

DVT occurs most commonly in people who are over 50 years in age. Certain conditions that alter how your blood moves through your veins can raise your risk of developing clots. These include:

•having an injury that damages your veins
•being overweight, which puts more pressure on the veins in your legs and pelvis
•having a family history of DVT
•having a catheter placed in a vein
•taking birth control pills or **undergoing hormone therapy**
•smoking (especially heavy usage)
•staying seated for a long time while you're in a car or on a plane, especially if you already have at least one other risk factor

Some diseases and disorders can increase your risk of having blood clots. These include hereditary blood clotting disorders, especially when you have at least one other risk factor. Cancer and inflammatory bowel disease can also increase the risk of developing a blood clot. Heart failure, a condition that makes it more difficult for your heart to pump blood, also occurs with an increased risk of clots.

What Are the Symptoms of Deep Vein Thrombosis?

According to the National Heart, Lung, and Blood Institute, symptoms of DVT only occur in about half of the people who have this condition. Common symptoms include:

•swelling in your foot, ankle, or leg, usually on one side
•cramping pain in your affected leg that usually begins in your calf
•severe, unexplained pain in your foot and ankle
•an area of skin that feels warmer than the skin on the surrounding areas
•skin over the affected area turning pale or a reddish or bluish color

People may not find out that they have deep vein thrombosis until they've gone through emergency treatment for a pulmonary embolism. A pulmonary embolism is a life-threatening complication of DVT in which an artery in the lung becomes blocked.

What Are the Complications Associated with Deep Vein Thrombosis?

A major complication of DVT is a pulmonary embolism. You can develop a pulmonary embolism if a blood clot moves to your lungs and blocks a blood vessel. This can cause serious damage to your lungs and other parts of your body. You should get immediate

medical help if you have signs of a pulmonary embolism. These signs include:
•dizziness
•sweating
•chest pain that gets worse with coughing or inhaling deeply
•rapid breathing
•coughing up blood
•rapid heart rate
•dry cough
•low grade fever

How Do I Prevent Deep Vein Thrombosis?

You can lower your risk of having DVT by making a few lifestyle changes. These include keeping your blood pressure under control, giving up smoking, and losing weight if you're overweight. Moving your legs around when you've been sitting for a while also helps keep your blood flowing. Walking around after being on bed rest can prevent clots from forming. Your risk of developing DVT during travel is low, but it becomes higher if you're sitting for more than four hours at a time while driving or flying. You can lower risk by moving around every so often — get out of your car and move around at intervals during long drives. Walk in the aisles if you're flying, taking a train, or riding a bus. Stretch your legs and feet while you're sitting; this keeps your blood moving steadily in your calves. Don't wear tight clothes that can restrict blood flow.

24. Consent Forms

ERbeta Consent Form

25. Attachments

LY500307 Investigator's Brochure