Study on Allopregnanolone and Depression in Perimenopausal Women

Detailed Protocol

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Institutional Review Board
Intervention/Interaction Detailed Protocol

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Project Title:	Using Allopregnanolone to Probe Behavioral and Neurobiological Mechanisms that Underlie Depression in Women during the Perimenopause
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1. BACKGROUND AND SIGNIFICANCE

Perimenopausal Depression: Midlife women are burdened with depression risk that is at least partly attributed to changing reproductive steroid dynamics across a prolonged reproductive transition. Perimenopause (Peri) confers a specific period of risk in women for subthreshold depressive symptoms and major depressive episodes (MDEs).[1] Indeed, women are twice as likely to experience MDEs in Peri than in late premenopause.[2,3] MDEs during Peri represent depression recurrence for most affected women, but 45%–68% of women without a history of major depressive disorder (MDD) develop at least subsyndromal depressive symptoms during Peri.[1] While less severe than MDE, subsyndromal depression also causes significant distress and impairs quality of life.[4,5] Perimenopausal depression (PeriDep) encompassing MDEs and subthreshold depression is common in women.

PeriDep shares risk factors with depression occurring at other life stages,[1] but also has unique risk factors including hot flashes [or vasomotor symptoms (VMS)], depression during other reproductive transitions, and sleep disturbance. Sleep disturbance is both a symptom of and causal for depression.[6–8] Sleep problems are prevalent among Peri women due to nocturnal VMS, which lead to increased time spent awake after sleep-onset (WASO), the most prominent Peri sleep problem.[9] However, sleep-onset problems and shorter total sleep time can also occur.[10] Selective association of PeriDep with nocturnal VMS[9] suggests that mechanistic processes underlying VMS at night[11] are especially disruptive to mood in Peri women.

PeriDep, postpartum depression (PPD), and premenstrual dysphoric disorder (PMDD) are reproductive–endocrine (repro-endo) mood disorders unique to women.[12] Each represents a vulnerable period for depression that is both temporally and causally linked to changing reproductive steroids. In all three disorders, estradiol (E2) and progesterone (P4) levels decline.[13–15] Because the P4 mechanism has been targeted effectively in PPD through its allopregnanolone (ALLO) metabolite,[16] the P4 withdrawal shared between disorders suggests that similar strategies may be effective therapy for PeriDep. Shared risk factors and parallel underlying reproductive steroid changes substantiate significant overlap between the reproductive-endocrine mood disorders of PeriDep, PPD, and PMDD. While synthetic P4 preparations anecdotally worsen mood in PeriDep, empiric studies find no adverse mood effects.[17] Our findings that ovulatory levels of P4 correlate with better mood[13] support the observation that natural P4 administration improves mood in PeriDep,[18,19] and our premise that ALLO administration improves PeriDep through the proposed behavioral and neurobehavioral mechanisms.

Changes in Endogenous Allopregnanolone (enALLO) as a Mechanism Underlying PeriDep: How P4 and its metabolites alter mood in PeriDep is unclear.[20] One plausible mechanism is through P4 metabolism to the endogenous neurosteroid ALLO.[21,22] Levels of enALLO mirror those of P4—increasing across pregnancy, in the menstrual cycle luteal phase, and when P4 is added to postmenopausal hormone therapy,[23] and decreasing postpartum and across menopause.[24,25] Through gaba-aminobutyric $(GABA)_A$ receptor activity, enALLO can rapidly impact mood, but other critical biological mechanisms discussed below may also translate these benefits. Changes in enALLO influence mood via regulation of neuronal function involved in both emotion and sleep/wakefulness. Ovulation triggers high systemic levels of P4, a precursor that stimulates

enALLO production; conversely, when P4 declines, reduced enALLO production may promote emergence of depression in repro-endo mood disorders, as suggested in studies of women who have PPD or PMDD, or across the menopause.[25,26] Symptoms of PMDD are averted by blocking P4 conversion to enALLO premenstrually,[27] and PPD symptoms are triggered when GABA receptor responsivity[28] to peripartum enALLO[29] activity is suppressed. enALLO levels are also lower in depressed than non-depressed women during premenopause, late Peri, and also postmenopause.[24,30–32] We hypothesize that falling enALLO levels across menopause underlies PeriDep. Taken together, evidence of analogous changes in endogenous progesterone and ALLO, alongside ALLO dysregulation during PPD, PMDD, and PeriDep, and data supporting the efficacy of exogenous ALLO for PPD, suggest that exogenous ALLO will exert its effects through similar behavioral and neural mechanisms in women with PeriDep.

Exogenous ALLO (exALLO) as a Mechanistic Probe in PeriDep: The gradual process through which P4 declines across Peri and the expected parallel changes in enALLO make exploration of ALLO's mechanistic effects on behavioral and neurobiological processes underlying depression challenging to quantify. Thus, we propose a mechanistic trial to amplify contrast between lower enALLO levels in Peri and higher levels experimentally induced by exALLO. We will follow the exALLO treatment studied in PPD patients using FDA-approved brexanolone,[16] which has proven antidepressant efficacy.[33] Brexanolone will be used as it is FDA-approved for the same indication of depression in a different population of women that has shared underlying neural mechanisms and for which substantial safety data are available. This specific source of exogenous ALLO was selected as the probe in this study because it is the pure form of ALLO mimicking the endogenous ALLO neurosteroid, which is the mechanism under investigation. Brexanolone optimizes the ability to test the mechanistic hypotheses in a controlled laboratory setting using a rigorous experimental design. By manipulating ALLO levels together with measurement of key depression-driving constructs, this powerful approach harnesses the endocrine biology of Peri to rigorously explicate behavioral and neurobiological mechanisms underlying depression in Peri women.

2. SPECIFIC AIMS AND OBJECTIVES

We propose to test the effects of exogenous ALLO in a well-characterized cohort of women with mild to severe depression presenting during early and late perimenopause. This project will use an experimental placebo-controlled, mechanistic trial to determine acute and durable effects of exogenous ALLO on behavioral, cognitive, circuit-level, physiological, and molecular processes centered on the Negative Valence Systems and Arousal and Regulatory Systems Domains of the Research Domain Criteria (RDoC) framework. We expect to uncover neurobiological mechanisms that underlie depression during early and late perimenopause via three aims:

Aim 1: To determine the impact of allopregnanolone on key behavioral and circuit-based processes that underlie depression in perimenopausal women.

Hypotheses: Compared to placebo, exogenous ALLO will improve the following: **1a) behavioral/cognitive** measures of rumination and attentional bias toward negatively valenced external stimuli; and **1b) neurocircuitry**, via resting-state connectivity underlying rumination and negative attentional bias within the default mode network and between the default mode and salience networks.

Aim 2: To determine the impact of allopregnanolone on key molecular and physiological processes that underlie depression by indexing markers of neuroprotection, peripheral inflammation, and sleep physiology in perimenopausal women.

Hypotheses: Compared to placebo, exogenous ALLO will improve the following: **2a) neuroprotection**, via circulating levels of brain-derived neurotrophic factor (BDNF), and levels of N-acetyl-aspartate (NAA) in the medial prefrontal cortex, measured with magnetic resonance spectroscopy; **2b) inflammation**, via circulating levels of pro-inflammatory molecules; and **2c) sleep physiology**, via EEG-derived wakefulness after sleep onset.

Exploratory Aim 3: To identify mediators and moderators of the impact of allopregnanolone on clinically-defined global measures of depression severity in perimenopausal women.

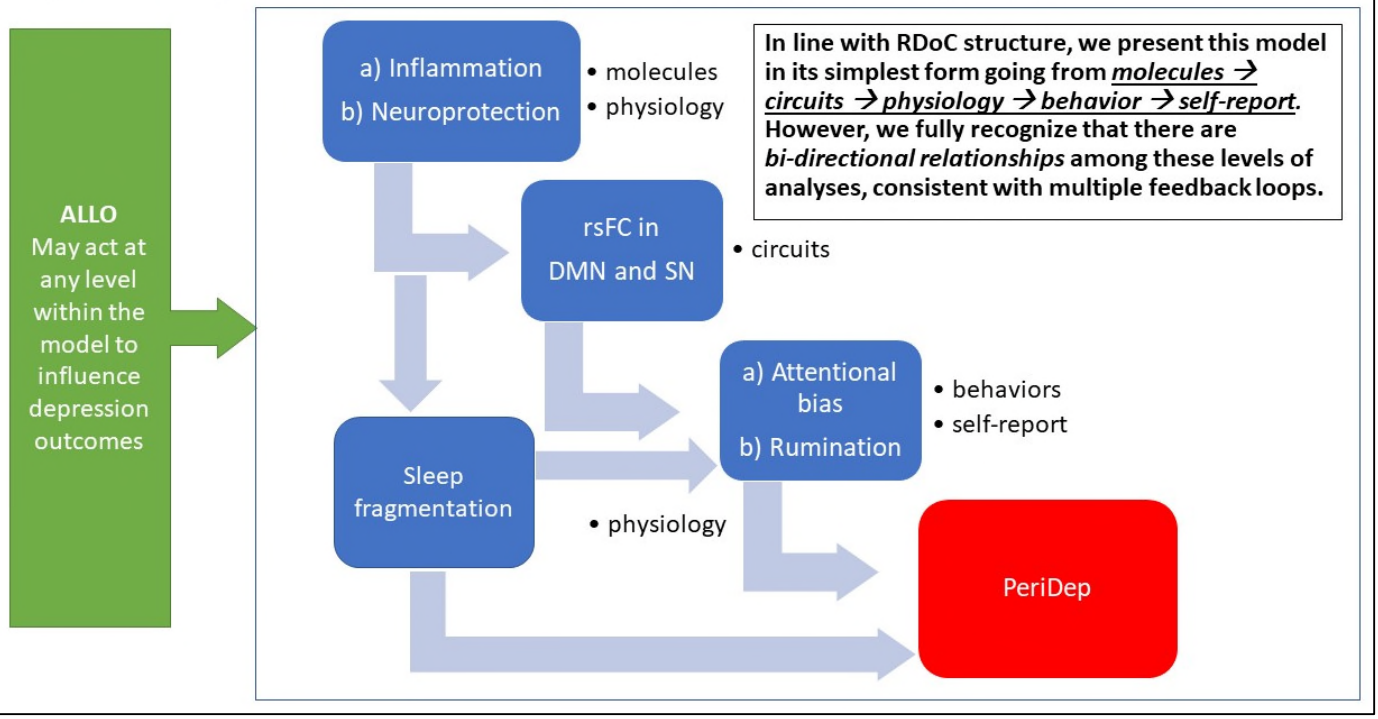
Exploratory Hypotheses: The magnitude of improvement in clinically-defined global measures of depression severity induced by exogenous ALLO will be: **3a)** mediated by changes in behavioral and circuit-based outcomes derived from Aim 1, and changes in neuroprotective, inflammatory, and sleep physiology outcomes derived from Aim 2; and **3b)** moderated by prior depression illness course and perimenopausal factors.

Alignment with the 2020 NIMH Strategic Plan for Research: To uncover behavioral and neurobiological mechanisms that underlie PeriDep, we will identify mechanisms targeted by exALLO (Aims 1 and 2) and evaluate the relationship of changes in these therapeutic targets (as mediators) and changes to depression symptom severity (Exploratory Aim 3). Using a clinically effective pharmacological probe (exALLO) with proven antidepressant action allows us to directly perturb systems to identify the mechanisms through which enALLO induces PeriDep.

We used the Research Domain Criteria (RdoC) Framework[1] to select key mechanistic targets in pathways believed to underlie depression, including behavioral, circuit-based, molecular, and physiological processes. Specifically, we will investigate constructs within Negative Valence Systems (NVS) and Arousal and Regulatory Systems (ARS).

Figure 1 illustrates our overarching theoretical model and the integration of our outcomes across our multiple levels of investigation. In this model, molecular mechanisms drive subsequent changes at the level of brain circuitry, physiology, and behavior, which together, result in the clinical syndrome of PeriDep. The figure shows that ALLO may act at any level within the model to influence depression outcomes in PeriDep.

Figure 1. Integrated Mechanistic Model of Perimenopausal Depression



NVS Behavioral Targets*:*

1) Rumination is focused attention on one's distress,[2] which contributes to depressed mood. Patients with MDD ruminate more frequently than healthy individuals.[3] Rumination is a risk factor for development and maintenance of MDEs,[2] and is associated with MDEs and subthreshold depression in women during Peri,[4] potentially due to diminished production of P4.

2) Attentional bias toward negative stimuli: Depressed patients display mood-congruent processing biases, with slower reaction times, less accuracy on tasks that use positively valanced stimuli, and heightened sensitivity to negative feedback. Similar to rumination, P4 is inversely related to negative attentional bias in midlife women, suggesting negative bias is a potential therapeutic target of exALLO.

NVS Circuit-based Targets: Neural networks implicated in rumination and negative attentional bias are themselves mechanistic targets.

1) DMN abnormal reactivity is linked to rumination, along with

2) abnormal connectivity between DMN and SN that also reflect biases in assignment of salience and failure to shift attention away from self-referential processes in depression. Convergent evidence, including our own,[5] suggests effects of enALLO on function in intrinsic networks related to rumination and negative attentional bias.

NVS Molecular and Physiological Targets:

1) Neuroprotection shields against cellular damage by processes such as excitotoxicity, mitochondrial dysfunction, neuroinflammation, and neurotrophin dysregulation.[6,7] Peri women exhibit evidence of decreased neuroprotection.[53,71] SSRIs exert neuroprotective effects,[8,9] upregulating serum enALLO levels in depressed patients[10–12] and increasing two key brain markers of neuronal health:

a) brain-derived neurotrophic factor (BDNF),[6,13] measured peripherally; and

b) N-acetyl aspartate (NAA) levels,[14–16] measured in vivo in the brain. enALLO boosts neuroprotection,[17–19] increasing neuronal viability via improved mitochondrial energetics,[20] oxidative stress,[21] apoptosis,[19,21–23] and BDNF levels in cell and animal models.[24–26]

2) Inflammation: Peri is a pro-inflammatory reproductive stage,[27] with increased rates of new-onset autoimmune diseases [e.g., multiple sclerosis (MS)]. Inflammatory responses during Peri also correspond with changes in brain structure[28] and function.[29,30] Inflammation plays an important role in depression across the lifespan,[31] at least in some individuals,[32] and may contribute to PeriDep.[4] ALLO has anti-inflammatory effects in both preclinical and human studie.[33] ALLO's anti-inflammatory action is demonstrated by reduced central nervous system measures in MS patients,[34] and indirectly by an inverse relationship of the ALLO precursor P4 with TNF-a, IL-6, and CRP in PeriDep. These findings suggest inflammation may be a therapeutic target of exALLO.

***ARS Physiological Targets*:** A multi-factorial mechanistic pathway links sleep disturbance to depression, including affect response,[35,36] neurotransmission,[37,38] and changes in brain connectivity associated with cognition and mood.

Mechanisms Linking Sleep to Depression: Sleep loss adversely affects function in intrinsic brain networks, including creating an imbalance in DMN activation and decreasing thalamocortical FC,[39,40] effects that may extend to impaired arousal, information integration, and cognition. Sleep disturbance may exacerbate depression severity by adversely altering inflammation[41] (e.g., increased CRP and IL-6 levels), which can be reversed by cognitive behavioral therapy for insomnia.

ALLO and Sleep: exALLO administration induces benzodiazepine-like effects on sleep, reducing time to onset of NREM sleep.[42]

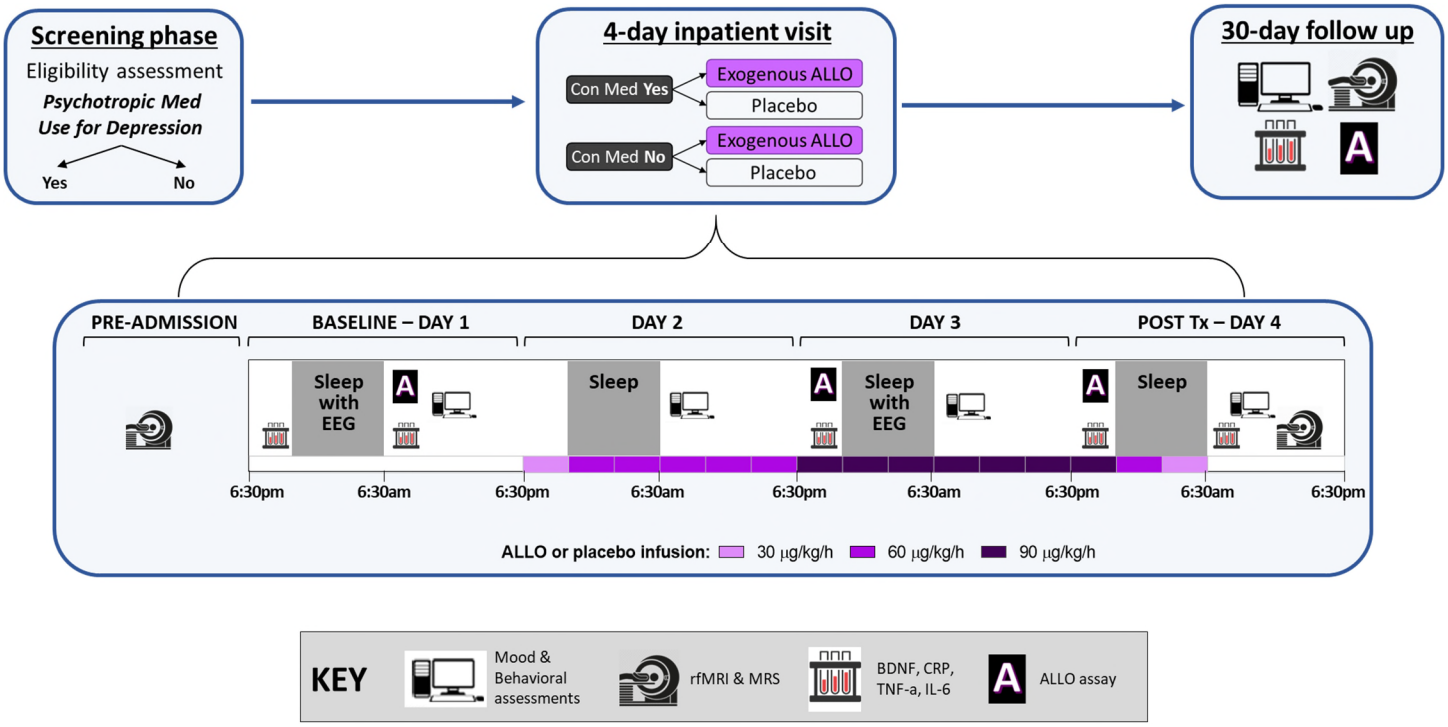
Impact: Despite evidence from preclinical models and clinical observational studies, there remains a significant gap in our understanding of behavioral and neurobiological mechanisms that drive PeriDep. Paralleling the postpartum period, P4 falls during Peri, potentially translating to a causal role for the P4 metabolite enALLO in PeriDep, similar to what has been observed in PPD. By applying an experimental therapeutic approach using a controlled mechanistic trial, we will directly interrogate key therapeutic targets of exALLO to identify the mechanisms through which ALLO exerts clinical benefits on mood. This protocol will determine how ALLO's effects on molecular mechanisms, brain circuitry, physiology, and behavior in NVS and ARS constructs relate to changes in depression severity to identify behavioral and neurobiological mechanisms that underlie PeriDep. Our novel approach will provide critical insights to guide future interventions using ALLO and similar agents while identifying key mechanistic targets that can be harnessed to develop novel treatment approaches for PeriDep.

3. GENERAL DESCRIPTION OF STUDY DESIGN

We propose a mechanistic trial enrolling up to 80 perimenopausal women with depression to complete an exALLO (as brexanolone) vs. placebo (saline) infusion using a 1:1 double-blind randomized design (n=up to 40/arm). This trial will be paired with data collection at screening, before (baseline) and following inpatient admission and infusion (4-day, acute effects), and at a 30-day follow-up to assess durability of ALLO effects.

Study participation will last approximately 5 weeks. Participants will undergo behavioral assessments and MRI scans to capture the impact of exALLO vs. placebo on key RdoC constructs thought to underlie depression (Aim 1). Blood will be drawn and optional MRS sequences from MRI scans will be used to measure the impact of exALLO vs. placebo on neuroprotective and pro-inflammatory biomarkers to assay key physiological and molecular mechanisms underlying ALLO's effect on depression (Aim 2). In addition, EEG sleep studies will be obtained to capture the acute effect of exALLO vs. placebo on physiological EEG measures of sleep continuity (Aim 2). Aim 3 analyses will integrate data collected in Aims 1 and 2 with measures characterizing each participant's prior course of depression and Peri status to identify mediators and moderators of ALLO effects on depression.

4. SUBJECT SELECTION



We aim to consent up to 100 women. Because we anticipate that approximately 20% will not meet eligibility criteria during the screening phase, this will achieve our accrual goal of 80 women (~40/treatment arm) who will complete the protocol and provide usable data for the acute primary outcomes. We also expect to have at least 72 women complete the 30-day follow-up period. These estimates derive from our experience with similar types of studies and populations.

***Sources of recruitment*:** We will recruit study participants via several key resources that exist within our health system, including:

- RALLY, an online platform that serves as a resource for disseminating research opportunities within the Mass General Brigham (MGB) health system.
- The MGB-wide Research Patient Data Registry (RPDR), a research registry that allows investigators to query eligible participants based upon specified inclusion/exclusion criteria.
- Patient Gateway letters (identified via RPDR, described above). If we do not receive a response to our first letter within one week, we will send a second letter; if there is still no response, our third and final letter will be sent at least two weeks after the first. Following that, we will call up to three times, and if we are still unable to reach the patient, we will mark them as not interested. Patients who opted out will be filtered out of the recruitment list and they will not be sent the Research Invitation. The PI will conduct ongoing monitoring of patient responses to ensure that our selection criteria are identifying the right patients, and complaints about this method of recruitment will be submitted to the IRB as an other event.
- Relationships with clinical leadership of the Departments of Psychiatry, Obstetrics and Gynecology, and the Division of Women's Health within the Department of Medicine.

- Partnerships with colleagues at primary care and gynecology outpatient community clinics, and with Community Health Centers throughout the greater Boston area.

To optimize our ability to reach a broad, diverse population in the Boston area, we also plan to use targeted advertisements on social media (e.g., Facebook, Instagram). Recruitment ads will be posted from our research lab's social media account, and staff will not interact with potential subjects via social media. If anyone asks questions through social media, staff will only respond with "Please reach out to a study coordinator for more information" with a link to the participant-facing webpage (IRB-approved text). This response will come from the research lab's account, not any staff's personal accounts. Consistent with procedures in ongoing protocols, we will use culturally sensitive targeted enrollment techniques to ensure minority participation. These approaches include cultural competency training available from our institution, engagement with potential participants by providing educational materials, and responsiveness to participants' needs (e.g., flexible scheduling for outpatient procedures). All patient-facing recruitment materials will be approved by the IRB prior to distribution.

Inclusion and Exclusion Criteria:

Eligible participants will be stratified by use of psychotropic medication to treat depression.

Inclusion Criteria:

1. Healthy women ages 40 to 60 years
2. Perimenopausal, determined clinically per Stages of Reproductive Aging Workshop (STRAW) +10 criteria
3. Depressive symptoms, as determined by Hamilton Depression Rating Scale-17 score >20
4. Current use of psychotropic medications is allowed if the following applies:
    - The dose of psychotropic medications or other centrally acting medications is stable and expected to be unchanged within the 14 days prior to screening, and
    - It is expected that the dose of the psychotropic medications will not change before the completion of the 4-night inpatient stay
5. Able to read Arabic numerals and perform simple arithmetic
6. Able to provide written informed consent

Exclusion Criteria:

Medications:

1. Current or recent use of systemic hormone therapy
2. Centrally active medications (e.g., opiates, benzodiazepines) expected to be sedating, may significantly interfere with study outcomes, and/or interact with brexanolone, as judged by study investigators

Psychiatric and health history:

3. Other psychiatric illness that are considered to be primary (comorbid conditions are allowed)
4. Depression symptoms judged by the investigators to be too severe for study participation
5. Current suicidal ideation (Columbia Suicide Severity Rating Scale, C-SSRS)
6. Current or recent substance use disorders
7. Medical conditions or treatments that preclude Peri determination
8. Obstructive sleep apnea
9. Other sleep conditions
10. Other medical conditions: pregnancy, lactation, auto-immune disorders, neurological disorders or any condition that might confound the diagnosis and/or interpretation of study data
11. Abnormal hepatic and renal function
12. Known allergy to progesterone, exALLO, or brexanolone
13. History of head injury resulting in loss of consciousness > 20 min

Other:

14. Inability to comply with barrier contraceptive methods
15. Intellectual disability
16. Investigator judgement that study participation will constitute substantial risk given medical or psychiatric condition

17. Current or recent participation in clinical trial involving experimental therapy judged by investigator to interfere with risk of or interpretation of data from our protocol
18. Inability to comply with study procedures
19. Inability to communicate in English

Additional exclusions for optional magnetic resonance imaging (MRI):

20. Medical contraindications to MRI (e.g., aneurysm clip, pacemaker)
21. Medical conditions that would interfere with MRI protocol or data interpretation (e.g., neurologic, cardiac, or pulmonary disease incompatible with lying flat for >90 min)
22. Claustrophobia or abnormal movements sufficiently severe to interfere with MRI scan

5. SUBJECT ENROLLMENT

Pre-Screening: Recruitment materials will direct interested participants to an electronic prescreen survey on REDCap. This prescreen survey consists of questions related to eligibility, including the PHQ-8. If participants are likely to be eligible based on pre-screen questions, they will be asked to provide contact information and notified that research staff will reach out to them to schedule and conduct a telephone screen.

Telephone Screen: Potentially eligible participants will be screened over the phone to determine if they continue to meet inclusion/exclusion criteria. Verbal consent to complete the telephone screen will be obtained at the start of the phone call. Before research staff asks any screening questions, they will inform the potential participants that the purpose of the call is to begin the process of pre-screening for eligibility and that all responses are confidential and voluntary. Interested participants who prefer not to answer questions will be thanked for their time and the phone call will end. They may ask to stop the screening phone call at any time. All potential participants who appear eligible based on the pre-screen and telephone screen will be scheduled for a Screening Visit.

Informed Consent and Assent: Participants will not undergo any study procedures until they understand the risks and benefits and provide written and informed consent to participate. At part 1 of 2 of the screening visit, the consenting study physician will review the consent form in its entirety with prospective participants utilizing REDCap. The consent form will include information about the risks and benefits of study participation and contact information for the study investigators and research staff. The consent will include information about all study risks, including those related to the infusion of brexanolone or saline. Participants enrolling in the study must understand the nature of the study, the discomforts/risks, and potential benefits. Any questions or areas of concern regarding the protocol will be addressed. The participants may choose to have a confidant with them during the consent procedure. If potential participants feel comfortable with the study procedures, they and the study physician will sign the consent form. Enrolled participants will be given a copy of the completed consent form before any study procedures are completed, and a copy of the signed consent form and documentation of the informed consent process will be stored in the participant's research file.

If participants have reservations about study participation at the first visit, they will have the option to take the consent form home to consider the decision to participate in the study and to consult with their family and health-care providers. They can return at a later date to discuss the study again before signing the consent form and initiating study procedures.

The study protocol, informed consent form, telephone and REDCap screens, and all other study procedures will be approved by the IRB before the study is initiated.

Double-blind Randomization: Subjects will be randomized 1:1 to brexanolone or saline infusion, stratified by use of psychotropic medication to treat depression status. Treatment allocation will be known only by the biostatistical consultant, Safety Monitoring Group, and BWH Investigational Drug Service, the latter of whom will determine the permuted block randomized assignment. Subjects and study staff will be blinded until primary analyses are done, unless unblinding is required for safety.

6. STUDY PROCEDURES

This study is a randomized, double-blind, mechanistic placebo-controlled trial that involves a 60-hour infusion of either brexanolone or placebo. All participants will undergo the same procedures, which include: remote (part 1) and in-person (part 2) screening visit, four-night inpatient visit, optional functional brain MRI studies, interim remote visit, and 30-day follow-up in-person visit.

***Screening Visit*:** All interested participants who appear eligible based on a telephone pre-screening will attend a remote visit to initiate the screening visit (part 1 of 2) followed by in-person screening visit (part 2 of 2) at a BWH Center for Clinical Investigation (CCI) outpatient unit.
At the remote visit, a study physician will first obtain written, informed consent from participants, who will then be assessed further for eligibility. Procedures will include:

- a medical evaluation to ensure medical appropriateness for the study
- administration of the Mini International Neuropsychiatric Interview (MINI)
- administration of the HDRS-17
- administration of the C-SSRS
- MRI safety screening
- completing self-administered questionnaires and surveys

At the in-person visit, assessment for eligibility will be finalized. Procedures will include:

- continuation of a medical evaluation to further ensure medical appropriateness for the study
- measurement of vital signs and anthropomorphic features
- having one blood draw for eligibility/safety labs and for reproductive hormones

Eligible participants will be asked to complete a week-long daily diary monitoring their sleep, vasomotor symptoms (VMS) and menstrual bleeding pattern at home.

***Optional pre-admission MRI Visit*:** resting-state functional MRI (rfMRI) / magnetic resonance spectroscopy (MRS) scans (before the infusion).

-1 Day to Inpatient Visit*: The HDRS-17 will be administered remotely prior to inpatient stay to confirm state of depressive symptoms. **If the pre-admission MRI is completed within a reasonable time frame to confirm state of depressive symptoms prior to inpatient stay the HDRS-17 can be done at this visit in lieu of -1 Day remote assessment.*

Four-Night Inpatient Visit: Participants will be admitted to BWH CCI Intensive Physiologic Monitoring Unit (IPM) for four consecutive nights, which will include the following procedures:

- The 60-hour brexanolone vs placebo infusion will begin in the evening of the 2nd inpatient night and end on the last morning immediately preceding post-treatment assessments
- Safety assessments will be conducted daily throughout the infusion and inpatient stay
- 2 electroencephalography (EEG) recordings (one before infusion and one during the final night of the infusion)
- At least once daily assessments of rumination, attentional bias, psychomotor vigilance, mood state (POMS), and depressive symptoms (before and after the infusion)
- Daily blood draws (before and after the infusion). Blood will be collected to run assays, including brain derived neurotrophic factor (BDNF), inflammatory biomarkers, reproductive hormones, serum ALLO levels, and serum for future analyses.
- One urine collection at admission for HCG, one at morning void after first night for melatonin. If there happens to be overnight voiding on first night additional samples will be requested but there is no scheduled collection, it will only be done if the participant is awake to void on their own.

***Optional post-admission MRI Visit*:** resting-state functional MRI (rfMRI) / magnetic resonance spectroscopy (MRS) scans (after the infusion)

Interim Follow-Up and Remote Visit: During the 1-month follow-up period after participants are discharged from the inpatient visit, participants will be asked to complete daily monitoring of their sleep, VMS, and menstrual cycle patterns.

One remote interim visit approximately 2 weeks after the inpatient visit will take place to monitor adverse events, administer the C-SSRS, and assess mood state (POMS) and depressive symptoms.

Follow-Up In-Person Visit: Approximately 30 days after treatment initiation, participants will have a final follow-up in-person visit, during which they will undergo:

- Adverse event and safety monitoring
- Optional rfMRI/MRS scanning procedures
- Behavioral assessments including depressive symptom severity, mood state (POMS), rumination, and attentional bias, psychomotor vigilance; and
- Blood draw for neuroprotective and inflammatory biomarkers, reproductive hormones, ALLO levels, and serum for future analyses.

Administration of brexanolone vs placebo infusion: Brexanolone is a 60-hour continuous infusion made by Sage Therapeutics that is FDA-approved for treatment of postpartum depression and available to investigators and clinicians. The dose and method of administration proposed in this protocol are identical to the agent that is available for prescription. The active agent will be provided by Sage Therapeutics. The control condition (placebo) will be furnished from BWH's Investigational Drug Services (IDS) as a 0.45% sodium chloride intravenous infusion.

BWH IDS will establish a randomization scheme using internal standardized procedures and will dispense the agents in a double-blinded fashion. Research nurses on the BWH CCI IPM will administer the infusion continuously over a 60-hour time-period following the proscribed titration schedule, which is FDA-approved: 30 mcg/kg/h x 4 h → 60 mcg/kg/h x 20 h → 90 mcg/kg/h x 28 h → 60 mcg/kg/h x 4 h → 30 mcg/kg/h x 4 h. Nurses will perform adverse event monitoring to titrate the infusion rate across the 60-hour infusion.

Investigational New Drug (IND) Exemption: Per FDA guidance (§ 312.2(b)), as the Sponsor-Investigator Dr. Joffe has determined that use of brexanolone in the proposed mechanistic trial meets all criteria required for an IND exemption (see below) and is requesting approval for an exemption from the Mass General Brigham Human Research Committee IRB, serving as proxy for the FDA:

1. The drug product is lawfully marketed in the United States.
   - *On March 19, 2019, brexanolone (Zulresso; Sage Therapeutics) was approved by the FDA for the treatment of postpartum depression in adults.*
2. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication, and there is no intent to use it to support any other significant change in the labeling of the drug.
   - *This investigation is using brexanolone to probe neurobiological mechanisms of depression and is not intended to support a new indication or any other significant change in the labeling of the drug.*
3. In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
   - *This investigation is not intended to support any changes in the advertising of the drug.*
4. The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).
   - *Brexanolone is FDA-approved for the same indication using the same dose and method of administration as is proposed in this protocol. The only difference in FDA approval and this protocol is the population being studied. Recent studies have demonstrated efficacy of zuranolone, the orally bioavailable sister drug to brexanolone, for treatment of PPD and for depression unrelated to the postpartum period in women and men ages 18-64*[43]*. Zuranolone now has an established efficacy and safety profile in over 500 individuals with depression in this broader age group*[44]*. As a result, this investigation does not significantly increase the risk associated with use of brexanolone.*

5. The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).
   - *This investigation will be conducted under the oversight of the Mass General Brigham IRB and in compliance with 21 CFR part 56 and 21 CFR part 50.*
6. The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).
   - *This investigation is not intended to promote or commercialize brexanolone.*

Remuneration: Study participants will be compensated for the time, effort, and study procedure risks involved in this intensive protocol. Participants will receive up to $1,000 upon completion of all study procedures. Participants will only be compensated for those procedures which they completed. Payment will be provided at the end of the study and will accumulate according to the following schedule:

- Screening Visit - $100 ($50 for remote, $50 for in-person)
- Pre-admission MRI visit, if MRI eligible - $100
- Four-Night Inpatient Visit - $500 ($125/night if the participant withdraws early during the Inpatient Visit)
- Post-admission MRI Visit, if MRI eligible - $100
- Follow-Up In-Person Visit - $100
- Follow-up MRI as part of Follow-Up In-Person Visit, if MRI eligible - $100

Reimbursement for transportation home after the inpatient visit will be provided. Parking vouchers or reimbursement up to $25 will be provided for outpatient in-person visits.

Table of Procedures:

		V1a	V1b	V2a	V2b	V2c					V2d	V3	V4
		Screening Visit (part 1of 2 remote)	**Screening Visit (part 2 of 2 in-person)**	**Pre-admission MRI visit**	**Remote Assessment***	**Inpatient Day 0**	**Inpatient Day 1**	**Inpatient Day 2**	**Inpatient Day 3**	**Inpatient Day 4**	**Post-admission MRI visit**	**Interim Visit**	**Final Visit**
	TIMEPOINT →			**Prior to Inpatient**	**Day prior to Inpatient**	**Evening Admission**	**Baseline**			**4-day**	**Day of Discharge**		**30-day Follow-up**
	DAY →				-1	0	1	2	3	4		19 (+/- 3 days)	30 (+/- 3 days)
INTERVENTION	Randomization						X						
	ALLO/placebo infusion						→	→	→	→			
SCREENING ASSESSMENTS	Demographics Form	X											
	Medical history	X											
	Physical exam		X										
	Eligibility/safety labs*		X										
	MINI	X											
	STOP-BANG		X										
	MRI screening	X											
SAFETY MONITORING	Adverse event monitoring		X				X	X	X	X		X	X
	Pulse oximetry						X	X	X	X			
	Sedation Scale (RASS)						X	X	X	X			
	Sleepiness assessment (SSS)		X			X	X	X	X	X			X
	Suicidal ideation assessment (C-SSRS)		X				X			X		X	X
SLEEP RECORDING	Inpatient EEG					X			X				
OPTIONAL BRAIN IMAGING	Pre-imaging Ingestion Questionnaire						X			X			X
	3T MRI scan			X							X		X
	Post-imaging VAS alertness						X			X			X
ASSAYS	Pregnancy screening (urine HCG)					X							
	Urine Melatonin (morning wake void)						X						
	Reproductive hormones		X			X	X			X			X
	BDNF levels					X	X	X	X	X			X
	Inflammatory biomarkers					X	X	X	X				X
	Future analysis						X		X	X			X
	Allopregnanolone levels					X		X	X	X			X
PSYCHOLOGICAL ASSESSMENTS	Ruminative Response Scale						X			X			X
	Attentional bias (Penn ER-40)						X			X			X
	Psychomotor Vigilance (PVT)						X	X	X	X			X
	PROMIS-Depression						X			X			X
	Hamilton Depression Rating Scale-17	X		X*	X*		X	X	X	X		X	X
	Snaith-Hamilton Pleasure Scale (SHAPS)						X			X		X	X
	Generalized Anxiety Disorder-7		X				X			X		X	X
	Profile of Mood States (POMS)					~~X~~	X	X	X	X		X	X
	Childhood trauma exposure (CTQ)						X						
	Holmes Rahe Stress Inventory						X						
	Perceived Stress Scale						X			X			X
	NIMH Lifechart - Illness course						X						X
SLEEP ASSESSMENTS	PROMIS Sleep Disturbance						X			X			X
	Pittsburgh Sleep Quality Index						X						X
	Daily sleep diaries		→				→	→	→	→		→	→
HOT FLASH MONITORING	Daily hot flash diaries w/ menstrual bleed tracking		→				→	→	→	→		→	→

* Remote Assessment not required if pre-admission MRI is scheduled within reasonable timeframe to confirm HDRS eligibility ** **Eligibility/Safety labs**: serum HCG; Comprehensive Metabolic Panel; Complete Blood Count
BDNF: Brain-Derived Neurotrophic Factor; **C-SSRS**: Columbia Suicide Severity Rating Scale; **EEG**: Electroencephalography; **MINI**: Mini International Neuropsychiatric Interview; **NIMH**: National Institute of Mental Health; **Penn ER-40**: Penn Emotion Recognition Task; **POMS**: Abbreviated Profile of Mood States; **PROMIS**: Patient-Reported Outcomes Measurement Information System; **PVT**: Assessment of Psychomotor Vigilance; **RASS**: Richmond Agitation Sedation Scale; **SSS:** Stanford Sleepiness Scale; **VAS**: Visual Analog Scale

***Data recorded on participants*:**

- rfMRI & MRS data will be collected in a Siemens 3T magnetic resonance imaging system located onsite at BWH. Alertness during the scan will be assessed by responsiveness to interactions during the scan and documented using visual analog scales after scan completion.
- Diagnostic and depression symptom severity interview data will be collected by trained research staff using semi-structured interviews, overseen by a licensed psychologist.
- Computerized testing will be implemented to assess attentional bias and psychomotor vigilance.
- Questionnaire data will be collected via self-report including measures of depression, rumination, and sleep disturbance.
- Sleep quality data will be collected using a standardized daily diary that will capture a series of sleep parameters.
- Objective sleep data will be collected using polysomnographic EEG recordings.
- VMS and menstrual bleeding pattern data will be collected using a daily diary.
- Urine will be collected per standard procedures and used to test for pregnancy and melatonin levels at baseline.
- Blood (7 draws, no more than 200mL over at least 5 weeks) will be collected by a trained phlebotomist per standard procedures and will be used to test for eligibility at screen, female reproductive hormones, neuroprotective and inflammatory biomarkers, and allopregnanolone levels. At four time points across the study serum will be collected and stored for future potential assays related to the focus on this protocol.

All data will be collected for research purposes only, and specimens and data will be available only to study investigators and research personnel. Data will either be collected on paper forms or entered directly into a password-protected, secure electronic database (REDCap) on an encrypted tablet or computer, depending on the measure. Data collected on paper will be entered into REDCap by a trained research assistant. Paper forms will be stored in research binders in locked cabinets. Biological specimens (serum) will be kept at -80ºC locked, monitored, and alarmed freezers and will be labeled by deidentified Participant ID number. MRI and EEG data will be stored on MGB secure servers.

***Linkages to Participants*:** Data, including EEG data, will be stored with a de-identified Participant ID number, and no identifying information will be entered in REDCap master database. Only the Principal Investigators, co-Investigators, and study staff will have access to the secure dedicated file area, databases, study binders, and biological specimens. MRI data will be stored on password-protected access-limited secure servers maintained by MGB and will be de-identified as part of the standard pre-processing pipeline.

7. Risks and Discomforts

Potential Risks:

1. ***Allopregnanolone (brexanolone):*** In previous trials, brexanolone has generally been well tolerated. However, this drug is associated with some adverse effects, most commonly sleepiness, xerostomia, altered or loss of consciousness, dizziness, and hot flushes. In postpartum depression trials, the most common treatment-emergent adverse events that were observed in ≥5% of individuals or at least twice the rate of placebo were sedation (13–21% on brexanolone vs 6% placebo), altered or loss of consciousness (3–5% vs 0%), flushing/hot flush (2–5% vs 0%), and dry mouth. Centrally active medications (e.g., opiates, benzodiazepines, alcohol) may enhance the risk of sedation-related side effects and should not be used in combination with brexanolone. Because of the sedation risk, driving or use of heavy equipment is not advised during or after brexanolone treatment until resolution of sedation.
   Reproductive and Lactation Risks: Effects on the fetus and on breastfed infants are not known. Animal studies suggest potential for fetal harm. Pregnancy exposure registries are ongoing.

2. ***Assignment of placebo:*** The risk associated with placebo includes the potential lack of clinical improvement potentially conferred by the active treatment. Because many people who receive placebo will report a variety of side effects consistent with the expectation that side effects may occur (nocebo effects), there are additional risks related to the perception of psychological and physical adverse events (side effects) that are common to those participants assigned to the active drug.

3. ***Treatment delay***: We will enroll women who are not receiving psychotropic medications targeting their depression episode at the time of enrollment and are asking them to make no changes to therapy throughout the trial. Therefore, given the duration of the trial, there is also a risk of delaying initiation of established, alternative treatments.

4. ***Possible exacerbation of clinical depression or emergence of suicidal ideation***: Depression may worsen as a result of natural symptom fluctuations that commonly occur in depression, in response to trial participation, or due to some other unknown cause. Likewise, suicidal thoughts may emerge, and suicidal behaviors may occur.

5. ***MRI:*** Participants undergoing a 3 Tesla (3T) MRI scan may be bothered by feelings of confinement (claustrophobia), and by the noise made by the magnet during the procedure. If participants move rapidly within or around the magnet, they may feel dizzy. We may discover an abnormality on the MRI exam that we are not expecting. Some findings may require additional tests to clarify them.
6. ***Venous blood sampling:*** The risks associated with venipuncture are minimal and include hematoma, pain, infection, and fainting spells.

7. ***In-dwelling IV for infusion administration:*** The risks associated with an indwelling IV are minimal and include bruising, clotting, bleeding, pain, and infection.

8. Inpatient sleep study procedures (4-night admission to the CCI IPM): Admissions of this length may be psychologically stressful and inconvenient or disruptive to daily routines.

9. Polysomnography: A minor rash may develop from the tape or adhesives used to attach electrodes. Participant may experience reduced quality of sleep if the monitoring equipment makes sleep somewhat more difficult than usual.

10. Emotional distress from completing questionnaires: Answering questionnaires may be upsetting given the sensitive nature of the information that is collected, including questions about depression and other mental health conditions as well as suicidal and other psychological symptoms.

11. Loss of confidentiality: Should there be a breach of confidentiality regarding psychiatric diagnosis, MRI findings, psychologic symptoms, or neurocognitive performance, the participant might be exposed to discrimination.

Alternative Treatments: Participants will be informed of other treatment options for depression before enrollment in this study. There are a number of FDA-approved medications that can treat depression, including SSRIs and SNRIs. Menopause-dosing of hormone therapy (estrogen with or without a progestin) can improve depression symptoms, especially when VMS are present, but this is not FDA-approved to treat depression and it is not recommended when depression symptoms are severe, persistent, and women meet criteria for a current episode of major depression. Of note, there are no medications that are specifically approved for the treatment of perimenopausal depression.

Protections Against Risk: There will be a number of different procedures in place to minimize the risks to participants. Thorough screening procedures will be implemented to exclude those for whom study treatments and procedures are contraindicated. Participants enrolled in the study protocol will be monitored closely for the development of any adverse events. All adverse event reports will be reviewed by the MPIs and by our Study Physicians and will be reported to our Safety Monitoring Group and our IRB in accordance with reporting guidelines. Criteria for withdrawing participants from the protocol in the event of an adverse event or serious health concern will be implemented. In the case of a serious adverse event, study procedures will be discontinued, and appropriate diagnostic and therapeutic measures will be taken. Potential risks to study participants during the inpatient admission will be further minimized by close monitoring and contact with the admitting physician, nurses, and other staff on the unit, as well as our protocol study staff, as required by the protocol design. In the event a participant discontinues early from the study, the study team will follow-up to document the outcome of any referral for follow-up clinical care.

1. Allopregnanolone (brexanolone): To rigorously monitor for adverse events and safety during the brexanolone vs. saline infusion, we will adhere to the FDA Risk Evaluation and Mitigation Strategy (REMS) program for brexanolone (see attached and [https://www.accessdata.fda.gov/scripts/cder/rems/index.cfm?event=IndvRemsDetails.page&REMS=387].
Consistent with REMS specifications for brexanolone, the 60-hour infusion will be administered by medical professional during a 4-day admission to an inpatient clinical research unit at Brigham and Women's Hospital, which is staffed 24 hours a day with nursing and medical care providers who can address immediate concerns. This is the same unit where our research group admitted and treated postpartum depression participants for the SAGE547 Phase 2b investigational trial (Partners IRB protocol #2015P002628).

Pursuant to the REMS program, potential risks of sedation and dizziness will be vigorously monitored through frequent assessments by trained nurses vigilant for these risks, frequent blood pressure checks, continuous oxygen saturation monitoring with a pulse oximeter, and frequent administration of a nurse administered arousal-sedation scale - the Richmond Agitation Sedation Scale (RASS) during wake hours throughout the period of the infusion. Use of intravenous programmable infusion pumps with alarms to alert when the pump malfunctions is standard. The CCI team along with our study team will continuously monitor participants and intervene as necessary. Participants will be counseled prior to the start of the infusion on signs/symptoms of excessive sedation so they know to report these immediately to the unit staff. Consistent with procedures in the SAGE547 trial, the brexanolone infusion rate will be reduced or suspended if any of these adverse events emerge during the administration of the intervention to protect

against further sedation and altered or loss of consciousness. The CCI has standard fall precaution protocols in place. Activity and movement around the inpatient unit during the infusion will be limited to facilitate close monitoring.

These safety monitoring procedures will be maintained during the daytime after completion of the infusion while study procedures are ongoing. Participants will not be discharged from the inpatient unit until any emergent sedation or adverse events are resolved. Transportation home after the inpatient stay will be provided where participants are unable to secure transportation themselves. They will not be permitted to drive home and will be advised not to use heavy machinery until the following day and when they are feeling fully alert. Concomitant medications that are known to be sedating will be disallowed to minimize cumulative sedation. All these steps will be carefully implemented as part of FDA REMS program.

Pregnancy and lactation risks: Women who are pregnant or breastfeeding will be excluded from the study. While the likelihood of pregnancy is low in this age group, a serum pregnancy test will be conducted at the screening visit to ensure that enrolled participants are not pregnant, and an additional urine pregnancy test will be conducted upon admission to the inpatient unit, immediately preceding the brexanolone/placebo administration. Anyone with a positive pregnancy test will be disqualified from participation in the study. While the likelihood of pregnancy is low, participants will be required to use a barrier method of contraception (e.g., condom) for the duration of the study if they are heterosexually active and at risk of pregnancy. These procedures will serve to minimize the unlikely possibility that study participants become pregnant during study procedures.

2. Assignment of placebo: There is a wide variability in placebo response, defined as an improvement in clinical symptoms, across patient groups. Response rates for placebo in antidepressant clinical trials range from 30% to 40% and among patients with milder forms of depression, the placebo response rate is close to 50%, at times in the range of response rates of participants assigned to the active treatment. As such, it is often argued that placebo is not “no treatment” and that participants may indeed benefit from placebo. These placebo-related risks are paralleled in the active treatment arm and will be mitigated in the same way. Specifically, potential side effects attributed to the study drug will be evaluated using the identical protocol for placebo as is used for brexanolone safety monitoring in this double-blinded trial. The infusion will be conducted on an inpatient basis, allowing for constant oversight throughout treatment exposure; after discharge we will assess mood and safety outcomes at an interim 2-week remote visit as well as at a 30-day in-person visit.

3. Treatment delay: We have mitigated the risk of delaying treatment through a short screening period and by not requiring a washout of existing medications. During the informed consent process, we will explain the alternative treatments available to each participant alongside the timeline of expected study participation. If there are exacerbations in symptoms or suicidality arises, we will ensure that the study participant receives immediate and appropriate clinical care. This may include the discontinuation of the trial for that participant.

4. Possible exacerbation of clinical depression or emergence of suicidal ideation: Depression and suicidal ideation will be assessed repeatedly in both treatment arms throughout the study as part of standard safety monitoring procedures using the HDRS-17 and the FDA-recommended tool for evaluation of suicidality, the C-SSRS. For women developing severe depression or who have suicidal or homicidal ideation, we will ensure that they are referred expeditiously for proper evaluation and treatment. Answers of “yes” to questions 4 or 5 of the C-SSRS will result in emergent evaluation. Depending on the determined risk, discontinuation of study participation and/or hospitalization may be deemed necessary. When possible, study discontinuation will trigger an end of study visit (mimicking all procedures from the 30-day visit). Study staff will review the case with the clinically licensed MPIs and the Study Physician or will make an emergency decision as needed to ensure that the participant receives emergency care or hospitalization. Our investigative team is experienced with operationalizing the C-SSRS in clinical studies and our MPIs are mental health clinicians equipped to make assessments and clinical referrals.

5. MRI: As part of this study, there will be several scans using "Magnetic Resonance Imaging" (MRI). MRI does NOT use ionizing radiation like x-rays. Instead it uses strong magnets and radio waves to make images of the inside of a person’s body. The MRI scan should not cause participants any pain. The MRI scan is not known to have any significant effect on health. Because MRI uses strong magnets and Radiofrequency certain kinds of implants or devices in or on the body may be affected by the MRI scan or create a significant risk for participants. The MRI technologist will

complete a safety questionnaire to make sure that participants don't have any such device and to see if it is safe to have an MRI scan. If participants have certain implants, tattoos, etc. that could be a problem, they may not be able to receive the MRI scan and they may not be able to participate in the study.

The MRI also produces loud beeping and hammering sounds when the scanner is collecting measurements. This is normal and participants will be given earplugs and/or other ear protection to reduce the noise.

The MRI scanner is a narrow tube and some people become uncomfortable inside the magnet. During the scan participants will be able to talk with the MRI technologist through an intercom system, so that any discomfort or anxiety they are experiencing can be immediately addressed and the scanning stopped if needed.

6. Venous blood sampling: All blood draws will be performed by trained phlebotomists (outpatient CCI) or RNs (inpatient CCI) who use standard sterile techniques and Universal Precautions (www.osha.gov). Infection is unlikely since stringent aseptic techniques are followed during all procedures, and appropriate technique is used to minimize bruising. Blood-sparing techniques will be used to minimize blood loss. The total volume of blood drawn for each participant will be no more than 200mL over at least 5 weeks and is not expected to lead to discomfort or health concerns.

7. In-dwelling IV for infusion administration: The IV will be placed by trained nurses who use standard sterile techniques and Universal Precautions (www.osha.gov). Infection is unlikely since stringent aseptic techniques are followed during all procedures, and appropriate technique is used to minimize bruising. IV lines will be flushed to maintain patency.

8. Inpatient sleep study procedures (4-night admission to the BWH IPM): The IPM is staffed 24 hours a day by nurses with research expertise who attend to the participant's medical needs and safety as if they are admitted to a routine medical unit, with frequent checks, vital signs, and status updates. Participants are also seen daily by the admitting physician. In the unlikely event that emergency treatment is required during the participant's stay in the IPM, treatment will be provided. Participants are free to withdraw consent from all study procedures at any time; if participants withdraw during the night, they may stay on the Unit to sleep and be discharged in the morning.

9. Polysomnography: PSG studies will be conducted according to standard clinical procedures by trained personnel experienced with the application of electrodes to the skin and staff will be available to attend to participants if any concerns emerge.

10. Emotional distress from completing questionnaires: We will use widely used and validated instruments. Participants will be informed that answering questions about personal matters may be upsetting. They will be told that they can skip questions that make them uncomfortable. Any woman who develops marked distress or psychiatric symptoms during the study will be evaluated and referred for treatment if warranted, including emergency evaluation if appropriate.

11. Loss of confidentiality: Every effort will be made to minimize this risk. All research records will be kept with maximum possible confidentiality, and all HIPAA regulations will be strictly followed. Results, including biological specimens, will be coded with deidentified study IDs, and the key to the code will be maintained in a locked file. No names will be used in presentation of any data. Source documents will be reviewed by study personnel. Identifying information will be made available on a "need to know" basis among study personnel.

8. Benefits

Participants may benefit from the close monitoring of their depression symptoms as well as related affective symptoms, sleep disturbance, and VMS. Participants may directly benefit from brexanolone administration since allopregnanolone has been shown to reduce depressive symptoms in women with postpartum depression.

However, this mechanistic intervention protocol aims to provide a meaningful benefit to perimenopausal women through knowledge gained about underlying behavioral and neurobiological mechanisms that drive risk and recurrence of depression. Some participants are curious about their brain anatomy and function and find having a brain MRI valuable because they will receive information if there are any structural abnormalities detected by the radiologist who reads the MRI scans. If an abnormality on the MRI, PSG recording, or laboratory studies is detected during the study, this information will be shared with the participant and her providers with her permission.

Many women experience studies focused on perimenopausal depression as validating of their menopause symptom experience. In addition, some women have reported that completing daily dairies to monitor sleep, VMS, and menstrual patterns has helped them understand their own perimenopausal symptoms better.

9. Statistical Analysis

Analysis Plan*:* Briefly, primary analyses will compare exALLO and placebo subject-level response profiles from baseline to 4-day in each primary outcome to examine acute effects. Secondary analyses will compare exALLO and placebo subject-level response profiles from baseline to 30-day in those same outcomes to examine durability of effects. Analyses of secondary outcomes will be conducted in parallel with those for primary outcomes. Key covariates (e.g., age, race, body mass index, socioeconomic status and stressful events) will be measured and considered a priori as potential predictors in multivariate analyses to reduce unexplained variability. Analytic outcomes can be transformed as needed to satisfy model assumptions such as normally distributed residuals for linear models or, alternatively, non-linear distribution models (e.g., negative binomial, lognormal, or gamma) can be applied in the generalized linear mixed model approach to model the observed distribution of the outcome. Model selection will be based on Akaike information criterion or quasi-likelihood under the independence model criterion values. We will use the False Discovery Rate (FDR) correction[45], which allows for robust retention of alpha to remain at 5% for each statistical test with the additional constraint of q-threshold also set to 5% (i.e., acceptable FDR of 5%). This analytic approach is advantageous over Bonferroni adjustment and other approaches that adjust the 5% alpha threshold as it will not increase the chance of inflating Type II error given the number of primary outcomes in Aims 1 and 2.

The primary analyses to test the hypotheses listed in Aims 1 and 2 will compare allopregnanolone and placebo subject-level response profiles from baseline to 4-day visit for each primary outcome using linear or generalized linear mixed models. Estimation of the fixed effects will allow comparison of means. Fixed effects will include treatment condition and time (baseline, 4-day, 30-day). Subject-level random effects will be modelled by including a random intercept. While linear or generalized linear mixed models provide a robust approach to handling inter-individual variability in response, we will also incorporate additional exploratory analyses to address this concern. To achieve this, we will examine our data using the interclass correlation coefficients (ICC) obtained from the R correlation matrix of the mixed-model regressions. A low ICC value will indicate that intra-individual variability in response is high relative to inter-individual variability, whereas a high ICC value will indicate that there is high inter-individual variation in response relative to intra-individual variability. Additionally, we will estimate subject-specific effects (i.e., slopes in response profiles of each subject from baseline to day 4, estimated by the random effect in the mixed models) and the distribution of subject-specific response magnitude. As exploratory analytic approaches, these subject-specific response magnitudes will be further modelled using baseline participant characteristics (e.g., age, prior history of depression) to elucidate potential physiological and neurophysiological predictors. We expect that these additional exploratory analyses will provide preliminary data on the distribution of participant-specific responses to ALLO, which will inform future studies and analyses. For **Aim 3,** the objective is two-fold: (1) we will assess mediation of improvement in clinically-defined global measures of depression severity by changes in Aim 1 and 2 outcomes, and (2) test effect modification of exogenous ALLO on improvement in clinically-defined global measures of depression severity by (i) the presence/absence of a history of depression recurrence, and separately, ii) perimenopausal factors (early vs. late perimenopause). In addition to the mediation pathways described above, we recognize that the possible pathways linking ALLO to depression are likely more numerous than these predefined mediation pathways. Therefore, we plan to further explore other putative pathways using partial least squares structural equation modeling. We will identify combinations of variables (features) that cluster together (factors) as latent variables, and then quantify the strength of their relationships based on factor loading (correlation between each feature and each factor). We will develop primary mediation models for 4-day effects and then 30-day secondary effects in

parallel. We will also compare mediator clustering (number of factors and combination of features) and strength of associations (factor loading) for 4-day and 30-day models to explore differences in mediation between the two time points. Comparing models at the two time points will help identify mediators that are consistent over time, indicating robustness of the findings, as well as identifying mediators that may be unique to acute and durable responses. Both vertical collinearity (predictor-predictor redundancy) and lateral collinearity (predictor-criterion) will be assessed with a variance inflation factor threshold of 3.3. Bootstrap routines will be used to determine significance of indirect effects to avoid parametric assumptions (i.e., normality). Effect size of the mediation will be assessed by Cohen's f. Assessed mediation types will include (i) full, (ii) partial mediation (complementary and competitive), (iii) only direct effect, and (iv) no effect. The significance of the difference between specific mediating effects will be assessed based on bootstrapped confidence intervals[46]. 4-day and 30-day models will be tested for differences using bootstrap-based tests for overall model fit[47]. To test effect modification of exogenous ALLO on improvement in clinically-defined global measures of depression severity by (i) the presence/absence of a history of depression recurrence, and separately, ii) early vs. late perimenopause, we will test for two-way interactions between each modifier with the main effect of treatment. These interaction models will be tested separately for 4-day and then 30-day effects; three-way interactions will not be tested.

The proposed sample size (n=80; 40 per arm) will yield more than 80% power to detect effect sizes 0.64 or larger in a two-tailed, two-sample contrast of group-level means, consistent with our primary analysis plan to assess acute treatment effect (4-day) of exogenous ALLO compared to placebo. Furthermore, this approach avoids a priori presumptions to be made on data distribution and impact of potential covariates (i.e., linear vs. non-linear relationship with the outcome). We conservatively based our anticipated estimates on published effect sizes for progesterone-associated or ALLO-induced changes in behavioral, circuit-based, molecular, and physiological measurements of the RDoC Negative Valence System and Arousal and Regulatory System domains, and when none were available, on our own pilot data. While the sample size justification uses a cross-sectional approach as if there is one measurement per participant, the data and analyses will be longitudinal – accounting for repeated measured per participant, which also will increase power and precision. Therefore, we expect that this mixed-model analytic approach will yield even greater statistical power than the conservative estimates from the simpler approach used for sample size determination.

For all primary contrasts, effect sizes estimated from either previous reports or our own preliminary data were large ($d \geq 0.8$). Specifically, for **Aim 1:** the effect of progesterone on rumination assessed using MADRS Item 9 (Pessimistic Thoughts) and the Beck Depression Inventory (BDI) subscore from Items 5 and 8 (Guilt and Self-incrimination, respectively) were large (Cohen's $d > 0.95$), an effect size that is consistent with previously reported[48], effects of exogenous ALLO on depression symptom ratings using the HDRS-17 being large (Cohen's $d > 1$), as will be investigated in **Aim 3**. Progesterone's effects on attentional bias to negative stimuli as well as functional connectivity are estimated from our preliminary data to be large ($d > 1$). **Aim 2:** Progesterone's effects are estimated from our preliminary data to be large on both BDNF and the inflammation index (derived from CRP, TNF-α, and IL-6 levels) ($d \geq 0.8$). Effects of progesterone on NAA levels in the medial prefrontal cortex, estimated from our preliminary data, and EEG-derived WASO, estimated from a previous report[49] of exogenous progesterone on sleep parameters in postmenopausal women, are both large ($d > 1$). For Aim 3: Based on prior reports and our preliminary data, our sample size (n=80; 40 per arm) will yield more than 80% power to detect select mediation as identified above.

10. Monitoring and Quality Assurance

This mechanistic intervention study will be conducted as a single-site inpatient randomized placebo-controlled trial using an agent that is FDA approved for women with a hormonally based depression in a new population of women who also have a hormonally based depression. The dose and method of administration—60-hour infusion of brexanolone—will be identical to the marketed agent. Research participants will be closely monitored for adverse events by physicians and nurses in the CCI research unit and by our experienced investigative team, which together participated as a site for the Sage Therapeutics PPD Phase 2 clinical trials using SAGE-547 (now FDA-approved as brexanolone). To minimize risks during the infusion, the FDA-approved Risk Evaluation and Mitigation Strategy (REMS) for brexanolone[1] will be strictly adhered to by our hospital clinical staff on the IPM Unit, consistent with procedures we implemented during the SAGE-547 trial. Safety monitoring will include frequent nursing checks, continuous oxygen saturation by pulse oximetry monitoring, and

administration of the Richmond Agitation Sedation Scale (RASS) for specific attention to sedation and loss of consciousness.

Data and Safety Monitoring Board (DSMB)

The Data and Safety Monitoring Board (DSMB) will have primary safety monitoring oversight for this protocol. As per guidance from the National Institute of Mental Health (NIMH) Human Research Protection Branch (HRPB), this protocol will be monitoring via a formal DSMB. The DSMB is comprised of a group of individuals who are qualified, not directly involved with the design and conduct of the study, and who are objective, without conflict of interest. Their focus is on participant safety, tolerability of study procedures, and adverse events.

To form the DSMB for this trial, our research team will expand upon an established Safety Monitoring Group (SMG) that Dr. Joffe used for prior studies involving mechanistic intervention and experimental procedures, consistent with these regulatory guidelines. The DSMB will review the proposed project at ongoing regular meetings held at least two times a year, approximately every 6 months. The DSMB will comprise two research physicians and one statistician who are familiar with clinical research, the study medications and their side effects but who are not collaborators or close colleagues of the MPIs or co-Investigators. The two physicians are [redacted] a neuro-endocrinologist at Massachusetts General Hospital with expertise in neurosteroids in relation to mood and reproductive transitions and [redacted] a psychiatrist at Weill Cornell Medicine with expertise in reproductive psychiatry. Drs. [redacted] will review all adverse events and unanticipated problems that arise during the study. A third DSMB member, [redacted], will join Drs [redacted] to round out the committee to include biostatistical expertise. Dr. [redacted] is an Associate Mathematician working in the Brigham and Women's Hospital Division of Sleep and Circadian Disorders.

Blinded outcome and safety data will be reported to the DSMB in open sessions when the study team is present. At the request of the DSMB, closed sessions will be scheduled to review unblinded data, adverse events, and unanticipated problems with only the unblinded statistical expert. The DSMB will also provide direction about early termination of the study should significant safety concerns arise to warrant such consideration. Consistent with local guidelines, all adverse events, including serious adverse events, and unanticipated problems will be managed and reported to our local Institutional Review Board (IRB).

We will adhere to the NIMH Policy on Reportable Events and ensure the timely report of any of the information that falls within this policy (https://www.nimh.nih.gov/funding/clinical-research/nimh-reportable-events-policy) to the NIMH Program Officer.

***Additional Safety Monitoring and Adverse Event Reporting*:** While the DSMB has the primary safety monitoring oversight role, this protocol will have additional safety checks that are routinely built into Dr. Joffe's experimental study procedures and those of the IPM Unit. These include routine weekly monitoring of all safety assessments, laboratory studies, and adverse events by designated Study Physicians for this study protocol. In addition, there is a team of physicians who round on all participants during inpatient study procedures in the CCI's IPM. This team coordinates research admissions for Dr. Joffe's studies, including previously for the SAGE-547 (now FDA-approved as brexanolone) PPD trial. The team includes an endocrinologist who will provide additional safety checks as the admitting physician and will be available for questions about safety concerns. During the inpatient admission, nurses will monitor the research participants by following the FDA-approved Risk Evaluation and Mitigation Strategy (REMS) for brexanolone as described above.

Monitoring of Accrual and Retention: Eligible participants will be pre-screened by phone using an IRB-approved questionnaire to inquire about the likelihood of meeting inclusion/exclusion criteria prior to scheduling an in-person screening visit. A tracking sheet with identifying information indicating whether or not they met eligibility will be kept separate from all other study files and saved on a secured server.

Once informed consent is documented, data will be acquired and stored as noted below. We will keep track of all participants who are consented and screened including those who are screen failures. A separate database will house the data from any participant who is eligible to be randomized, as these data will be included in final statistical analyses. Any attrition over the course of the trial will be documented with the reason for discontinuation if known. If not known, these participants will be coded as lost to follow up. These numbers will be reported to the Safety Monitoring Group at each

convened session.

Once randomized, we will be vigilant to minimize loss of data due to the failure of participants to complete all measures. Research staff are trained to check all measures at the time the data are collected to ensure that all items are filled out completely and correctly. For missing data identified at the time data are collected, participants will be asked to promptly provide the necessary missing information unless that indicate their desire not to answer those items.

Data Integrity: All data management activities will be centralized. Standard procedures are used for protocol and form development, data entry, data reconciliation, data editing, database updating, database closure, data retrieval, statistical computing, data security, and confidentiality. An electronic database (REDCap) will serve as the master database for this study. All questionnaire data are collected using standard measures. Those questionnaires completed on paper will be entered by research staff into the REDCap database in a timely manner. Discrepancies will be identified and reconciled. Data that are entered directly into the electronic database either by research staff or by participants via electronic survey mode are validated against appropriate formats and acceptable ranges of responses set for each variable that assure data are accurately entered. Data entry procedures within REDCap will use data checking routines that are executed automatically during the entry process. Finally, summary reports of descriptive statistics will be generated on a regular basis to examine for statistical outliers. All data will be archived and backed-up on a nightly basis on secure servers.

11. Privacy and Confidentiality

- ☒ Study procedures will be conducted in a private setting
- ☒ Only data and/or specimens necessary for the conduct of the study will be collected
- ☒ Data collected (paper and/or electronic) will be maintained in a secure location with appropriate protections such as password protection, encryption, physical security measures (locked files/areas)
- ☒ Specimens collected will be maintained in a secure location with appropriate protections (e.g. locked storage spaces, laboratory areas)
- ☒ Data and specimens will only be shared with individuals who are members of the IRB-approved research team or approved for sharing as described in this IRB protocol
- ☒ Data and/or specimens requiring transportation from one location or electronic space to another will be transported only in a secure manner (e.g. encrypted files, password protection, using chain-of-custody procedures, etc.)
- ☒ All electronic communication with participants will comply with Mass General Brigham secure communication policies
- ☒ Identifiers will be coded or removed as soon as feasible and access to files linking identifiers with coded data or specimens will be limited to the minimal necessary members of the research team required to conduct the research
- ☒ All staff are trained on and will follow the Mass General Brigham policies and procedures for maintaining appropriate confidentiality of research data and specimens
- ☒ The PI will ensure that all staff implement and follow any Research Information Service Office (RISO) requirements for this research
- ☐ Additional privacy and/or confidentiality protections

Confidentiality Protections
Since information of a personal nature is being collected, there is a risk of loss of confidentiality. All subject data will be entered into Research Electronic Data Capture (REDCap), a HIPAA compliant web-based application hosted by Mass General Brigham, Research Information Science & Computing (RISC). Only research staff listed on the approved IRB protocol with specific permissions will have access to the database. Participants will only be identified with a numerical code in compliance with HIPAA regulations. Moreover, participants will be apprised of their ensured confidentiality and told that clinical data collected from them will be included in an electronic database for research analysis using only a deidentified subject ID.

As with any electronic data storage and transmission, there is a possibility that databases may be hacked. Given that REDCap encrypts all data transmissions and data will be de-identified, these risks are very low.

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APPENDIX A

Data Monitoring Committee / Data and Safety Monitoring Board Appendix

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A Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) will be convened for safety monitoring of this research study. The following characteristics describe the DMC/DSMB convened for this study (Check all that apply):

☒ The DMC/DSMB is independent from the study team and study sponsor.

☒ A process has been implemented to ensure absence of conflicts of interest by DMC/DSMB members.

☒ The DMC/DSMB has the authority to intervene on study progress in the event of safety concerns, e.g., to suspend or terminate a study if new safety concerns have been identified or need to be investigated.

☒ Describe number and types of (i.e., qualifications of) members:

The Data and Safety Monitoring Board (DSMB) comprises 3 members: two research physicians at Massachusetts General Hospital and Harvard Medical School who are familiar with clinical research and the study medications and their side effects, and one biostatistician in the Brigham and Women's Hospital Division of Sleep and Circadian Disorders. None of the DSMB members are collaborators or close colleagues of the MPIs or co-Investigators. The two physicians will serve as safety monitors. The two physicians are a neuro-endocrinologist with expertise in neurosteroids in relation to mood and reproductive transitions [redacted] and a psychiatrist with expertise in reproductive psychiatry [redacted]. Drs. [redacted] will review all adverse events and unanticipated problems that arise during the study. The biostatistician is [redacted] who will provide biostatistical expertise to the DSMB.

☒ Describe planned frequency of meetings:
The DSMB will incorporate review of the proposed project into ongoing regular meetings held at least two times a year, approximately every 6 months to review the progress of Dr. Joffe's studies.

☒ DMC/DSMB reports with no findings (i.e., "continue without modifications") will be submitted to the IRB at the time of Continuing Review.

☒ DMC/DSMB reports with findings/modifications required will be submitted promptly (within 5 business days/7 calendar days of becoming aware) to the IRB as an Other Event.