Study Title: **Dynamic neural mechanisms of brexanolone-induced antidepressant effects in postpartum depression**

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Summary of Changes from Previous Version:

Previous Version No.	Affected Section(s)	Summary of Revision(s)	Reason for Change(s)
v1	Title Page; 1.0 Protocol Synopsis; 5.2 Eligibility; 6.1 Drug Preparation & Administration; 7.1 Table of Events; 7.4 Study Visits; 10.1 Risk/Benefit Assessment; 10.2 Assessment of Safety; 10.3 UP, AE, SAE	Title Page – Sponsor is UNC-CH, Sage is source of funding; 1.0 Target population – onset of PPD within 4 weeks of delivery (rather than 8); 5.2 – renal and hepatic impairment (in addition to failure) added to list of exclusion criteria; 6.1 – Drug Preparation & Administration – UNC will destroy unused study drug locally at the end of the study (rather than Sage); 7.1 – AEs changed from q4h to q2h (while awake) during infusion; 7.4 – monitoring for loss of consciousness in addition to excessive sedation; 10.1 – added "serious risks of BRX" language from informed consent (in second person) and additional information about the two cases of accidental overdose that were recorded in the clinical trial UPSI; added "pain" to risks associated with phlebotomy; 10.2 – aligned language in Risks Associated with BRX in this section with 10.1; 10.3 – added IQVIA email address for reporting of AEs;	Suggestions after initial protocol review by Sage Therapeutics
v2	Abbreviations; 4.3 Measurement Design; 5.2 Eligibility; 5.3 Strategies for Recruitment and Retention; 6.1 Test Article modification; 6.3 Intervention Procedural; 6.4 Concomitant Therapy; 7.1 Table of Events; 7.3-7.7 Study Procedures; 7.8 Premature Discontinuation; 9.2 Description	Abbreviations – removed unused abbreviations (e.g., CIOMS, CRO, QA); 4.3 – added column to Table 1A to included scoring conventions and ranges of study measures; added Tables 1B and 1C to include details specific to EEG and iMotions software outcomes; 5.2—removed use of anti-inflammatory agents from exclusion criteria and clarified breastfeeding cessation for 4 days of hospitalization (rather than infusion) and added caregiver of child(ren) to inclusion criteria; added rationale for HAM-D severity threshold to inclusion criteria; added documented proof of full COVID-19 vaccination; 5.3—clarified the difference between clinical procedures and research procedures for potential subjects; provided examples of re-screening for eligibility; 6.1 – removed language about PK analysis in "modification" section; included protocol for reducing infusion rate and continuing if infusion is stopped; 6.3 – included REMS training and certification for prescribers and pharmacists; 6.4 –	Suggestions following protocol review by UNC Scientific Review Committee

	OF STUDY COHORT; 10.1 RISK/BENEFIT ASSESSMENT; 10.2 ASSESSMENT OF SAFETY; 10.4 SAFETY MONITORING & 11.7 CLINICAL MONITORING; 10.5 STUDY SUSPENSION & 11.2 STUDY DISCONTINUATION AND CLOSURE; 11.8 QA AND QC; 11.9.1 DATA COLLECTION AND MANAGEMENT	CLARIFIED "MEDICATIONS" AND "PSYCHOTROPIC MEDICATIONS"; 7.1 – ITEM "M" IN FOOTNOTES CORRECT TO STABLE DOSE OF PSYCHOTROPICS 14 DAYS PRIOR TO ENROLLMENT; REVISED H24 TO H28; REMOVED COLUMN FOR H48; REMOVED AFFECTIVE STROOP TASK/PROBE FROM STUDY PROCEDURES; 7.3-7.7—INCLUDED DETAILS ABOUT STUDY VISIT PROCEDURES; 7.8—INCLUDED PROTOCOL IF SUICIDALITY IS ENDORSED AT PREMATURE DISCONTINUATION; 9.2 – INCLUDED RATIONALE FOR USE OF MOOD MEASURES TO DESCRIBE DIFFERENCES IN THE TRAJECTORY OF TREATMENT RESPONSE ACROSS INDIVIDUALS; 10.1 – REMOVED LANGUAGE ABOUT RISKS ASSOCIATED WITH AFFECTIVE PROBE TASKS; CLARIFIED SUICIDALITY MONITORING RECOMMENDATIONS FOR SUBJECTS WHEN THEY ARE OUTPATIENT; ADDED DISCUSSION OF POTENTIAL RISK TO INFANT CHILDREN OF SUBJECT AS A RESULT OF HOSPITALIZATION AND TEMPORARY CESSATION OF BREASTFEEDING; ADDED PROTOCOL FOR SUICIDALITY; 10.2 – INCLUDED USE OF NC TRACS IN RECRUITMENT OF HISTORICALLY UNDERREPRESENTED IN RESEARCH AND INCLUDED CLARIFICATION THAT RECRUITMENT WILL INCLUDE PATIENTS FOR WHOM BRX TREATMENT IS ALREADY CLINICALLY INDICATED; INCLUDED TABLE 5 TO DESCRIBE AROUSAL SCALE TO MONITOR FOR EXCESSIVE SEDATION/ LOSS OF CONSCIOUSNESS; 10.4&11.6 – CLARIFIED IQVIA'S ROLE IN RECEIVING SAE REPORTS, BUT NOT PROVIDING CLINICAL MONITORING; 10.5 & 11.2 – REMOVED CRITERIA FOR STUDY STOPPING THAT ARE NOT APPLICABLE (I.E. DEMONSTRATION OF EFFICACY AND DETERMINATION THAT PRIMARY ENDPOINT HAS BEEN MET); 11.8 – REMOVED LANGUAGE SUGGESTING MULTIPLE SITES IN THIS STUDY; 11.9.1—STUDY COORDINATOR RESPONSIBLE FOR CREATION, MAINTENANCE, AND CODING OF REDCAP DATABASE.	
V3	TITLE PAGE, 10.2 ASSESSMENT OF SAFETY; 11.5 KEY ROLES AND STUDY GOVERNANCE	DR. RUBINOW WAS REMOVED FROM APPLICABLE SECTIONS WHERE HE WAS REFERENCED AS PI AND REPLACED AS CO-I	SATISFYING UNC IRB STIPULATION BASED ON COI DETERMINATION
V4	PROTOCOL SYNOPSIS; 4.3 MEASUREMENT DESIGN; 5.2	REDUCED HAMD ELIGIBILITY SCORE TO ≥20; REMOVED COVID-19 VACCINATION FROM INCLUSION CRITERIA; INCREASED POSTPARTUM PERIOD FROM 6 TO ≤8 MONTHS; ALLOW FOR OUTSIDE LABORATORY TESTS TO BE USED FOR	INCREASE RECRUITMENT SUCCESS; DECREASE SUBJECT BURDEN

	ELIGIBILITY; 7.1 TABLE OF EVENTS	**SCREENING IF DONE WITHIN 14 DAYS OF INFUSION; REMOVED CLINICAL LAB ASSESSMENTS FROM H72; INCLUDED CONTINUOUS HRV MEASURE THROUGHOUT INFUSION**	

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Statement of Compliance

This study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6) and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The statistical analysis plans will be consistent with guidances such as the CONSORT Statement [1] or STROBE Statement [2], ICMJE recommendations [3], the 2016 and 2019 statements of the American Statistical Association [4,5], and recommendations in Nature [6,7].[1] All personnel involved in the conduct of this study have completed human subjects protection training.

[1] [1] www.consort-statement.org [2] www.strobe-statement.org [3] www.icmje.org [4] Wasserstein RL, et al. (2016), The ASA's Statement on p-Values, ***The American Statistician***, 70:2, 129-133 [5] Wasserstein RL, et al. (2019), Moving to a World Beyond p < 0.05, ***The American Statistician***, 73:sup1, 1-19 [6] Amrhein, et al. (2019) Scientists rise up against statistical significance, ***Nature*** 567, 305-307 [7] Editorial (2019) It's time to talk about ditching statistical significance: Looking beyond a much used and abused measure would make science harder, but better. ***Nature*** 567, 283-283.

Abbreviations and Definitions of Terms

Abbreviation/Acronym	Definition
AE	Adverse Event/Adverse Experience
ALLO	Allopregnanolone
AMICA	Adaptive Mixture of Independent Component Analyses
BIMF	Barkin Index of Maternal Functioning
BRX	Brexanolone
CI	Confidence Interval
CO-I	Co-Investigator
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
C-SSRS	Columbia Suicide Severity Rating Scale
CTRC	Clinical & Translational Research Center
CTSA	Clinical and Translational Science Awards
DCC	Data Coordinating Center
dFC	Dynamic Functional Connectivity
dlPFC	Dorsolateral Prefrontal Cortex
DSMB	Data and Safety Monitoring Board
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EEG	Electroencephalography
EPDS	Edinburgh Postnatal Depression Scale
FC	Functional Connectivity
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FMT	Frontal Midline Theta
GABA	Gamma Aminobutyric Acid
GCP	Good Clinical Practice
HAM-D	Hamilton Depression Rating Scale
HIPAA	Health Insurance Portability and Accountability Act
HRSA	Health Resources and Services Administration
IAPS	International Affective Picture System
IB	Investigator's Brochure
ICA	Independent Component Analysis
ICF	Informed Consent Form

ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IDS-SR	Inventory of Depression Symptoms – Self-Report
IDS	Investigational Drug Services
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous Line
MDD	Major Depressive Disorder
N	Number (typically refers to subjects)
NC TraCS	North Carolina Translational and Clinical Sciences Institute
NIH	National Institutes of Health
OB-GYN	Obstetrics-Gynecology
OHRP	Office for Human Research Protections
PDF	Probability Density Function
PFC	Prefrontal Cortex
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PPD	Postpartum Depression
PPIU	Peripartum Inpatient Unit
QC	Quality Control
REMS	Risk Evaluation and Mitigation Strategies
RRS-10	Rumination Response Scale - 10
rsEEG	Resting State Electroencephalography
SAE	Serious Adverse Event/Serious Adverse Experience
SCID-V	Structured Clinical Interview for DSM-5 Disorders
SCS	State Complacency Scale
SD	Standard Deviation
SE	Standard Error
SHAPS	Snaith-Hamilton Pleasure Scale
SMDDS	Symptoms of Major Depressive Disorder Scale
SOP	Standard Operating Procedures
STAI	State-Trait Anxiety Inventory
STROBE	STrengthening Reporting of OBservational studies in Epidemiology
SVM	Support Vector machines
UNC-CH SOM	University of North Carolina at Chapel Hill School of Medicine
UP	Unanticipated Problem

1. Protocol Synopsis

Study Title	**Dynamic neural mechanisms of brexanolone-induced antidepressant effects in postpartum depression**
Objectives	**Primary: To evaluate the feasibility of performing five serial EEG recordings and frequent affective state assessments over 72 hours in women with postpartum depression receiving inpatient brexanolone (BRX) infusion treatment.** **Exploratory:** **1. To calculate resting state nodal flexibility from five serial EEG recordings.** **2. To identify rapid brain state configurations and their probability density function from five serial EEG recordings.** **3. To assess the ability of iMotions Affectiva software to identify affective states using facial expression recognition analysis.** **4. To assess the amplitude of left frontal midline theta oscillations by performing spectral analyses of data collected from five serial EEG recordings.**
Target Population	**Key Inclusion Criteria** • **Women aged 18-45** • **Moderate to severe postpartum depression (DSM-V; HAM-D ≥20)** • **Onset of PPD in 3rd trimester or within 4 weeks of delivery** **Key Exclusion Criteria** • **Current pregnancy** • **Pregnancy that resulted in a stillbirth, termination, or child that was placed for adoption.** • **History of thyroid disorder, renal impairment, hepatic impairment, anemia, or seizure disorder** • **History of psychotic symptoms, concurrent substance use disorder, or suicide attempt this episode.** • **Use of anticonvulsant agents or benzodiazepines.**
Numbers of Participants	**Number to be recruited for screening: n=20** **Number of eligible participants enrolled: n=12**
Clinical Phase	**Not applicable**
Intervention	**Enrolled subjects will receive a 60-hour infusion of BRX according to FDA approved protocol for administration. A programmable peristaltic infusion pump will be used to ensure accurate delivery.**
Study Description	**This is a feasibility study of performing repeated EEG recordings and assessment of affective states during open-label administration of BRX to**

	women with postpartum depression. Study phases will include screening, enrollment, intervention, and follow-up. Subjects will be screened for study eligibility criteria through clinical assessments and self-report. Enrolled subjects will be admitted to the UNC Women's Hospital, where five serial EEG recordings will be obtained, along with frequent assessments of affective state, before, during, and after a 60-hour IV infusion of BRX. Follow-up procedures will include assessments of PPD and affective symptoms, as well as an exit interview with the study team. If feasibility outcomes are achieved, exploratory EEG analyses will be performed with AMICA (adaptive mixture independent component analysis), community detection, and microstate assessment. Exploratory analyses of data collected by facial expression detection software (iMotions Affectiva) are also planned.
Outcome Measures	**Primary outcome measures of feasibility:** **1. Number of successfully analyzed EEG recordings.** **2. Number of subjects completing the entire study protocol through the follow-up phase.** **3. Number of subjects withdrawn from the protocol due to adverse events and/or participation burden.**
Study Duration	**2 years**
Subject Participation Duration	**7 weeks**
Estimated Time to Complete Enrollment	**20 months**
Statistical Analysis Plans	**This study will determine the feasibility of the subject's participation in the protocol. Consequently, we intend to generate descriptive statistics of the number of subjects who complete the entire protocol, the number of subjects who are withdrawn from the study, and the quality of the EEG recordings and affective assessments collected over the course of the protocol.**

2. Introduction: Background and Scientific Rationale

2.1. Background Information

PPD is a great public health problem: it affects approximately 12% of women, adversely impacts mother and child, and is the leading cause of maternal death in the first year postpartum[1]. Recently we demonstrated that a 60-hour IV infusion of the neurosteroid (and progesterone metabolite) allopregnanolone (ALLO) (the active molecule in brexanolone [BRX]) rapidly (within 8-24 hours) reversed the depressed state in hospitalized women with PPD. Moreover, the antidepressant efficacy lasted for at least 30 days[2,3]. These exciting findings introduce an entirely new compound to the antidepressant armamentarium (and the first specifically for women with PPD). Several elements of ALLO's function have been identified, notably strong positive allosteric modulation of GABA receptor activity[4] and, more recently, blockade of proinflammatory immune signaling[5–7], but how and if the effects of ALLO on these molecular systems translate into its rapid therapeutic efficacy in PPD remain unknown.

GABAergic enhancement by ALLO suggests that its rapid antidepressant effects may be mediated through modulation of neuronal networks. Multiple lines of evidence suggest that GABA receptor signaling is compromised in depression[8]. Deficient GABA signaling produces abnormal neural synchronization and emergence of dominant network states[9]. Yet, the network features of a change in affective state remain unknown due, in part, to the long timescale of symptom improvement for most treatments. The rapid clinical response to ALLO in PPD thus provides a unique opportunity to delineate the evolution of network dynamics associated with the affective state switch. *Understanding the network dynamics of symptom remission is highly significant since it will enable the future design of targeted interventions for correcting aberrant network signaling associated with depression.* Studies of networks with fMRI and EEG have commonly generated summary measures that presume that network activity is stationary over time. In contrast, recently developed analytic methods for EEG and fMRI identify moment-to-moment brain states, recurrent patterns of interaction between brain regions that map onto and predict behavioral states[10–12]. These dynamic measures have been described as explaining more of the variance in cognitive and emotional state than more static measures[13–15]. We propose that PPD results from disordered coordination of rapid interactions between widely distributed brain regions, and that positive GABAergic modulation by ALLO restores normal network synchronization. *We hypothesize that BRX-induced rapid emergence from the depressed state in PPD will be accompanied by changes in time-varying measures of brain coordination dynamics.*

Significance of the Expected Research Contribution: Upon successful completion of this pilot study, we expect our contribution will advance efforts to illuminate the physiology underlying the rapid antidepressant actions of a novel neurosteroid treatment for PPD. By studying women while they transition between affective states, rather than focusing solely on the symptomatic state, we will gain novel and fundamental insight into a transdiagnostic phenomenon - the transition between affective states. This pilot study will be the first step in the identification of the disturbed networks and network kinetics in women with PPD and restored by ALLO, insights that will ultimately be particularly helpful for

developing neuromodulatory therapies designed to destabilize persistent dysphoric states. These therapies may be particularly important as alternatives to pharmacotherapies during conditions like pregnancy, when medications might otherwise be avoided.

INNOVATION: Studies of the kinetics of brain dynamics during remission from depression – and particularly in PPD - are virtually absent from the literature, as are investigations of the physiology underlying the transition between markedly different affective states. Our proposed study is innovative for several reasons: it employs a novel, rapidly acting, neurosteroid antidepressant in a highly morbid, relatively homogeneous form of depression (PPD); its study design exploits the rapidity of response to BRX, thus narrowing the window during which changes in both mood state and EEG characteristics are anticipated; it focuses on rapid, network dynamics at different temporal scales during induced affective state changes. Critically, the unique, within-subject study design creates the opportunity to identify novel electrophysiological characteristics of PPD.

2.2.Supporting Pilot / Unpublished Data

We will use high-density EEG to examine functional interactions via cortical synchronization. Spectral analyses are commonly used to infer inter-regional connectivity, with synchrony of neural oscillations in specific frequency bands critically involved in long-range communication. Theta (4-8Hz) oscillations link PFC and amygdala[72], are involved in cognitive regulation of affect[73], and are decreased (both power and FC) in major depression[74]. The relevance of this static measure for ALLO is suggested by unpublished data from Dr. Jamie Maguire et al showing disturbed theta synchrony activity in the PFC and amygdala in animal models that share behavioral features with patients with PPD. Strikingly, administration of ALLO restored both normal network activity and normal behavior in these rodent models. Modulation of network theta oscillations by ALLO as a potential therapeutic mechanism is also indirectly suggested by preliminary findings by CO-I Frohlich showing a positive correlation between increased amplitude of frontal midline theta (FMT) oscillations and plasma levels of progesterone, the ALLO precursor. *We hypothesize an increase in FMT oscillations will be seen in association with remission from the depressed state*.

Application of dynamic (rather than aggregate) measures takes greater advantage of the temporal precision of EEG. Three methods described in the literature form the basis of the exploratory measures to be employed in this pilot study.

2.3.Scientific Rationale

The main justification for the proposed pilot study is twofold: 1) Given the severity of the consequences of PPD, it is critical that we increase our understanding of the pathophysiology of the disorder; 2) Greater understanding of the brain mechanisms underlying the switch between affective states will advance the goal of identifying targets for novel therapeutics.

Modulation of GABAergic signaling by ALLO and implications for network dynamics of affective state in PPD: Three early observations support the premise of Exploratory Aim 1, which investigates changes in

network dynamics in response to ALLO administered in patients with PPD: 1) Both ALLO levels and GABA receptor composition change during pregnancy and postpartum[18–20]; 2) ALLO (but not benzodiazepines)[21] activates delta subunit-containing GABA receptors, those responsible for tonically inhibiting and synchronizing cortical excitatory tone[22,23]; and 3) knockout of delta subunit-containing GABA receptors creates an animal model in which female mice develop depressive-like symptoms restricted to the postpartum and deficits in maternal care[22], behaviors reversed by an ALLO analogue[21]. In parallel, recent clinical trials showed the antidepressant efficacy of a 60-hour infusion of BRX in women with PPD, with 50% remitting at 60 hours[24]. Given both recent conceptualization of depression as a discoordination of neural networks[25–27] and the ability of ALLO to modulate GABA signaling,[4,28,] the alteration by ALLO of network communication in PPD is a logical but untested inference. Few if any EEG studies of PPD exist, but the utility of EEG in studies of depression is well demonstrated by recent machine learning-based demonstrations that rsEEG robustly predicts antidepressant response[33] and that spatio-temporal network dynamics identify susceptibility to depression[34]. Most fMRI and EEG studies measure average functional organization over the entire scan. Convergent evidence suggests, however, that even in the resting state, functional brain networks demonstrate significant temporal variability and dynamic reconfiguration not captured by aggregate "static" measures[35,13,36]. Rapid, dynamic fluctuations in time-varying network connectivity (dFC) are believed to be essential for continuous updating and efficient flow of neuronal information and have been increasingly implicated in a range of cognitive and affective processes and psychiatric disorders, including, most recently, depression[37–41]. Common to these studies is the observation that it is the number and characteristics of the dynamic transitions between states that differ as a function of diagnosis or behavioral state[40,35]; i.e., affective behavior arises from the rapid but coordinated interactions among multiple brain circuits, not from one, isolated circuit. As shown in our preliminary studies of several psychiatric populations (see above), dFC measures offer unparalleled tools for mapping the network changes underlying the antidepressant effects of BRX.

Multiple approaches exist for assessing dFC, each of which segments the time courses from spatial or voltage signals into a set of temporal windows, characterizes connectivity (e.g., a correlation matrix) in each window, and decomposes information by identifying a smaller number of recurrent patterns. The power of these techniques lies in their ability to both preserve the temporal information and yet reduce or decompose the data to manageable and interpretable dimensions. Notably, these dynamic techniques have only rarely been studied in depression[37] and not at all in PPD or during a medication-induced change in affective state. **We propose, therefore, to apply multiscale (aggregate and time varying/dynamic) EEG measures in women with PPD undergoing BRX infusion to identify the network dynamic changes that accompany recovery from the depressed state**. We will characterize rapid brain state configurations and transitions/kinetics at baseline, track changes in state landscape during BRX infusion, and compare changed dynamics (nodal flexibility) both pre and post infusion and in those who do and do not remit. By interrogating our dataset with these methods, we will have an unprecedented ability to detect the formation, dissolution, and reconfiguration of global brain network activity during the transition to a remitted state.

The first dynamic method, **adaptive mixture independent component analysis (AMICA),** can, in an unsupervised fashion, detect brain dynamic state changes associated with changes in cognitive state[11].

AMICA assumes that different segments of the data will be best characterized by distinct ICA models, Different models dominate at different time points, and the probabilities of activation and brain source characteristics of these models have been shown to successfully identify even rapid changes in behavioral state[11] *We hypothesize that BRX-induced remission will exhibit different model topographies and probabilities compared to baseline and to non-remission.*

Network-based **community detection** (developed by CO-I Mucha)[75], generates measures sensitive to mood, diagnosis (schizophrenia) and medication-induced change in cognitive states[10,38,76]. A community is a collection of brain regions that preferentially communicate with one another. Community detection creates a graph that assesses the reconfiguration of networks over time. This method permits determination of how often nodes change networks (network flexibility), which is presumed essential for adaptive behavior[10,77]. In a novel application of this methodology to model EEG dynamic network connectivity, CO-I Campbell has identified differences in the formation, strength, and trajectory of networks in response to cognitive tasks in patients with prodromal psychotic symptoms vs. controls. Remarkably, the "flexibility" of network nodes (i.e., their joining other networks) over the course of several seconds predicts with high precision both individual exposure to stressors and group membership (high risk group or controls). *We predict an increase in global flexibility* [37,78]*) following BRX in those who emerge from the depressed state (remission at hour 60).*

Finally, EEG resting state data can be decomposed into sub-second (60-100ms), transiently stable and recurrent components called **microstates**[79]. The microstates can be clustered into a small number (usually four or five) of canonical classes, which repeatedly recur in individuals and the characteristics of which (i.e., specific topography of potentials, sequence, mean duration, number/second) have repeatedly been found to be altered in patients with schizophrenia[80–82,] . *We predict the BRX-induced switch will show disruption of the depression-associated microstate temporal dynamics, resulting in altered frequency, duration and transition probability of the two states described by Koenig*[83] *that have been linked to rsfMRI changes in brain regions pertinent to frontal-amygdala connectivity*[84,85] .

3. Objectives

3.1. Specific Aim

To Evaluate the feasibility of obtaining serial EEG recordings and affective state measures in women with PPD who receive inpatient BRX infusion treatment.

3.2. Exploratory Aims

Exploratory Aim 1
To calculate resting state nodal flexibility from five serial EEG recordings.
Exploratory Aim 2
To identify rapid brain state configurations and their probability density function from five serial EEG recordings.
Exploratory Aim 3
To assess the ability of iMotions Affectiva software to identify affective states using facial expression recognition analysis.
Exploratory Aim 4
To assess the amplitude of left frontal midline theta oscillations by performing spectral analyses of data collected from five serial EEG recordings.

4. Study Design

This is a feasibility study of performing repeated EEG recordings and assessments of affective states during open-label administration of BRX to women with postpartum depression. Study phases will include screening, enrollment, intervention, and follow-up. Subjects will be screened for eligibility criteria through clinical assessments and self-report. Enrolled subjects will be admitted to the UNC Women's Hospital, where five serial EEG recordings will be obtained, along with frequent assessments of affective state, before, during, and after a 60-hour IV infusion of BRX. Follow-up procedures will occur 30 days after completion of the drug infusion, and will include assessments of PPD and affective symptoms, as well as an exit interview with the PI. If feasibility outcomes are achieved, exploratory EEG analyses will be performed with AMICA (adaptive mixture independent component analysis), community detection, and microstate assessment. Exploratory analyses of data collected by facial expression detection software (iMotions Affectiva) are also planned.

4.1. Treatment Design

Based on an FDA-approved regimen, the 60-hour infusion will be administered as follows: 30 mcg/kg/hour (Hour 0 to 4), then 60 mcg/kg/hour (Hour 4 to 24), then 90 mcg/kg/hour (Hour 24 to 52), followed by 60 mcg/kg/hour (Hour 52 to 56), and 30 mcg/kg/hour (Hour 56 to 60). A programmable peristaltic infusion pump will be used to ensure accurate delivery of BRX.

4.2. Experimental / Observational Design

All enrolled participants receive the same intervention.

4.3. Measurement Design

Table 1: Measurement Design

Name	Time Frame	Brief Description	Scoring
Hamilton Depression Rating Scale (HAM-D)	Baseline, q4h while awake during infusion, 12 hours post-infusion, Day 30 (follow-up)	17-item rating scale to measure severity of depression. Will be used to define remission as scale score equal to or less than 7. This assures comparability with the original clinical trials of BRX (which used this scale as the primary endpoint).	Range: 0-55 Eligibility Score: ≥20 based on original clinical trials of BRX Remission Score: ≤7
Inventory of Depression Symptoms – Self Report (IDS-SR)	Baseline; 12 hours post-infusion	30 item self-report questionnaire that assesses depressive symptoms, including sad mood, irritability, lassitude, vegetative symptoms, well-being, and suicidality. Items reflecting anhedonia (inverse of the well-being items 21 [pleasure], 20 [energy], 19 [engagement], and 8 [reactivity]) will be summed to provide a subscale of depression symptoms	Range: 0-84 Total score obtained by summing the ratings of 28 of the 30 items. Either weight loss or gain, appetite loss or gain, is scored as only one member of each pair is applicable to any given respondent. Each of the 28 items is scored on a 0 to 3 scale (0—absence; 3—severe pathology)
Symptoms of Major Depressive Disorder Scale (SMDDS)	Baseline, q4h while awake during infusion, 12 hours post-infusion, Day 30 (follow-up)	16-item, rigorously developed patient reported outcome measure for depression, the only PRO measure for depression approved by the FDA to assess clinical trial endpoints.	Range: 0-64 Total score obtained by summing the ratings of the 16 items. Response scale for items 1-9: Not at all/ A little bit/ Moderately/ Quite a bit/ Extremely. Response scale for

			items 10-16: Never/ Rarely/ Sometimes/ Often/ Always
Edinburgh Postnatal Depression Scale (EPDS)	Baseline, q4h while awake during infusion, 12 hours post-infusion, Day 30 (follow-up)	21 item clinician-rated standardized scale assessing depressive symptom severity in women with PPD; widely used as a measure of symptom change.	Range: 0-30 Questions 1,2, &4 are scored 0, 1, 2, or 3 in order of appearance. Questions 3, 5-10 are reverse scored.
Ruminative Responses Scale-10 (RRS-10)	Baseline, 12 hours post-infusion, Day 30 (follow-up)	10-item measure of reflective pondering and brooding, minus the depression items contained in the 22-item version. Prominent sx of PPD; believed related to treatment efficacy.	Range: 10-40 Response items on a scale of 1 “almost never” to 4 “almost always” are summed. Two subscales (brooding and reflection) contain five items each.
Snaith-Hamilton Pleasure Scale (SHAPS)	Baseline, 12 hours post-infusion, Day 30 (follow-up)	A 14-item clinician administered scale to assess anhedonia, the inability to experience pleasure. Items cover the domains of: social interaction, food and drink, sensory experience, and interests/pastimes.	Range: 0-14 Either of the “disagree” responses receives a score of 1 and either of the “agree” responses receives a score of 0. A higher score is indicative of higher levels of anhedonia. An “abnormal” score is defined as 3 or more.
State Complacency Scale (SCS)	Baseline, q2h while awake during infusion, 12 hours post-infusion, Day 30 (follow-up)	100mm line scale ratings for depressed mood, anxiety, irritability, mood stability, satisfaction with current mood, belief that mood is about to change over time.	Range: 0-100 for each item

State-Trait Anxiety Inventory (STAI) State = Y-1 Trait = Y-2	State: Baseline, q4h while awake during infusion, 12 hours post-infusion, Day 30 (follow-up) Trait: Baseline, 12 hours post-infusion, Day 30 (follow-up)	40-item measure used in clinical settings to diagnose anxiety and distinguish it from depressive symptoms. 20 items for assessing trait anxiety and 20 items for state anxiety.	Range: 20-80 (each form) Sum the scoring weights (1-4) for each response item. See manual for reverse scoring guide.
Barkin Index of Maternal Functioning (BIMF)	Baseline, 12 hours post-infusion, Day 30 (follow-up)	20-item self-report measure to assess overall functioning in the context of new motherhood.	Range: 0-120 Items 16 and 18 are reverse scored, then all 20 items are summed to generate the total score. Higher total scores are associated with greater levels of functioning.
Resting State EEG Recording (EEG-RS)	Baseline, during infusion (Hours 4, 8, and 28), 12 hours post-infusion	8-minute EEG recording where subject focuses on neutral visual target while alternating eyes open and closed every 2 minutes	See Table 1B for description of EEG outcomes of interest
iMotion Affectiva Facial Expression Analysis Software	Baseline, during infusion (Hours 4, 8, and 28), 12 hours post-infusion	Software that measures 20 facial expression metrics (e.g., affective valence, engagement, facial landmarks) known to display altered configurations that map onto emotions. Changes in facial expression will provide a non- verbal complement to self-ratings in detecting BRX-induced alterations in affective state.	See Table 1C for description of iMotions software outcomes of interest.
Semi-structured Interview	Baseline, during infusion (Hours 4, 8, and 28), 12 hours post-infusion	Administered simultaneously with iMotions recording to elicit emotional response	Qualitative responses to questions about recent experiences

		in lieu of an affective probe task	of enjoyment, guilt, and irritability will be compared to software output of detected facial expression (see Table 1C).

Table 1B: EEG Outcomes of Interest (Exploratory Aims)

Exploratory Aim	**Variable of Interest**	**Brief Description of Measurement**
Calculation of resting state nodal flexibility (community detection)	Nodal flexibility: Frequency with which a nodal hub changes its community identity	Measured by allegiance of nodes to modules: the number of times that each node changes module allegiance normalized by the total possible number of changes. The flexibility of the network as a whole is equal to the mean flexibility over all the nodes
Identification of (A) rapid state brain configurations (global brain states) and (B) their probability density function	(A) Microstate: four quasi-stable, recurring topographic patterns of voltage (i.e., different configurations of electric field lasting 80-120 milliseconds). (B) Duration: frequency of occurrence of microstates reflects the tendency of its underlying neural sources to become activated	Frequency = the number of times a microstate is present relative to the total number of microstates recorded. Average duration = average time in milliseconds. Duration = proportion of time that state is activated relative to the sampling recording.
Calculation of amplitude of frontal midline theta oscillations (spectral analysis)	Frontal Midline Theta: 4-7 Hz reflecting activity from the limbic system and hippocampal regions	Extracted theta power (4-7Hz) from FCz electrode during resting state recordings. Unit = dB.

Table 1C: iMotions Affectiva Software Outcomes of Interest (Exploratory)
Exploratory Aim: To assess the ability of iMotions Affectiva software to identify affective states using facial expression recognition analysis.

Variable of Interest	**Brief Description of Measurement**	**Unit of Measurement**

Action Unit (most sensitive level of detection)	Tiny muscular changes in a facial expression (e.g., inner brow raiser, brow lowered, lip corner depressor, etc.)	Evidence score represents the probabilistic odds in logarithmic scale (base 10) of a target action unit being present.
Emotion Score	Percent of time spent in a certain emotional state (Eckman's core 7 emotions)	The weighted average of action units (e.g., cheek raiser, lips part, and lip corner puller act in concert to create a smile during the state of enjoyment)
Engagement and valence (least sensitive levels of analysis)	Threshold of expressiveness in general and positive or negative expression	Probability (percentage) that a human assessor would rate the emotion equally to the software. Expressions that don't meet the predetermined thresholds are considered "neutral"

4.4. Outcome Measures

Primary Outcome(s):

1. Number of successfully analyzed EEG recordings.
2. Number of subjects completing the entire study protocol through the follow-up phase.
3. Number of subjects withdrawn from the protocol due to adverse events and/or participation burden.

Tertiary/Exploratory Outcome(s):

1. Calculation of resting state nodal flexibility (community detection) at H0, 4, 8, 28, 72.
2. Identification of (A) rapid state brain configurations (global brain states) and (B) their probability density function at H0, 4, 8, 28, 72.
3. Identification of affective states with iMotion Affectiva software at H0, 4, 8, 28, 72.
4. Calculation of amplitude of frontal midline theta oscillations (spectral analysis) at H0, 4, 8, 28, 72.

Other Measures: Baseline Characteristics

1. Age
2. Race
3. Ethnicity
4. History of depression (SCID-5)
5. Comorbid anxiety disorder(s) (SCID-5)

6. Concurrent psychotropic medication use
7. Pregnancy history (# pregnancies, # births, methods of delivery, birth complications, past PPD episodes)

5. Study Participants

5.1. Number of Participants

Number to be recruited for screening: n=20

Number of eligible participants enrolled: n=12 women with moderate to severe PPD, medically eligible to receive FDA-approved BRX infusion, recruited from current inpatient and outpatient population in the Women's Mood Disorders Clinic at UNC and through local advertisements. Subjects recruited from outpatient setting will be individuals for whom BRX is the chosen recommended course of treatment. 20 subjects will be screened for eligibility, 12 will be enrolled, and 10 will complete the protocol in its entirety.

5.2. Eligibility

Inclusion Criteria:

To be eligible to participate in this study, an individual must meet **all** the following criteria:

- Signed informed consent.
- Ambulatory, female, aged 18-45
- ≤ 8 months postpartum
- Agrees to adhere to the study requirements.
- Onset of depression in 3rd trimester or within 4 weeks of delivery
- Meets DSM-V criteria for major depressive disorder with peripartum onset.
- 17-item HAM-D total score ≥20 at screening
- Stopped breastfeeding or agrees to temporarily stop for 7 days including 4 days of hospitalization and 3 days after
- No new psychotropic drugs during screening and active treatment of study
- Stable use of any current psychotropic drugs for at least 28 days prior to enrollment, with stable dosage for at least 14 days prior to enrollment
- Must be on documented contraceptive.
- Must have a caregiver or family member with them to help care for the subject's child(ren) during the infusion and to be in the room with the subject if the child(ren) are present during the infusion

Exclusion Criteria:

Any individual who meets **one or more** of the following criteria will be excluded from participation:

- Positive pregnancy test at screening or day 1

- Pregnancy that resulted in a stillbirth, termination, or child that was placed for adoption.
- Renal impairment or failure, hepatic impairment or failure, or anemia
- Untreated or inadequately treated hypothyroidism or hyperthyroidism
- Known allergy to progesterone or allopregnanolone.
- Suicide attempt at this episode
- Medical history of schizophrenia, and/or schizoaffective disorder
- Current psychotic symptoms including delusions, hallucinations, or formal thought disorder.
- Concurrent substance abuse
- Exposure to another investigational medication or device within 30 days
- Has previously participated in any study employing brexanolone or SAGE-217.
- Subject is investigative site personnel, sponsor personnel, or an immediate member of their family.
- Has received electroconvulsive therapy during current episode.
- History of seizure disorder
- On anticonvulsant agents
- On benzodiazepines

5.3.Strategies for Recruitment and Retention

Recruitment Strategy:

Potential subjects will be selected among patients admitted to the PPIU or seen in our Women's Mood Disorders outpatient program, for whom BRX is an appropriate course of treatment. Thus, we will capitalize on the existing BRX program at UNC and invite women who are interested and eligible to receive BRX treatment for PPD to participate in this research study. The BRX treatment protocol in this study will align with the clinical course of treatment at UNC, with the addition of serial EEG recordings, affective assessments, iMotions facial expression recordings to satisfy research aims. Should subjects decline to participate in the research study, they may still be able to receive BRX treatment through the traditional route at UNC.

Similarly, we will recruit through patients referred as part of our outreach to community OB-GYN practices; through advertisements in local newspapers, parent magazines, public transportation and online; and through our consultation with the UNC CTSA, the North Carolina Translational and Clinical Sciences Institute (NC TraCS). Through the NC TraCS Data Access and Informatics Core, we can identify a cohort of UNC Hospital patients for recruitment. UNC uses the EPIC electronic medical record system, which enables communication to potential research participants via email and through the MyChart patient interface. This makes communication with many potential research participants cost effective and highly efficient, because it will enable potential participants to access our online screening tool simply by clicking a link in their email or MyChart electronic message. Additionally, we will capitalize on the TraCS Research Recruitment Service's expertise in enrolling members of communities historically under-represented in research. We additionally can use the following widely accessed entities: email

listservs that are directed to staff, faculty, and students on campus; public transportation in Chapel Hill, which is free and widely used; the local newspaper in which we advertise, and online advertising through sites like Facebook, which reaches many women of reproductive age.

Retention Strategy:

These strategies include the following: providing clear descriptions of all study procedures and burdens; enrolling only fully informed subjects; and using a financial bonus linked to completion in full compliance. Although we recognize that side effects of any treatment may influence retention, the side effects associated with BRX led to no dropouts in our previous experience. Non-compliance should not be an issue in this short-term inpatient study. Nonetheless, our sample size of 12 allows for a 20% drop-out rate. Finally, the study team will conduct exit interviews with participants at the final study visit to solicit candid feedback about participants' experiences in the study. We will use this feedback to make improvements to study procedures. Most of past participants have taken advantage of this optional session to review their own data and to discuss implications for managing affective symptoms in future pregnancies. During the final exit interview, many participants described the opportunities to learn more about the effects of hormones on their mood and to help others with similar problems as important motivations for their enrolling in and completing the study. We have strategies in place in case of unexpected difficulties. The main such strategy will be use of the state-wide, HRSA-funded, education and assessment hotline for perinatal depression, the North Carolina Maternal Health Matters program. Through this program, staff in the UNC Perinatal Psychiatry Program have access to patients and providers throughout the state of North Carolina.

Screen Failures:

Of the 20 women screened for eligibility, we anticipate 12 will meet inclusion criteria (60%). Subjects who consent to and complete screening protocols (including physical examination, clinical lab assessments, ECG, and structured clinical interview for DSM-5 psychiatric disorders) will be compensated for their willingness and time, regardless of ultimate eligibility determination. Should subjects fail to meet eligibility criteria upon initial screening, re-screening may occur on a case-by-case basis, depending on the reason(s) for initial exclusion. Examples may include stabilized regimen of psychotropic medications for 28 days and stable dose for 14 days prior to enrollment; cessation of breastfeeding; HAM-D score severity in the required range at rescreening.

5.4. Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent will be obtained by either the PI or the study coordinator. The consent process will occur in either the outpatient clinic, the CTRC, or the inpatient unit (for subjects recruited after admission). Consent forms will be Institutional Review Board (IRB)-approved, and the participant will be asked to read and review the document. The research personnel will explain the research study to the participant and answer any questions that may arise. If there are unanswered questions, the PI will be available to provide further responses to the participant's questions. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants.

Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document, witnessed by the study personnel, prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. Each participant will sign a HIPAA authorization form.

6. Study Intervention

6.1. Intervention – Test Article

Description:

As supplied, the brexanolone drug product is a sterile, clear, colorless 5 mg/mL solution of brexanolone and 250 mg/mL sulfobutyl-ether-beta-cyclodextrin buffered with 10 mM citrate at a pH of 6.0, supplied in single-dose 20 mL vials for IV administration. The composition and pharmaceutical quality of the investigational product will be maintained according to the current Good Manufacturing Practice and Good Clinical Practice (GCP) guidelines and available for review in the study medication documentation.

Acquisition:

Brexanolone will be provided to the site by the sponsor and to the investigator by IDS.

Formulation/Packaging/Labeling:

The product is aseptically processed, sterile filtered, and filled into 20 mL Type 1 parenteral glass vials with West FluroTec® coated stopper container closure systems, under current Good Manufacturing Practice conditions. Brexanolone is intended to be used as a single-use vial.

Storage and Stability:

The investigator or designee should refer to the Pharmacy Manual for instructions on acknowledging receipt of study drug.

Study drug vials should be stored under refrigerated conditions (2 to 8°C). The vials must be carefully stored safely and separately from other drugs. The study drug may not be used for any purpose other than the present study.

The Investigator or designee will be responsible for ensuring appropriate storage, dispensing, inventory, and accountability of all clinical supplies. An accurate, timely record of the disposition of all clinical supplies must be maintained. The supplies and inventory must be available for inspection by the

designated representatives of the Sponsor or the Sponsor's representatives on request, and must include the information below:

• The identification of the subject to whom the drug was dispensed

• The date(s) and quantity of the drug dispensed to the subject

• The product lot/batch number

The preparation of the study drugs must be documented on a 'Drug Preparation and Dispensing Log Form' or similar form.

The drug inventory and any clinical supplies that have been destroyed must be documented. This documentation must include at least the information below or as agreed with the Sponsor:

- The number of prepared units
- The number of administered units
- The number of unused units
- The number of units destroyed at the end of the study
- The date, method, and location of destruction

Preparation and Administration:

The Pharmacist or designee will be responsible for preparing study drug for subject dosing. The prepared admixture will be administered at room temperature.

Refer to the Pharmacy Manual for specific instructions regarding requirements for IV bags and labeling, infusion sets, infusion preparation and administration instructions.

This is an open-label study. Subjects will receive a 60-hour continuous IV infusion of brexanolone.

The specific infusion dose of study drug will be calculated based on weight (obtained at screening) for each subject and administered according to the dose regimen in Table 2.

Table 2: **Infusion Rate**

Time point	**Day 1 0 to 4 hours**	**Day 1 4 to 24 hours**	**Day 2 to 3 24 to 52 hours**	**Day 3 52 to 56 hours**	**Day 3 56 to 60 hours**
Dose	30 mcg/kg/hour	60 mcg/kg/hr	90 mcg/kg/hr	60 mcg/kg/hr	30 mcg/kg/hr

Dosing will begin in the morning (on Day 1) to avoid the dose increase to 90 mcg/kg/hour late in the day and to avoid awakening subjects during the night for completion of study assessments.

Refer to the Pharmacy Manual for complete details on preparation and administration.

The pharmacist or designee for drug accountability is to document the date and time of preparation of test article and for which subject the study drug was intended (i.e., record subject initials and birth date or another unique identifier).

At the end of the study, any unused study drug will be destroyed locally per the site's procedures; disposition of study drug will be documented.

Modification:

If the UNC Hospitals Arousal Score = 3 (see Table 5), the dose will be reduced by 50% and the doctor will be contacted. If there are mild symptoms (e.g., dizzy, lightheaded), the dose will be held at the current rate rather than increased. Typically, these mild symptoms will resolve with more time at the lower infusion rate, and then subjects can tolerate an increase up to 90mcg/kg. The infusion will be immediately stopped if there is loss of consciousness, or if the UNC Hospitals Arousal Scale score is less than or equal to 2, or if pulse oximetry reveals hypoxia (oxygen saturation below 90%).

For patients who do not tolerate the 90 mcg/kg/hour dose, a reduction to 60 mcg/kg/hour may be considered during the time that the 90 mcg/kg/hour is scheduled to occur.

If other intolerable adverse events occur, the infusion will be stopped until the symptoms resolve. The infusion may be resumed at the same or lower dose as clinically appropriate.

Accountability:

The Pharmacist or designee will maintain accurate records of all investigational drug product supplies received, stored, dispensed, and discarded. Accurate records will be kept regarding the volume of study drug used for each IV preparation, as well as the required infusion dose (or doses), and the date and time of preparation. Reasons for departure from the expected dosing regimen must be recorded. To satisfy regulatory requirements regarding drug accountability, all study medication needs to be reconciled in full.

6.2. Assignment Procedures

Matching and Stratification:

Not applicable

Randomization, Concealment, and Blinding:

Not applicable

Masking (Blinding):

Not applicable

6.3. Intervention – Procedural (if applicable)

Description:

Refer to the Pharmacy Manual for specific instructions regarding requirements for IV bags and labeling, infusion sets, infusion preparation and administration instructions.

Training on Procedural Intervention:

For individuals administering, it will be the unit chief from the PPIU, who has extensive experience administering brexanolone according to the proposed regimen. Nurses on both the PPIU and the Women's Hospital inpatient unit have extensive experience with exactly the administration regimen proposed in this protocol. Prescribers and pharmacists will have been trained and certified in the BRX REMS program.

6.4. Concomitant Therapy:

Subjects may receive standard of care for-patients diagnosed with PPD, including psychosocial interventions. Any concomitant medication determined medically necessary for the welfare of the subject may be given at the discretion of the Investigator at any time during the study, with the caveat that changes to psychotropic medications may be made only as outlined in the paragraph below.

Subjects that are receiving medications administered to treat the symptoms of depression or anxiety (such as antidepressants) must have been initiated at least 14 days prior to dosing and must remain at a stable dose from at least 14 days prior to dosing until completion of the 72-hour assessments. Any new medication for depression or anxiety initiated after the 72-hour assessments are completed must be preceded by a discussion with the investigator and designated REMS physician.

The following interventions will be recorded:

• All medications taken from 60 days prior to informed consent through the final study visit

• All psychotropic medications (including anxiolytics or antidepressants) taken in the previous 6 months prior to informed consent through the final study visit

• All medications used to treat the current episode of PPD regardless of timing through the final study visit

• All nonpharmacological interventions used to treat the current episode of PPD regardless of timing through the final study visit

6.5. Rescue Medications and Procedures (if applicable)

Since subjects will be hospitalized inpatients, and since they will be treated with a GABA positive allosteric modulator, which has known anxiolytic effects, it is not anticipated that anyone would experience a worsening of symptoms; if there was a concern that rescue was required, the response would take the form of decreasing or discontinuing the brexanolone and treating the subject with conservative measures until the response to drug discontinuation could be assessed.

6.6. Compliance Checks

Study drug will be prepared by a pharmacist or designee, administered as a continuous IV infusion, and the dose received will be documented in the study record. There should be no adjustments in dosing except as described in Section 6.1.

6.7. Withdrawal / Discontinuation of Enrolled Participants

Enrolled participants may be withdrawn if new or worsening symptoms or any adverse event suggest that continued participation would place the subject at risk and not be in the subject's best interest.

Subjects may withdraw from the study at any time for any reason without compromising the subject's medical care. The Investigator will also withdraw subjects upon the request of Sage Therapeutics or upon termination of the study.

Subjects who withdraw should, if possible, complete an early termination visit, which includes the assessments for the Hour 72 visit (see Table 3).

The reasons for protocol discontinuation and/or subject withdrawal from the study will be recorded in the study record.

6.8. Voluntary Withdrawal (Drop-Out) of Enrolled Participants

The subjects may voluntarily withdraw participation at any time, for any reason, with no penalty or loss of rights. Reasons for discontinuation will be documented in the database.

7. Study Procedures and Schedule

7.1. Table of Events

Table 3: Schedule of Events

Study Procedure	Screening Period	Treatment Period/Inpatient Stay (Day 1 to Day 4)						Follow-up
Visit Days	**D-14 to 1**	**D1**	**D1**	**D1**	**D2**	**D3**	**D4**	**D30 (±3d)**
Hour		**H0***	**H4**	**H8**	**H28**	**H60**	**H72**	
Informed Consent	X							
SCID-5	X							
Demographics	X							
Medical History	X							
Height		X						
Body Weight		X						
Physical Examination[a]	X							
Drug and Alcohol Test[b]	X	X						
Pregnancy Test[c]	X	X						
Clinical lab assessments[d]	X							
ALLO blood sample[e]		X			X		X	
12-lead ECG[f]	X							
Vital Signs[g]	X	X	q4h (while awake) during infusion				X	
C-SSRS[h]	X	X					X	
HAM-D[i]	X	X	q4h (while awake) during infusion				X	X
SMDDS[i]		X	q4h (while awake) during infusion				X	X
EPDS[i]		X	q4h (while awake) during infusion				X	X
STAI (state Y-1)[i]		X	q4h (while awake) during infusion				X	X
SCS[i]		X	Q4h (while awake) during infusion				X	X
BIMF[i]		X					X	X
IDS-SR[i]		X					X	X
RRS-10[i]		X					X	X
SHAPS[i]		X					X	X
STAI (trait Y-2)[i]		X					X	X
EEG Recording[i]		X	X	X	X		X	
RS Eyes Open/Closed (8min)[i,j]		X	X	X	X		X	

iMotion Recording[i,j]		X	X	X	X		X	
Semi-structured Interview [i,k]		X	X	X	X		X	
Brexanolone infusion		X						
Continuous pulse oximetry[l]		X						
Continuous HRV collection		X						
Adverse Events		q2h (while awake) during infusion					X	X
Monitor for excessive sedation		q2h (while awake) during infusion						
Prior/Concomitant medications[m]	X	X					X	X
Nonpharmological interventions[n]	X	X					X	X
Exit Interview								X
Compensation (total $750)	$50	$100	$100	$100	$100		$100	$200

AE= adverse event; ALLO= allopregnanolone; BIMF= Barkin Index of Maternal Functioning; C-SSRS = Columbia-Suicide Severity Rating Scale; D= Day; ECG= electrocardiogram; EEG= electroencephalogram; EPDS= Edinburgh Postnatal Depression Scale; HAM-D= Hamilton Depression Rating Scale; H=Hour; IDS-SR= Inventory of Depression Symptoms Self-Report; Q= every; RRS-10= Ruminative Responses Scale-10; RS= Resting State; SCID-5= Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; SCS= State Complacency Scale; SHAPS= Snaith-Hamilton Pleasure Scale; SMDDS= Symptoms of Major Depressive Disorder Scale; STAI= State-Trait Anxiety Inventory;

* = All H0 procedures to be completed prior to dosing

a Full physical examination at screening. Symptom-directed physical examination may be conducted at subsequent time point

b Drug and alcohol testing will occur at screening and Day 1 (predose; H0). Drug testing will be done via urine dipstick; alcohol use will be tested via urine dipstick or breath test.

c Serum pregnancy test at screening and serum or urine pregnancy test at Day 1 (predose; H0)

d Safety laboratory tests will include hematology and serum chemistry at all scheduled time points. Coagulation to be assessed at screening only. Laboratory assessments documented in subject's medical record will be considered valid for screening purposes if done within 30 days of infusion

e Laboratory tests for hormone parameters are to be completed within ±30 minutes of the scheduled timepoint.

f Performed within ±1 hour of the scheduled time point on Day 4

g At all time points, vital signs to include oral temperature (°C), respiratory rate, heart rate, and blood pressure. Heart rate and blood pressure to be collected in supine position at all scheduled time points. Vital signs collected after the initiation of brexanolone will be obtained within ±30 minutes of the scheduled time point, unless the subject is asleep between 23:00 h and 06:00 h.

h The "Baseline/Screening" C-SSRS form will be completed at Screening. The "Since Last Visit" C-SSRS form will be completed at subsequent time points
i Hour 0 assessments to be completed on Day 1 prior to the start of the infusion. Subsequent assessments during the inpatient period to be initiated within ±1hour of the scheduled time point.
j Performed during EEG recording
k Performed during iMotion Affective Facial Expression Analysis Software recording
l Continuous pulse oximetry to occur for the duration of the infusion. Oxygen saturation need only be recorded in the event of hypoxia (<92% SpO2), in which case, the event is to be recorded as an AE
m At screening to include all medications taken within 60 days, all psychotropic medications taken within 6 months, and all medications used to treat the current episode of PPD. At visits subsequent to screening all changes to any medication should be captured. To be eligible, subjects must be on a stable dose of any psychotropic drugs for at least 14 days prior to enrollment.
n All nonpharmacological interventions (eg, psychosocial, psychotherapeutic) used to treat the current episode of PPD should be captured at screening, and all changes should be captured at subsequent visit.

7.2. Pre-Screening / Screening (Day -14 to Day 0)

PRE-SCREENING: will be performed by research coordinator, either by phone or in person, following verbal consent from the participant. Information obtained will include self-reported information relevant to inclusion and exclusion criteria (to be confirmed by objective assessment during screening).

SCREENING* (Day –14 to Day 1):
**Sequence of screening procedures not pre-defined after informed consent and HIPAA authorization are obtained.*

Informed Consent and HIPAA Authorization
To be obtained by PI or Study Coordinator prior to any other procedures listed herein.

Demographic/Medical History

Medical history will be collected via a checklist completed by the subject. Age, race, and ethnic origin will be recorded. The diagnosis of PPD will be determined using the SCID-5. Subjects will be specifically asked about the following: depression, anxiety, and other diagnoses per DSM-5. Pregnancy history (including number of pregnancies, number of births, methods of delivery, and birth complications) and PPD episodes will be recorded.

Vital Signs

Vital signs include oral temperature (°C), respiratory rate, heart rate, and blood pressure. Heart rate and blood pressure to be collected in supine position at all scheduled time points as well as in the standing position at specific time points listed in Table 3.

Weight, Height and Body Mass Index

Body weight and height will be measured; body mass index will be programmatically calculated in the study record.

Physical Examination

Physical examinations of major body systems will be undertaken and recorded. Major body systems to be included in the screening physical examination are head, eye, ear, nose and throat, heart, lungs, abdomen, and extremities; as well, cognitive, neurological, and mental status examinations will be performed.

Electrocardiogram

Twelve-lead ECGs will be performed. The Investigator will record if the ECG is normal; abnormal, not clinically significant; or abnormal, clinically significant (CS). If abnormal, details of the abnormality will be provided (e.g., first-degree AV block, bundle branch block). The standard intervals (heart rate, PR, QRS, QT, and QTcF) will be recorded for all ECGs.

Laboratory Assessments

Blood samples will be collected for hematology, serum chemistry, and coagulation. Analytes to be evaluated are summarized in Table 4.

All blood samples, including those taken for eligibility screening, will be sent to the central laboratory. Laboratory assessments obtained by outside medical clinic will be considered valid if collected within 30 days of infusion start date.

Table 4: Summary of Laboratory Analytes

Hematology	complete blood count (red blood cells, white blood cells with differentiation and absolute values, hemoglobin, hematocrit, reticulocytes, and platelets)
Coagulation (at Screening only)	activated partial thromboplastin time, prothrombin time, and international normalized ratio
Biochemistry	serum electrolytes (sodium, potassium, chloride, bicarbonate or total carbon dioxide, calcium, and phosphorus) renal function tests (creatinine and blood urea nitrogen); liver function tests (total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and gamma glutamyl transferase); thyroid stimulating hormone; total protein; albumin and glucose (fasting or non-fasting)

All clinical laboratory test results outside the reference range will be interpreted by the Investigator as abnormal, not clinically significant; or abnormal, CS. Screening results considered abnormal, and CS at the Screening Visit may make the subject ineligible for the study pending review by the Investigator. Clinically significant abnormal results after screening will be considered and reported as AEs.

Pregnancy Test

All subjects will be tested for pregnancy by serum or urine human chorionic gonadotropin at the during screening.

Drugs of Abuse and Alcohol

Urine assessment for select drugs of abuse will be performed (including amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, and phencyclidine). A positive urine drug screen is exclusionary, unless deemed by the investigator to reflect a prescribed medication. Alcohol will be assessed via breathalyzer or urine dipstick.

Hormones and Biochemistry

Blood samples will be collected and may be analyzed for estrogen, progesterone, progesterone metabolites, thyroid function (TSH, T4, T3), and markers of inflammation.

Columbia-Suicide Severity Rating Scale

Suicidality will be monitored during the study using the C-SSRS (Posner 2011). This scale consists of a baseline evaluation that assesses the lifetime experience of the subject with suicidal ideation and behavior, and a post-baseline evaluation that focuses on suicidality since the last study visit. The C-SSRS includes 'yes' or 'no' responses for assessment of suicidal ideation and behavior as well as numeric ratings for severity of ideation, if present (from 1 to 5, with 5 being the most severe).
The "Baseline/Screening" C-SSRS form will be completed at screening (lifetime history and past 24 months). The "Since Last Visit" C-SSRS form will be completed at all subsequent time points, as outlined in Table 1.

Hamilton Depression Rating Scale
The HAM-D will be administered by PI or Study Coordinator at screening to confirm eligibility based on a score of ≥20, which was the threshold used in the original BRX clinical trials.

Structured Clinical Interview for DSM-V Disorders
The SCID-V will be administered by PI or Study Coordinator at screening to confirm eligibility based on diagnosis of Major Depressive Disorder with Peripartum Onset. The SCID-V will also be used to diagnose and record concurrent anxiety disorders, and to rule out current suicide attempt, concurrent alcohol and/or substance use disorder, and history of psychosis or mania.

7.3. Enrollment / Baseline (Day 0)

Refer to Tables 1 and 3 for schedule of procedures at Baseline. Procedures to be performed by the following personnel: PI, Study Coordinator, Research Nurse, and/or EEG technician.

Baseline (Hour 0)

- Subject will be admitted to UNC Hospital on a Monday or Tuesday morning. Prior to administration of drug intervention, the subject will undergo the following procedures:
    - Measure subject's height and weight, record, and submit to IDS to calculate drug dosage
    - Negative drug/alcohol and pregnancy test (if positive, subject will be withdrawn from the study)
    - Baseline blood sample to measure circulating allopregnanolone levels
    - Vital signs
    - Review of current concomitant medications and nonpharmacological interventions
- **First EEG recording:**

  - EEG cap will be fitted by technician and electrodes filled with gel solution. Impedance will be measured to ensure proper adherence to scalp. Cap set-up will take approximately 20 minutes.
  - Recording of 8-minute resting state EEG, eyes alternating open and closed at regular intervals.
  - Cap will remain on the subject until the end of the Hour 8 session, so as not to disrupt the electrode placement over the course of the serial recordings. EEG will be recorded for 8-minute sessions at Hours 4 and 8 on Day 1 (not continuously throughout the day).
- **Self-Report measures:** Electronic versions of the following surveys will be administered: C-SSRS, EPDS; STAI (Y1&Y2); SCS; BIMF; IDS-SR; RRS-10; SHAPS.
- **Semi-Structured interview & iMotions Recording:** Facing a laptop equipped with a standard camera and microphone and iMotions Affectiva software, the subject will be administered a semi-structured interview about depressive symptoms and affect by study personnel. Interview questions will include the HAM-D and additional questions to assess the subject's experiences of enjoyment, guilt, and irritability. The interview and recording will last 10-20 minutes. A memory probe task will be completed, in which a subject will be asked to think of a sad event in her life and focus on that memory for 30 seconds while the laptop camera records her facial expressions. Then they will be asked to rate their emotional experience during that recall. This procedure will be repeated with a prompt to recall a happy memory from her life. Subjects will then be asked to come up with a word or phrase that can be used to recall each event during later recording sessions.
- **Heart Rate Variability:** Using a non-invasive device, heart rate variability data will be collected throughout the infusion. The device will be adhered to the subject's torso by two electrodes and will begin collecting data once it is adhered to the skin. The device will be removed following completion of the brexanolone infusion

7.4.Study Visits (Days 1-4)

Refer to Tables 1 and 3 for schedule of procedures at study visits following Baseline. Procedures to be performed by the following personnel: PI, Study Coordinator, Research Nurse, and/or EEG technician.

Hour 4 Visit

- **Second EEG recording:** With the cap in place from the baseline recording, electrode impedance will be measured, and electrode gel refilled to ensure proper fit. A second EEG will be recorded during an 8-minute resting state with eyes opened and closed at regular intervals. The cap will remain on the subject until the end of the Hour 8 recording but will not be recording continuously between sessions.
- **Self-Report measures:** Electronic versions of the following surveys will be administered: SMDDS, EPDS, STAI (Y1), and SCS.

- **Semi-Structure interview & iMotions Recording:** Facing a laptop equipped with a standard camera and microphone and iMotions Affectiva software, the subject will be administered a semi-structured interview about depressive symptoms and affect by study personnel. Interview questions will include a modified version of the HAM-D (items suitable for repeated measure within the same day) and additional questions to assess the subject's experiences of enjoyment, guilt, and irritability. The interview and recording will last 10-20 minutes. The memory probe task will be completed, in which a subject will be told the predetermined word or phrase to cue recall of the sad and happy events, while the camera records her facial expressions for 30 seconds for each event and the subject rates her emotional experience.

Hour 8 Visit

- **Third EEG recording:** With the cap in place from the Hour 4 recording, electrode impedance will be measured, and electrode gel refilled to ensure proper fit. A third EEG will be recorded during an 8-minute resting state with eyes opened and closed at regular interviews. Following completion of this recording, the cap will be removed.
- **Self-Report measures:** Electronic versions of the following surveys will be administered: SMDDS, EPDS, STAI (Y1), and SCS.
- **Semi-Structured interview and iMotions Recording:** Facing a laptop equipped with a standard camera and microphone and iMotions Affectiva software, the subject will be administered a semi-structured interview about depressive symptoms and affect by study personnel. Interview questions will include a modified version of the HAM-D (items suitable for repeated measure within the same day) and additional questions to assess the subject's experiences of enjoyment, guilt, and irritability. The interview and recording will last 10-20 minutes. The memory probe task will be completed, in which a subject will be told the predetermined word or phrase to cue recall of the sad and happy events, while the camera records her facial expressions for 30 seconds for each event and the subject rates her emotional experience.

Hour 28 Visit (4 hours after highest dose of brexanolone begins)

- **Blood sample:** to measure and document peak circulating levels of allopregnanolone
- **Fourth EEG recording:** EEG cap will be fitted by technician and electrodes filled with gel solution. Impedance will be measured to ensure proper adherence to scalp. Cap set-up will take approximately 20 minutes. A fourth EEG will be recorded dur an 8-minute resting state with eyes opened and closed at regular intervals. The cap will be removed following this recording.
- **Self-Report measures:** Electronic versions of the following surveys will be administered: SMDDS, EPDS, STAI (Y1), and SCS.
- **Semi-Structured interview and iMotions Recording:** Facing a laptop equipped with a standard camera and microphone and iMotions Affectiva software, the subject will be administered a semi-structured interview about depressive symptoms and affect by study personnel. Interview questions will include the HAM-D and additional questions to assess the subject's experiences of

enjoyment, guilt, and irritability. The interview and recording will last 10-20 minutes. The memory probe task will be completed, in which a subject will be told the predetermined word or phrase to cue recall of the sad and happy events, while the camera records her facial expressions for 30 seconds for each event and the subject rates her emotional experience.

Hour 60

- **Brexanolone infusion concludes**

SAFETY MONITORING DURING INTERVENTION PHASE (Every 2 hours, while awake, during infusion)

Continuous Pulse Oximetry

Subjects will be monitored for hypoxia throughout intervention phase using continuous pulse oximetry equipped with an alarm. Oxygen saturation need only be recorded in the event of hypoxia, in which case, the event is to be recorded as an AE. The infusion will be immediately stopped if pulse oximetry reveals hypoxia. After an episode of hypoxia, the infusion should not be resumed.

Monitoring for Excessive Sedation and Loss of Consciousness

For the duration of the infusion, subjects must be assessed for excessive sedation and loss of consciousness by a healthcare provider every 2 hours during planned, non-sleep periods. If excessive sedation or loss of consciousness occurs at any time during the infusion, the infusion will be stopped until the symptoms resolve. The infusion may be resumed at the same or lower dose as clinically appropriate (see Section 6.1), and an AE is to be recorded.

Vital Signs

Vital signs include oral temperature (°C), respiratory rate, heart rate, and blood pressure. Heart rate and blood pressure to be collected in supine position at all scheduled time points as well as in the standing position at specific time points listed in Table 3.

7.5. Phone Contact

Subjects will be provided phone and email contact information for PI and Study Coordinator, should the participant have questions or concerns that may be addressed over the phone between in-person visits.

7.6. Final Visit (Hour 72)

Refer to Tables 1 and 3 for schedule of procedures at final inpatient visit. Procedures to be performed by the following personnel: PI, Study Coordinator, Research Nurse, and/or EEG technician.

Hour 72 Visit (12 hours after the end of brexanolone infusion)

- **Fifth EEG recording:** EEG cap will be fitted by technician and electrodes filled with gel solution. Impedance will be measured to ensure proper adherence to scalp. Cap set-up will take approximately 20 minutes. A fifth and final EEG will be recorded dur an 8-minute resting state

with eyes opened and closed at regular intervals. The cap will be removed following this recording.

- **Self-Report measures:** Electronic versions of the following surveys will be administered: C-SSRS, EPDS; STAI (Y1&Y2); SCS; BIMF; IDS-SR; RRS-10; SHAPS.
- **Semi-Structured interview and iMotions Recording:** Facing a laptop equipped with a standard camera and microphone and iMotions Affectiva software, the subject will be administered a semi-structured interview about depressive symptoms and affect by study personnel. Interview questions will include the HAM-D and additional questions to assess the subject's experiences of enjoyment, guilt, and irritability. The interview and recording will last 10-20 minutes. The memory probe task will be completed, in which a subject will be told the predetermined word or phrase to cue recall of the sad and happy events, while the camera records her facial expressions for 30 seconds for each event and the subject rates her emotional experience.
- **Blood sample**: to measures and document a return to baseline circulating levels of allopregnanolone
- **Safety Check prior to discharge from UNC Women's Hospital**
    - Vital signs
    - C-SSRS ("since last visit")
    - Review of concomitant medications and nonpharmacological interventions administered during hospital stay

In the unlikely event that there is imminent risk of suicidal behavior at the time of the final safety check, the subject will be withdrawn from the research study, discharged from the Women's Hospital, and referred to the UNC Emergency Department and/or other appropriate inpatient hospitalization. The study doctor will provide referrals for continued care, which may include hospitalization, but will not be responsible for providing such care following withdrawal from the research study.

7.7. Follow-Up Contact (Day 30 ± 3)

Refer to Tables 1 and 3 for schedule of procedures at final inpatient visit. Procedures to be performed by PI and Study Coordinator.

Day 30 Visit

This follow-up visit will occur in-person, on the phone, or over HIPAA-secured Zoom call, depending on the subject's preference.

- **Self-Report Measures:** Electronic versions of the following surveys will be administered: EPDS; STAI (Y1&Y2); SCS; BIMF; IDS-SR; RRS-10; SHAPS.
- **Semi-Structured interview:** Study coordinator or PI will administer the HAM-D
- **Exit Interview:** an informal interview with PI to include items such as:
    - What is your mood like today?
    - Do you feel the same as, worse than, or better than you did when you left the hospital? (If worse than, when did your mood start to decline?)
    - Have you had any trouble with your thinking?

- How stressed do you feel? Have you had any stressful life events since leaving the hospital?
- Do you have any current illnesses?
- How would you describe your experience participating in this research study?
- Do you have any feedback you'd like to share about your experience in this study or with the study drug?

7.8. Premature Discontinuation

If a subject withdraws or the investigator discontinues subject participation, the subject will be offered the optional exit interview session with PI to discuss her experience in the study protocol and assessment conducted while enrolled. Safety assessments will be performed on subjects who withdraw prematurely following initiation of brexanolone and include the following: clinical lab assessments, vital signs, 12-lead ECG, C-SSRS, and evaluation of adverse events.

In the unlikely event that there is imminent risk of suicidal behavior at the time of the final safety check, the subject will be withdrawn from the research study, discharged from the Women's Hospital, and referred to the UNC Emergency Department and/or other appropriate inpatient hospitalization. The study doctor will provide referrals for continued care, which may include hospitalization, but will not be responsible for providing such care following withdrawal from the research study.

7.9. Collection and Management of Tissue Specimens

Sample Preparation:

Not applicable.

Record Keeping and Monitoring:

Record keeping and monitoring will be managed by Study Coordinator using secure, encrypted, and HIPAA-compliant data management system (REDCap) provided by UNC. Data will be recorded in real time and verified by Study Coordinator for accuracy and completion. Study records and data collection will be reviewed in regularly scheduled meetings by Study Coordinator and PI.

Sample Storage and Security:

Experimental samples are not applicable.

8. Study Measurements and Evaluations

See Tables 2-4 for descriptions and schedule of study measurements and evaluations.

8.1. Outcome Measures for Evaluation of Feasibility / Tolerability

1. Number of successfully analyzed EEG recordings.
2. Number of subjects completing the entire study protocol through the follow-up phase.
3. Number of subjects withdrawn from the protocol due to participation burden.

8.2. Outcome Measures for Evaluation of Efficacy

Not applicable

8.3. Outcome Measures for Evaluation of Safety

1. Number of subjects withdrawn from the protocol due to adverse events.

8.4. Other Outcomes in the Causal Pathway

Not applicable

8.5. Baseline Characteristics of the Participants

1. Age
2. Race
3. Ethnicity
4. History of depression (SCID-5)
5. Comorbid anxiety disorder(s) (SCID-5)
6. Concurrent psychotropic medication use
7. Pregnancy history (# pregnancies, # births, methods of delivery, birth complications, past PPD episodes)

8.6. Variables Representing Treatment

Not applicable to feasibility study.

9. Statistical Analysis Plans

9.1. Strategies that Apply to all the Specific Aims

To help ensure replicability of the research, the analysis plans will be reviewed and finalized prior to collection of data (a priori). As this is a feasibility study, descriptive rather than inferential statistics will

be employed. All statistical estimates of population parameters will be tabulated along with corresponding confidence intervals (CI) or standard errors (SE). Where appropriate (i.e., in aggregate measures where we might observe a change from pre to post infusion, such as nodal flexibility, spectral power, and symptom rating scales) means and confidence intervals will be calculated for the differences in means pre and post infusion. Point estimates and CI will be used to inform effect size where appropriate. All missing values and drop-outs will be detailed in the database. Descriptive graphical and tabular methods will be used to characterize the participants, visualize the data, and examine relationships among variables.

9.2. Description of the Study Cohort

Descriptive methods to characterize the study cohort will include use of graphical figures, sample means, sample standard deviations, minimum, maximum, frequencies, and counts. Parameters examined will include age, parity, history of MDD, latency of onset, symptom rating scales (EPDS, HAM-D, anhedonia, rumination, complacency state scale, side effects), race, ethnicity, concurrent meds, concurrent anxiety disorder. For ordinal or ordered metric variables (symptom ratings), we will estimate means and confidence intervals. Mood measures will be used to see if the trajectory of treatment response is monotonic or whether it shows different trajectories by different symptom domains across individuals.

9.3. Aim-Specific Plans

Plans for Aim 1:

To evaluate the feasibility of obtaining serial EEG recordings in women with PPD who receive inpatient BRX infusion treatment. Measures obtained will include (1) the number of successfully completed EEG recordings, with particular interest in the quality of EEG data from hours 4 to 8 (to assess the viability of electrode net for extended time period); (2) the number of subjects who complete the entire study protocol through the 30-day follow-up period; and (3) the number of subjects withdrawn from the protocol due to adverse events and/or participant burden. (Quality of EEG data is determined by visual inspection to identify missing or corrupted signal. An automated process will be used to detect poor electrodes and flag them for interpolation. An infomax independent component analysis (ICA) will be performed to separate plausible neural activity from eye blinks, eye movement, muscle activity, heartbeats, and channel noise.)

Plans for Exploratory Aims:

Exploratory Aim 1: To calculate nodal flexibility at baseline, during, and following BRX infusion. As per the published work of Co-I Peter Mucha, nodal flexibility is a measure of how often major neural hubs change the networks (communities) in which they participate. We will display, in tabular and graphic form, the nodal flexibility during the affective probe and resting state across time points in each subject, in the group as a whole, and in the groups of those who do and do not respond to BRX. We will calculate and display means and confidence intervals (in tables) and means and standard errors (in graphs). Point

estimates and confidence intervals will be used to inform subsequent calculations of effect sizes (pre vs post differences in nodal flexibility). Despite the small sample size, we are confident that we will be able generate meaningful population estimates, as prior work by Dr. Mucha and colleagues identified very robust effect sizes in small samples of subjects at risk for schizophrenia vs controls. This study included 6 different conditions (before/after stress by N=0,1,2) with 64 electrodes. Data were time-averaged flexibilities calculated from multilayer modularity community detection on the temporal network of electrodes defined by theta band correlations over 3 second intervals. Estimating effect size with Cohen's d with the combined s = sqrt(s1^2+s2^2) of whole-brain flexibility (which averages overall electrodes equally) yielded d for each of the 6 tests individually ranging from 0.8 to 1.2. Further, with concatenated data, a support vector machine (SVM) supervised classification method successfully separated the two subject groups with accuracy 91.2% +/- 9.8%.

Exploratory Aim 2: To characterize rapid brain state configurations (global brain states) and their probability density function at baseline, during, and following the BRX infusion. Brain states will be determined with two assessment methods. 1) Microstate analysis generates four canonical patterns of correlative voltage distribution. Parameters generated include the duration of each microstate (which we will examine) and the sequence of its occurrence (which we will not examine in this pilot). We will calculate, and display in a table, means and confidence intervals for the duration of each microstate during the affective probe and resting state across time points in the group as a whole and in the groups of those who do and do not respond to BRX. 2) AMICA is an unsupervised means of decomposing EEG signals to generate recurring, quasi-stable patterns of correlated voltage distribution (global brain state). The EEG signal is divided into time windows (one second), each of which serves as a correlation matrix. The matrices are decomposed with independent component analysis to yield brain states that account for much of the variance in the EEG signal, and the probability of appearance of the models generated is then displayed as a probability density function (PDF). We will first create this template, and then graphically display the template PDF for each EEG session.

Exploratory Aim 3: To assess the ability of the iMotions Affectiva software to identify differences in affective state (determined by symptom ratings). Our previous data demonstrate delayed self-recognition of change in affective state, which suggests that affect state changes occur and manifest (to others) prior to a subject's labeling (and recognizing) the state change as having occurred. Affectiva is a software that permits detection of emotion in facial expression by examining and integrating key alterations in facial musculature. The software calculates emotion scores, or the percent time spent in a certain affective state, as the weighted average of action units (tiny musculature changes). We will display in tabular form the emotion scores during the affective probe in each session across all five time points and will plot the percentage of time spent in each affective state against the HAM-D ratings and responses to the semi-structured interview.

Exploratory Aim 4: To assess the amplitude of left frontal midline theta oscillations by performing spectral analyses of data collected from five serial EEG recordings. Fast Fourier transform will be applied to each two-second EEG epoch, and the average spectral power will be calculated within the canonical theta frequency band from 4 to 7 Hz. To estimate frontal midline theta (FMT), theta power in electrode FCz will be log-transformed and then normalized for each participant using the z-transformation across the 90 scalp data channels. Point estimates and CI will be presented in tabular form.

9.4. Planned Interim Analyses (If Applicable)

No interim analyses are planned, but electrode impedance will be assessed at each EEG session to decrease the likelihood that degraded signal will be obtained.

9.5. Plans for Coping with Withdrawals and Loss-To-Follow-Up

Given that these are hospitalized inpatients, we anticipate that no more that 20% (two subjects) will fail to complete the study through the inpatient phase (5 EEG sessions). As such, we will recruit 12 subjects in order to obtain 10 completers. In the event that subject discontinuation exceeds our expected limits, we will calculate and display point estimates for as many sessions as the subject completes. Similarly, in the event of unusable EEG data, we will calculate and display point estimates for those sessions with reliable EEG readings.

9.6. Sample Size Rationale

The choice of sample size was based on expert opinion, availability of subjects, and funding limit; we predict that 10 participants may be sufficient for two purposes: 1) to determine the feasibility of obtaining multiple, high quality EEGs during a BRX infusion; and 2) to provide adequate precision for the estimators of variance components and other parameters for which information is needed to plan a future study. With a conservative expected drop-out rate of 20%, we will enroll 12 subjects to attain 10 completers.

10. Safety Monitoring and Management

10.1. Risk / Benefit Assessment

Potential Risks:
(Per informed consent form)

Serious Risks of BRX:
BRX can cause serious side effects, including:

- Excessive sedation and sudden loss of consciousness: BRX may cause subjects to feel very sleepy (excessive sedation) or pass out (loss of consciousness). This is different from normal sleep where one drifts into sleep naturally and can wake up easily. Sudden loss of consciousness (passing out) can occur in some people.
- Because this could cause serious harm, a medical professional will monitor the subject for symptoms of excessive sleepiness every 2 hours while they are awake.
- During the infusion, the subject will be instructed to tell the healthcare provider right away if they feel like they cannot stay awake during the time they are normally awake or if they feel like they are going to pass out. The healthcare provider, under direction of the study doctor, may lower the dose or stop the infusion until the symptoms go away.

•Subjects must have a caregiver or family member with them to help care for the subject's child(ren) during the infusion and to be in the room with the subject if the child(ren) are present during the infusion.

The subject will be directed to tell the healthcare provider if they have any of the following symptoms while getting BRX:

- Feeling more sleepy than usual (they cannot stay awake when they are trying to stay awake)
- Having a hard time paying attention
- Having trouble following simple instructions
- Feeling lightheaded or dizzy or like they are going to pass out

If the subjects start to feel the symptoms listed above, they will be instructed to:

- Tell the healthcare provider right away
- If they are holding a baby, to put their baby down
- Sit or lie down

The healthcare provider and study doctor may decide to stop the infusion. During clinical trials, once BRX was stopped, patients who passed out woke up after a short time. The subject and the study doctor will decide whether to continue BRX.

BRX can cause other serious side effects, including:

- Increased risk of suicidal thoughts or actions. BRX and other antidepressant medicines may increase suicidal thoughts and actions in some people 24 years of age and younger. Depression or other serious mental health illnesses are the most important causes of suicidal thoughts or actions.

Subjects will be advised to monitor suicidal thoughts and behaviors while outpatient in the following manner:

- Pay close attention to any changes, especially sudden changes in mood, behavior, thoughts, or feelings, or if they develop suicidal thoughts or actions.
- Tell the healthcare provider or study doctor right away if they have any new or sudden changes in mood, behavior, thoughts, or feelings during the study.
- Keep all follow-up visits with the subject's regular doctor.
- Contact the study or regular doctor if they have concerns about these symptoms after the infusion has been completed.

Subjects will be instructed to tell the study or regular doctor right away if they have any of the following symptoms, especially if they are new, worse, or worrying the subject:

- attempts to commit suicide and/or harm themself
- new or worse depression
- other unusual changes in behavior or mood

If the subject has thoughts of harming themself or others, they will be instructed to go to the nearest emergency department (ED) or call 911. They can also call the National Suicide Prevention Lifeline 24 hours a day at 1-800-273-8255.

If there is imminent risk of suicidal behavior at the time of the final safety check (Hour 72 prior to discharge), the subject will be withdrawn from the research study, discharged from the Women's Hospital, and referred to the UNC Emergency Department and/or other appropriate inpatient hospitalization. The study doctor will provide referrals for continued care, which may include hospitalization, but will not be responsible for providing such care following withdrawal from the research study.

Risks Associated with BRX: The following adverse events in the PPD clinical program are considered the most common (more than 1 in 20 people and at least twice the rate of placebo) side effects of brexanolone:
• Sedation (drowsiness) /somnolence (sleepiness)
• Dry mouth
• Passing out (loss of consciousness)
• Flushing of the skin or face (flushing/hot flush)

Other side effects that have occurred in ≥ 2% and > placebo include:
• Faster than normal heart rate (tachycardia)
• Diarrhea
• Upset stomach (dyspepsia)
• Dizziness, feeling faint (presyncope), feeling of losing one's balance (vertigo)
• Mouth and throat pain (oropharyngeal pain)

Two cases of accidental overdosage due to malfunction of the infusion pump were reported (Meltzer-Brody et al, Lancet, 392:1058-1070, 2018). The result was a transient loss of consciousness, with normal consciousness restored within approximately 15 minutes without supportive measures with discontinuation of the infusion. After full resolution of symptoms, both patients subsequently resumed and completed treatment.

Risks Associated with EEG: No medical risks are associated with the EEG. There may be slight discomfort while the electrodes are attached to the scalp, and subjects may not like the smell of the paste or the glue remover. The conductive gel sometimes causes mild scalp irritation. The risk level for adverse impact is minimal.

Risks Associated with Phlebotomy: There may be some discomfort, pain and bruising at the site of needle entry. There is a very small risk of fainting. Infection in the area of the needle insertion is rare. The risk level for adverse impact is low.

Psychological Risks: 1) The structured interview to assess current and lifetime psychiatric illness may be associated with some psychological distress. Some items in the questionnaires may

provoke some negative emotion in some individuals. 2) Additionally, the frequency of administration of mood ratings may prove irritating for some subjects. 3) Subjects may worry about the confidentiality of data collected, including the video samples for processing of emotion in facial expression. Overall, we believe the risk level for adverse impact to be minimal.

Risks to infant children of the subjects as a result of hospitalization and treatment of the mother are mitigated by the requirement of alternative childcare arrangements for the duration of the mother's hospitalization, as outlined in the eligibility criteria of this study. During clinical trials, women were not permitted to breastfeed while receiving brexanolone and for four days following the end of the infusion. Data show that brexanolone is transferred to breastmilk, though the relative infant dose is low (1.3%) and many insurance companies state that breastmilk cannot be used for feedings, in line with the clinical trial criteria. For this reason, we have included the temporary cessation of breastfeeding in the eligibility criteria for this study.

Potential Benefits:

This study is not designed for the benefit of the individuals participating. The primary purpose of this study is to answer research questions. Benefits may accrue, however, as follows: 1) subjects will gain access to what is currently an expensive, effective, approved treatment for PPD; 2) subjects may also benefit by knowing that they are contributing to research aimed at enhancing our understanding of PPD.

10.2. Assessment of Safety

Adequacy of Protection against Risks

Recruitment and Informed Consent:

Subjects will be recruited from inpatients on the UNC PPIU or from the UNC Women's Mood Disorder Clinic. Subjects enrolled in this study will be individuals for whom BRX treatment is the appropriate and recommended course of treatment. Additionally, subjects will be sought from ads seeking volunteers for research on the mechanisms of action of BRX in PPD. For recruitment of women from rural North Carolina, recruitment will be made via contacts with regional gynecologists, county health departments, and through contacts made via the HRSA-sponsored NC Maternal Health MATTERS hotline. Through the NC TraCS Data Access and Informatics Core, we can identify a cohort of UNC Hospital patients for recruitment. UNC uses the EPIC electronic medical record system, which enables communication to potential research participants via email and through the MyChart patient interface. This makes communication with many potential research participants cost effective and highly efficient, because it will enable potential participants to access our online screening tool simply by clicking a link in their email or MyChart electronic message. Additionally, we will capitalize on the TraCS Research Recruitment Service's expertise in enrolling members of communities historically under-represented in research

Trained members of the research team will obtain informed consent from each participant as follows: 1) the subject will have the protocol described; 2) the research assistant will be thoroughly trained in the procedures administered and will, therefore, be able to answer questions that arise. However, if there are unanswered questions, the PI will be available to provide further responses to participants' questions; 3) the subject will sign the consent form, witnessed by the research assistant. A copy of the consent form will be provided to the subject. Subjects will also be consented to access their medical records for information related to relevant medical history. Each will sign a HIPAA authorization form.

Risks Associated with BRX:

The serious risks of BRX include excessive sedation and sudden loss of consciousness. Other risks are described in Section 10.1. These risks will be addressed by the progressive, stepped, infusion rate (with lower doses early), with the dose held rather than increased if the subject experiences dizziness or profound sedation. Patients will be monitored for the duration of the infusion and will also have consistent pulse oximetry to monitor and ensure adequate oxygenation. Additionally, possible side effects of BRX will be monitored with a Likert scale (1=Absent, 2=Mild, 3=Moderate, 4=Severe) that we have used in prior studies of BRX. If the participant reports side-effects of Moderate to Severe intensity, the study coordinator or research assistant will contact the study nurse to determine if adjustment or cessation of the infusion is required.

The UNC Hospitals Arousal Scale (see Table 5). will be administered every 2 hours during planned, non-sleep periods. If a score is equal to 3 on the Arousal Scale, the dose will be reduced by 50% and the inpatient prescriber or psychiatric on-call MD will be notified (216-3834). If the score is less than or equal to 2 on the Arousal Scale, the infusion will be discontinued, and the MD will be contacted.

Table 5: UNC Hospitals Arousal Scale

5	Fully awake
4	Arouses easily
3	Arouses with tactile stimuli
2	Arouses to vigorous stimuli
1	Responsive to painful stimuli
0	Unresponsive

Time	**Score**	**Time**	**Score**
Hour 0:		Hour 32:	
Hour 2:		Hour 34:	
Hour 4:		Hour 36:	
Hour 6:		Hour 38:	
Hour 8:		Hour 40:	
Hour 10:		Hour 42:	
Hour 12:		Hour 44:	
Hour 14:		Hour 46:	
Hour 16:		Hour 48:	

Hour 18:		Hour 50:	
Hour 20:		Hour 52:	
Hour 22:		Hour 54:	
Hour 24:		Hour 56:	
Hour 26:		Hour 58:	
Hour 28:		Hour 60:	
Hour 30:			

Risks Associated with EEG: The possibility of rash will be addressed by monitoring the scalp for signs of irritation.

Risks Associated with phlebotomy: Subjects will be seated during the procedure to decrease the risk of fainting, and sterile technique will be employed to decrease the risk, albeit rare, of infection in the area of needle insertion.

Psychological Risks: 1) Structured interview - The risk of discomfort during assessment will be minimized via the use of clinically trained interviewers and highly competent and professional staff. 2) Mood ratings - The risk of irritation with frequency of administration of mood ratings will be addressed by carefully informing potential subjects during the informed consent process about this possibility. In our experience, frequent mood ratings have not resulted in any subject wishing to discontinue the protocol, in part because most subjects in our studies are very dedicated to collecting this information. Subjects will also be informed of their right to withdraw from the study at any time. 3) Multiple measures will be undertaken to ensure the confidentiality and integrity of the data obtained, including clinical information, mood ratings and video samples. All data collected for analysis in this study will be de-identified at collection and will stay on encrypted, password-protected computers. Only the PI and the study coordinator will have access to individually identifiable patient information. Each subject will receive a code number, and the code will be stored - separately from data collected - in a locked file the Study Coordinator's locked office. Additionally, the video samples will be obtained on a dedicated, encrypted, password protected laptop, which will be returned after use and stored in a locked filing cabinet in the Study Coordinator's locked office.

10.3. Unanticipated Problems, Adverse Events, Serious Adverse Events

Unanticipated Problems:

An unanticipated problem is any incident, experience or outcome that meets all three OHRP criteria (1) unexpected (in severity, specificity, frequency, or nature), (2) related or possibly related to the research, and (3) suggests the research places subjects or others at greater risk than previously known or recognized. Only a subset of adverse events will meet criteria for unanticipated problems.

Adverse Event (AE) Definitions:

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product whether or not related to the medicinal product. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered. A treatment-emergent adverse event (TEAE) is an AE that occurs after the first administration of any study drug. The term study drug includes any Sage Therapeutics investigational product, a comparator, or a placebo administered in a clinical trial.

Serious Adverse Events (SAE) Definition:

An SAE is any untoward medical occurrence that at any dose:
• Results in death
• Is immediately life-threatening (Note: The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
• Requires inpatient hospitalization or prolongation of existing hospitalization
• Results in persistent or significant disability or incapacity
• Results in a congenital abnormality or birth defect
An SAE may also be any other medically important event that, in the opinion of the Investigator, may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above. (Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization).

Grading the Severity of Adverse Events and Events of 'Special Interest':

We will employ the grading scale adopted by the UNC IRB to grade and report AEs. Adverse events will be classified according to severity as either mild, moderate, or severe.
Mild AE: is defined as having no effect on activities of daily living, such as transient lightheadedness or sweating with venipuncture; headache; mild skin irritation; or something of equal significance that requires no medical intervention and is of marginal clinical relevance.
Moderate AE: would be associated with temporary (minutes to a few days) disruption in activities of daily living, such as temporary loss of consciousness with venipuncture; worsening of migraines or headache that require bed rest; an increase in depressive symptoms above baseline of more than five points on the HAM-D; dizziness that precludes ambulation; or something of equal significance.
Severe AE: would include any event that acutely threatens the patient's health, is life-threatening, or potentially permanently disabling, or an event of equal significance.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 10.3. An AE of severe intensity may not be considered serious.

The following events are considered AESIs (Adverse Events of Special Interest) and should be reported on the AESI form within 72 hours.
• Excessive sedation
• Loss of consciousness
• Any sedation-related AE that leads to dose reduction, interruption, or termination
If the AESI also qualifies as an SAE an SAE form should be submitted per the guidelines above.

Relatedness Definition:

The Investigator must make the determination of relationship to the study drug for each AE (not related or related). The Investigator should decide whether, in their medical judgment, there is a reasonable possibility that the event may have been caused by the drug intervention. If no valid reason exists for suggesting a relationship, then the AE should be classified as "not related." If there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered at least "possibly related."

Not Related: No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or subject's clinical state.
Possibly Related: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the subject's clinical state or other modes of therapy administered to the subject, but this is not known for sure.
Probably Related: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.

If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable", the event will be considered related to the investigational product for the purposes of expedited regulatory reporting. AEs will be reported per Sage Therapeutics guidelines as "related" or "not related".

Expectedness Definition:

The PI will be responsible for determining whether an adverse event is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency or the event is not consistent with the risk information previously described for the study intervention.

AE and SAE Assessment, Follow-up Procedures:

The study coordinator or research nurse will monitor side effects and AEs every two hours when the patient is awake and receiving the infusion. Adverse events forms will also be completed after each EEG session and will included the assessment of EEG-related AEs, including discomfort and irritation from the EEG net and electrode gel. Side effects consistent with moderate or severe AEs or that are troublesome to the subject will also be immediately reported to the inpatient unit nurses and to Dr. Patterson, who will be responsible for determining the next course of action, which might include slowing or discontinuing the infusion or discontinuation of the subject from the study. Dr. Patterson will review all protocol data monthly, including enrollment and retention statistics and aggregate reports of side effects/AEs. As the contact PI, Dr. Girdler will be the one responsible to report all AEs reported as severe to the IRB within one week. The study sponsor will be notified of any study modifications or suspension imposed by the local IRB in response to an AE. Finally, it should be noted that the subjects will be hospitalized on an inpatient unit during the entire administration of BRX and will not be discharged until clinically cleared by the physician.

Reporting and Documentation Procedures:

All SAEs (regardless of causality) will be recorded from the signing of the consent form(s) until the final visit of the study for that subject. Serious adverse events occurring after the subject's final study visit should be reported to the Sponsor or Sponsor's designee only if the investigator considers the SAE to be related to study drug.

All SAEs must be reported to Sage Therapeutics, or designee, immediately. A written account of the SAE must be sent to Sage Therapeutics within 24 hours of the first awareness of the event by the Investigator and/or his/her staff to the IQVIA email address: **sage.safety@IQVIA.com**. The Investigator must complete, sign and date the SAE report form, verify the accuracy of the information recorded on the SAE report form with the corresponding source documents, and send a copy to Sage Therapeutics, or designee.

Additional follow-up information, if required or available, must be sent to Sage Therapeutics, or designee. within 24 hours of receipt; a follow-up SAE form should be completed and placed with the original SAE information and kept with the appropriate section of the study file.

Sage Therapeutics, or designee, is responsible for notifying the relevant regulatory authorities of certain events. It is the Principal Investigator's responsibility to notify the IRB of all SAEs that occur at his or her site. Investigators will also be notified of all suspected, unexpected, serious, adverse reactions (SUSARs) that occur during the clinical study or from other studies with brexanolone. Each site is responsible for notifying its ethics committee of these SUSARs.

Participant Notification of New Information:

Corrective actions to be considered in response to New Safety Information include:

- Modification of inclusion or exclusion criteria to mitigate the newly identified risks.
- Implementation of additional safety monitoring procedures

- Suspension of enrollment of new participants or halting of study procedures for enrolled participants.
- Modification of informed consent documents to include a description of newly recognized risks.
- Provision of additional information about newly recognized risks to previously enrolled participants.

10.4. Safety Monitoring

The proposed research is not a phase III clinical trial requiring a Data and Safety Monitoring Board (DSMB), and because it represents an unblinded, small scale, single site, clinical trial involving relatively low risk interventions in a non-vulnerable population, the NIH policy on Data and Safety Monitoring stipulates that, in most such studies, the PI would be expected to perform some monitoring functions as part of the general oversight and scientific leadership of the study. This is the strategy that we intend to employ in the proposed research according to the data and safety monitoring plans outlined above. IQVIA will receive SAE reports but will not be providing any clinical monitoring.

10.5. Study Suspension / Early Termination of the Study

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

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

11. Supporting Documentation and Operational Considerations

11.1. Regulatory, Ethical, and Study Oversight Considerations

11.1.1. Informed Consent Process

11.1.1.1. Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

11.1.1.2. Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.2. Study Discontinuation and Closure

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

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and IRB.

11.3. Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigator, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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

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

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will stored in the study database. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the research site.

Certificate of Confidentiality (if applicable)

Not appliable.

11.4. Future Use of Stored Specimens and Data

Not applicable.

11.5. Key Roles and Study Governance

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11.6. Safety Oversight

The proposed research is not a phase III clinical trial requiring a Data and Safety Monitoring Board (DSMB), and because it represents an unblinded, small scale, single site, clinical trial involving relatively low risk interventions in a non-vulnerable population, the NIH policy on Data and Safety Monitoring stipulates that, in most such studies, the PI would be expected to perform some monitoring functions as part of the general oversight and scientific leadership of the study. This is the strategy that we intend to employ in the proposed research according to the data and safety monitoring plans outlined above. IQVIA will receive SAE reports but will not be providing any clinical monitoring.

11.7. Clinical Monitoring

Not Applicable.

11.8. Quality Assurance and Quality Control

The study team perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system, and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the research team for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), Good Laboratory Practices (GLP).

The investigational site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

11.9. Data Handling and Record Keeping

11.9.1. Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be converted to electronic records provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a data capture system provided by the North Carolina Translational and Clinical Sciences Institute. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. The study coordinator will be responsible for programming and computations for data management in the REDCap database, with support from consultation services through NC TraCS.

11.9.2. Study Records Retention

Study documents should be retained for a minimum of 3 years have elapsed since the formal discontinuation of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

11.10. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

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

It is the responsibility of the site investigator to use continuous vigilance to identify and record deviations in study source documents. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

11.11. Publication and Data Sharing Policy

Not applicable.

11.12. Conflict of Interest Policy

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

12. Additional Considerations

Not applicable

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14. List of Appendices

Not applicable.