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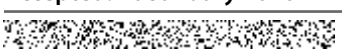

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Irina Balan, Cecilia Isabel Sousa Pearson, Holly Krohn, Todd K. O'Buckley, Kai Xia, Samantha Meltzer-Brody, A. Leslie Morrow & Riah Patterson

Brexanolone Infusion Produces Sustained Anti-inflammatory and Neurotrophic Effects in Patients with Postpartum Depression that Predict Symptom Improvement

Authors: Irina Balan[1], Cecilia Isabel Sousa Pearson[2], Holly Krohn[2], Todd K. O'Buckley[1], Kai Xia[2], Samantha Meltzer-Brody[2], A. Leslie Morrow[1,2,3*], Riah Patterson[2*]

[1] Bowles Center for Alcohol Studies, School of Medicine, The University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC, USA
[2] Department of Psychiatry, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[3]Department of Pharmacology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

*** Correspondence: riah_patterson@med.unc.edu and morrow@med.unc.edu**

Running title: Sustained Effects of Brexanolone in Postpartum Depression

Abstract.

Postpartum depression (PPD) is linked to neuroimmune dysregulation. Brexanolone, an intravenous formulation of the neurosteroid allopregnanolone and the first FDA-approved treatment for PPD, produces rapid and sustained antidepressant effects. However, its long-term mechanisms of action remain unclear. This study evaluated brexanolone's prolonged impact on two groups of biomarkers in whole blood: inflammatory mediators and growth/differentiation/neurotrophic factors. Whole blood was also maintained in culture (4 hours) and subjected to lipopolysaccharide (LPS) stimulation of the TLR4 inflammatory pathway. Ten individuals with moderate-to-severe PPD received brexanolone and were assessed before, and at 6 hours, ~7, and ~30 days post-infusion. BDNF significantly increased and remained elevated through 30 days, representing a sustained neurotrophic response. In contrast, inflammatory mediators CCL11, IL-6, TNF-α, and IL-18 showed rapid reductions by 6 hours. TNF-α suppression lasted up to 7 days, while CCL11 and IL-6 remained suppressed through 30 days. These changes were associated with reductions in Hamilton Depression Rating Scale (HAM-D) scores over time. LPS-stimulated whole blood cultures revealed suppression of TLR4-induced CCL11, IL-1β, IL-6, IL-8, IL-18, TNF-α, HMGB1, and MIP-1β at 6 hours. IL-8, IL-18, and TNF-α remained suppressed through 7 days, while IL-1β and CCL11 remained suppressed through 30 days, aligning with sustained HAM-D score improvements. Biomarker × time interactions suggested dynamic regulation of inflammatory and neurotrophic pathways. Given the small sample size, these findings should be interpreted as a pilot study, but they indicate that brexanolone promotes both rapid and sustained anti-inflammatory and neurotrophic effects supporting lasting symptom remission in PPD.

Introduction.

Postpartum depression (PPD) is a major depressive episode occurring during late pregnancy or within the first few weeks postpartum, affecting ~15% of new mothers. Although prevalent, its underlying mechanisms remain unclear, and treatment options have been limited. In 2019, brexanolone, an intravenous formulation of allopregnanolone, became the first Food and Drug Administration (FDA) approved treatment for PPD, offering rapid and sustained antidepressant effects [1-5]. Allopregnanolone exerts its effects via positive allosteric modulation of γ-aminobutyric acid (GABA) type A receptors, regulation of the hypothalamic-pituitary-adrenal (HPA) axis, and bidirectional immune modulation, suppressing pro-inflammatory mediators while enhancing anti-inflammatory responses [6-19].

Neuroinflammation is increasingly recognized as a central mechanism in PPD. Elevated levels of proinflammatory cytokines, including interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), and IL-1β have been observed in PPD patients [20-23]. Our previous work showed that brexanolone rapidly suppresses IL-6 and TNF-α and dampens immune cell responses to toll-like receptor (TLR) agonists such as lipopolysaccharide (LPS; TLR4) and imiquimod (TLR7), with changes observed as early as 6 hours post-infusion (Post-6h). Furthermore, these effects predicted reductions in Hamilton Depression Rating Scale (HAM-D) scores [20]. However, the durability of these anti-inflammatory effects beyond the immediate post-infusion phase remains unclear.

Although allopregnanolone is rapidly metabolized, typically within hours following infusion, brexanolone produces antidepressant effects lasting weeks to months [5]. Preclinical data suggest that allopregnanolone inhibits IL-6, TNF-α, IL-1β, monocyte chemoattractant

protein-1 (MCP-1), and high-mobility group box 1 (HMGB1); suppresses glial activation; and increases anti-inflammatory mediators such as IL-10, fractalkine and transforming growth factor-beta [8-11, 15, 16, 18]. It also enhances neuroplasticity by increasing brain-derived neurotrophic factor (BDNF), a key regulator of synaptic function and mood [16, 24, 25]. These combined effects may contribute to allopregnanolone's sustained therapeutic benefits, although the long-term impact of brexanolone on immune and neurotrophic pathways in PPD remains poorly understood.

BDNF is essential for neuronal survival, plasticity, and mood regulation, and its levels are often reduced in depression [26-28]. Reduced BDNF is associated with impaired neurogenesis and lower stress resilience [29], both of which are highly relevant to PPD. Inflammatory mediators, especially IL-6, TNF-α, IL-1β, and IL-18, are elevated in PPD and major depression and correlate with symptom severity [20-22, 30-35]. Other molecules, such as C-C motif chemokine ligand 11 (CCL11), MCP-1, IL-8, and macrophage inflammatory protein-1β (MIP-1β), are linked to cognitive decline, immune cell trafficking, and chronic inflammation [36-38]. In contrast, cytokines like IL-3, IL-5, IL-7, IL-17A, and neurotrophic factors such as BDNF, regulate immune cell function, differentiation, and neuroimmune signaling linking immune activity with plasticity [39-45]. By examining inflammatory cytokines and chemokines as well as growth/differentiation/neurotrophic factors, it becomes possible to capture the dual mechanisms through which brexanolone may exert its therapeutic effects, inhibiting excessive inflammation while supporting neuroplastic and trophic pathways [6, 9, 10, 12, 15-17, 20].

This pilot study expands upon previous findings [20] by examining 14 immune biomarkers, including both inflammatory mediators and growth/differentiation/neurotrophic factors, at four

time points to assess the sustained neuroimmune effects of brexanolone in PPD. Whole blood samples were collected at baseline (Pre), Post-6h, and approximately 7 days (Post-7d), and 30 days (Post-30d) after brexanolone infusion to capture acute, subacute, and long-term changes in biomarker levels and their relationship to depression symptom severity. Additionally, we assessed peripheral immune cell responsiveness to LPS stimulation at each time point, as activation of TLR4 by LPS provides a well-established ex vivo challenge that unmasks latent inflammatory reactivity and reveals functional changes in immune responsiveness not observable under resting conditions [20, 31].

Brexanolone was withdrawn from the market in December 2024, forcing us to terminate the study with a sample size of 10. After its initial clinical use, brexanolone infusion was discontinued and replaced by zuranolone, an oral neurosteroid with a distinct structure and pharmacokinetic profile [5]. Structural differences among neurosteroids may influence their anti-inflammatory and neurotrophic capacity, and thus their clinical effects [8, 46]. Despite the limited sample size, the integration of Friedman and Generalized Estimating Equations (GEE) models allowed us to detect neuroimmune changes that persisted over time and to link these effects with symptom improvement. Together, these approaches provided complementary insights into the sustained immunoregulatory and clinical effects of brexanolone in PPD.

Subjects and Methods

Participant Recruitment and Characteristics

Ethics approval and consent to participate: This study was approved by the Institutional Review Board (IRB) at the University of North Carolina (UNC) at Chapel Hill, North Carolina, USA, IRB# 23-1288. Written informed consent was obtained from all participants. All clinical

procedures and experimental methods were performed in accordance with the relevant guidelines and regulations.

Eligible individuals were enrolled through UNC's Clinical Brexanolone Treatment Program, which provides access to brexanolone for patients with moderate to severe PPD, defined as a HAM-D score ≥18 [4].

A total of 10 participants were included in the analysis (Table 1). Enrollment was limited due to the market withdrawal of brexanolone in December 2024. All participants were diagnosed with PPD, with symptom onset occurring during the third trimester or within four weeks postpartum. At the time of infusion, participants had a mean age of 33 years (range: 23–47). The majority were married (80%) and identified as non-Hispanic white (70%); the remaining participants identified as Hispanic white (10%), non-Hispanic Black (10%), and non-Hispanic Pacific Islander (10%).

Eighty percent of participants had a documented history of psychiatric comorbidity and were receiving concurrent psychiatric treatment at the time of infusion. Most participants (70%) were taking two psychotropic medications, and one participant (10%) was taking six. Medications spanned multiple psychotropic classes, including serotonin antagonist and reuptake inhibitors (SARIs), selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), norepinephrine-dopamine reuptake inhibitors (NDRIs), typical and atypical antipsychotics, anxiolytics, mood stabilizers, benzodiazepines, beta blockers, antihistamines, stimulants, and anticonvulsants (Table 1).

Brexanolone Infusion Protocol

Brexanolone (ZULRESSO®), an intravenous formulation of the neurosteroid allopregnanolone, was administered using the FDA-approved 60-hour infusion schedule: 1) 0–4 hours: 30 μg/kg/h; 2) 4–24 hours: 60 μg/kg/h; 3) 24–52 hours: 90 μg/kg/h; 4) 52–56 hours: 60 μg/kg/h; 5) 56–60 hours: 30 μg/kg/h.

Participants remained in a medically supervised inpatient unit for 72 hours, which included the 60-hour drug infusion and 12 additional hours for monitoring and post-treatment assessments. Blood samples were collected at four time points: approximately 1 hour before infusion initiation (Pre; 9–10 a.m.), Post-6h (10–11 a.m.), ~Post-7d (10–11 a.m.), and ~Post-30d (10–11 a.m.). Meals were provided during the inpatient stay; however, participants did not eat prior to blood collection on the day of admission, discharge, or follow-up visits.

Depression Symptom Assessment

Depression severity was assessed using the 17-item HAM-D, which evaluates domains such as mood, guilt, suicidal ideation, insomnia, anxiety, and somatic symptoms [47]. The HAM-D was administered at baseline (~2 hours prior to infusion), at 6 hours post-infusion initiation, and again at ~7 and ~30 days post-infusion.

Brexanolone treatment was associated with a substantial and sustained reduction in depressive symptoms. Mean HAM-D scores declined from 22 (SD = 7) at baseline to 5 (SD = 4) at Post-6h, 4 (SD = 4) at Post-7d, and 3 (SD = 4) at Post-30d.

Preparation of Cell Lysate and Supernatant Fractions from Whole Blood and In Vitro LPS Stimulation of Whole Blood Cultures

Preparation of cell lysate and supernatant fractions from whole blood and in vitro LPS stimulation of whole blood cultures were as previously described [48, 49]. Full details are available in the Supplementary Materials.

Luminex Multiplex Immunoassays

Quantification of 14 immune biomarkers (IL-1β, IL-3, IL-5, IL-6, IL-7, IL-8, IL-17A, IL-18, TNF-α, MCP-1, MIP-1β, CCL11, BDNF, and HMGB1) was carried out using the ProcartaPlex™ Multiplex Immunoassay Kit (Catalog #PPX-14-MXCE7DR, Thermo Fisher Scientific, Vienna, Austria) [48]. See Supplementary Materials for further details on this method.

Statistical Analysis

All statistical analyses were conducted in R (version 4.4.3) within the RStudio environment (version 2024.12.1). We applied the Friedman test to evaluate overall differences in biomarker levels in whole blood (combined cell lysates and supernatants) across four time points (Pre, Post-6h, Post-7d, and Post-30d) after brexanolone infusion. Before analysis, normality was evaluated using the Kolmogorov–Smirnov test.

Effect sizes were calculated using Kendall's W (coefficient of concordance). Effect size interpretations followed conventional thresholds: 1) $W < 0.30$ = small; 2) 0.30-0.49 = moderate; 3) 0.50-0.69 = strong; 4) ≥ 0.70 = very strong.

To examine the association between individual biomarkers and HAM-D scores over time following brexanolone infusion, we employed GEE for repeated measures model.

False discovery rate (FDR) corrections were applied across all biomarkers within each analytic framework (Friedman tests for longitudinal changes and GEE models for associations with

HAM-D scores). Both unadjusted and FDR-adjusted p-values are reported in the Supplementary Tables S1- S4. FDR adjusted p-values ≤ 0.05 were considered statistically significant.

Detailed statistical methods and reproducible R code, including packages and functions used, are provided in the Supplementary Materials.

Results.

Sustained Suppression of Inflammatory Mediators and Increase in BDNF Following Brexanolone Treatment.

A total of 14 immune biomarkers were measured in whole blood samples (cell lysates plus supernatants) collected at four time points (Pre, Post-6h, Post-7d, and Post-30d) following brexanolone infusion, using Luminex multiplex assays. Biomarkers were grouped into inflammatory mediators (CCL11, HMGB1, IL-1β, IL-6, IL-8, IL-18, MCP-1, MIP-1β, TNF-α) and growth/differentiation/neurotrophic factors (BDNF, IL-3, IL-5, IL-7, IL-17A). Friedman tests assessed overall longitudinal changes, followed by Wilcoxon signed-rank post-hoc tests. FDR adjusted $p \leq 0.05$ was considered statistically significant.

Among the inflammatory mediators, CCL11, IL-6, TNF-α, and IL-18 showed suppression following brexanolone infusion (Figure 1a; Table S1). CCL11 declined significantly ($\chi^2 = 10.6$, *adj. p* = 0.02), from 47.2±8.1 pg/mL at Pre to 35.5±5.8 pg/mL at Post-30d), with post-hoc comparisons showing reductions at Post-6h (-35.2±7.2%), Post-7d (-30.3±4.9%), and Post-30d (-36.7±5.4%) ($p \leq 0.05$), indicating a rapid and sustained suppression of CCL11 following infusion.

IL-6 also decreased significantly ($\chi^2 = 11.1$, *adj. p* = 0.02), declining from 8.4 ± 2.6 pg/mL at Pre to 1.4 ± 0.5 pg/mL at Post-30d, with post-hoc tests confirming suppression at Post-6h (-

46.5±8.2%), Post-7d (-32.5 ± 10.6%), and Post-30d (-67.1± 4.9%) ($p \leq 0.05$), indicating a rapid and sustained suppression of IL-6.

TNF-α was significantly reduced at Post-6h and Post-7d ($\chi^2 = 10.6$, *adj. p* = 0.02), declining from 4.1±1.8 pg/mL at Pre to 2.0±0.7 pg/mL at Post-7d; post-hoc tests confirmed reductions from Pre to Post-6h (-57.5 ± 9.7%) and Pre to Post-7d (-46.4±9.6%) ($p \leq 0.05$), indicating an early and sustained suppression of TNF-α through day 7. However, no significant difference was observed between Pre and Post-30d ($p > 0.05$).

IL-18 showed only transient suppression ($\chi^2 = 8.0$, *adj. p* = 0.05), decreasing from 437.2±62.5 pg/mL at Pre to 280.6±61.1 pg/mL at Post-6h (–36.5±13.5%), but returning toward Pre levels by Post-7d and Post-30d, with no significant pairwise differences at later time points (Figure 1a, Table S1). Other inflammatory mediators did not show significant longitudinal changes, including HMGB1, IL-1β, IL-8, MCP-1, and MIP-1β (Table S1).

Effect size estimates supported these findings. CCL11, IL-6, and TNF-α exhibited very strong effects (Kendall's $W \geq 0.70$), IL-18 showed strong effects (0.57), and all others had small or nonsignificant effects (Table S1).

Among the growth/differentiation/neurotrophic factors, only BDNF showed significant changes across time ($\chi^2 = 8.9$, adj. p = 0.04) (Figure 1b). Levels increased from 682.1 ± 162.8 pg/mL at Pre to 1628.8 ± 348.6 pg/mL at Post-30d. Wilcoxon post-hoc analysis confirmed significant increases from Pre to Post-6h (+152.0 ± 62.0%), Pre to Post-7d (+212.1 ± 105.4%), and Pre to Post-30d (+154.8 ± 51.7%), all $p \leq 0.05$. Kendall's W effect size indicated a strong effect (0.63), consistent with a sustained neurotrophic response. Other analytes in this group (IL-3, IL-5, IL-7, IL-17A) did not exhibit significant changes (Table S1).

Sustained association between baseline biomarker levels and HAM-D scores following brexanolone infusion.

We applied a GEE model to evaluate the relationship between baseline biomarker levels and HAM-D scores over time following brexanolone infusion. The analysis focused on main effects, reflecting the independent association of biomarker levels with HAM-D scores, and biomarker × time interactions, reflecting whether these associations changed across post-infusion time points. FDR-adjusted p-values ≤ 0.05 were considered statistically significant.

Although initial Spearman correlations suggested that CCL11, HMGB1, IL-6, and MCP-1 levels were significantly associated with age ($p < 0.05$), these associations did not remain significant after FDR correction (Table S2).

Within the inflammatory mediator group, several markers showed significant associations with HAM-D scores. Lower CCL11 levels following brexanolone infusion were associated with lower HAM-D scores (Estimate = 0.22±0.09, Z = 5.54, adj. p = 0.05) (Figure 2a), with no significant time interaction (Table S2), indicating a stable relationship over time.

The IL-6 main effect was significant (Estimate = 0.66±0.13, Z = 25.69, adj p = 3.0E-06) (Figure 2a), indicating that lower IL-6 levels following brexanolone infusion were associated with lower HAM-D scores. The biomarker × time interaction was also significant (Estimate = -0.11±0.03, Z = 14.68, adj p = 0.001), indicating that the association between IL-6 levels and HAM-D scores weakened over time.

The IL-1β main effect was significant (Estimate = 6.2, Z = 39.68, adj. p = 4.0E-09) (Figure 2a), indicating that lower IL-1β levels following brexanolone infusion were associated with lower HAM-D scores. The IL-1β × time interaction was also significant (Estimate = –0.47, Z = 47.00, adj.

p = 1.0E-10), indicating that the association between IL-1β levels and HAM-D scores weakened over time. Although the Friedman test did not reach statistical significance after FDR correction (Friedman test: χ^2 = 7.1, adj. p = 0.07; Kendall's W = 0.51) (Table S1), it showed a trend toward group-level reduction in IL-1β.

Within the growth/differentiation/neurotrophic group, BDNF showed a robust inverse association with HAM-D scores (Estimate = –0.009±0.003, Z = 10.93, adj. p = 0.004) (Figure 2b), indicating that higher BDNF levels following brexanolone infusion were associated with lower HAM-D scores. The biomarker × time interaction was not significant, indicating that this inverse relationship remained stable across all post-infusion time points.

Although IL-5 levels did not significantly change at the group-level following brexanolone infusion (Friedman test: χ^2 = 0.20, adj. p = 0.89; Kendall's W = 0.02) (Table S1), the GEE model revealed a significant main effect (Estimate = 0.37, Z = 7.86, adj. p = 0.02) (Figure 2b), indicating that lower IL-5 levels were associated with lower HAM-D scores across individuals. No significant IL-5 × time interaction was observed, suggesting a stable association between IL-5 levels and HAM-D scores over time.

Together, these results indicate that brexanolone's effects in PPD involve sustained suppression of inflammatory mediators (CCL11, IL-6, IL-1β), neurotrophic support through BDNF elevation, and modulation of growth/differentiation pathways (IL-5), with some inflammatory associations (IL-6 and IL-1β) weakening as symptom improvement stabilized.

Sustained brexanolone suppression of TLR4 inflammatory responses in whole blood in vitro.

To evaluate the effect of brexanolone on sustained blood cell responsiveness to the TLR4 agonist LPS, whole blood was stimulated in vitro at each time point (Pre, Post-6h, Post-7d, Post-30d), and 14 immune biomarkers were quantified. Responses were normalized by subtracting paired unstimulated baseline (in culture) values. Friedman tests assessed overall longitudinal changes, with post-hoc Wilcoxon signed-rank tests used for pairwise comparisons. FDR-adjusted p-values ≤ 0.05 were considered statistically significant.

Among inflammatory mediators, brexanolone significantly suppressed multiple LPS-induced responses. Significant overall reductions were observed for CCL11 (χ^2 = 12.3, adj. p = 0.03), HMGB1 (χ^2 = 10.3, adj. p = 0.03), IL-1β (χ^2 = 11.1, adj. p = 0.03), IL-6 (χ^2 = 7.1, adj. p = 0.049), IL-8 (χ^2 = 8.0, adj. p = 0.04), IL-18 (χ^2 = 8.9, adj. p = 0.04), MIP-1β (χ^2 = 7.1, adj. p = 0.049), and TNF-α (χ^2 = 8.0, adj. p = 0.04) (Figure 3), indicating that brexanolone significantly suppressed LPS-induced biomarker responses over time. Based on Kendall's W, IL-6, IL-8, IL-18, MIP-1β, and TNF-α showed strong effect sizes (W = 0.50-0.69), while CCL11, HMGB1, and IL-1β showed very strong effect sizes (W≥0.70), reflecting stable longitudinal changes across individuals (Table S3).

CCL11 and IL-1β showed the most sustained suppression. CCL11 was reduced from Pre to Post-6h (–191.1 ± 33.2%), Post-7d (–72.1 ± 21.9%), and Post-30d (–103.5 ± 8.2%) (all p < 0.05), indicating long-lasting inhibition. IL-1β declined by –62.9 ± 5.4% at Post-6h, –55.5 ± 3.4% at Post-7d, and –47.7 ± 4.2% at Post-30d (all p < 0.05), demonstrating rapid and sustained suppression through 30 days.

IL-18, IL-8, and TNF-α showed subacute suppression. IL-18 decreased by –116.7 ± 33.7% at Post-6h and –102.1 ± 57.7% at Post-7d (p < 0.05), but returned toward Pre levels by Post-30d. IL-8 was reduced by –40.4 ± 21.5% at Post-6h and –48.6 ± 14.1% at Post-7d (p < 0.05), again

normalizing by Post-30d. TNF-α decreased by –67.0 ± 11.0% at Post-7d ($p < 0.05$), with no significant suppression persisting at Post-30d.

IL-6, HMGB1, and MIP-1β exhibited only transient inhibition, with significant reductions at Post-6h (IL-6: –64.1 ± 5.2%; HMGB1: –118.4 ± 20.1%; MIP-1β: –65.5 ± 6.7%, all $p < 0.05$), but not at later time points (Figure 3).

In contrast, no significant longitudinal changes were detected among growth/differentiation/neurotrophic factors (BDNF, IL-3, IL-5, IL-7, IL-17A) (Table S3), indicating that brexanolone's effects on LPS-induced immune responses were largely confined to the inflammatory mediator group.

Sustained brexanolone-induced suppression of TLR4 inflammatory responses correlates with lower HAM-D scores in whole blood in vitro.

To examine whether sustained suppression of TLR4-driven inflammatory responses by brexanolone correlates with improvements in depressive symptoms, we applied a GEE model. FDR-adjusted p-values ≤ 0.05 were considered significant.

Initial Spearman correlation revealed a significant association between LPS-induced IL-7 and age ($p < 0.05$). This association remained significant in the GEE model and after FDR correction (Estimate = 1.31 ± 0.45, Z = 8.52, adj. p = 0.02; Table S4).

Among the inflammatory mediators, lower LPS-stimulated levels of CCL11 (Estimate = 0.58 ± 0.14, Z = 17.66, adj. p = 0.0002), HMGB1 (Estimate = 0.00004 ± 0.00002, Z = 7.81, adj. p = 0.02), IL-1β (Estimate = 0.005 ± 0.0007, Z = 37.43, adj. p = 1.0E-08), IL-6 (Estimate = 0.0004 ± 0.0001, Z = 16.58, adj. p = 0.0007), IL-8 (Estimate = 0.002 ± 0.0005, Z = 13.14, adj. p = 0.002), IL-18 (Estimate = 0.005 ± 0.001, Z = 17.08, adj. p = 0.0002), and MIP-1β (Estimate = 0.001 ± 0.0003, Z = 21.75,

adj. p = 0.00004) were significantly associated with lower HAM-D scores over time following brexanolone treatment (Figure 4).

Several of these biomarkers also showed significant biomarker × time interactions, including CCL11 (Estimate = –0.07 ± 0.02, Z = 11.12, adj. p = 0.004), HMGB1 (Estimate = –0.000004 ± 0.000001, Z = 15.95, adj. p = 0.0005), IL-8 (Estimate = –0.0002 ± 0.00002, Z = 87.68, adj. p = 1.0E-12), and MIP-1β (Estimate = –0.0001 ± 0.00003, Z = 9.44, adj. p = 0.01), indicating that these associations weakened over time (Table S4).

Among the growth/differentiation/neurotrophic factors, significant associations with lower HAM-D scores were observed for IL-3 (Estimate = 0.56 ± 0.19, Z = 9.28, adj. p = 0.02), IL-7 (Estimate = 7.61 ± 2.27, Z = 11.19, adj. p = 0.01), and IL-17A (Estimate = 4.02 ± 1.30, Z = 9.58, adj. p = 0.01) (Figure 4). IL-7 also demonstrated a significant time interaction (Estimate = –0.30 ± 0.11, Z = 7.55, adj. p = 0.03), indicating that this association diminished over time (Table S4).

Together, these findings suggest that sustained suppression of TLR4-driven inflammatory responses is closely linked to reductions in depressive symptom severity, with additional modulation of growth and differentiation pathways.

Discussion

This study provides new evidence that brexanolone treatment in individuals with PPD leads to sustained modulation of both inflammatory mediators and growth/differentiation/neurotrophic factors, measured in whole blood across basal and LPS-stimulated conditions. Brexanolone's effects were predominantly anti-inflammatory, with CCL11, IL-6, TNF-α, and IL-18 showing rapid and sustained suppression. In contrast, the growth/differentiation/neurotrophic group was represented predominantly by BDNF, which

showed a sustained increase. These biomarker changes were significantly associated with improvements in HAM-D scores over time. These findings suggest that future neurosteroid treatments, including zuranolone and other next-generation compounds, may achieve therapeutic benefit in PPD by replicating the dual profile of anti-inflammatory activity and neurotrophic support observed with brexanolone.

Complementing these findings, in vitro LPS-stimulated whole blood cultures revealed that brexanolone suppressed TLR4-driven inflammatory responses for up to 30 days after infusion. Sustained reductions in LPS-induced CCL11 and IL-1β were evident through day 30, while IL-18, IL-8, and TNF-α showed significant reductions through day 7. IL-6, HMGB1, and MIP-1β were transiently suppressed at 6 hours post-infusion. These data show that brexanolone reduces baseline immune tone and changes blood cell responsiveness to innate immune challenge, supporting the dual regulation of resting and inducible immune pathways. The reductions in LPS-induced responses were also tracked by improvements in HAM-D scores over time. These findings support the hypothesis that brexanolone's antidepressant efficacy is mediated, at least in part, by prolonged and dynamic neuroimmune regulation.

Baseline BDNF showed the most robust and sustained change, with levels significantly elevated at 6 hours, 7 days, and 30 days post-brexanolone infusion. In the GEE model, higher BDNF levels were associated with lower HAM-D scores, consistent with preclinical studies showing that allopregnanolone enhances BDNF expression and promotes neuroplasticity [24, 25, 29]. These findings also align with clinical reports linking low BDNF to major depression and with evidence that BDNF mediates antidepressant responses [26-28]. The lack of a significant BDNF × time interaction suggests that the inverse association between BDNF and HAM-D scores remained

stable during the post-infusion period, reinforcing its role as a sustained marker of treatment response.

Importantly, BDNF measured in whole blood may derive from multiple cellular sources, including platelets, lymphocytes, monocytes, and vascular endothelial cells, which can release or store BDNF. While platelets are a major peripheral reservoir, a fraction of circulating BDNF may also originate in the brain, as BDNF can cross the blood–brain barrier bidirectionally [50-53]. Thus, the observed elevation in whole-blood BDNF following brexanolone infusion may reflect both peripheral immune/platelet contributions and central neurotrophic signaling, providing a broader index of neuroimmune regulation.

Baseline IL-6 and CCL11 levels decreased significantly by 6 hours after infusion and remained suppressed at 7 and 30 days, indicating a long-term anti-inflammatory effect. IL-6 is a central cytokine elevated in both PPD and major depression, where it contributes to neuroendocrine dysregulation and symptom severity [20, 21, 32]. CCL11 is linked to neuroinflammation, reduced neurogenesis, and cognitive impairment [36, 54]. Both IL-6 and CCL11 showed consistent associations with HAM-D scores over time. Notably, we previously demonstrated a positive association between IL-6 and HAM-D scores in individuals with PPD as early as 6 hours after brexanolone infusion [20]. In the current study, IL-6 exhibited a gradually weakening association with HAM-D scores over time, suggesting that its contribution to the antidepressant response may be more prominent during the early phase following infusion. In contrast, CCL11 maintained a stable association with HAM-D scores throughout the observation period, suggesting that persistent suppression of this chemokine may reflect ongoing resolution

of neuroinflammation and could serve as a clinically relevant biomarker for monitoring sustained treatment response in PPD.

Baseline TNF-α exhibited a rapid but time-limited reduction, with significant decreases to 6 hours and 7 days after infusion, followed by a return to baseline by 30 days. This subacute suppression aligns with the known rapid induction of TNF-α following TLR4 activation and its critical role in mediating early-phase inflammatory responses [55]. In our previous work, lower TNF-α levels at 6 hours post-infusion were significantly associated with reduced HAM-D scores in individuals with PPD [20], suggesting that early suppression of TNF-α may contribute to the rapid improvement in depressive symptoms observed following brexanolone treatment.

Baseline IL-18 also showed an early reduction that rebounded by 7 days and remained elevated at 30 days, suggesting a transient effect. IL-18 is involved in inflammasome activation and chronic stress-induced inflammation [34]. The short-lived nature of its suppression may indicate limited modulation by brexanolone or the presence of compensatory immune activation [31].

Although baseline IL-1β did not reach FDR-adjusted statistical significance in group-level analysis, the effect size was strong, and individual-level analyses revealed a robust positive association with HAM-D scores. The biomarker × time interaction for IL-1β indicated that this association diminished over time, potentially reflecting a progressive normalization of immune activity in response to brexanolone treatment. Given its established role in treatment-resistant depression and persistent immune activation [56, 57], IL-1β may represent an important target of brexanolone's immunomodulatory effects.

Although baseline IL-5 and LPS-induced IL-3, IL-7, and IL-17A (Table S3) did not exhibit significant group-level changes following brexanolone infusion, they were positively associated with HAM-D scores at the individual level. This indicates that growth/differentiation factors may also contribute to symptom severity and LPS-induced response in specific subgroups, even if not uniformly regulated by brexanolone treatment. IL-5, a Th2-associated cytokine involved in eosinophil regulation and immune modulation, has been linked to an increased likelihood of major depressive disorder (MDD), suggesting its relevance to mood disorders [58]. IL-3, a hematopoietic growth factor that influences myeloid lineage differentiation and immune signaling, has also been found to be elevated in individuals with depression [59]. IL-7 has been reported to be significantly reduced in drug-free MDD patients and inversely correlated with depression severity, indicating a role in impaired immune regulation in depression [60]. IL-17A, a key effector of Th17-driven inflammation, has been implicated in neuroimmune dysregulation, and elevated peripheral levels have been reported in depressed individuals [61]. These findings highlight the importance of considering individual immune phenotypes when evaluating treatment responses in PPD and suggest that IL-5, IL-3, IL-7, and IL-17A may serve as informative biomarkers of symptom severity in select patient subgroups.

Baseline IL-7 levels (Table S2), as well as LPS-induced MCP-1 levels (Table S4), were not significantly associated with HAM-D scores. However, significant biomarker × time interactions suggest that their relationships with depressive symptoms varied over the post-brexanolone infusion period. IL-7 is a key cytokine supporting T cell survival and immune homeostasis [40], and may reflect ongoing adjustments in lymphocyte dynamics as the immune system transitions toward regulation after brexanolone treatment. Notably, LPS-induced IL-7 responses were

significantly associated with age, suggesting that older individuals may exhibit heightened IL-7 activity following TLR4 activation. MCP-1, a chemokine involved in monocyte recruitment and linked to neuroinflammation and stress-related disorders [62], also showed a significant biomarker × time interaction, indicating that its association with depression symptoms varied post-brexanolone infusion. This may reflect shifts in peripheral monocyte activation or chemotactic signaling as inflammation resolves after brexanolone treatment. Although MCP-1 did not show significant longitudinal changes, its inclusion remains relevant given its dual role as a chemokine regulating monocyte trafficking and as an inflammatory mediator implicated in neuroinflammation and stress-related disorders.

The current findings build on previous evidence that brexanolone rapidly suppresses LPS-induced inflammatory responses in vitro [20], including reductions in IL-6, IL-1β, and TNF-α as early as 6 hours post-infusion. Here, we confirmed and extended these findings, demonstrating that suppression of LPS-induced IL-1β and CCL11 was not only rapid but also sustained through 30 days after infusion. Similarly, LPS-induced elevations in IL-18, IL-8, and TNF-α were significantly reduced by 6 hours and remained suppressed through 7 days, indicating a subacute anti-inflammatory effect. In contrast, suppression of LPS-induced elevations in IL-6, HMGB1, and MIP-1β was transient, with reductions evident only at 6 hours. These distinct temporal patterns in response to LPS stimulation suggest that brexanolone exerts both immediate and sustained immunomodulatory effects on TLR4-driven innate immune responses. Importantly, because LPS-induced activation of TLR4 mimics microbial infection [55], sustained reductions in LPS-induced responses suggest a potential reprogramming of innate immune cells toward a more homeostatic and less reactive phenotype [63, 64].

Brexanolone (allopregnanolone) exerts broad immunomodulatory effects by targeting multiple nodes within the TLR signaling network. It disrupts protein–protein interactions critical for TLR4 activation, including those with its co-receptor myeloid differentiation protein 2 (MD2) and adaptor proteins myeloid differentiation primary response 88 (MyD88) and TIR domain-containing adaptor protein (TIRAP), thereby blocking initiation of the MyD88-dependent proinflammatory cascade [9, 10, 17, 46]. These actions dampen downstream kinase activity and inhibit key transcription factors, resulting in reduced expression of proinflammatory mediators. In parallel, allopregnanolone promotes TIRAP degradation and facilitates the redistribution of TLR4 from the proinflammatory plasma membrane complex to the endosomal TRIF-related adaptor molecule (TRAM) and TIR-domain-containing adapter-inducing interferon-β (TRIF) signaling axis. This shift enhances TRIF-dependent IL-10 production, as evidenced by increased phosphorylated TRAM [11, 16].

Brexanolone also stimulates the accumulation of Ras-related protein Rab-7a (Rab7) and upregulates the phosphoinositide 3-kinase catalytic subunit delta isoform (PI3K p110δ), promoting TLR4 internalization and trafficking toward anti-inflammatory compartments. Notably, brexanolone increases fractalkine, a chemokine that supports neuron–microglia communication and neuroimmune homeostasis, and enhances BDNF expression, which may further promote neurotrophic signaling and IL-10 upregulation [11, 16, 18]. Through the coordinated suppression of proinflammatory signaling and promotion of anti-inflammatory mediators, these TLR-targeted actions may help establish and sustain immune homeostasis.

In addition to modulating TLR signaling, brexanolone may exert lasting effects through epigenetic reprogramming, including DNA methylation, histone acetylation, and chromatin

remodeling. These modifications, such as histone H3 lysine 4 trimethylation (H3K4me3), can reshape transcriptional programs governing cytokines and neurotrophins, alter TLR surface expression, and affect adaptor protein stability, thereby promoting sustained immune homeostasis [64-66]. These epigenetic changes may underlie trained immunity or immune tolerance, where proinflammatory genes are selectively silenced while protective genes remain inducible. As shown by Foster et al. [65], inflammatory mediators like IL-6 and IL-1β become transcriptionally silenced in tolerant macrophages through loss of activating marks such as H3K4me3 and histone acetylation. Similarly, β-glucan has been shown to reverse LPS-induced tolerance by restoring active histone marks, reinforcing the role of gene-specific chromatin remodeling in innate immune memory [66]. In addition, DNA methylation within promoters of proinflammatory genes has been shown to contribute to transcriptional silencing during endotoxin tolerance, via coordinated recruitment of histone methyltransferases and methyl-DNA-binding proteins [67]. These epigenetic processes may underlie the sustained biomarker changes observed here, where brexanolone durably suppressed inflammatory mediators while enhancing BDNF expression.

Finally, brexanolone's effects may extend to stress-response systems, including modulation of HPA axis activity, further supporting its capacity to restore immune and emotional balance [7, 11]. Together, these mechanisms position brexanolone as a multifaceted therapeutic agent capable of establishing durable anti-inflammatory and neuroprotective states.

In addition to neuroimmune pathways, brexanolone's effects may extend to neuroendocrine systems, including oxytocin and cortisol regulation. Oxytocin, a neuropeptide involved in maternal bonding, social cognition, and stress buffering, has been shown to interact

with immune signaling and may contribute to mood stabilization in the postpartum period [68, 69]. Preclinical studies further demonstrate that oxytocin and BDNF pathways are functionally interconnected: oxytocin can transactivate TrkB receptors in neurons and modulate BDNF signaling [70], while BDNF in oxytocin neurons contributes to maternal behaviors in mice [71]. These findings suggest that oxytocin and BDNF may act synergistically to support sustained remission of depressive symptoms. Cortisol, the primary glucocorticoid of the HPA axis, is frequently dysregulated in postpartum depression and exerts potent immunomodulatory effects by suppressing proinflammatory cytokine production [72, 73].

This study has several strengths, including repeated within-subject sampling, classification of biomarkers into two functional groups, and the integration of both Friedman and GEE statistical approaches. The nonparametric Friedman test is well-suited for detecting within-subject changes across multiple time points without assuming normality, while GEE allows for modeling population-averaged effects and time-dependent associations between biomarkers and clinical outcomes. Together, these methods provide complementary insights into both group-level trends and individual-level associations. Additionally, the use of whole blood biomarker profiling captures both intracellular and extracellular components, offering a more physiologically relevant and comprehensive view of immune cell function. The use of whole blood also preserves the native cellular environment, allowing assessment of cytokines in the context of natural cell-to-cell signaling interactions.

Key limitations must also be acknowledged. This was a small, proof-of-concept study, with 10 participants and 4 completing the 30-day follow-up. Early termination reflected the discontinuation of brexanolone infusion therapy, which was replaced in clinical settings by oral

zuranolone, a structurally distinct neurosteroid with different pharmacokinetic characteristics and potentially different anti-inflammatory properties [5, 8, 46, 74]. The absence of a control group prevents attribution of observed effects solely to brexanolone, and participant heterogeneity, including concomitant psychotropic treatments, introduces additional confounders. These constraints reduce generalizability and highlight the need for replication in larger, controlled trials. In addition, while peripheral rather than central biomarker measurements were used, several of the measured cytokines and chemokines are also expressed and functionally active in the central nervous system. Finally, the findings should be interpreted with caution, given the limited sample size and single-center design, which restricts generalizability to the broader population of women with PPD. Despite these limitations, the findings provide important mechanistic insight into how brexanolone may exert sustained clinical effects in PPD.

Conclusion. The sustained neuroimmune effects observed following brexanolone treatment suggest that its clinical benefits in PPD extend well beyond acute symptom relief. By modulating both basal and stimulus-evoked inflammatory responses and enhancing neurotrophic support, brexanolone appears to recalibrate immune function toward long-term homeostasis. The involvement of TLR4 signaling disruption, increased BDNF, and possible epigenetic reprogramming points to a multifactorial mechanism of action distinct from traditional antidepressants. Although brexanolone has been withdrawn from the market, the biomarker profiles identified here, sustained BDNF elevation and suppression of TLR4-driven inflammatory mediators, could inform the development of next-generation neurosteroid treatments. Future work integrating neuroendocrine markers such as oxytocin and cortisol with immune readouts

will be important to clarify the broader biological pathways engaged by neurosteroid therapy and to strengthen translational relevance.

Data availability

Deidentified participant data, study protocol, and informed consent forms are available upon reasonable request.

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Author Contributions

Study design: IB, RP, SMB and ALM; Clinical approvals and data collection: RP and HK; Data collection: IB, CISP and TKO; Data verification: IB, RP, CISP, KX, ALM. Drafted manuscript: IB, RP, ALM; All authors edited and approved the manuscript.

Conflict of Interest

ALM and IB declare a U.S. provisional patent on the anti-inflammatory effects of allopregnanolone and related steroids. ALM and SMB have previously received research funding from Sage Therapeutics for other projects. SMB has received consulting fees from Ancora Bio, Modern Health and Web MD. The authors declare no other potential conflicts of interest.

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Figure legends

Figure 1. Time course of baseline inflammatory and neurotrophic biomarker changes following brexanolone infusion.

Whole blood (cell lysates and supernatants) was collected from 10 individuals with postpartum depression (PPD) before treatment (Pre) and at 6 hours (Post-6h), ~7 days (Post-7d), and ~30 days (Post-30d) after brexanolone infusion. Fourteen immune biomarkers were quantified using Luminex multiplex assays. (a) Among inflammatory mediators, CCL11 and IL-6 were rapidly and durably suppressed through Post-30d, TNF-α was reduced through Post-7d, and IL-18 exhibited only transient suppression at Post-6h. (b) Among growth/differentiation/neurotrophic factors, BDNF significantly increased at Post-6h, Post-7d, and Post-30d. Post hoc comparisons were performed using Wilcoxon signed-rank tests. Data are presented as mean ± standard error of the mean (SEM). Each dot represents an individual subject. *FDR-adjusted $p \leq 0.05$.

Figure 2. Associations between baseline biomarker levels and HAM-D scores over time following brexanolone infusion.

Generalized estimating equation (GEE) models were used to evaluate relationships between baseline levels of 14 biomarkers and Hamilton Depression Rating Scale (HAM-D) scores across post-infusion time points (Post Brex Day). (a) Within inflammatory mediators, lower CCL11, IL-6, and IL-1β were significantly associated with reduced HAM-D scores; IL-6 and IL-1β showed time interactions, indicating weakening associations over time, while CCL11 maintained a stable inverse association. (b) Within growth/differentiation/neurotrophic factors, higher BDNF and lower IL-5 were significantly associated with reduced HAM-D scores. BDNF maintained a stable association across all time points, while IL-5 also showed a consistent association. Data are

shown as scatterplots with regression lines and 95% confidence intervals (gray shading). Color gradient reflects the days since brexanolone infusion. FDR-adjusted p-values are ≤ 0.05.

Figure 3. Brexanolone durably suppresses TLR4-induced inflammatory responses in whole blood cultures. LPS-induced levels of 14 immune biomarkers were quantified in LPS-stimulated whole blood cultures from individuals with PPD at four time points: before infusion (Pre), and at 6 hours (Post-6h), ~7 days (Post-7d), and ~30 days (Post-30d) following brexanolone infusion. Biomarker values were normalized by subtracting paired unstimulated culture values. Friedman tests with FDR correction were followed by Wilcoxon signed-rank post-hoc tests. Among inflammatory mediators, CCL11 and IL-1β were rapidly and durably suppressed through Post-30d. IL-8, IL-18, and TNF-α showed subacute suppression through Post-7d, while IL-6, HMGB1, and MIP-1β exhibited only transient reductions at Post-6h. Data are presented as mean ± SEM. Each dot represents an individual subject. *FDR-adjusted p ≤ 0.05.

Figure 4. Sustained suppression of TLR4-induced inflammatory responses by brexanolone is inversely associated with HAM-D scores in whole blood cultures.

Generalized estimating equation (GEE) models assessed associations between LPS-induced immune biomarker levels and HAM-D scores across post-brexanolone infusion time points (Post-6h, Post-7d, Post-30d). Among inflammatory mediators, lower levels of CCL11, HMGB1, IL-1β, IL-6, IL-8, IL-18, and MIP-1β were significantly associated with reduced HAM-D scores over time, with time interactions for CCL11, HMGB1, IL-8, and MIP-1β, indicating weakening associations over time. Among growth/differentiation/neurotrophic factors, lower levels of IL-3, IL-7, and IL-17A were significantly associated with reduced HAM-D scores, with IL-7 showing a time interaction. Data are shown as scatterplots with regression lines and 95% confidence intervals

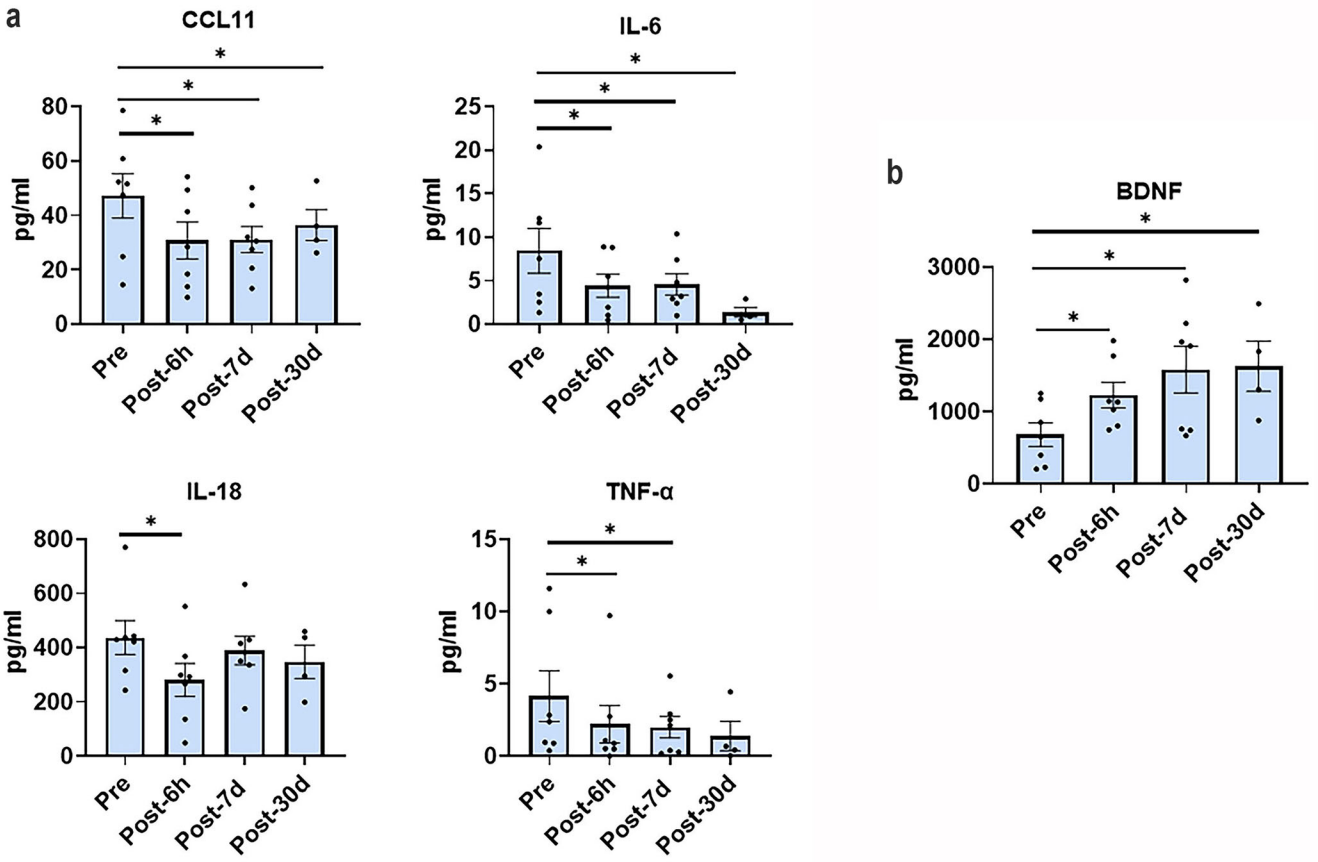
(gray shading). Color gradient reflects the days (Post Brex Day) since brexanolone infusion. FDR-adjusted p-values are ≤ 0.05.

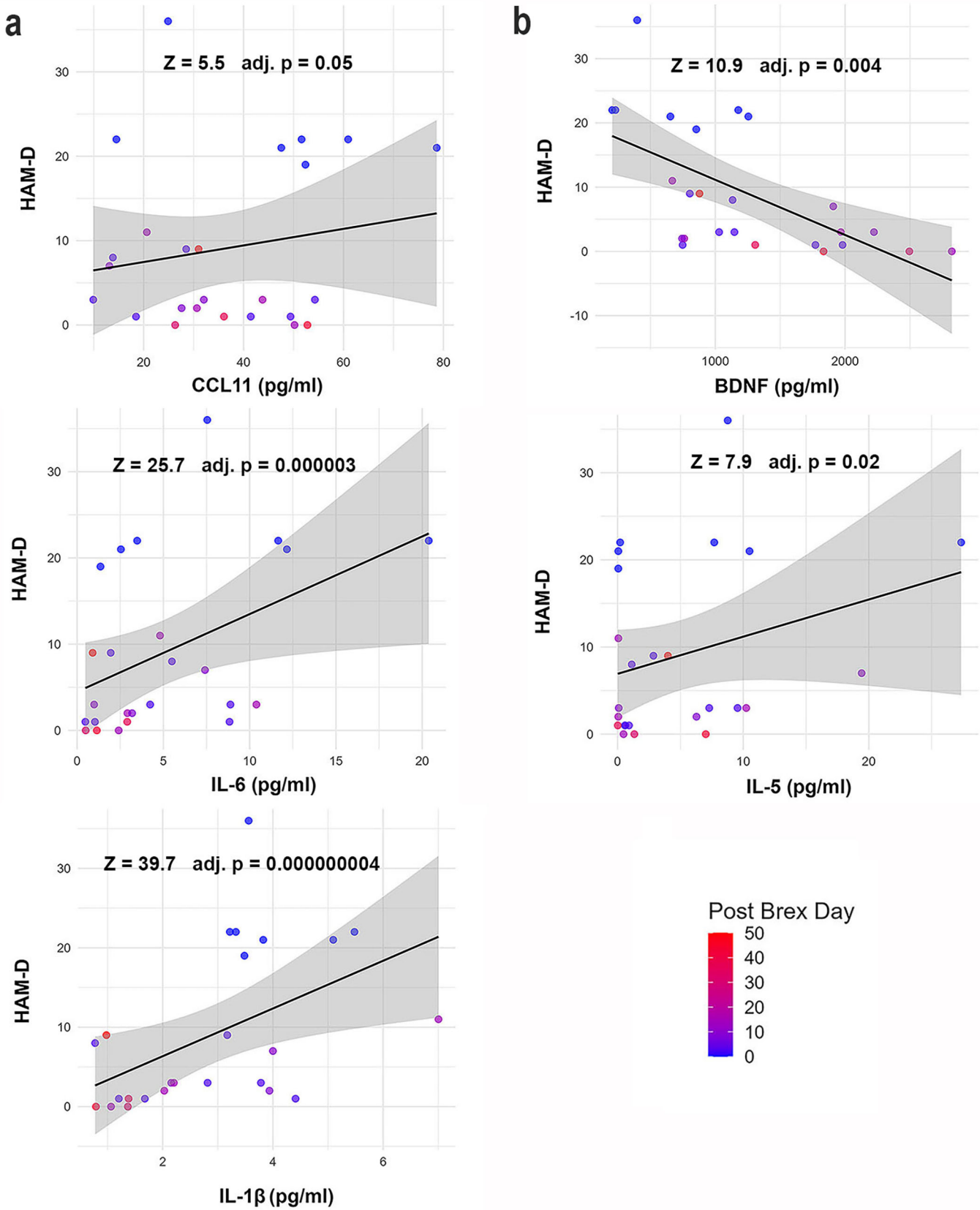
Table 1. Participant Demographic and Clinical Characteristics

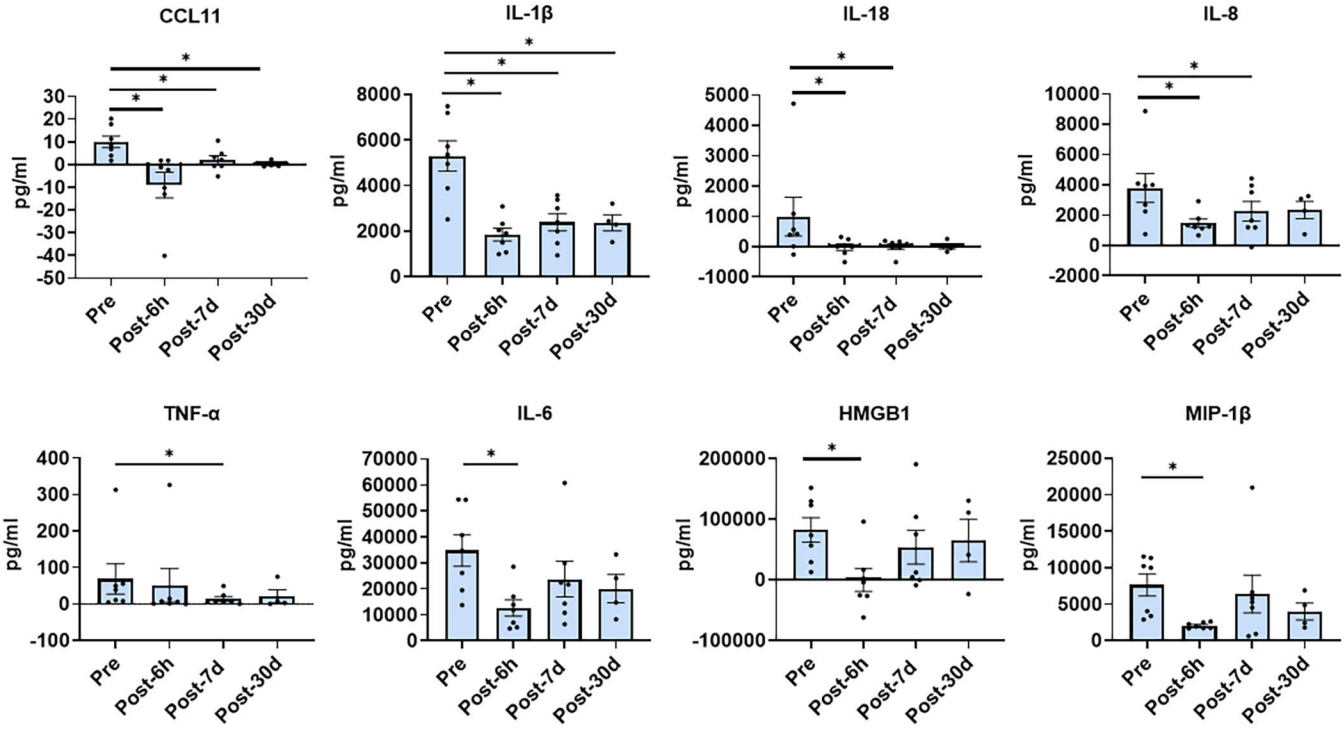
This table summarizes demographic information, clinical history, baseline depression severity, and psychotropic medication use for 10 individuals with postpartum depression who received brexanolone treatment. The median

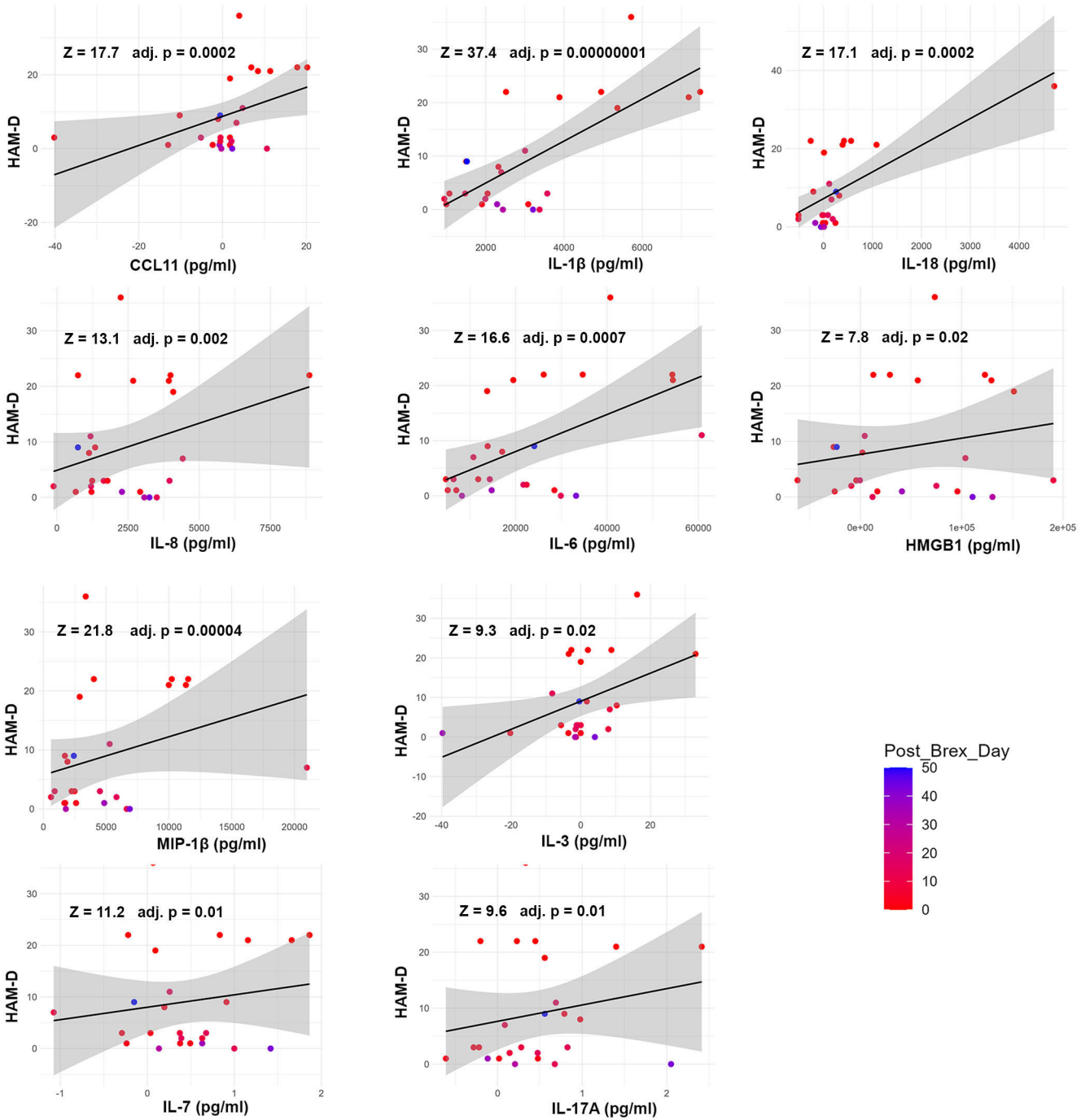
Mean age (years)	33 (23-47)		
	N = 10 (%)		
Race/ethnicity			
Non-Hispanic white	7 (70%)		
Hispanic white	1 (10%)		
Non-Hispanic black	1 (10%)		
Non-Hispanic pacific islander	1 (10%)		
Marital status			
Married	8 (80%)		
Single	2 (20%)		
Patient characteristics	Statistic	HAM-D score baseline (SD)	HAM-D score 6 h post infusion (SD)
	N = 10 (%)	22 (7)	5 (4)
Presence of psychiatric comorbidity/history			
Yes	8 (80%)		
No	2 (20%)		
Concurrent psychiatric treatment			
None	2 (20%)		
Two psychotropics	7 (70%)		
Six psychotropics	1 (10%)		
Psychotropic class type			
SARI	2 (20%)		
SSRI	5 (50%)		
SNRI	1 (10%)		
NDRI	2 (20%)		
Antipsychotic	2 (20%)		
Atypical antipsychotic	1 (10%)		
Anxiolytic	1 (10%)		
Mood stabilizer	1 (10%)		
Benzodiazepine	1 (10%)		
Beta blocker	1 (10%)		
Antihistamine	1 (10%)		
Stimulant	1 (10%)		
Anticonvulsant	1 (10%)		

age was 33 years (range: 23-47). The cohort was predominantly non-Hispanic White (70%), with most participants being married (80%) and having a history of psychiatric comorbidity (80%). At baseline, the mean HAM-D score was 22 (SD = 7), which decreased to 5 (SD = 4) at 6 hours post-infusion. Most participants (80%) were receiving concurrent psychotropic treatment, including selective serotonin reuptake inhibitors (SSRI), serotonin-norepinephrine reuptake inhibitors (SNRI), serotonin antagonist and reuptake inhibitors (SARI), norepinephrine-dopamine reuptake inhibitors (NDRI), and other adjunctive agents.


a
CCL11
pg/ml
0
20
40
60
80
Pre
Post-6h
Post-7d
Post-30d
IL-6
pg/ml
0
5
10
15
20
25
IL-18
pg/ml
0
200
400
600
800
TNF-α
pg/ml
0
5
10
15
b
BDNF
pg/ml
0
1000
2000
3000


a
Z = 5.5 adj. p = 0.05
HAM-D
CCL11 (pg/ml)
b
Z = 10.9 adj. p = 0.004
HAM-D
BDNF (pg/ml)
Z = 25.7 adj. p = 0.000003
HAM-D
IL-6 (pg/ml)
Z = 7.9 adj. p = 0.02
HAM-D
IL-5 (pg/ml)
Z = 39.7 adj. p = 0.000000004
HAM-D
IL-1β (pg/ml)
Post Brex Day
50
40
30
20
10
0


CCL11
IL-1β
IL-18
IL-8
TNF-α
IL-6
HMGB1
MIP-1β
pg/ml
Pre
Post-6h
Post-7d
Post-30d
*


Z = 17.7 adj. p = 0.0002
HAM-D
CCL11 (pg/ml)
Z = 37.4 adj. p = 0.00000001
IL-1β (pg/ml)
Z = 17.1 adj. p = 0.0002
IL-18 (pg/ml)
Z = 13.1 adj. p = 0.002
IL-8 (pg/ml)
Z = 16.6 adj. p = 0.0007
IL-6 (pg/ml)
Z = 7.8 adj. p = 0.02
HMGB1 (pg/ml)
Z = 21.8 adj. p = 0.00004
MIP-1β (pg/ml)
Z = 9.3 adj. p = 0.02
IL-3 (pg/ml)
Z = 11.2 adj. p = 0.01
IL-7 (pg/ml)
Z = 9.6 adj. p = 0.01
IL-17A (pg/ml)
Post_Brex_Day
50
40
30
20
10
0