Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Allopregnanolone

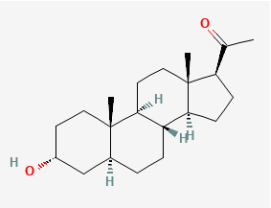
Evidence Summary

Preclinical evidence for neuroprotection is strong. Efficacy and safety of allopregnanolone in Alzheimer's patients are under investigation in the ongoing phase 2 trial.

Neuroprotective Benefit: Allopregnanolone restores cognition, promotes neurogenesis, reduces inflammation, and lowers Aβ in preclinical models. The phase 1b/2a study in AD patients was not powered for cognitive effects, but a larger phase 2 study is ongoing.

Aging and related health concerns: Preclinical studies have shown benefits in models of Parkinson's disease, neuropathic pain, ischemia, neurogenic hypertension, and traumatic brain injury, but no studies have confirmed these effects in humans yet.

Safety: The continuous infusion to treat postpartum depression has a boxed warning of excessive sedation. The lower and intermittent dosing tested in Alzheimer's disease has been well tolerated with rash, fatigue, and dizziness as common adverse events.

Availability: Rx for postpartum depression; in clinical development for AD	**Dose**: Doses tested in a phase I study in MCI and mild Alzheimer's patients were 2 mg, 4 mg, and 6-18 mg once per week (i.v.) for 12 weeks (NCT02221622). For postpartum depression, it is given for 60 hours at doses ranging from 30-90 µg/kg/hour, i.v., via continuous infusion.	**Chemical formula:** $C_{21}H_{34}O_2$ **MW**: 318.5  Source: PubChem
Half-life: 30 minutes	**BBB**: penetrant	
Clinical trials: The two phase 3 postpartum depression clinical trials included 138 subjects in one study and 108 subjects in the other.	**Observational studies**: none available	

What is it? Allopregnanolone is a neurosteroid that is a metabolite of progesterone and is synthesized in the central nervous system, adrenal cortex, and gonads (Irwin et al., 2014). It directly binds to and is a potent positive allosteric modulator of GABA-A receptors (Locci and Pinna, 2017). In 2019, allopregnanolone was approved for the treatment of postpartum depression. As a medication, it is referred to as brexanolone and sold under the brand name Zulresso. Allopregnanolone levels increase during pregnancy and decrease substantially after childbirth (Meltzer-Brody et al., 2018). Allopregnanolone has been shown to modulate anxiety, depression, seizure activity, sedative activity, cholesterol homeostasis, and the immune system (Chen et al., 2011; Irwin et al., 2014). Based on some preclinical evidence, therapeutic efficacy of allopregnanolone has been suggested for Alzheimer's disease, Parkinson's disease, multiple sclerosis, Niemann-Pick, diabetic neuropathy, epilepsy, and traumatic brain injury. A phase 2 study testing allopregnanolone is ongoing in Alzheimer's patients (NCT04838301).

Neuroprotective Benefit: Allopregnanolone restores cognition, promotes neurogenesis, reduces inflammation, and lowers Aβ in preclinical models. The phase 1b/2a study in AD patients was not powered for cognitive effects, but a larger phase 2 study is ongoing.

Types of evidence:

- 1 double-blind RCT testing acute effects of allopregnanolone in healthy women
- 1 phase 1b/2a double-blind RCT in early Alzheimer's patients
- 1 pilot open-label study in Fragile X-associated tremor/ataxia syndrome
- 2 observational studies showing lower levels of allopregnanolone in dementia patients
- 1 observational study evaluating the relationship between allopregnanolone and emotional memory in women with PTSD
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

A double-blind RCT of 28 healthy women tested the effects of a single dose of allopregnanolone (0.07 mg/kg) that produced serum concentrations of allopregnanolone equivalent to those in third-trimester pregnant women (Kask et al., 2008). Women receiving this dose of allopregnanolone had impaired episodic memory when compared to those receiving placebo. However, there were no effects of allopregnanolone on semantic memory or working memory with this dose. There was also no correlation between the change in episodic memory task performance and serum concentration of allopregnanolone. The negative effect of allopregnanolone on episodic memory may have been due to its sedative effects. Physiological levels of allopregnanolone are highest in the third trimester of pregnancy (Luisi et al., 2000).

Fragile X-associated tremor/ataxia syndrome is a late-onset neurodegenerative disorder affecting approximately 45% of male and 16% of female carriers of the FMR1 premutation over the age of 50 years. In a pilot open-label study in 6 men with Fragile X-associated tremor/ataxia syndrome who are carriers of the FRM1 premutation, allopregnanolone treatment for 12 weeks improved executive functioning, episodic memory, and learning (Wang et al., 2017). Allopregnanolone (2 mg) was intravenously infused at a concentration of 0.5 mg/ml in 6% sulfobutylether-β-cyclodextrin with 0.9% NaCl (dispensed by the UC Davis Investigational Drug Pharmacy) over 30 minutes, followed by a flush of 30 min; the second week of treatment the infusion increased to 4 mg, and in weeks 3-12, the dose was increased to 6 mg of allopregnanolone. The majority of patients showed improvement in cognitive

function (MMSE), executive function (BDS-2 and CANTAB One Touch Stockings), memory (WMS-IV and CANTAB Paired Associates Learning), anxiety (SCL-90-R), and intention tremor (CATSYS Dot-to-Dot left-hand tremor intensity). The improvement in BDS-2 and CANTAB Paired Associates Learning was statistically significant (p=0.009 and p=0.042, respectively). MRI analyses were performed on 5 out of 6 patients. Before allopregnanolone treatment, patients showed significant progressive atrophy in the hippocampus, amygdala, and the corpus callosum. After the treatment, MRI changes in both directions were seen in individual patients. As a group, none of the MRI changes reached statistical significance.

In the same pilot study in Fragile X-associated tremor/ataxia syndrome patients, pharmacometabolomics studies of plasma samples showed that allopregnanolone treatment affected GABA metabolism, oxidative stress, Parkinsonism or involuntary movements, and mitochondria-related outcomes (Napoli et al., 2019). Patients treated with allopregnanolone when compared to before treatment had significantly lower levels of 3,4-dihydroxybutyric and 4,5-dihydroxyhexanoic acids ($p < 0.031$) with higher values of Glu/Gln (1.34-fold), suggesting an improvement in GABA metabolism. Allopregnanolone also lowered the levels of 3 markers of oxidative stress (IAA, methionine sulfoxide, and 2-hydroxybutyric acid). 2-hydroxybutyric acid is an early marker for insulin resistance and impaired glucose regulation resulting from increased lipid peroxidation and oxidative stress. Other metabolites related to oxidative stress damage or response (i.e., cysteine disulfide over cysteine, alpha-tocopherol, vitamin C, taurine) failed to show an effect with allopregnanolone treatment. None of the mitochondrial function biomarkers (complex I, II–III, IV, V, and citrate synthase activities; ratios of lactate-to-pyruvate, lactate, pyruvate, ratio of alanine and lactate to pyruvate; short-chain organic acids levels) showed an allopregnanolone treatment effect (pre- versus post-allopregnanolone treatment).

Because of the small size of the study and the open-label design, further studies are needed to validate the efficacy of allopregnanolone in Fragile X-associated tremor/ataxia syndrome patients.

Human research to suggest benefits to patients with dementia:
In a phase 1b/2a double-blind randomized controlled single ascending and multiple ascending dose study, 24 subjects with early Alzheimer's disease (mild cognitive impairment due to Alzheimer's or mild Alzheimer's) were treated with allopregnanolone (2, 4, and 6 ascending to 18 mg; i.v.) once weekly for 12 weeks (Hernandez et al., 2020). The phase 1b/2a was a safety study (data discussed in the "Safety" section). Exploratory endpoints included cognitive and MRI outcomes, but the studies were not powered to detect treatment effects. Accordingly, after 12 weeks of treatment, no statistically significant differences were observed amongst treatment groups on the ADAS-Cog14 total score, MoCA

total score, and Cogstate Brief Battery composite score. Baseline and post-treatment MRI imaging data were available for 23 subjects and there was no statistically significant difference in hippocampal volumes among the 4 groups (placebo, allopregnanolone 2 mg, allopregnanolone 4 mg, allopregnanolone 6-18 mg). Analysis of change in left and right hippocampal volumes showed a trend of decreased atrophy in allopregnanolone-treated subjects. Analyses of other MRI structural volumes, resting state functional MRI, diffusion tensor imaging, and other exploratory outcomes are ongoing. A phase 2 double-blind randomized controlled trial is currently underway (NCT04838301).

Mechanisms of action for neuroprotection identified from laboratory and clinical research

Postmortem studies have shown that allopregnanolone levels are reduced on average by over 50% in the prefrontal cortex (Marx et al., 2006) and temporal cortex (Naylor et al., 2010) of Alzheimer's disease patients compared to control subjects, though there is a high level of variability within each group. In both studies, low allopregnanolone levels correlated with worse Braak neuropathological disease stage. Blood levels of allopregnanolone are also reduced in both Alzheimer's and vascular dementia patients compared to controls (Bernardi et al., 2000).

In an observational study of 143 US military veterans, circulating levels of allopregnanolone was positively correlated with gray matter thickness in multiple regions of cingulate, parietal, and occipital association cortices, even after statistical correction (FDR-correction)(Morey et al., 2019). Allopregnanolone levels had significant associations with gray matter cortical thickness in 59 cortical regions ($p<0.05$; FDR-corrected). In assessing biological variables, age was the only variable that contributed significantly to predicting gray matter cortical thickness, and other variables, including depression score, alcohol use, PTSD diagnosis, childhood trauma exposure, antidepressant medication use, and smoking status were not significantly associated with cortical thickness.

Allopregnanolone is blood-brain-barrier penetrant (Irwin et al., 2015). There have been many studies examining the effects of allopregnanolone in preclinical models of Alzheimer's. In the 3xTgAD mouse model, allopregnanolone increases neurogenesis (by inducing CREB and neuronal differentiation factor NeuroD) and the survival of newly generated neurons, restores cognitive function, and reduces Aβ generation in the hippocampus, cortex, and amygdala (Wang et al., 2005; Wang et al., 2010; Chen et al., 2011; Singh et al., 2012; Irwin et al., 2015). Allopregnanolone also reduces microglia activation (expression of OX42) and increases CNPase, an oligodendrocyte myelin marker (Chen et al., 2011). Also in 3xTgAD mice, allopregnanolone treatment (10 mg/kg) significantly reversed the deficits in mitochondrial respiration and biogenesis, while also reducing lipid peroxidation (Wang et al., 2020).

Pathway analyses predicted that allopregnanolone induced PPAR-γ and coactivator 1-alpha (PPARGC1A) pathways while simultaneously inhibiting the presenilin 1, phosphatase and tensin homolog (PTEN), and TNF pathways to reduce Alzheimer's pathology. In 3xTgAD mice treated with allopregnanolone (10 mg/kg, s.c.) once per week for 2 weeks, proliferation and differentiation of neural stem cells were significantly increased, which were paralleled by increases in IGF-1 and IGF-1 receptor expressions (Chen et al., 2020). Allopregnanolone treatment also increased oligodendrogenesis. In a hippocampal neuronal culture, allopregnanolone significantly increases dendritic spine density (Shimizu et al., 2015). Administration of allopregnanolone prior to and during the early stages of AD pathology was optimal in increasing regenerative responses and reducing AD pathology, while allopregnanolone treatment initiated at or after the point of Aβ plaque generation was not protective (Chen et al., 2011). Thus, for translation to human patients, targeting the early stages of the disease is likely optimal.

Allopregnanolone has also shown benefit in other models of cognitive impairment. In a rat model of cognitive deficit (induced by ketamine), allopregnanolone treatment (8 or 16 mg/kg, i.p.) for 5 days reversed the impairment in spatial learning and memory, while upregulating the PGRMC1/EGFR/GLP-1R/PI3K/Akt pathway (Cao et al., 2021). In rats subjected to metabolic syndrome (induced by a high-fructose diet), allopregnanolone treatment (20 mg/kg, orally, dissolved in ethanol and saline) for 8 weeks improved spatial memory, hypertension, and biochemical markers of metabolic syndrome (serum glucose, insulin, insulin resistance, and lipid profile) and synapses (synaptophysin)(Amin et al., 2021). Because of the way allopregnanolone was delivered in this study, further studies are needed to tease apart the potential effects of the diluent (alcohol) from allopregnanolone.

It is worth emphasizing that the dosing regimen for allopregnanolone is a critical factor. Brain regenerative properties of allopregnanolone in rodents are achieved with a once per month or once per week regimen at 10 mg/kg, per i.v. injection, whereas a constant infusion treatment regimen (such as those used in postpartum depression) is anti-regenerative (Chen et al., 2011; Hernandez et al., 2020). Chronic allopregnanolone treatment results in cognitive dysfunction and decreased hippocampal weight in wild-type mice (Bengtsson et al., 2016). In mouse models of Alzheimer's disease, chronic allopregnanolone treatment also resulted in impaired learning and memory and increased levels of soluble (toxic) Aβ in the brain (Bengtsson et al., 2012, 2013).

APOE4 interactions: Patients carrying the APOE4 allele have reduced allopregnanolone levels in the temporal cortex compared to non-carriers (Naylor et al., 2010). Although allopregnanolone treatment may be more effective in APOE4 carriers than non-carriers, this possibility has not been validated yet.

Aging and related health concerns: Preclinical studies have shown benefits in models of Parkinson's disease, neuropathic pain, ischemia, neurogenic hypertension, and traumatic brain injury, but no studies have confirmed these effects in humans yet.

Types of evidence:

- 0 clinical trials
- Numerous laboratory studies

Parkinson's disease: POTENTIAL BENEFIT BASED ON RODENT STUDIES
In two rodent models of Parkinson's disease, allopregnanolone treatment (5 and 20 mg/kg, oral gavage or 10 mg/kg, s.c. once per week for 2 weeks) significantly restored cognitive dysfunction (Nezhadi et al., 2016; Adeosun et al., 2012). Allopregnanolone treatment (10 mg/kg, s.c., once per week for 2 weeks) also promoted neurogenesis and restored the number of dopaminergic neurons in the substantia nigra in these mice (Adeosun et al., 2012). These neuroprotective effects have not been confirmed in Parkinson's disease patients yet.

Neuropathy/Pain: POTENTIAL BENEFIT BASED ON RODENT STUDIES
In a rat model of chemotherapy (oxaliplatin)-induced peripheral neuropathy, prophylactic or corrective allopregnanolone treatment (4 mg/kg, every other day) prevented/abolished biochemical and functional alterations in peripheral nerves (Meyer et al., 2011). Allopregnanolone restored sciatic nerve conduction velocity and action potential peak amplitude drastically reduced by chemotherapy. Protective effects of allopregnanolone have also been demonstrated in a rat and cell culture model of diabetic neuropathy. Allopregnanolone reduced sensitivity to pain (hyperalgesia) and decreased markers of apoptosis (caspase 3 and Bax)(Afrazi et al., 2014). No studies have confirmed these protective effects of allopregnanolone in neuropathy patients yet.

Neurosteroids are considered the most potent endogenous modulators of GABA-A receptors and their levels decrease in acute and chronic painful conditions (Nair and Diwan, 2019). Downregulation of GABA-A receptors or impairment in spinal GABA-A receptors have been found to play a role in chronic pain later in a model of peripheral neuropathy (Janssen et al., 2011). Allopregnanolone and other neurosteroids that modulate GABA-A receptors have shown benefits in addressing neuropathic pain and acute surgically-mediated pain. However, repeated exposure to allopregnanolone may lead to tolerance.

In a rat model of postoperative pain (hind paw plantar incision model), allopregnanolone treatment (0.16 or 1.6 mg/kg, s.c., dissolved in 5% polysorbate 80 and 16% polyethylene glycol in physiological saline) inhibited mechanical allodynia (Fujita et al., 2018). Allopregnanolone treatment decreased the plantar incision-evoked NK-1 receptor internalization in the dorsal horn, suggesting inhibition of substance P release from the primary afferent fiber.

Traumatic brain injury: POTENTIAL BENEFIT BASED ON RODENT STUDIES
In a rat model of traumatic brain injury, allopregnanolone treatment (4 mg/kg/day, i.p.) for 5 days improved memory performance, reduced neuronal death, and reduced inflammation (levels of IL-1β, TNFα)(He et al., 2004a, 2004b). Dose and treatment regimen that are recommended are immediate and continuous exposure, given the high levels of glutamate excitotoxicity present during an acute injury. A phase II double-blind, placebo-controlled RCT of allopregnanolone in treating traumatic brain injury was conducted (NCT01673828). In this trial, allopregnanolone was started within 8 hours after injury and given i.v. continuously for a 4-day treatment period, followed by a one-day dose de-escalation. No significant difference in the primary outcome (Extended Glasgow Outcome Scale Score) was seen between allopregnanolone (n=7) and placebo (n=6) groups, but the study was small.

Cerebral ischemia: POTENTIAL BENEFIT BASED ON RODENT STUDIES
In a rat model of ischemia, allopregnanolone treatment (8 mg/kg, i.v.) significantly reduced the impairment in spatial learning and memory, as well as in reference and working memory, and prevented the narrowing of the hippocampus, otherwise induced by ischemia (Morali et al., 2011). This better performance of allopregnanolone-treated rats compared to the vehicle group occurred in spite of a loss of pyramidal neurons in the hippocampus. No studies have tested allopregnanolone in ischemia/stroke patients.

Cholesterol homeostasis: POTENTIAL BENEFIT BASED ON PRECLINICAL STUDIES
Preclinical studies have shown that allopregnanolone regulates cholesterol homeostasis via increasing liver-X-receptor (LXR) and pregnane-X-receptor (PXR). LXR activation increases cholesterol efflux through increased ABCA1 and ApoE expression, and prevents overactivation of gamma secretase and overproduction of Aβ (Whitney et al., 2002; Shenoy et al., 2004). No studies have confirmed these effects in humans yet.

Neurogenic hypertension: POTENTIAL BENEFIT BASED ON RODENT STUDIES

Neurogenic hypertension refers to hypertension in which the sympathetic nervous system plays a significant role. Increased sympathetic vasomotor tone to the heart, kidney, and blood vessels has been suggested as a major influence on the development of hypertension, and neurogenic hypertension is associated with chronic stress, obesity, and chronic kidney disease (Head et al., 2019). Up to 50% of patients with hypertension are thought to have the neurogenic form (Esler, 2014). In a mouse model of neurogenic hypertension, allopregnanolone treatment (5 mg/kg/day, s.c.) reduced mean arterial pressure and inhibited stress responses (Stevenson et al., 2017). It is hypothesized that impaired GABAergic inhibition may be characteristic of neurogenic hypertension.

Safety: The continuous infusion to treat postpartum depression has a boxed warning of excessive sedation. The lower and intermittent dosing tested in Alzheimer's disease has been well tolerated with rash, fatigue, and dizziness as common adverse events.

Types of evidence:

- 1 meta-analysis in postpartum depression
- 2 phase 3 double-blind RCTs in postpartum depression
- 1 clinical trial follow-up in postpartum depression
- 4 clinical studies testing acute effects of allopregnanolone
- 1 phase 1b/2a double-blind RCT in early Alzheimer's patients
- 1 pilot open-label study in Fragile X-associated tremor/ataxia syndrome
- Numerous review papers
- Numerous laboratory studies

Allopregnanolone has been studied in multiple indications and safety may depend on the dosing regimen appropriate for each indication. Zulresso, the brand name product for postpartum depression, comes with a boxed warning stating the risk of excessive sedation or sudden loss of consciousness during administration (Drugs.com). The doses tested in Alzheimer's disease clinical trials are significantly lower and the dosing schedule is intermittent (once weekly). It is recommended that people treated with allopregnanolone (e.g., Zulresso) not engage in activities that require high levels of alertness, such as operating a motor vehicle (Edinoff et al., 2021). Allopregnanolone should also be avoided in people with end-stage renal disease, who can have difficulty clearing the solubilizing agent of Zulresso (betadex sulfobutyl ether sodium), causing further damage to the kidneys.

Alzheimer's disease patients: In a phase 1b/2a study in 24 subjects with early Alzheimer's disease (mild cognitive impairment due to Alzheimer's or mild Alzheimer's), allopregnanolone treatment (2, 4, and 6 ascending to 18 mg; i.v.) once weekly for 12 weeks was safe and well-tolerated in all study participants (Hernandez et al., 2020). Adverse events were reported by 83% of participants in the placebo arm and 61% of participants in the allopregnanolone arm. No differences were observed between treatment arms in the occurrence and severity of adverse events. The most common adverse events were mild to moderate in severity and included rash (n=4; 22%), fatigue (n=3; 17%), and dizziness (11%). A single non-serious adverse event, dizziness, which occurred within 24 hours after the infusion, was attributed to allopregnanolone. There was one serious adverse event (rectal hemorrhage) that occurred in a participant receiving 2 mg of allopregnanolone, but this was determined not to be related to treatment and the participant completed the study without interruption. No subjects discontinued treatment due to adverse events. There were no clinically significant changes in echocardiograms, physical exams, or clinical laboratory assessments. Additionally, imaging analyses did not detect any amyloid-related imaging abnormalities (ARIA) across cohorts.

For all doses tested in the phase 1b/2a study in Alzheimer's patients, the apparent half-life occurred at approximately 30 minutes (Hernandez et al., 2020). The pharmacokinetics indicated a linear dose-response in plasma concentration of allopregnanolone after intravenous administration over 30 minutes. The maximum tolerated dose was established based on evidence of allopregnanolone-induced sedation at the highest doses.

The threshold for sedation was 10 mg for men and 14 mg for women (Hernandez et al., 2020). No indicators of sedation were observed at the 2 mg and 4 mg doses of allopregnanolone. Mental and physical sedation was dose dependent. The incidence of Stanford Sleepiness Scale (SSS) scores consistent with sedation (≥7) was directly proportional to allopregnanolone dose. In the allopregnanolone dose 6–18 mg group, the highest rated SSS score (≥7) indicated "sleep onset soon" or asleep. In female participants, no sedation was observed at either the 2 mg, 4 mg, 6 mg or 10 mg dose but was observed at 14 mg and 18 mg. In contrast, male participants demonstrated sedation at doses at and above 6 mg.

Postpartum depression: In a meta-analysis of 3 randomized controlled trials in a total of 267 patients with postpartum depression, allopregnanolone infusion led to a significantly higher rate of discontinuation for any reasons (RR=2.68; 95% CI, 1.35 to 5.32)(Zheng et al., 2019). Discontinuation due to intolerability and adverse drug reactions was comparable between allopregnanolone and placebo

groups (2.1% in allopregnanolone group, 1.3% in placebo). Meta-analysis of adverse drug reactions were not significantly different between allopregnanolone and placebo.

Two phase 3 double-blind randomized controlled trials in postpartum recruited 138 subjects in one study and 108 in the other (Meltzer-Brody et al., 2018). The most common treatment-emergent adverse events in the allopregnanolone groups were headache (n=7, 60 µg/kg/hr group; n=6, 90 µg/kg/hr group; n=7, placebo group for study 1; n=9, 90 µg/kg/hr group; n=6, placebo group for study 2), dizziness (n=6, 90 µg/kg/hr group; n=6, 90 µg/kg/hr group; n=1, placebo group for study 1; n=5, 90 µg/kg/hr group; n=4, placebo group for study 2), and somnolence (n=7, 60 µg/kg/hr group; n=2, 90 µg/kg/hr group; n=3, placebo group for study 1; n=4, 90 µg/kg/hr group; n=2, placebo group for study 2). In Study 1, one patient in the 60 µg/kg/hr group had 2 serious adverse events (suicidal ideation and intentional overdose attempt during follow-up). This participant had 3 previous suicide attempts before the study, and the event was not considered drug-related by the investigator. Another patient in the 60 µg/kg/hr group had 2 severe adverse events (somnolence and loss of consciousness) during treatment but completed treatment following interruption of dosing and rapid resolution of the adverse events. In Study 2, one patient (out of 51) in the 90 µg/kg/hr group had 2 serious adverse events (altered state of consciousness and syncope), which were moderate in intensity considered to be treatment related. Two (4%) of 51 patients in the 90 µg/kg/hr group had severe adverse events (1 case of fatigue and 1 case of presyncope).

Overall, of the 130 patients who received allopregnanolone across Studies 1 and 2, 5 patients (4%) patients had excessive sedation considered to be due to allopregnanolone; in all cases, the patients alerted staff and infusion was stopped immediately (Meltzer-Brody et al., 2018). Patients who lost consciousness regained consciousness within 15 minutes of pausing infusion, and all excessive sedation events were completely resolved within 90 minutes. No respiratory or hemodynamic problems were associated with these cases of excessive sedation. No clinically significant differences in laboratory parameters, vital signs, or ECGs were observed between the allopregnanolone treatment and placebo groups.

Healthy people: A pharmacokinetic study in 10 healthy women reported that allopregnanolone given in a cumulative dose of 0.09 mg/kg (three i.v. injections of 0.015, 0.03, and 0.045 mg/kg) did not have any adverse effects other than fatigue, mild nausea, and sedation (Timby et al., 2006). Self-rated sedation was affected until 55 min after the last dose. Three women reported mild feelings of alcohol-like

intoxication, 3 women experienced mild nausea, and 1 woman reported flushing. One woman reported an anxiety attack 24 hours after the allopregnanolone injection.

In a clinical study of 9 men and 9 women (on oral contraceptives), allopregnanolone (three i.v. injections of 0.015, 0.03, and 0.045 mg/kg) did not result in serious adverse effects, but 3 men and 2 women reported sleepiness and tiredness, 3 men and 4 women reported feeling as if they had taken alcohol, 1 man reported flushing, and 1 woman complained of a mild frontal headache. (van Broekhoven et al., 2007). Also, men scored higher on "sedation" than women after allopregnanolone.

Epilepsy patients: In an open-label study of 25 patients with super-refractory status epilepticus, allopregnanolone infusion showed tolerability and was associated with a high rate of successful third-line agent weaning (Rosenthal et al., 2017). A total of 207 adverse events were reported in 23 patients (92%). The most common adverse events were fever, hypotension, diarrhea, peripheral edema, anemia, and blood urea nitrogen (BUN) increase. Within these most common AEs, 2 events (fever and BUN increase, in 1 patient each) were deemed by the investigators to be "possibly related" to the administration of allopregnanolone, although both of these events resolved without dose changes. No serious adverse events were attributable to allopregnanolone, as determined by the Safety Review Committee. Sixteen patients (64%) experienced 1 or more severe adverse events, including respiratory failure, pulmonary embolism, sepsis, convulsion, cardiac arrest, and acute renal failure. Six deaths occurred, all deemed related to the medical conditions and not to the drug. Four patients died after completing the allopregnanolone infusion, in each case from causes related to underlying medical conditions. The cause of death for the 2 patients who died before completion of treatment were metastatic brain cancer and fulminant cardiac sequelae of organophosphate ingestion. Twenty-two patients underwent third-line agent wean attempt. Seventeen (77%) patients were successfully weaned off third-line agents before tapering allopregnanolone. Treatment with allopregnanolone did not have a significant effect on vital signs such as heart rate and systolic/diastolic blood pressure.

In a pilot clinical study of 2 super-refractory status epilepticus patients, 120 hours of continuous allopregnanolone infusion effectively weaned them off of pentobarbital (Vaitkevicius et al., 2017). Patient 1 was a 23-year-old man who presented with generalized convulsions and myoclonus, preceded by a week of malaise and headache. The patient underwent intravenous general anesthesia and there were 8 unsuccessful attempts to discontinue pentobarbital while multiple antiepileptic and immunologic interventions were administered. Allopregnanolone infusion was used as a bridge therapy to wean the patient off pentobarbital. The allopregnanolone intravenous solution contained 6% hydroxypropyl-β-

cyclodextrin (Trappsol; Cyclodextrin Technologies Development, Alachua, FL) in 0.9% sodium chloride injection, USP, and was manufactured by the Good Manufacturing Products Laboratory at the University of California, Davis. The patient received 670.8 mg of allopregnanolone at 86 µg/kg/h (5.6 mg/h) by continuous infusion for 5 days, during which the patient was successfully weaned from pentobarbital without recurrence of status epilepticus. At the 3-year follow-up, he was cognitively intact and seizure-free. Patient 2 was a 28-year-old man who developed super-refractory status epilepticus of unknown cause. Status epilepticus recurred during 3 attempts to wean the patient from pentobarbital. Allopregnanolone was administered over 5 days as bridge therapy during the successful wean from pentobarbital. The formulation in this case contained sulfobutylether-β-cyclodextrin (Captisol; CyDex Pharmaceuticals, Division of Ligand Pharmaceuticals, Lenexa, KS). At the 3-year follow-up, the patient demonstrated good cognitive function with occasional breakthrough seizures.

Fragile X-associated tremor/ataxia syndrome: In a pilot open-label study in 6 men with Fragile X-associated tremor/ataxia syndrome who are carriers of the FRM1 premutation, weekly intravenous infusions of allopregnanolone (2-6 mg over 30 minutes) for 12 weeks were well-tolerated in all subjects (Wang et al., 2017).

Premenstrual dysphoric disorder: In a double-blind RCT of 16 women with premenstrual dysphoric disorder (and 12 healthy controls), a single injection of allopregnanolone (0.05 mg/kg, i.v.) significantly increased self-rated sedation (Kask et al., 2009).

Safety data from animal models: In a pharmacokinetic, pharmacodynamic, and safety study in dogs (3 healthy, 2 with history of epilepsy), a single intravenous allopregnanolone treatment (1-4 mg/kg) showed dose-proportional increases in exposure and based on pharmacokinetic/pharmacodynamic simulations, a 2 mg/kg dose infused over 5 minutes was predicted to achieve plasma concentrations above the EC50 while being below concentrations associated with heavy sedation (Vuu et al., 2021). In the same study, a single intramuscular allopregnanolone treatment (1-6 mg/kg) showed incomplete absorption and volume-dependent bioavailability. In dogs, allopregnanolone was safe and well-tolerated when administered at 1-3 mg/kg intravenously and up to 2 mg/kg intramuscularly. With an intravenous allopregnanolone dose of 4 mg/kg, dogs were immobile and briefly unarousable even with pain stimulation for 1-3 minutes with stable vital signs. Following intravenous infusion, ataxia occurred within 1.5-3 minutes following infusion start and persisted for 10-18.5 minutes. In healthy dogs, onset of sedation occurred at 3.5-5 minutes following infusion initiation and lasted up to 6 minutes at doses greater than 2 mg/kg. Following intramuscular injection, onset of ataxia without sedation occurred 3-5

minutes following the 6 mg/kg dose and lasted for 17-19 minutes. The intramuscular 6 mg/kg dose was associated with pain, which may have been due to the volume infused (19.1-20.6 mL).

In a mouse model of Alzheimer's disease (3xTgAD mice), allopregnanolone treatment (10 mg/kg) did not result in uterine weight changes, while estradiol treatment significantly increased uterine weight (Wang et al., 2020).

Drug interactions: Zulresso, the approved treatment for postpartum depression, has 2 major and 262 moderate drug interactions (for full list, see Drugs.com). Concomitant use of Zulresso with opioids, benzodiazepines, or antidepressants may increase the likelihood or severity of adverse reactions related to sedation. Zulresso also has a moderate interaction with alcohol and taking Zulresso with alcohol may increase side effects such as dizziness, drowsiness, confusion, and difficulty concentrating (Drugs.com). Allopregnanolone can inhibit CYP2C9, which is a cytochrome p450 enzyme that is responsible for the metabolism of certain drugs and compounds. Thus, care should be taken when allopregnanolone is co-administered with drugs that are metabolized by CYP2C9 (Edinoff et al., 2021).

Sources and dosing: Allopregnanolone is approved for the treatment of postpartum depression. As a medication, it is referred to as brexanolone and sold under the brand name Zulresso. For the treatment of postpartum depression, allopregnanolone (brexanolone) is given for 60 hours at doses ranging from 30-90 µg/kg/hour, intravenously via continuous infusion, under medical supervision.

Allopregnanolone is also being studied for the treatment of Alzheimer's disease. Dosing regimen is a critical factor with allopregnanolone due to its actions on the GABA-A receptor, producing sedative effects. Like other neurosteroids, allopregnanolone has an inverted U-shaped dose-response profile, where too high or two low of an allopregnanolone dose leads to suboptimal responses (Wang et al., 2005). In aged and Alzheimer's model mice, treatment once per week at sub-sedative doses (10 mg/kg, i.v.) was optimal for regeneration and reduction in Alzheimer's pathology (Chen et al., 2011; Irwin et al., 2014). The phase 2 double-blind randomized controlled trial (REGEN-BRAIN©) in mild Alzheimer's disease is testing allopregnanolone at a dose of 4 mg (i.v., 30-minute infusion) once per week for 12 months (NCT04838301).

Different routes of administration are being investigated for allopregnanolone treatment. In a study in mice subjected to seizure tests, intranasal allopregnanolone (16 mg/kg) showed anti-seizure effects comparable to those of benzodiazepines while causing minimal sedation or motor toxicity (Zolkowska et

al., 2021). Intranasal allopregnanolone did not cause loss-of-righting reflex in most mice, but when the same dose was administered intramuscularly, all animals became impaired. Intranasal allopregnanolone (10 mg/kg) caused a rapid increase in brain allopregnanolone with a Tmax of 5 min. High levels of allopregnanolone were present in the olfactory bulb (Cmax, 16,000 ng/mg) whereas much lower levels (Cmax, 670 ng/mg) were found in the forebrain, midbrain, cerebellum. For intranasal delivery, sulfobutylether-β-cyclodextrin was used as the solvent. Cyclodextrins are believed to enhance nose-to-brain permeability by extracting membrane cholesterol on the nasal epithelium, leading to the opening of tight junctions. Intranasal delivery of a drug with cyclodextrin leads to preferential delivery of the drug in certain brain regions, including the olfactory bulb and the hypothalamus (Nonaka et al., 2008). However, it is worth noting that toxicities have been associated with certain cyclodextrins, including ototoxicity with hydroxypropyl-β-cyclodextrin, often used as the solvent for allopregnanolone in animal studies (Crumling et al., 2017). Before pursuing the intranasal route for allopregnanolone, nasal and other toxicities of the solvent needs to be investigated. For example, a sulfobutylether-β-cyclodextrin formulation of midazolam in humans caused mild to moderate transient irritation of nasal and pharyngeal mucosa (Gudmundsdottir et al., 2001). Aside from local irritation, cyclodextrins may also travel to the brain and therefore, broader adverse events including impacts to the brain need to be investigated.

Research underway: According to ClinicalTrials.gov, there are 6 ongoing clinical trials testing allopregnanolone and 2 clinical trials testing ganaxolone, a 3β-methylated synthetic analog of allopregnanolone, as of January 2021. For allopregnanolone, there are 2 trials in Alzheimer's disease patients (NCT03748303; NCT04838301), 1 trial in postpartum depression, 1 trial in alcohol use disorder, 1 trial in PTSD, and 1 trial in traumatic brain injury. For ganaxolone, 1 trial is in PCDH19-related epilepsy and 1 trial is in CDKL5 deficiency disorder (a genetic seizure disorder).

The phase 2 double-blind randomized controlled trial (REGEN-BRAIN©) in mild Alzheimer's disease is led by Roberta Diaz Brinton, PhD, at University of Arizona (NCT04838301). The trial will test allopregnanolone treatment at a dose of 4 mg (i.v., 30-minute infusion) once per week for 12 months against a placebo (normal saline) in 200 participants with mild Alzheimer's disease who are APOE4 carriers. The primary outcome is hippocampal volume. The secondary outcome measures include cognitive assessments (Cambridge Cognition's paired associates learning test, CANTAB, ADAS-Cog), functional outcomes (ADCS-iADL), safety, and tolerability. Exploratory outcomes include regional brain volumes, diffusion tensor imaging, resting state functional MRI, arterial spin labeling, blood-based

biomarkers of Alzheimer's pathology, neurogenesis, and inflammation, Clinical Dementia Rating, ADAS-Cog14, MMSE, Neuropsychiatric Inventory-Questionnaire, quality of life, and others.

Studies of allopregnanolone analogs are also underway. Mensah-Nyagan and colleagues have modified the chemical structure of allopregnanolone to synthesize a new set of compounds by introducing either an oxo-group or an O-allyl as substitute of the 3-hydroxyl group. These modifications have resulted in more stable compounds and individually designated BR053 (12 oxo-epiAP), BR297 (O-allyl-epiAP), BR351 (O-allyl-AP), and BR338 (12 Oxo-AP) (Mensah-Nyagan et al., WO2012127176A1). In a cell culture model, BR297 exhibited advantages over allopregnanolone with regards to both protection of mitochondrial functions and reduction of oxidative stress (Lejri et al., 2017). Under conditions of high oxidative stress, BR297 decreased reactive oxygen species, improved mitochondrial respiration, and promoted cell survival in ways that appeared more robust compared to allopregnanolone. However, these studies in cell culture are still preliminary and it is not known whether BR297 or other analogs of allopregnanolone will be more neuroprotective.

Search terms:

Pubmed, Google: allopregnanolone

- + clinical trial, + Alzheimer's, + cognitive, + meta-analysis, + safety, + lifespan, + neuropathy, + Parkinson's

Clinicaltrials.gov: allopregnanolone, ganaxolone