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A REVIEW ARTICLE ON BREXANOLONE DRUG-ANTI DEPRESSANT

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Abstract:

ZULRESSO (Brexanolone) is a novel FDA-approved treatment for moderate-to-severe postpartum depression. Postpartum depression may be diagnosed in women experiencing depressive symptoms which can manifest as cognitive, behavioural, or emotional disturbances as early as the third trimester to 4 weeks following delivery. The efficacy of brexanolone suggests that neurosteroids such as allopregnanolone are important to treat PPD. However, it is currently unclear if brexanolone provides lasting relief of depressive symptoms at or beyond 30 days following administration. Further studies are necessary to make this determination.

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INTRODUCTION:

BREXANOLONE is an antidepressant drug called a neuroactive steroid gamma-aminobutyric acid (GABA)A receptor positive modulator indicated for the treatment of postpartum depression (PPD). It works by balancing substances in your brain that help regulate mood.The medication is for infusion into a vein.

fig:1

Common Brand Name:ZULRESSO

- ★ The reduction in symptoms of PPD in two phase 3 clinical trials. These results coincide with previous studies that found that low allopregnanolone concentration during pregnancy can predict postpartum depression and that its concentrations are negatively correlated with postpartum depressive symptoms.
- ★ It is postulated that an interplay between genetics (diathesis) and environmental (stress)factors contribute to the development of PPD with a heritability cited as high as 40%. In addition to this stress diathesis paradigm hormonal fluctuations and sleep deprivation during the peripartum period are associated with PPD. implicated endocrinological changes include variations in estrogen,progesterone, cortisol, oxytocin, and allopregnanolone- a progesterone derivative. The presenting symptomatology of PPD includes anhedonia, anergia, low mood, suicidality, and disturbances in sleep, appetite, and concentration.

DRUG DISCOVERY

Brexanolone is a medication primarily used to treat postpartum depression {ppd}-It's a medical condition that many women get after having a baby. It's a strong feeling of sadness, anxiety[worry] and tiredness that lasts for a long time after giving birth.

- Its discovery involved extensive research and development . Small molecule, neuroactive steroids GABA-A receptor positive allosteric modulator that was developed by SAGE THERAPEUTICS under licence to the university of CALIFORNIA for the treatment of {PPD} Here's a brief overview of the drug discovery process for brexanolone
- Drug discovery typically begins with the identification of a biological target associated with a specific disease or condition. In the case of brexanolone , researchers focused on the role of the neurotransmitter gamma - aminobutyric acid [GABA] in postpartum depression.
- brexanolone , marketed as zulresso, received FDA approval in march 2019 the treatment of postpartum depression. It's administered intravenously under medical supervision and has shown promise in providing rapid relief for PPD symptoms
- Please note that drug discovery is a complex and lengthy process that can take many years and involves a significant investment in research and development.
- Once clinical trials demonstrate safety and efficacy the pharmaceutical company submits a New Drug Application {NDA} to the U.S. Food and Drug Administered {FDA} or relevant regulatory agencies in other countries. If approved, the drug can be marketed and sold.
- The mechanism of brexanolone involves activation of both synaptic and extrasynaptic GABA-Areceptors, which promote tonic inhibition and serve as a key target for PPD and related conditions.

CHARACTERISATION OF BREXANOLONE

Characterisation of pharmaceutical compounds like brexanolone involves a comprehensive assessment of

its physical, chemical, and biological properties. Here are some aspects of brexanolone's characterisation:

1. **CHEMICAL STRUCTURE:** Determining the exact chemical structure of brexanolone is a fundamental step. This involves techniques such as nuclear magnetic resonance [NMR] spectrometry to confirm its molecular formula and structural arrangement.
2. **PHYSICOCHEMICAL PROPERTIES:** Various physicochemical properties of brexanolone such as solubility, melting point, boiling point and stability under different conditions [eg:Temperature, pH], are studied. These properties help determine its formulation and storage requirements
3. **SPECTROSCOPIC ANALYSIS**: Infrared rays[IR] and ultraviolet [UV] spectroscopy are used to study brexanolone's absorption characteristics in the infrared and uv-visible spectra. These techniques provide information about its functional group and uv absorbance profile.
4. **CHROMATOGRAPH PURITY:** High performance liquid chromatography[HPLC] or other chromatographic methods are employed to assess the purity of brexanolone, ensuring that it meets regulatory standards
5. **STABILITY STUDIES: Stability** testing involves exposing brexanolone to various conditions [eg:temperature humidity] over to evaluate its self life and degradation pathways.
6. **PARTICLE SIZE AND MORPHOLOGY:** The size and morphology of brexanolone particles can impact its formulation and bioavailability. Techniques such as scanning electron microscopy [SEM] are used for particle characterisation.
7. **FORMULATION DEVELOPMENT**: Determining the appropriate formulation for brexanolone, whether it's for intravenous administration as in the case of zulresso or other routes, is part of its characterisation.
8. **BIOAVAILABILITY AND EQUIVALENCE:** For orally administered forms of brexanoone bioavailability studies assess how much of the drug reaches the bloodstream, and bioequivalence studies compare different formulations to ensure they have similar effects.
9. **REGULATORY APPROVAL:** Characterization data , along with clinical trial results, are submitted to regulatory agencies like the FDA for approval. The regulatory process involves rigorous evaluation of the drug's safety and efficacy based on the collected data.

FORMULATION OF BREXANOLONE

★ Brexanolone is aqueous and chemically identical formulation of endogenous allopregnanolone, a neuroactive steroid gamma-aminobutyric acid {GABA}A receptor modulator. The medication given as an infusion administered intravenously in a healthcare facility over a 60-hours period

- 5mg of brexanolone
- 250mg of sulfobutylether -B-cyclodextrin
- 0.265mg of citric acid monohydrate
- 2.57mg of sodium citrate dihydrate
- Water for injection
- Hydrochloride acid or sodium hydroxide may be used during manufacturing to adjust pH.
- Its chemical name is 3α-hydroxy-5α-pregnan-20-one.
- Molecular formula:C21H34O2

PHARMACOKINETIC OF BREXANOLONE

The pharmacokinetics of brexanolone, like any drug, involves its absorption, distribution, metabolism,and elimination within the body. Here's a brief overview of it's pharmacokinetics properties:

1. **ABSORPTION:** Brexanolone is rapidly cleared in the blood and has an oral bioavailability of less than 5%, making it administered as an intravenous infusion, which means it's directly introduced into the bloodstream. This method ensures rapid and complete absorption.
2. **DISTRIBUTION:** After entering the bloodstream, brexanolone is distributed throughout the body, including the central nervous system[CNS] where it exerts its effects. It is approximately 3L/kg. Plasma protein binding is >99% and independent of plasma concentrations. The drug half life is approximately 9 hours and total plasma clearance is approximately 1 L/h/kg.
3. **METABOLISM:** Brexanolone undergoes metabolism by 3 main, non-cyp based routes: keto-reduction, glucuronidation and sulfation.3 major metabolites circulate; however, they are pharmacologically inactive and do not contribute to the efficacy of the drug. in the liver and is converted into various metabolites through enzymatic pathways and metabolites . The only CYP enzyme which had any evidence of inhibition by brexanolone was CYP2C9, and even then there were no clinically significant differences in phenytoin pharmacokinetics when it was used with brexanolone.
4. **ELIMINATION**:

Brexanolone and its metabolites are eliminated primarily through the feces and urine , with 47% of radiolabeled drugs recovered in feces and 42% in urine renal [Kidney] excretion. The exact half-life and clearance rates can vary between individuals.

5. **SPECIAL CONSIDERATIONS**: Brexanolone's pharmacokinetics may be necessary based on these factors.

- Brexanolone has an oral bioavailability of less than 5% in adults.
- Brexanolone is extensively bound to plasma protein approximately 99%.
- It has an elimination half-life of 9 hours.

DRUG DEPOSITION

- The drug is administered through an intravenous [IV] infusion over a 60- hour period. The drug is thought to work by affecting the GABA neurotransmitter system in the brain.

PRECLINICAL TOXICITY STUDIES OF BREXANOLONE

- These studies are typically conducted in animals before a new drug like brexanolone is tested in humans.
- These studies assess the potential adverse effects of the drug on various organs and systems. Brexanolone has a rapid onset of action within 60 hours of infusion and has a sustained response for up to 30 days after infusion. Patients in clinical trials were notafter a 30 days follow up period limiting data on toxicity from long term use . Long term efficacy of brexanolone when compared with other antidepressants, is unknown.
- The manufacturer's labelling describes two cases of accidental overdose. These were due to confusions. Patients recovered after 15 mins of discontinuation without the need for supportive measures. The infusion was resumed, and patients completed treatment. It is recommended to stop the infusion immediately in case of overdose and initiate supportive measures as needed.
- Clinical studies on exogenous allopregnanolone administration have reported sedation, intoxication, flushing and headaches, however, there are no reports of severe adverse events.
- Researchers assess whether brexanolone has the potential to cause damage to an organism's genetic material. This helps determine if the drug might increase the risk of cancer or genetic mutations.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES: These studies evaluate the impact of brexanolone on reproduction and the developing foetus. They help identify potential risks to pregnant women and their unborn children.

CARDIOVASCULAR AND NEUROLOGICAL ASSESSMENT: Given the involvement of the GABA system, specific attention might be given to cardiovascular and neurological effects in animals.

APPLICATIONS OF BREXANOLONE

- It i is used to treat postpartum depression [PPD]
- It can provide rapid relief from the symptoms of PPD, such as mood swings, sadness, and anxiety. It works by affecting certain neurotransmitters in the brain.

BIOANALYTICAL TESTING OF BREXANOLONE

- It involves the quantification and analysis of this medication in biological samples, typically in plasma or serum, to assess its concentration in a patient's body. This type of testing is crucial to ensure proper dosing, efficacy, and safety. Here are some key aspects of bioanalytical testing for brexanolone:

1. **SAMPLE COLLECTION**:Blood staples are collected from patients who have received brexanolone treatment.these samples are usually collected at specific time points after administration.
2. **SAMPLE ANALYSIS**:The collected blood samples are processed to extract the brexanolone and prepare them for analysis. This may involve techniques like centrifugation, protein, precipitation, or liquid-liquid extraction.
3. **ANALYTICAL TECHNIQUES:** High-performance liquid chromatography [HPLC]coupled with mass spectrometry [MS] is commonly used for brexanolone analysis. HPLC separates the compound in the sample, and NS identifies and quantifies brexanolone based on its mass-to-charge ratio.
4. **CALIBRATION STANDARDS**: Known concentrations of brexanolone are used to create calibration standards. These standards are used to establish a calibration curve that is

used to quantify the concentration of brexanolone in patient samples.

5. **QUALITY CONTROL SAMPLES**: Quality control samples with known brexanolone concentrations are analysed alongside patient samples to ensure the accuracy and precision of the assay.
6. **DATA ANALYSIS**: The data generated from the bioanalytical testing is analysed to determine the concentration of brexanolone in the patient samples. This information helps healthcare providers adjust dosages as needed.
7. **REPORTING**: The results of the bioanalytical testing are reported to health care professionals, who use this information to make treatment decisions.
8. **REGULATORY COMPLIANCE: Bioanalytical** testing of brexanolone must adhere to regulatory guidelines and quality assurance standards to ensure the reliability and validity of the results.

CLINICAL TRIALS OF BREXANOLONE

- The first drug approved for clinical use in PPD. in 2021 clinical trials four studies:Two in phaseII and Two in phase lll.BX is the first drug approved for clinical use in PPD. AP's role in postpartum blues was first reported in 2001. Serum AP levels were significantly lower in women with postpartum blues than in those who did not meet PPD criteria. Progesterone levels, however, did not differ significantly between the 2 groups.
- These results provided evidence that maintaining stable ap levels in postpartum women was important . Neurosteroids play a key role in regulating menstrual conditions which promoted the development of NRT,i.e., administering exogenous neurosteroids to levels that activate GABA-A receptors . These studies ultimately led to clinical development of BX IN 2014 , clinical trials were initiated to evaluate the potential use of BX in PPD .
- These studies showed clinically beneficial effects of BX, with improvement in depressive symptoms on the Hamilton Depression Rating Scale[HAM-D] that is consistent with other antidepressants and faster than other treatments. The HAM-D remission criteria , which requires a total score of <10 and>50% reduction, also support the efficacy of bx, additionally , the clinical global impression of improvement[CGI-I] showed statistically significant and clinically meaningful differences in BX from the placebo.The first phase II study enrolled 21 women with severe PPD—defined in the study as a Hamilton Depression Rating Scale (HAM-D) compared mean score reductions between the 10 subjects receiving brexanolone and 11 controls receiving placebo .They found that those receiving brexanolone experienced a mean reduction of 21 points, compared with 8.8 points for those receiving placebo. After 60 hours, seven of the 10 women receiving brexanolone had remission of symptoms. No serious adverse events, discontinuations of the trial, or deaths of participants were reported. Although eight of the 11 women receiving placebo experienced adverse events, only four of the 10 women receiving brexanolone reported the symptoms of dizziness and somnolence .
- Thereafter, two phase III trials ensued). Of the 138 women in one study, 45 were assigned to receive intravenous brexanolone 60 µg/kg/hour, 47 to receive intravenous brexanolone 90 µg/kg/hour, and 46 to receive placebo. After 60 hours, the least-squares mean reductions in the HAM-D score were 19.5 (SE=1.2), 17.7 (SE=1.2), and 14.0 (SE=1.1), respectively . The second phase III study enrolled 108 participants randomly assigned to either intravenous brexanolone 90 µg/kg/hour or placebo . In contrast to a least-squares mean reduction in the HAM-D score of 12.1 among those receiving placebo, a 14.6 (SE=0.8) decrease was observed among those receiving brexanolone

NEW APPROACHES IN DRUG DISCOVERY HIGH THROUGHPUT SCREENING

- The GABAA1-CHO cell line stably expressing α1β2γ2L was constituted by cotransfection of α1, β2 and γ2L subunits into CHO-T-Rex cells. The high-throughput screening method of membrane potential targeting GABAAR was established and optimised. The optimised method was used to screen the compound library, and the compounds with high activity were obtained.
- The active compounds were confirmed in vitro by electrophysiological detection technique, and the sleep effects of compounds in vivo were detected by pentobarbital sodium sleep model in mice.
- Results: A stable cell line expressing human GABAA1 receptor in CHO-T-Rex cells was generated and used to establish a functional high-throughput screening assay based on the

measurement of membrane potential changes in living cells by fluorometric imaging plate reader (FLIPR). The assay was further used to detect the dose-effect relationships of tool compounds, the EC50 values of agonist GABA (137.42 ± 26.31 nM), positive allosteric modulator diazepam (3.22 ± 0.73 μM), and antagonist gabazine (0.16 ± 0.04 μM), blocking agents bicuculline (0.47 ± 0.06 μM) and PTX (6.39 ± 1.17 μM).

- In the meanwhile, the compounds were screened from a compound library (10000) by the membrane potential dye assay. Selected 4 active compounds were further identified for their EC50 values in vitro by electrophysiological method, the EC50 values of 4 compounds were further determined as 1.37 ± 0.43 μM, 0.69 ± 0.17 μM, 0.77 ± 0.16 μM, and 1.62 ± 0.29 μM. Furthermore, the pentobarbital sleep rate and the sleep time of mice pretreated with 4 active compounds by oral administration were significantly increased compared with mice pretreated with a negative control in vivo experiment.

[CPCSEA] GUIDELINES FOR THE USE OF THE CARE AND USE OF LABORATORY ANIMALS

Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and safety of animals used in laboratory studies while conducting biomedical and behavioural research and testing of products.

GOAL: The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioural research and testing with the basic objective of providing specifications that will enhance animal well being, quality in the pursuit of advancement of biological knowledge that is relevant to humans and animals.

VETERINARY CARE: Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine.

ANIMAL PROCUREMENT

All animals must be acquired lawfully as per the CPCSEA guidelines.

- A health surveillance program for screening incoming animals should be carried out to assess animal quality. Methods of transportation should also be considered (Annexure – 4).
- Each consignment of animals should be inspected for compliance with procurement specifications, and the animals should be quarantined and stabilised according to procedures appropriate for the species and circumstance.

QUARANTINE, STABILISATION AND SEPARATION

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimises the chance for introduction of pathogens into an established colony. Minimum duration of quarantine for small lab animals is one week and larger animals is 6 weeks (cat, dog and monkey)

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE All animals should be observed for signs of illness, injury, or abnormal behaviour by animal house staff. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis (Annexure 1 and 2)

- Unexpected deaths and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony.

ANIMAL CARE AND TECHNICAL PERSONNEL

- Animal care programs require technical and husbandry support. Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training to ensure effective implementation of the program .

PERSONAL HYGIENE

- It is essential that the animal care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided e.g. showers, change of uniforms, footwears etc.
- Clothing suitable for use in the animal facility should be supplied and laundered by the institution.

- A commercial laundering service is acceptable in many situations; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. In some circumstances, it is acceptable to use disposable wear such as gloves, masks, head covers, coats, coveralls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene.
- Outer garments worn in the animal rooms should not be worn outside the animal facility. Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics in animal rooms.

ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

- Institutions should have policies governing experimentation with hazardous agents. Institutional Biosafety Committee whose members are knowledgeable about hazardous agents are in place in most of the higher level education, research institutes and in many pharmaceutical industries for safety issues.
- This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions Since the use of animals in such studies requires special consideration, the procedures and the facilities to be used must be reviewed by both the Institutional Biosafety Committee and Institutional Animal Ethics Committee (IAEC).

MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

- Multiple surgical procedures on a single animal for any testing or experiment are not to be practised unless specified in a protocol only approved by the IAEC.

DURATIONS OF EXPERIMENTS

- No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

PHYSICAL RESTRAINT

- Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimise stress and avoid injury to the animal.

PHYSICAL RELATIONSHIP OF ANIMAL FACILITIES TO LABORATORIES

- Good animal husbandry and human comfort and health protection require separation of animal facilities from personnel areas such as offices, conference rooms, and most laboratories.
- Laboratory animals are very sensitive to their living conditions. It is important that they shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds.
- The building, cages and environment of animal rooms are the major factors, which affect the quality of animals.
- The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilised for services such as stores, washing, office and staff, machine rooms, quarantine and corridors.

SPECIALIZED LABORATORIES

- Pharmaceuticals and biologics and supplies
- Space for administration, supervision and direction of the facility
- Showers, sinks, lockers and toilets for personnel
- An area for washing and sterilisation of equipment and supplies,
- An autoclave for equipment
- Food and bedding and separate areas
- For holding soiled and unclean equipment
- An area for repairing cages and equipment
- An area to store waste prior to incineration or removal

PHYSICAL FACILITIES

a) Building materials

- It should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

b) Corridors(s)

- It should be wide enough to facilitate the movement of personnel as well as equipment and should be kept clean.

C)Utilities

- Such as water lines, drains pipes and electrical connections should preferably be

accessible through service panels or shafts in corridors outside the animal rooms.

d)Animal rooms doors

- Doors should be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

(e) Exterior windows

- Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate sources of light and ventilation. In primate rooms, windows can be provided.

(f) Floors

- Floors should be smooth, moisture proof, nonabsorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with a minimum number of joins.

(g)Drains

- Floor drains are not essential in all rooms used exclusively for housing rodents. The floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying

h) Walls and ceilings

- Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners. Surface materials should be capable of withstanding scrubbing with detergents and disinfectants and the impact of water under high pressure.

i)Storage areas

- Separate storage areas should be designed for feed, bedding, cages and materials not in use. Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste.

ENVIRONMENT

a) Temperature and humidity control

- Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions or differences in the number and kind of room occupants. Ideally, capability should be provided to allow variations within the range of approximately 18 to 29°C (64.4 to 84.2øF), which includes the temperature ranges usually recommended for common laboratory animals.

(b) Ventilation

- In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals primary enclosures
- Heating, ventilation ,and air-conditioning systems should be designed so that operation can be continued with a stand by system. The animal facility and human occupancy areas should be ventilated separately.

(c) Power and lighting

- The electrical system should be safe and provide appropriate lighting and a sufficient number of power outlets. It is suggested that a lighting system be installed that provides adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals.

(d) Noise control

- The facility should be provided with a noise free environment. Noise control is an important consideration in designing an animal facility. Concrete walls are more effective than metal or plaster walls in containing noise because their density reduces sound transmission.

ANIMAL HUSBANDRY

a) Caging or housing system The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal well being, meet research , and minimise experimental variables.

- The housing system should: provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species;
- provide a comfortable environment
- provide an escape proof enclosure that confines
- keep the animals dry and clean, consistent with species requirements

facilitate research while maintaining good health of the animals.

(b) Sheltered or outdoor housing

- When animals are maintained in outdoor runs, pens, or other large enclosures, there must be protection from extremes in temperature or other harsh weather conditions and adequate protective and escape mechanisms for submissive animals, as in case of monkeys by way of an indoor portion of a run, should be provided. Shelter should be accessible to all animals, have sufficient ventilation, and be designed to prevent build up of waste materials and excessive moisture.
- Houses, dens, boxes, shelves, perches, and other furnishings should be constructed in a manner and made of materials that allow cleaning or replacement in accordance with generally accepted husbandry practices

(c) Social environment

- The social environment includes all interactions among individuals of a group or among those able to communicate. The effects of the social environment on caged animals vary with the species and experience of the animals. In selecting a suitable social environment, attention should be given to whether the animals are naturally territorial or communal and whether they will be housed singly or in groups. When appropriate, group housing should be considered for communal animals.

ACTIVITY

- Provision should be made for animals with specialised locomotor patterns to express these patterns, especially when the animals are held for long periods. For e.g., ropes, bars, and perches are appropriate for branching jon-human primates. Cages are often used for short-term (up to 3 months) housing of dogs and may be necessary for postsurgical care, isolation of sick dogs, and metabolic studies. Pens, runs, or other out-of-cage space provide more opportunity for exercise, and their use is encouraged when holding dogs for long periods

FOOD

- Animals should be fed palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise
- . Feeders should allow easy access to food, while avoiding contamination by urine and faeces.
- Food should be available in amounts sufficient to ensure normal growth in immatureness, and behaviour. Group composition should be held as stable as possible, particularly for canine, non-human primates, and other highly social mammals, because animals and maintenance of normal body weight, reproduction, and lactation in adults.
- Food should contain adequate nutrition, including formulation and preparation; freedom from chemical and microbial contaminants; bioavailability of nutrients should be at par with the nutritional requirement of the animal.

BEDDING

- Bedding should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes.

WATER

- Ordinarily animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their particular requirements. Periodic monitoring of microbial contamination in water is necessary. Watering devices, such as drinking tubes and automatic waterers if used should be examined routinely to ensure their proper operation . Sometimes it is necessary to train animals to use automatic watering devices. It is better to replace water bottles than to refill them, however, if bottles are refilled care should be taken that each bottle is replaced on the cage in which it was removed.

SANITATION AND CLEANLINESS

- Sanitation is essential in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris , and harmful contamination. Cleaning utensils, such as mops, pails, and brooms, should not be transported between animal rooms.
- Where animal waste is removed by hosting or flushing, this should be done at least twice a day. Animals should be kept dry during such procedures. For larger animals, such as dogs, cats, and non-human primates, soiled litter material should be removed twice daily. Cages should be sanitised before animals are placed in them.
- Animal cages, racks, and accessory equipment, such as feeders and watering devices, should be washed and sanitised frequently to keep them clean and contamination free.
- Rodent cages for all other animals should be washed at least every 2 weeks. It is good

practice to have extra cages available at all times so that a systematic cage-washing schedule can be maintained.
- Cages can be disinfected by rinsing at a temperature of 82.2oC (180oF) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms.

ASSESSING THE EFFECTIVENESS OF SANITATION
- Monitoring of sanitation practices should be appropriate to the process and materials being cleaned; it can include visual inspection of the materials, monitoring of water temperatures, or microbiological monitoring.
- The intensity of animal odours, particularly that of ammonia, should not be used as the sole means of assessing the effectiveness of the sanitation program
- A decision to alter the frequency of cage – bedding changes or cage – washing should be based on such factors as the concentration of ammonia, the appearance of the cage, the condition of the bedding and the number and size of animals housed in the cage.

WASTE DISPOSAL
- Wastes should be removed regularly and frequently. All waste should be collected and disposed of in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Incinerators should be in compliance with all central , state and local regulations.
- Hazardous wastes should be rendered safe by sterilisation, contamination, or other appropriate means before they are removed from an animal facility for disposal.

PEST CONTROL
- Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in an animal environment.

EMERGENCY, WEEKEND AND HOLIDAY CARE
- Animals should be cared for by qualified personnel every day, including weekends and holidays, to safeguards their well-being including emergency veterinary care. In the event of an emergency, institutional security personnel and fire or police officials should be able to reach people responsible for the animals.
- That can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animal facilities or by placing them in the security department

RECORD KEEPING

The animal house should maintain the following records:
- Animal house plans, which includes typical floor plans, all fixtures etc.
- Animal house staff record-both technical and non-technical
- Health record of staff/ animals
- All standard operating procedures (SOPs) relevant to the animals
- Breeding, stock, purchase and sales records
- Minutes of institute Animals Ethics Committee Meetings
- Records of experiments conducted with the number of animals used (copy of Form)
- Death Record
- Clinical record of sick animals

STANDARD OPERATING PROCEDURES (SOPs)/GUIDELINES

The Institute shall maintain SOPs describing procedures / methods adapted about animal husbandry, maintenance, breeding, animal house microbial analysis and experimentation records.

A SOP should contain the following items:
- Name of the Author
- Title of the SOP
- Date of preparation
- Reference of previous SOP on the same subject and date (Issue no and Date)
- Location and distribution of SOPs with sign of each recipient
- Objectives
- Detailed information of the instruments used in relation with animals with methodology (Model no., Serial no. and Date of commissioning)
- The name of the manufacturer of the reagents and the methodology of the analysis pertaining to animals
- Normal value of all parameters
- Hazard identification and risk assessment

PERSONNEL AND TRAINING
- The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility. The staff must be provided with all required protective clothing (masks, aprons,

gloves and gumboots and other footwear) while working in animal rooms. Facilities should be provided for change over with lockers, wash basin, toilets and bathrooms to maintain personal hygiene.

- It is also important that a regular medical check-up is arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the animals.

TRANSPORT OF LABORATORY ANIMALS

The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are, the mode of transport, the containers, the animal density in cages, food and water during transit, protection from transit infections, injuries and stress. The mode of transport of animals depends on the distance, seasonal and climatic conditions and the species of animals

- . Animals can be transported by road, rail or air taking into consideration the above factors.

ANAESTHESIA AND EUTHANASIA

The scientists should ensure that the procedures, which are considered painful, are conducted under appropriate anaesthesia as recommended for each species of animal. It must also be ensured that the anaesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the experiment.

Anaesthesia

- Unless contrary to the achievement of the results of study, sedatives, analgesics and anaesthetics should be used to control pain or distress under experiment. Anaesthetic agents generally affect cardiovascular, respiratory and thermo-regulatory mechanisms in addition to the central nervous system.
- Before using actual anaesthetics the animal is prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of the cardio-pulmonary system and reduce salivary secretion. Atropine is the most commonly used anticholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure.
- Local anaesthetics are used to block the nerve supply to a limited area and are used only for minor and rapid procedures. This should be carried out under expert supervision for regional infiltration of surgical sites, nerve blocks and for epidural and spinal anaesthesia.
- A number of general anaesthetic agents are used in the form of inhalants. General anaesthetics are also used in the form of intravenous or intramuscular injections such as barbiturates. Species characteristics and variation must be kept in mind while using an anaesthetic.
- Side effects such as excessive salivation, convulsions, excitement and disorientation should be suitably prevented and controlled. The animal should remain under veterinary care till it completely recovers from anaesthesia and postoperative stress.

Euthanasia

- Euthanasia is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety.
- For accepting an euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal to be killed The method should in all cases meet the following requirements:
  (a) Death, without causing anxiety, pain or distress with minimum time lag phase.
  (b) Minimum physiological and psychological disturbance.
  (c) Compatibility with the purpose of study and minimum emotional effect on the operator.
  (d) Location should be separate from animal rooms and free from environmental contaminants. Tranquillisers have to be administered to larger species such as monkeys, dogs and cats before an euthanasia procedure.

PRECLINICAL STUDY

- Unlike reproductive steroids, neurosteroids do not cause hormonal activity but do have rapid nervous system effects, including hypnotic, anxiolytic, and anticonvulsant actions . In 1986, the adrenal-derived neurosteroid tetrahydro-deoxycorticosterone (THDOC) was shown to interact with the GABA-A receptor in a manner similar to barbiturates, allowing benzodiazepines, leading to faster receptor binding . This activity prompted researchers to analyse THDOC more thoroughly, as its behavioural effects are similar to those of

established anxiolytics . and the preclinical anxiolytic profile of BX is outlined in Initial attempts to study the anxiolytic effects of BX showed that animals in stressful environments had lower serum AP levels . This observation was originally thought to be a result of stress, as opposed to its cause, but decades of neurosteroid research proved this to be untrue. Bitran and team reported anxiolytic effects after treating ovariectomized rats with progesterone and AP . While studying the mechanism by which progesterone alters behavioural anxiety phenotypes, they found that progesterone-induced anxiolytic responses correlated highly with increased AP levels in the blood and brain. It was thought that a reduction in the amount of AP is the cause of excessive anxiety and excitability. However, the precise mechanism remained unexplored for years.

- In 1996, fluoxetine was shown to increase local AP levels in the brain but not in plasma. Anxiolytic effects were observed in animals treated with fluoxetine, and while researchers did not credit these observations to increased AP levels, they concluded that AP activation could be critical to the anxiolytic and anti-dysphoric effects of fluoxetine. In 1997, we evaluated the differential anxiolytic effect of neurosteroids using a mirrored chamber test of anxiety following injections of AP, progesterone, and 4′-chlordiazepam, a specific ligand for the mitochondrial diazepam-binding inhibitor receptor. A clear and robust dose-dependent anxiolytic response was seen in animals treated with progesterone or AP. The anxiolytic effects of progesterone occur when it is converted to neurosteroids in the brain . Extensive studies conducted by our lab and others on psychopharmacological effects of neurosteroids have paved the way for further research on the pharmaceutical potential of neurosteroids to treat depressive conditionsWe developed neurosteroid replacement therapy (NRT) as a rational approach for treating PPD and other conditions related to neurosteroid deficiency, unveiling the power of neurosteroids as novel anxiolytic-antidepressants. The neurosteroid, brexanolone (BX), is a progesterone-derived allopregnanolone that rapidly relieves anxiety and mood deficits by activating GABA-A receptors, making it a transformational treatment for PPD.
- In a brain slice preparation, zuranolone produced a sustained increase in GABA currents consistent with metabotropic trafficking of GABAA receptors to the cell surface. In vivo, zuranolone exhibited potent activity, indicating its ability to modulate GABAA receptors in the central nervous system after oral dosing by protecting against chemo-convulsant seizures in a mouse model and enhancing electroencephalogram β-frequency power in rats. Together, these data establish zuranolone as a potent and efficacious neuroactive steroid GABAA receptor positive allosteric modulator with drug-like properties and CNS exposure in preclinical models. Recent clinical data support the therapeutic promise of neuroactive steroid GABAA receptor positive modulators for treating mood disorders; brexanolone is the first therapeutic approved specifically for the treatment of postpartum depressionLevels of allopregnanolone, the major metabolite of progesterone, increase commensurate with the rise in progesterone during pregnancy, with highest physiologic levels in the third trimester , and then subsequently decline precipitously after childbirth, when progesterone levels decrease . Allopregnanolone is a neuroactive steroid that has been shown in animal models to modulate neuronal excitability through direct action on synaptic and extrasynaptic GABAA receptors . It appears to play a significant role in affective disturbances that occur with changes in reproductive endocrine function, such as during the postpartum period .

➔ Failure of GABAA receptors to adapt to abrupt changes in allopregnanolone levels at parturition may play a part in triggering PPD . Allopregnanolone has been implicated in preclinical models of anxiolysis and mood improvement, with evidence that elevated allopregnanolone levels may protect against depressed mood during pregnancy .Preclinical data suggest anxiogenic effects of low allopregnanolone concentrations . On the basis of preclinical models, we hypothesise that the abrupt postpartum decline in allopregnanolone may be associated with symptoms of PPD. This supports the rationale for examining the effects of treatment of PPD patients with therapeutic doses of allopregnanolone equivalent to third trimester levelAlthough selective serotonin reuptake inhibitors are commonly used as first-line PPD treatment, there is limited evidence for their use in the postpartum period specifically, and the proportion of PPD patients treated successfully with SSRIs has a wide range .

Considering the pathophysiology of depression in the perinatal period and the negative consequences of untreated PPD, development of efficacious new treatments with more targeted mechanisms of action is warranted. Brexanolone , a proprietary, aqueous formulation of allopregnanolone in sulfobutylether-β-cyclodextrin, was evaluated in an open-label, proof-of-concept study for treatment of severe PPD, with the primary objective of evaluating safety and tolerability. Secondary and exploratory objectives included assessment of the effects of brexanolone on depression and anxiety symptoms.

➔ This open-label, proof-of-concept study enrolled healthy females 18–45 years old admitted to the University of North Carolina at Chapel Hill Perinatal Psychiatry Inpatient Unit (PPIU) for a major depressive episode beginning no earlier than the third trimester and no later than 12 weeks following delivery. Admission to the PPIU occurred 14 days to 20 weeks postpartum. At the screening visit, a score of ≥20 on the 17-item Hamilton Rating Scale for Depression (HAMD; Hamilton, 1960) was required for inclusion. If patients were taking antidepressants for longer than 2 weeks on a stable dose, they were allowed to continue their medications during the brexanolone dosing period. Two patients were taking sertraline (50 and 100 mg) during brexanolone administration. Three of the women had prior PPD episodes, and two also experienced prior major depressive episodes outside of the peripartum period.

➔ All women participated in the standard PPIU program, which included psychotherapy and has been previously described . Permanent weaning from breastfeeding prior to Visit 1 was required. Written informed consent was obtained prior to screening, and the study was approved by the UNC institutional review board. The first patient was enrolled January 9, 2015; last patient follow-up was June 8, 2015.

BIOSTATISTICS PRECLINICAL STUDY

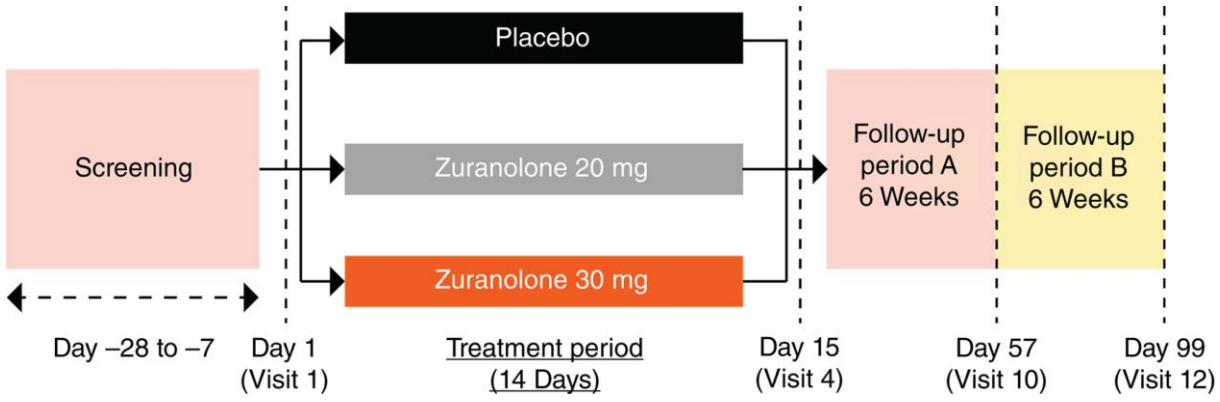

fig:3

Patients receiving BRX90 (n = 102) versus placebo (n = 107) achieved a more rapid HAMD-17 response (median, 24 vs 36 h; p = 0.0265), with an Hour-60 cumulative response rate of 81.4 % versus 67.3 %; results were similarDescriptive statistics were calculated and summarised for all endpoints. Baseline values were defined as the last value prior to study drug infusion. Primary outcomes were safety and tolerability measures, including treatment-emergent AEs and changes from baseline in physical and psychiatric evaluations. Secondary outcomes were assessments of efficacy. The key efficacy measure was change from baseline in HAMD total score. A paired t test was performed to evaluate whether the change in HAMD from baseline to the end of infusion (Hour 60) was nonzero. Exploratory endpoints included EPDS, GAD-7, PHQ-9, and Stanford Sleepiness Scale scores

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