Protocol

Acute Effect of Lemborexant on CSF Amyloid-Beta and Tau

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Protocol Title: Acute Effect of Lemborexant on CSF Amyloid-Beta and Tau (Lemborexant Trial)

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IRB SUMMARY OF REVISIONS

Date	Protocol Version	Regulatory Submission	Revision History
10/10/2022	2	IRB initial review	Protocol V2 (9.28.2022) - Added additional information to section 3.4 Interim Analysis - Updated section 8.1.4 Physical Exam - Updated the safety related criteria for stopping study under section 13.2 Plan for Adverse Events - Changed safety labs listed on the summary of events table. - Blood sampling change from 6 to 12 ml.
06/08/2023	3	IRB Amendment 1	Protocol V3 (06.08.2023) - Clarified bedtime and study drug administration time at V4 - Moved APOE sample collection from V3 to V1 due to processing time constraints - Included intent to share participant data with ADRC/SEABIRD studies - Included data shared with study team by SEABIRD and ADRC at time of referral - Updated exclusion criteria to include marijuana use at PI's discretion - Added information on participant referrals/recruitment from ADRC - Changed time point for PSQI administration to occur at prescreen or Visit 1 - Updated lemborexant drug interactions - Informed consent form (ICF) V3 (06.08.2023) ▪ Updated the ICF's purpose to include the current FDA approved dosages for lemborexant the dose used in this research. ▪ Added ADRC data sharing language

			▪ Moved APOE sample collection from V3 to V1 ▪ Included a Schedule of Events to enhance participant understanding of what each visit entails ▪ Updates to correct spelling & formatting to improve readability

Protocol Title:
Acute Effect of Lemborexant on CSF Amyloid-Beta and Tau
(Lemborexant trial)

Signature: _____ **Date**: _____

Brendan P. Lucey, MD, MSCI
Principal Investigator

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SUB-INVESTIGATORS

None

ABBREVIATIONS

AD- Alzheimer's Disease
ADL- Activities of Daily Living
ADRC- Knight Alzheimer's Disease Research Center
AE- Adverse event
AIC- Akaike information criterion
APOE- Apolipoprotein Epsilon
APP- Amyloid Precursor Protein
Aβ- Beta Amyloid
CNS- Central Nervous System
CSF- Cerebral Spinal Fluid
C-SSRS- Columbia Suicide Severity Rating Scale
CTRU- Clinical Translational Research Unit
DORA- Dual Orexin Receptor Antagonist
DSMC- Data Safety Monitoring Committee
DSM- Data Safety Monitoring
ECG- Electrocardiogram
EEG- Electroencephalogram
FDA- Food and Drug Administration
GABA- Gamma-Aminobutyric Acid
GUID- Global Unique Identifier
HRPO- Human Research Protection Office
ICTS- Institute of Clinical and Translational Sciences
IMEs-Important Medical Events
IND- Investigational New Drug
IRB- Institutional Review Board
ISI- Insomnia Severity Index
LEM- Lemborexant
LME- Linear Mixed Effect
MAD- Multiple Ascending Dose
MEQ- Morning/Eveningness Questionnaire
MMRM- Mixed Model for Repeated Measures
MMSE- Mini Mental Status Examination
NCRU- NeuroClinical Research Unit
NCT- National Clinical Trial
NIH- National Institute of Health
OSA- Obstructive Sleep Apnea
OXRI- Orexin Receptor 1
OXR2- Orexin Receptor 2
PBO-Placebo
PD- Pharmacodynamic

PET- Positron Emission Tomography
PHI- Protected Health Information
PI- Principal Investigator
PK- Pharmacokinetics
PSG- Polysomnography
PSQI- Pittsburgh Sleep Quality Index
PVT- Psychomotor Vigilance Testing
SAE- Serious Adverse Event
SAAMII- Simulation Analysis and Modeling
SEABIRD- Study to Evaluate Amyloid in Blood and Imaging Related to Dementia
SILK- Stable Isotope Labeling Kinetics
SOP- Standard Operating Procedures
SSS- Stanford Sleepiness Scale
TEAE Treatment Emergent Adverse Event
UP- Unanticipated Problem
VFH- Volunteers for Health
WU- Washington University

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1. BACKGROUND AND SIGNIFICANCE

1.1 Background:
Alzheimer's disease (AD) is characterized by deposition of amyloid-β (Aβ) as insoluble plaque (i.e., amyloid-positive), tau aggregation and hyperphosphorylation, increased immune response, neuronal degeneration, synaptic loss, and eventual cognitive dysfunction, dementia, and death. Soluble forms of Aβ and tau, proteins critical to Alzheimer's disease pathogenesis, change in cerebrospinal fluid (CSF) with sleep-wake activity: 1) CSF Aβ and tau increase during wakefulness and decrease during sleep in both mice[1] and humans[2]; 2) overnight sleep deprivation increases CSF Aβ and tau levels by ≥30% via increased production/release[3-5]; 3) tau hyperphosphorylation (p-tau), an early step in tau-mediated neurodegeneration, is also affected by sleep deprivation depending on the specific site of phosphorylation[5]. Based on these findings, sleep disturbances are recognized as a potentially modifiable risk factor for Alzheimer's disease[6, 7].

1.2 The Orexin System:
Orexin-A and orexin-B (also known as hypocretin-1 and hypocretin-2) are two neuropeptides of 33 and 28 amino acids encoded by a common precursor polypeptide, prepro-orexin[8]. Neurons producing orexin are exclusively localized to the perifornical area and the lateral and posterior hypothalamic area and project to the brainstem nuclei, amygdala, hippocampus, and cerebral cortex[9-12]. Orexins bind to two G protein-coupled receptors, orexin receptor 1 (OXR1) and orexin receptor 2 (OXR2)[8]. The orexin system regulates sleep-wake activity, feeding behavior, energy homeostasis, and the reward system[8]. Orexins are wake-promoting neuropeptides and orexin deficiency causes narcolepsy, a sleep disorder resulting in excessive daytime sleepiness, sleep paralysis, sleep-related hallucinations, and cataplexy. Further, blockade of orexin function with a dual orexin receptor antagonist (DORA) increases sleep. Based on its role in narcolepsy pathophysiology, DORAs have been pursued as a treatment of insomnia.

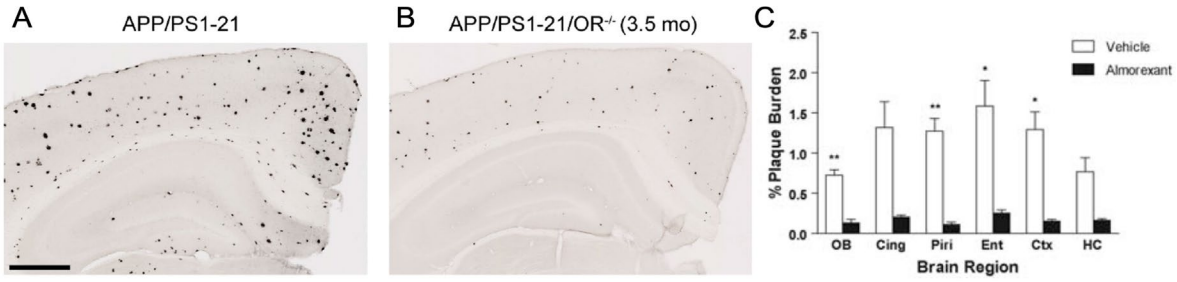


Figure 1. Marked reduction of amyloid pathology in mice when orexin is reduced. The difference in amyloid pathology between APP/PS1-21 mice (A) and APP/PS1-21 orexin knock out mice (B) is shown. A dual orexin receptor antagonist, almorexant, administered to mice daily for 8 weeks via intraperitoneal injection significantly decreased amyloid deposition in the brain (C). Panels A and B from: Roh JH, et al., J Exp Med. 2014; 211: 2487. Panel C from: Kang JE, et al., Science. 2009; 326: 1005.

1.3 Orexin and Alzheimer's Disease:
Substantial evidence supports a role for the orexin system in the development of amyloid deposition. Knocking out the orexin gene in amyloid precursor protein (APP) transgenic mice led to a marked decrease in amyloid pathology in the brain while over-expression of

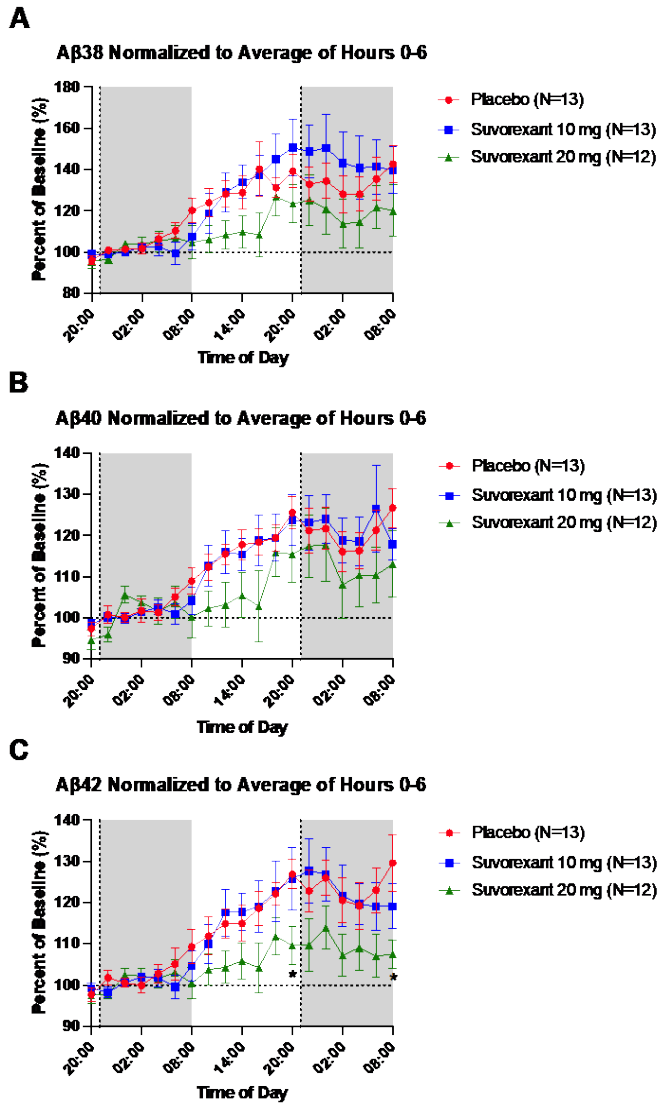


Figure 2: Effect of suvorexant on cerebrospinal fluid (CSF) Aβ. Participants received placebo, suvorexant 10 mg, or suvorexant 20 mg. In all graphs, Aβ concentrations at each time are normalized to the average concentration of hours 0-6. Aβ38 (A), Aβ40 (B), and Aβ42 (C) show that the suvorexant 20 mg group is decreased compared to placebo. Blue: placebo; Red: suvorexant 10 mg; Green: suvorexant 20 mg. Error bars indicate standard error. The vertical dashed lines show when placebo/drug was administered. The horizontal dashed line is at 100% of baseline. Shaded area is 08:00-20:00. *p<0.05 after Bonferroni correction. Unpublished data.

orexin in the hippocampus did not alter amyloid pathology[13] (Fig 1A-B). Studies in APP transgenic mice that develop amyloid deposition found that treatment with a DORA, almorexant, decreased soluble Aβ concentrations while intra-cerebroventricular administration of orexin increased them[1]. Further, prolonged treatment with almorexant for 8 weeks decreased amyloid deposition (Fig 1C); this effect was recently replicated in mice with suvorexant[14]. In humans, patients with narcolepsy (i.e., with orexin deficiency) have reduced CSF Aβ, tau, p-tau, and amyloid deposition on amyloid PET compared to age- and sex-matched controls[15, 16]. Finally, there are no preclinical studies in animal models or human clinical studies showing that other sleep-inducing drugs (e.g., zolpidem, sodium oxybate) affect CSF AD biomarkers. These findings strongly suggest that blocking orexin will modulate amyloid pathology in the brain.

1.4 Preliminary Data:

Our preliminary data shows that a first-in-class DORA, suvorexant, acutely decreases tau phosphorylation and Aβ in human CSF within hours. In this study, cognitively normal adults aged 45-65 years old were screened for poor sleep quality (defined by sleep efficiency <85% as measured by actigraphy) and then randomized to suvorexant 10 mg, suvorexant 20 mg, or placebo. After admission to our Clinical Translational Research Unit (CTRU) on Day 1, an in-dwelling lumbar catheter was placed at ~8pm and CSF sampled every 2 hours for 36 hours. Participants received their blinded tablet at 9pm and the lights were turned off so they could sleep as they are able. Upon waking on Day 2 (~6-7am), participants remained awake until ~9pm when they received their second

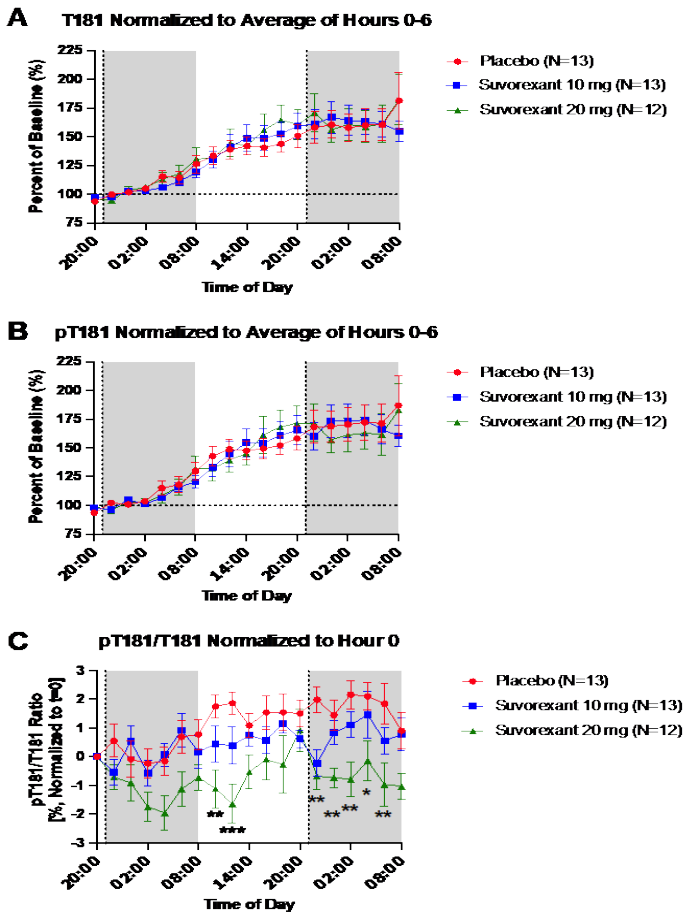


Figure 3: Effect of suvorexant on cerebrospinal fluid (CSF) tau threonine-181 (T181) phosphorylated T181 (pT181). Participants received placebo, suvorexant 10 mg, or suvorexant 20 mg. In A and B, concentrations at each time are normalized to the average concentration of hours 0-6. There is no difference between groups for T181 (A) or pT181 (B). The pT181/T181 ratio was normalized to the hour zero concentration (C) shows that the suvorexant 20 mg group is decreased compared to placebo. Blue: placebo; Red: suvorexant 10 mg; Green: suvorexant 20 mg. Error bars indicate standard error. The vertical dashed lines show when placebo/drug was administered. The horizontal dashed line is at 100% of baseline. Shaded area is 08:00-20:00. *p<0.05; **p<0.01; ***p<0.001 after Bonferroni correction. Unpublished data.

blinded tablet; there is no change in treatment with the second tablet. At hour 36 on Day 3 (~8am), the lumbar catheter was removed and the study ended. There was no sleep manipulation other than the intervention conditions. Thirteen participants completed the placebo group, thirteen participants completed the suvorexant 10 mg group, and twelve participants completed the suvorexant 20 mg group. We normalized the average Aβ concentration to hours 0-6 because these time points do not represent the effect of the intervention due to the ~5 hour transit time from the brain to the lumbar catheter[2, 3]. We found that CSF Aβ38, Aβ40, and Aβ42 concentrations decreased in the suvorexant 20 mg group compared to the placebo group (Fig 2). For Aβ42, the suvorexant 20 mg group was significantly decreased after Bonferroni correction for multiple comparisons at hour 24 (17.33% mean difference, 95% confidence intervals 1.852-32.8, p=0.0253) and 36 (22.12% mean difference, 95% confidence intervals 0.79-43.45, p=0.041).

We also measured different forms of tau and phosphorylated tau. The unphosphorylated tau forms of threonine-181 (T181), serine-202 (S202, and threonine-217 (T217) as well as the phosphorylated forms of T181 (pT181), S202 (pS202), and T217 (pT217) were normalized to the average concentration of hours 0-6 as described above. There were no significant differences between groups after Bonferroni correction for multiple comparisons (Fig 3, 4, 5). However, there was a significant decrease of the pT181/T181 ratio in the suvorexant 20 mg group compared to placebo (Fig 3C). The pTau/Tau ratios indicate the occupancy rate of tau phosphorylation[17] and are a measure of the phosphorylation rate. pS202/S202 shows a trend toward decreasing in suvorexant group compared to placebo but is not significant. pT217/T217 shows no difference between groups. pS202/S202 has a lower occupancy rate than pT181/T181 and pT217/T217 has a lower occupancy rate than pS202/S202 suggesting that prolonged treatment or increase occupancy such as is seen in amyloidosis would result in a larger effect.

1.5 Study Rationale:

Prior studies showed that sleep loss increased CSF Aβ and tau in humans[3-5, 18, 19], however treatment with sodium oxybate (a GABA-B receptor agonist) did not decrease CSF Aβ or tau compared to controls[3]. Our preliminary data shows that treatment with suvorexant, a DORA, will decrease the CSF pT181/T181 phosphorylation rate and Aβ concentration acutely over hours. These findings and the studies performed in animal models suggest that the beneficial effects measured by orexin receptor blockade are not simply due to sedation or sleep and depends on specific neurotransmitter networks. Treatment with DORAs may lead to lower CSF levels of Aβ and tau phosphorylation, and therefore decreased deposition of Aβ as insoluble plaques, decrease development of tauopathy, and ultimately lower the risk of AD. However, we do not know if the effect on CSF tau phosphorylation and Aβ is specific to suvorexant. A second DORA, lemborexant, was recently approved by the Food and Drug Administration[20] and has not been shown to decrease CSF pT181/T181 and Aβ. **We hypothesize that the effect of lemborexant 25 mg on CSF pT181/T181 and Aβ will be similar to or greater than suvorexant 20 mg compared to placebo**. Understanding if lemborexant has a similar effect on CSF Alzheimer biomarkers is critical to designing future clinical trials with the ultimate goal of designing a phase III clinical trials using lemborexant to prevent or delay AD.

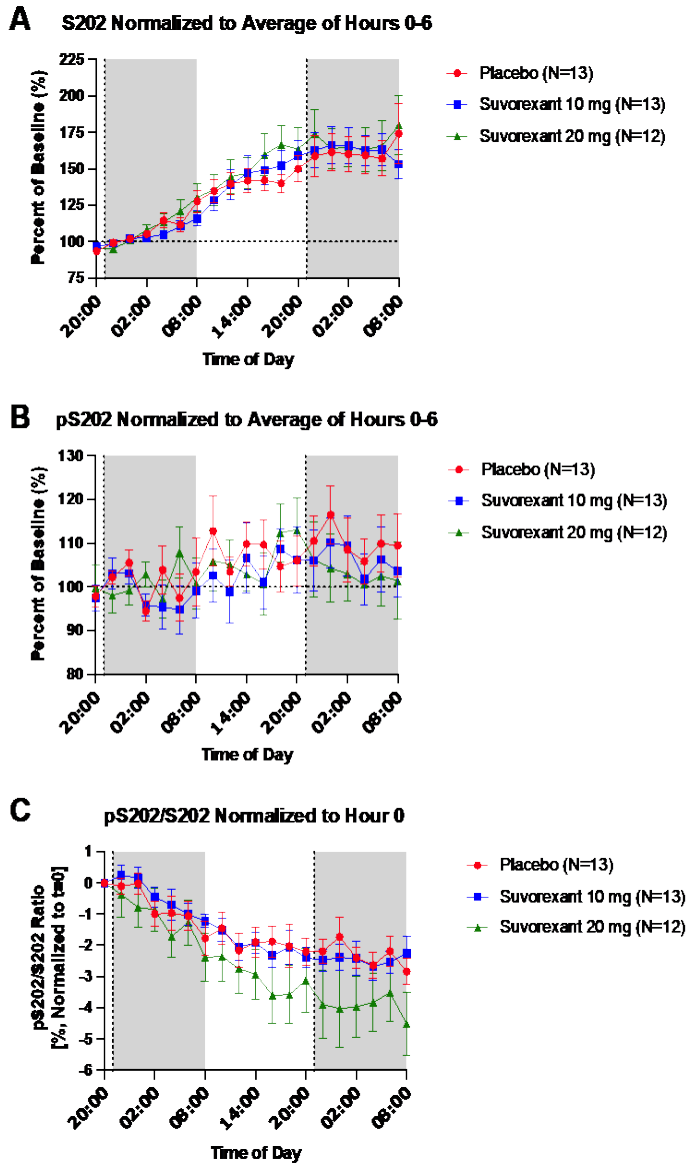


Figure 4: Effect of suvorexant on cerebrospinal fluid (CSF) tau serine-202 (S202) phosphorylated S202 (pS202). Participants received placebo, suvorexant 10 mg, or suvorexant 20 mg. In A and B, concentrations at each time are normalized to the average concentration of hours 0-6. There is no difference between groups for S202 (A) or pS202 (B). The pS202/S202 ratio was normalized to the hour zero concentration (C) shows that the suvorexant 20 mg group is decreased compared to placebo. Blue: placebo; Red: suvorexant 10 mg; Green: suvorexant 20 mg. Error bars indicate standard error. The vertical dashed lines show when placebo/drug was administered. The horizontal dashed line is at 100% of baseline. Shaded area is 08:00-20:00. Unpublished data.

2. SPECIFIC AIMS:

To determine the acute effect of lemborexant on CNS tau phosphorylation in individuals with poor sleep quality. Cognitively normal amyloid-positive adults aged 60-80 years old with a Pittsburgh Sleep Quality Index (PSQI) >5 will be randomized to lemborexant 25 mg (N=20) or placebo (N=10). All participants will have indwelling lumbar catheter placed to sample CSF every 2 hours for 48 hours. Each participant will be infused with $^{13}C_6$-leucine for future analyses of Aβ stable isotope labeling kinetics.

Aim 1: To test the hypothesis that treatment with lemborexant 25 mg will decrease the CSF pT181/T181 ratio compared to placebo (primary outcome).

Aim 2: Measure the effect of lemborexant on other CSF AD biomarkers such as pS202/S202, pT217/T217, and Aβ (secondary outcome).

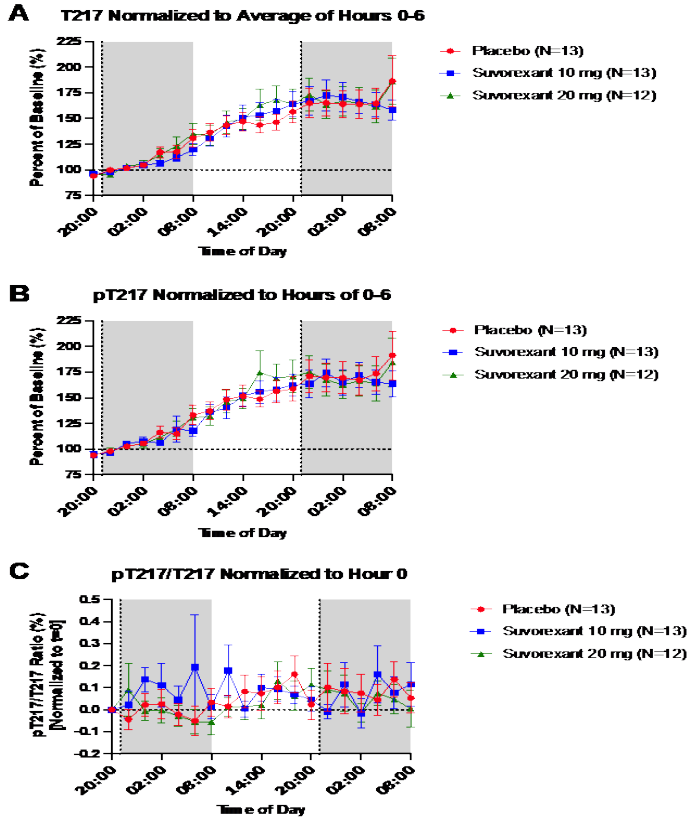


Figure 5: Effect of suvorexant on cerebrospinal fluid (CSF) tau threonine-217 (T217) phosphorylated T217 (pT217). Participants received placebo, suvorexant 10 mg, or suvorexant 20 mg. In A and B, concentrations at each time are normalized to the average concentration of hours 0-6. There is no difference between groups for T217 (A) or pT217 (B). The pT217/T217 ratio was normalized to the hour zero concentration (C) shows that the suvorexant 20 mg group is decreased compared to placebo. Blue: placebo; Red:

3. APPROACH:

3.1. Experimental Design:

To investigate the acute (over hours) effect of lemborexant on CSF pT181/T181, we will recruit participants who are cognitively normal amyloid-positive. Participants age 60-80 years will be screened for normal cognitive function (Mini-Mental Status Examination[21] score ≥27), poor sleep (PSQI>5), and will be evaluated for sleep disorders by history, questionnaires (STOP-Bang[22]), and an attended polysomnogram performed at the Washington University Sleep Medicine Center. **Please see Table 1 for the full inclusion/exclusion criteria.**

Initially, twelve participants with a PSQI >5 will be randomized to receive placebo (N=4) or lemborexant 25 mg (N=8). Amyloid status will be determined by a plasma Aβ test. All participants will undergo attended polysomnography (PSG) during the screening period to assess for moderate or severe obstructive sleep apnea (OSA), which is exclusionary. Randomized participants will be admitted to the CTRU in the early afternoon (Night 1). All participants will have their sleep monitored with unattended full-montage PSG (TrackIt™; Lifelines, Troy, IL) that will allow for sleep staging according to the gold standard American Academy of Sleep Medicine criteria[23] and has already been used in

similar studies to monitor sleep for 36-48 hours[2, 3]. Participants will be monitored with PSG throughout the CTRU admission.

At approximately 20:00, a lumbar catheter and two IVs will be placed for collecting 6 ml of CSF every 2 hours for 48 hours. Sampling start time will begin ~1 hour prior to the typical bedtime defined by sleep logs for each participant in order to allow for $^{13}C_6$-leucine infusion and frequent sampling of blood prior to bedtime. Up to 12 ml of blood will be collected at the following time points: 0, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 26 hours, 28 hours, 30 hours, 32 hours, 34 hours, 36 hours, 38 hours, 40 hours, 42 hours, 44 hours, 46 hours, and 48 hours (Table 2). Approximately 1 hour before the habitual bedtime on Day 1 (t=0), all participants will start an infusion of 800 mg labeled $^{13}C_6$-leucine to label proteins *in vivo* during intracellular translation in order to monitor Aβ kinetics[3, 24, 25]. Aβ stable isotope labeling kinetics (SILK) is an innovative approach to measure Aβ production and clearance rates, and we have used the above protocol in the Sleep Quality study with acceptable labeling of Aβ (Suppl Fig 1).

Table 1: Inclusion/Exclusion Criteria

Inclusion criteria
1. Age 60-80
2. Mini-Mental score ≥ 27
3. Pittsburgh Sleep Quality Index >5
4. Positive plasma Aβ test (i.e., amyloid-positive)

Exclusion criteria
1. Sleep disorders other than insomnia and mild obstructive sleep apnea
   -STOP-Bang score >5
   -ex. restless legs syndrome, narcolepsy
2. Cardiovascular or cerebrovascular disease
3. Hepatic or renal impairment
4. Pulmonary disease
5. Psychiatric disorder requiring medication
6. Alcohol or tobacco use (PI discretion)
7. Use of sedating medications
8. Abnormal movement of the non-dominant arm
9. Contraindications to lumbar catheter
   -Anticoagulant
10. Body Mass Index <35

Participants will be allowed to sleep as they are able immediately with the lights turned off at their habitual bedtime (bedtime is at hour 1, approximately 22:00-00:00). Nursing staff will use dim red lights (safelights) when collecting CSF and blood in the dark. The lumbar catheter ports will be placed on the outside of the gown sleeve for easy access to minimize disturbance during fluid collection. Participants will then sleep until final awakening in the morning. During Day 2, all participants will be in well-lit rooms with regular monitoring for staying awake and naps will not be permitted. At ~22:00 on Day 2 (hour 25), all participants will follow the same sleep routine except that there will be no infusion of labeled $^{13}C_6$-leucine. However, the time of hour 25 will depend on the participant's regular bedtime. Participants will receive the same placebo or lemborexant dose as the previous night (Fig 6). The study will end at ~20:00 on Day 3 (hour 48) when the IVs and lumbar catheters will be removed. All participants will then sleep overnight and will be monitored for at least 8 hours after catheter removal and then discharged. CSF tau, phosphorylated tau, and Aβ kinetics will be quantified by mass spectrometry as was done for our previous work[3, 5].

Table 2: Timeline of Catheter Placement and Removal, Bedtime, and Sampling														
Hour	0	1	2	4	6	8	10	12	14	16	18	20	22	24
Event	**C**, X	**B**	X	X	X	X	X	X	X	X	X	X	X	X
Hour	25	26	28	30	32	34	36	38	40	42	44	46	48	49
Event	**B**	X	X	X	X	X	X	X	X	X	X	X	X, **R**	**B**

C: catheter placement; B: bedtime; X: CSF/blood sampling; R: catheter removal

Table 3: Recruitment Using Plasma Aβ			
	Amyloid+	Amyloid-	Total
Tests positive	**20**	**13**	**33**
Tests negative	5	63	68
Total	25	76	101

3.2 Screening for amyloid-positivity with plasma Aβ:
We plan to screen 100 participants to enroll 30 participants in this trial. Cognitively normal participants who are amyloid-positive on the plasma Aβ test are eligible for the study. The plasma Aβ test has an 80% sensitivity, 83% specificity for amyloid-positivity after accounting for age and ApoE genotype. Assuming that 25% of the cognitively normal population will be amyloid-positive, we plan to screen 100 cognitively normal participants with an PSQI>5 to obtain ~20 amyloid-positive cognitively normal individuals (Table 3). We expect to recruit the remaining participants from the Knight Alzheimer's Disease Research center (ADRC) and on-going studies at Washington University such as the SEABIRD study.

3.3 Power Calculation:
A maximum of 30 participants will be recruited for this study and will be randomized in a 2:1 (treatment:placebo) ratio with lemborexant 25 mg (N=20) and placebo (N=10). Our preliminary data was used to estimate the effect size between the placebo arm and suvorexant 20 mg arm. Effect sizes were calculated based on two-sided independent two-sample *t* test with type I error of 0.05 and 80% power. G*power 3.1 was used to calculate effect sizes and

Table 4. Power Analysis and Change from Baseline Detected with Different Sample Sizes			
	pT181/T181 [norm t=0; average t=14-18]	pS202/S202 [norm t=0; average t=36]	Aβ42 [norm average t=0-6; average t=22-26]
Placebo Group Mean (SD)	1.56 (1.41)	-2.83 (1.37)	123.97 (13.16)
Suvorexant 20 mg Group Mean (SD)	-1.09 (2.20)	-4.52 (3.01)	110.21 (16.66)
Effect Size of Suvorexant 20 mg Compared to Placebo	1.43	0.72	0.92
Power (10 participants/group)	86%	33%	50%
Power (15 participants/group)	95%	47%	65%
Power (20 participants/group)	99%	60%	80%

perform the power calculation. Table 4 shows the power analysis for CSF pT181/T181, pS202/S202, and Aβ42.

Effect sizes were calculated based on the preliminary data in Figures 2-4 and assume equal intervention groups. For CSF pT181/T181, the average of hours 14-18 in Figure 3C was used to determine means, standard deviations, and effect size. For CSF pS202/S202, the average of hour 36 in Figure 4C was used to determine means, standard deviations, and effect size. For CSF Aβ42, the average of hours 22-26 in Figure 2C was used to determine means, standard deviations, and effect size. Table 4 shows the power to detect these effect sizes with 10 participants/group, 15 participants/group, and 20 participants/group. Note, CSF Aβ42 may increase in amyloid-positive participants with amyloid plaque accumulation is decreased.

3.4 Interim Analysis:
After 12 participants have completed the study (placebo N=4; lemborexant N=8), an interim analysis will be performed for efficacy to see if there is a significant difference in CSF pT181/T181 between lemborexant 25 mg and placebo groups. If there is a statistically significant ($p<0.05$) or trend towards statistically significant ($p<0.1$) reduction in the primary outcome (CSF pT181/T181 ratio) or secondary outcome (CSF amyloid-beta), then the study may be continued at lemborexant 25 mg. If there is not a trend towards a statistically significant ($p>0.1$) reduction in the primary outcome (CSF pT181/T181 ratio) or secondary outcome (CSF amyloid-beta), or power analysis suggests that statistical significance will not be achieved by the fully enrolled study, then the study will be terminated.

3.5 Data Analysis Plan:
For each participant, AD biomarkers (Aβ38, Aβ40, Aβ42, T181, S202, T217, pT181, pS202, pT217) will be normalized to the average of the first 6 hours (t=0-6, 20:00-02:00) before changes from the intervention will be seen in CSF. We will also normalize to the hour 0 concentration. Phosphorylated tau ratios (pT181/T181, pS202/S202, pT217/T217) will be normalized to the first time point (hour 0). The trajectory of changes from these baselines over time will then be plotted by treatment arms to examine whether the change in concentrations or ratios is linear. If the linearity assumption is valid, (linear mixed effects) LME models with random intercept and slope, similar to our previous work[3, 5], will be used for analysis, otherwise mixed model for repeated measure (MMRM) will be used. Fixed effect in the model will include treatment group, time and their interaction. Baseline will be included as a covariate. The normality assumption will be examined using residual plot and appropriate transformation (e.g., log) will be considered. Unstructured covariance matrix will be used and if there is convergence issue, various other covariance matrix structure (e.g., compound symmetry, First-order Autoregressive) will be compared and the best fit structure will be selected for final analysis based on Akaike information criterion (AIC).

3.6 Study Outline and Timeline:

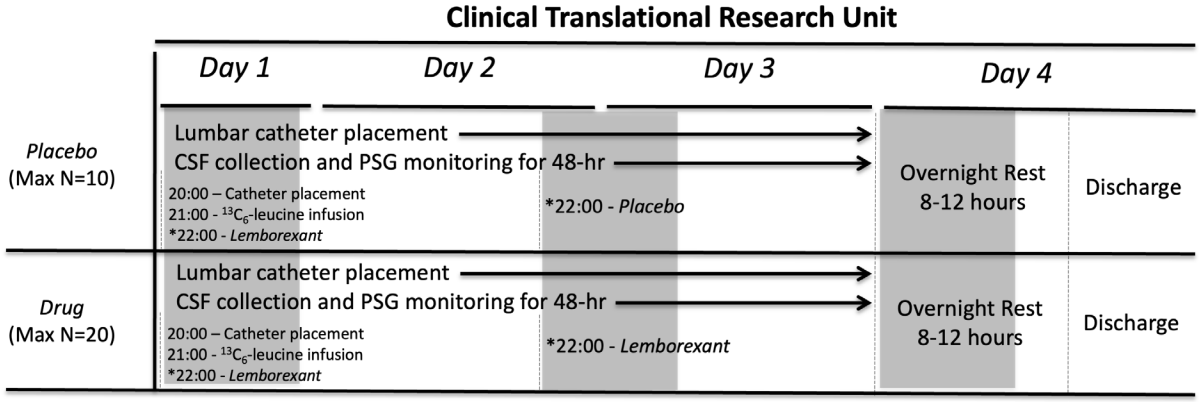


Figure 6 shows an overview of the study design with a habitual bedtime of 22:00. Table 5 shows the study timeline. We anticipate that this study will take 2 years to complete.

Figure 6: Overview of Study Design. Overnight periods are shaded.

Table 5: Proposed Timeline for Trial

	Year 1				Year 2			
Months	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Start-Up								
Enrollment								
IP/MS								
Analysis								
Manuscript Prep/Submission to Conferences								

4. SCIENTIFIC RIGOR, TRANSPARENCY, AND DATA SHARING:

We will ensure scientific rigor by working closely with the biostatisticians collaborating on this project to implement adequate database control, statistical procedures, data analysis methods, and unbiased and accurate interpretation of findings from all publications of this project. We will consider all relevant biological variables by performing statistical analyses on sex, race, age, and other variables as covariates in statistical models. We will assess their effects in association with other main predictors on main outcomes through appropriate interactions in the models. We will register the study with ClinicalTrials.gov and will follow CONSORT Guidelines in reporting the study findings. Selected data will be shared with the ADRC and SEABIRD for participants referred to our study; specifically, this will consist of amyloid status, CSF tau and Aβ at hour 0, and screening MMSE. Finally, we will share data and biospecimens with qualified investigators.

5. POTENTIAL PROBLEMS AND ALTERNATIVE APPROACHES:

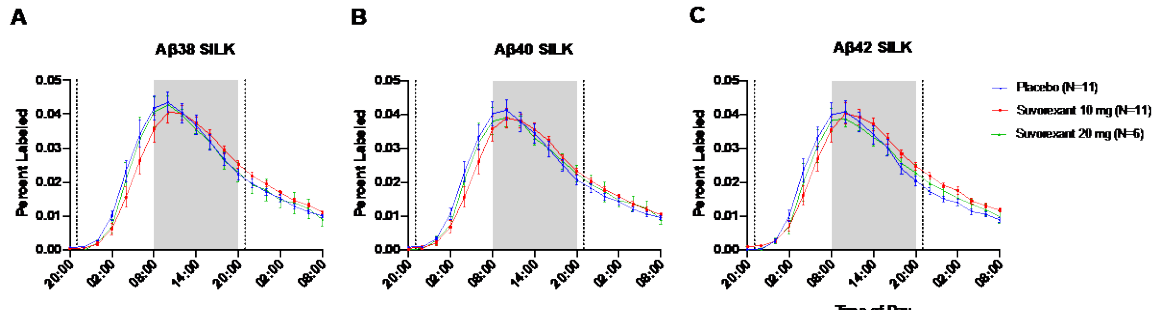
We plan to recruit participants from the community, the ADRC, and from an ongoing study testing the plasma Aβ test (SEABIRD, PI: Randall Bateman). We have received permission

to recruit participants from the ADRC and SEABIRD study and expect to recruit the majority of the participants from the community. We have budgeted to screen 100 participants with plasma Aβ tests and to perform 50 polysomnograms to evaluate for moderate/severe obstructive sleep apnea from a database of >10,000 research volunteers maintained at Washington University (Volunteers for Health). We have successfully recruited from the database for our previous studies. We plan on 1 participant/month completing the study and have performed at most 15-18 lumbar catheter admissions per year in previous studies. The COVID-19 pandemic may result in delays both recruiting participants and bringing them into the CTRU as well as processing samples.

6. FUTURE DIRECTIONS

This project tests the hypothesis that CSF pT181/T181 is decreased with lemborexant, a DORA, compared to placebo. The proposed study may suggest innovative AD prevention and treatment approaches that involve sleep therapies such as DORAs in older individuals with poor sleep quality. If successful, future studies will include an interventional trial to prevent amyloidosis (or slow amyloid growth) and tauopathy in those identified by clinical criteria. Such a prevention trial could dramatically impact the incidence and prevalence of AD.

SUPPLEMENTAL MATERIAL



Supplementary Figure 1: Aβ labeling curves for stable isotope labeling kinetics (SILK) for A38 (A), Aβ40 (B), and Aβ42 (C). 800 mg of 13C6-leucine is infused over 10 minutes at 20:00 (t=0) in participants treated with placebo (N=11), suvorexant 10 mg (N=11), and suvorexant 20 mg (N=6). Note, this data has not been normalized to the plasma free leucine concentration and Aβ kinetics cannot be determined. This data is presented to demonstrate feasibility. Standard error bars are shown. Unpublished data.

7. STUDY POPULATION AND ENROLLMENT

7.1 Justification for human subject involvement:
Alzheimer Disease (AD) is a current and growing public health problem characterized by progressive cognitive impairment resulting in dementia. AD is estimated to afflict millions of people in the coming decades. Multiple lines of evidence suggest a role for sleep disturbances in the development of AD. Further, there is evidence in mice that improving sleep parameters such as sleep efficiency (i.e. sleep quality) may prevent the initial pathological changes seen in the brain with AD. These findings need to be replicated in humans if sleep interventions are to be deployed to prevent or delay AD. In this study, I propose to translate the findings to humans that improving sleep efficiency has similar effects

as in mice. The specific objective of this study is to translate basic science findings to humans for the long-term improvement in human health, therefore the proposed study must be carried out in human subjects.

7.2 Subject population characteristics:

The goal is to enroll healthy, cognitively normal amyloid-positive adults 60-80 years old with no medical comorbidities that would affect the risk of AD and with no conditions, medications, or substance use that may affect outcome measures or contraindicate study procedures (e.g. cerebrospinal fluid (CSF) amyloid-β (Aβ) levels obtained by lumbar catheter). Participants will be recruited from the Volunteers for Health registry of >10,000 research study volunteers maintained at Washington University, the ADRC, as well as an ongoing study of a plasma Aβ test (SEABIRD, PI: Randall Bateman). Individuals from both sexes and any race or ethnicity will be included. Minors will not be included because AD does not occur in individuals <18 years old, even if predisposed to develop AD through an autosomal dominant mutation. Further, it is not known if the diurnal Aβ pattern is present in children. The relevance of determining if sleep alters Aβ in children to the pathogenesis of a neurodegenerative disorder that will potentially develop decades later is also unknown at this time. As a result, we will not include any children in this study.

7.3 Eligibility criteria:

7.3.1 Inclusion criteria:

- Age 60-80 years
- Any sex
- Any race/ethnicity
- Mini-Mental Status Examination score (MMSE) ≥ 27
- Positive plasma Aβ test (i.e., amyloid-positive)
- Pittsburgh Sleep Quality Index >5

7.3.2 Exclusion criteria:

- Cognitive impairment as determined by history of MMSE < 27
- Inability to speak or understand English
- Any sleep disorders other than insomnia
  - No history of moderate-to-severe sleep-disordered breathing and STOP-Bang score > 5
  - History or reported symptoms suggestive of restless legs syndrome, narcolepsy or other sleep disorders
  - No more than mild sleep apnea (AHI <16) on PSG
- Sleep schedule outside the range of bedtime 22:00-midnight
- Contraindication to lumbar catheter (anticoagulants; bleeding disorder; allergy to lidocaine or disinfectant; prior central nervous system or lower back surgery)
- Cardiovascular disease requiring medication except for controlled hypertension (PI discretion)
- Stroke
- Hepatic or renal impairment

- Pulmonary disease (PI discretion)
- Type 1 diabetes
- HIV or AIDS
- Neurologic or psychiatric disorder requiring medication (PI discretion)
- Suicidal ideations
- Alcohol, tobacco or marijuana use (PI discretion)
- Use of sedating medications (PI discretion)
- Inability to get out of bed independently
- In the opinion of the investigator, the participant should be excluded due to an abnormal physical examination.
- Current pregnancy
- Body Mass Index >35
- History of migraines (PI discretion)
- History of drug abuse in the last 6 months
- History or presence of any clinically significant medical condition, behavioral or psychiatric disorder (including suicidal ideation), or surgical history based on medical record or patient report that could affect the safety of the subject or interfere with study assessments or in the judgment of the PI participant is not a good candidate.
- Urinary or fecal incontinence
- Concurrently enrolled in another trial of an investigational drug or device

We will enroll up to 30 participants to complete the study. **Participants will receive either placebo or lemborexant 25 mg, a dual orexin receptor antagonist. Lemborexant is approved by the Food and Drug Administration (FDA) for the treatment of insomnia (approved doses 5-10 mg).** Lemborexant 25 mg will be used in this study to see if there is an effect on CSF pT181/T181 and Aβ. The use in this study will be considered investigational and is not approved by the Food and Drug Administration (FDA).

Participants that screen fail may be invited back to rescreen at a later date.

Sampling plan and justification:

- Power calculations are shown in "C3. Power Calculation" portion of the research plan above. Due to concerns about recruitment if participants have to repeat the study, the study power calculation is based on each participant completing the study once without repeating.
- Children will be excluded and age range will be limited to 60-80, as detailed in the previous section "Subject population characteristics".

Vulnerable populations: We will not enroll participants from vulnerable populations.

Assignment to a study group: Participants will be randomized to receive placebo or lemborexant 25 mg at the Clinical Translational Research Unit (CTRU) admission. We will randomize 8 participants to receive 25 mg of lemborexant for 2 nights and 4 participants to receive placebo for two nights. After an interim analysis for safety and futility, a decision will be made to either: 1) continue the study with lemborexant 25 mg, 2) continue the study

with a lower dose of lemborexant, or 3) stop the study. If the study is continued, then 12 participants will be randomized to lemborexant and 6 participants will be randomized to placebo. In addition to being a FDA approved drug for the treatment of insomnia, lemborexant is also a drug with the same mechanism of action as was used in experiments to decrease Aβ with sleep in mice, therefore this will be a direct translation of this finding. The drug's mechanism of action is to block orexin-A and orexin-B receptors. The orexin neuropeptide acts in the brain to promote wakefulness. Through antagonism of this system, sleep is induced.

Collaborating sites: None

7.4 Description of Study Visits:
At all study visits, safety assessments will consist of monitoring and recording all adverse events (AEs) at each visit.

7.4.1 Visit 1:
Participants must provide informed consent prior to any study related assessments take place. Screening assessments include review of demographics, medical history, and medications. Vital signs and a physical and neurological exam are performed. Blood is collected for the amyloid blood test and APOE testing (APOE processing will not occur until later in the screening period and may not be processed at all should participants screen fail) and questionnaires are administered. If the participant was referred by SEABIRD, the amyloid blood test may not need to be repeated. However, if the participant was referred by the ADRC, the amyloid blood test will be repeated.

Additional safety assessment at this visit include an electrocardiogram (ECG), routine blood tests, and administration of the Columbia Suicide Rating Scale (CSSRS).

Participants will be sent home with a sleep log to complete for two weeks.

7.4.2 Visit 2:
Participants will undergo polysomnography (attended sleep study) at the Washington University Sleep Medicine Center for sleep apnea screening. Participants will have the option to have the sleep study results added to their medical record. All participants will receive a generic letter stating if the results were normal or abnormal. Participants with abnormal results will be encouraged to follow up with their primary care physician.

7.4.3 Visit 3:
This visit will occur one week prior to the Clinical Translational Research Unit (CTRU) admission. Eligibility criteria, and changes to medical conditions or medications will be reviewed. Weight will be measured, blood will be collected for a coagulation panel and participants will be sent home with a sleep log.

7.4.4 Visit 4:
Participants will be admitted to the Clinical Translational Research Unit (CTRU) for a 3-night stay. Questionnaires and psychomotor vigilance testing (PVT) will be administered at admission and discharge.

Throughout the CTRU admission, participants will be monitored with a full 10-20 EEG montage as well as bilateral EOG and chin EMG to stage sleep. EEG monitoring will occur throughout the CTRU stay while CSF and blood sampling occur.

The participant's habitual bedtime is based on the sleep log completed after visit 1. The leucine infusion (T=0) will begin approximately one hour prior to the habitual bedtime (~21:00-23:00).

Lemborexant 25mg or placebo will be administered ~ the participant's habitual bedtime on day 1 (hour 1) and day 2 (hour 25), and the lights will be turned off to allow for sleep.

Blood and CSF samples will be collected over 48 hours as specified below. CSF safety labs will be collected at the end of the sampling period. The nursing staff will monitor participants throughout the stay, and all food and water intake will be recorded during the sampling period.

Participants stay 8-12 hours after the study is completed for observation to monitor for adverse events. Participants will contact the study team after discharge if they develop any physical complaints. In addition, the physician or a member of the research staff will contact participants by phone 24 and 48 hours after discharge.

8. STUDY PROCEDURES AND METHODS

8.1 Specimens, records, or data:
We will obtain the following from our participants at visit 4

8.1.1 Cerebrospinal fluid (CSF) specimens:
Each participant regardless of study aim or group will have an indwelling lumbar catheter placed for the collection of CSF every 2 hours for 48 hours (hours 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48). At each time point, 6 ml of CSF will be collected. At the end of the study an additional 2 ml of CSF (when available) will be sent to the lab to check for any signs of infection. Therefore, a total of 152 ml of CSF will be collected from each participant over 48 hours. It is expected that the catheter will fail to draw CSF during the study for some participants. This will result in missing CSF samples and missing CSF safety labs.

8.1.2 Blood specimens:
Each participant will have an intravenous (IV) catheter placed for collection of blood. At the start of the study, blood will be collected 0, 5, 10, 15, and 30 minutes after the

lumbar catheter is placed. Then, blood will be collected every 30 minutes until hour 4. After hour 4, blood will be collected every 2 hours until hour 48. Approximately 1-3 teaspoons of blood will be collected at 34 time points over 48 hours. The total amount of blood taken over 24 hours is 120-265 ml (half of a standard blood donation).

8.1.3 Polysomnography:
Polysomnogram recordings to determine sleep stages will be obtained from all participants. This includes standard electrodes for electroencephalography, electromyogram, and electrooculogram. These recordings are not identifiable unless a participant requests the recordings to be placed in his/her medical record.

8.1.4 Physical Exam:
Prior to discharge, a medically qualified professional will perform a brief physical examination that consists of a review of the major body systems (i.e., skin, head/ears/eyes/nose/throat (HEENT), cardiovascular, pulmonary, abdomen, musculoskeletal, and extremities).

8.2 Data from human subjects:
Our participants will provide the following data:

8.2.1 Personal identifiers:

- Name, date of birth, phone number, mailing address, and email (if they want). These are for the purposes of proper identification for the study and communication about research activities. Additionally, social security number is collected only for purposes of participant reimbursement.
- GUID IDs will be obtained for the purpose of data sharing. Additional information collected to create GUID IDs include the place of birth and legal last name at birth.
- Answers to screening questions for inclusion/exclusion criteria.
- Demographic information: date of birth, sex, race/ethnicity, education, handedness
- Medical history: Comorbidities, surgeries, medications, allergies, alcohol use
- Medical record numbers may be documented on medical records obtained for the study.

All personal identifying data are stored separately from all other data. Only team members trained in the protocol and approved by the Human Research Protection Office (HRPO) will have access to private identifiable information, including the Principal Investigator, research coordinator/polysomnographic technicians, and the mentor.

Data that do not include PHI will be coded with a study ID number not derived from personal identifiers. This data will contain dates of assessments.

8.2.2 Sleep log:
The sleep log is a daily record that logs sleep patterns over time. The sleep log will be used to assess habitual bedtimes.

8.2.3 Mini Mental State Exam (MMSE):
The MMSE is a brief, frequently used screening instrument to assess cognitive function[28]. The MMSE scale evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two overlapping pentagons. The MMSE is scored as the number of correctly completed items with a lower score indicative of poorer performance and greater cognitive impairment. The total score ranges from 0 (worse) to 30 (perfect performance).

8.2.4 Columbia- Suicide Severity Rating Scale (C-SSRS):
Consistent with FDA regulatory guidance[26], occurrence of suicide-related thoughts and behaviors will be assessed. The C-SSRS is a scale that captures the occurrence, severity, and frequency of suicide-related thoughts and behaviors during the corresponding assessment period[27]. The scale includes suggested questions to elicit the type of information needed to determine if a suicide-related thought or behavior occurred. If a suicide-related thought or behavior is identified at any time during the study, a thorough evaluation will be performed by a study physician, and appropriate medical care will be provided, such as referring the participant to their primary physician or therapist, or getting the participant to a medical facility for safety. The number to a suicide prevention hotline is also provided in the informed consent document.

8.2.5 STOP-Bang Questionnaire:
The STOP-Bang Questionnaire is a screening instrument used to assess risk for Obstructive Sleep Apnea (OSA).

8.2.6 Pittsburgh Sleep Quality Index (PSQI):
This is an assessment tool of overall sleep quality and collects information about multiple sleep disorders to generate an overall sleep quality score.

8.2.7 Stanford Sleepiness Scale (SSS):
A self-assessment that documents how alert an individual is feeling.

8.2.8 Morningness/ Eveningness Questionnaire (MEQ):
A self-assessment to measure whether a person's circadian rhythm produces peak alertness in the morning, in the evening or in between.

8.2.9 Psychomotor Vigilance Testing (PVT):
An assessment that measures speed responses to visual stimuli via a thumb operated device.

8.2.10 Electrocardiogram (ECG):
An appropriately qualified individual will conduct a standard 12-lead resting ECG. The ECG report must be reviewed, signed, and dated by the site PI (or a medically-qualified individual delegated by the site PI).

8.2.11 Physical and Neurological Examination:
A medically qualified professional will perform a brief physical examination that consists of a review of the major body systems (i.e., skin, head/ears/eyes/nose/throat (HEENT), cardiovascular, pulmonary, abdomen, musculoskeletal, and extremities) and a brief neurological examination which will include an assessment of cranial nerves, strength, coordination, reflexes, sensation, tremor, gait and mental status. Assessments of height, weight, and vital signs (systolic and diastolic blood pressure, pulse, temperature, and respiration) are included

8.2.12 Blood Collection:
All participants will be screened with a blood test for Aβ status unless they have been referred by the SEABIRD study. The plasma Aβ test has been shown to be very sensitive and specific for Aβ deposition in the brain with an area under the curve of 0.88[29,30].

Blood will also be obtained for genetic testing, plasma AD biomarkers and other blood-based biomarkers, and routine labs for safety purposes.

8.3 *Scale to Inform the Disclosure of Amyloid Results*
The Concern's About AD Scale[31] allows participants to indicate their level of agreement with six statements about developing AD. The scale will be used only to inform the participant's concerns about AD during the disclosure of amyloid results.

8.4 Disclosure of Amyloid Results
Participants will receive implied amyloid results. Eligible participants are cognitively normal and amyloid-positive. Risk of having elevated brain amyloid will be screened using a plasma amyloid-beta blood test.

Using a standard operating procedure (SOP) for the disclosure of amyloid results, the study team will convey the results of the plasma amyloid-beta test. Disclosure of amyloid results will be informed by the Concern's About Alzheimer's Disease Scale,[31] This scale will not affect participant eligibility and is not part of the inclusion/exclusion criteria. The purpose of the scale is to inform disclosure of the amyloid results. Please see the SOP for the Disclosure of Amyloid Results.

9. Potential risks

9.1 Lumbar Catheter:

- Discomfort during the procedure is common and mild. Some people may have discomfort sitting in the same position for the duration of the lumbar catheter

placement. Lidocaine will be used for local anesthesia, which feels like a small pinch followed by burning sensation (<10 seconds). There is pressure but not pain during the insertion of the lumbar puncture needle. During the lumbar catheter procedure, participants may experience momentary (<1 second) cramping or pain in a leg, due to the needle or the catheter briefly touching a floating nerve ending.

- Back soreness is common and mild. Some people have soreness of the area where the needle and catheter were inserted, especially when the lidocaine wears off. This soreness resolves by itself over 2-3 days.
- Feeling faint is common and mild. As with any medical procedure, some people feel faint. This is a normal response and is *not* a physical effect of the needle.
- Post-lumbar catheter headache occurs frequently (50-80% of participants) and is mild to moderate. Another possible symptom is ringing in the ears. Individuals may have a headache following placement of a lumbar catheter, due to the decreased volume of CSF. For the majority of individuals, the headache resolves when the catheter is removed. Occasionally (30% or less), this headache persists after removal of the catheter and requires treatment with a "blood patch."
- Bleeding is extremely rare, but is moderate to severe when it occurs. In individuals with problems of blood clotting, a lumbar catheter may cause a large amount of bleeding.
- Infection is an extremely rare risk of a lumbar catheter and can be moderate-to-severe. Very rarely, the lumbar puncture needle and catheter may introduce pathogens internally, leading to infection.

9.2 Intravenous Catheter:

- Discomfort from minor pain, bleeding, bruising, or swelling caused by the needle or intravenous catheter are common and mild.
- Bruising at the phlebotomy site is infrequent and mild. Some individuals may have bruising due to leakage of a small amount of blood from the vein into the surrounding tissue. This will spontaneously resolve.
- Feeling faint is common and mild. As with any medical procedure, some people feel faint. This is a normal response and is *not* a physical effect of the needle.

9.3 Lemborexant:

- This study will use lemborexant 25 mg, a dose greater than the 10 mg dose approved by the FDA for the treatment of insomnia. Treatment-emergent adverse events for lemborexant 25 mg were similar to lemborexant 10 mg, however. See Appendix 1 for a summary of treatment-emergent adverse events for different doses of lemborexant.
- Somnolence is a common, generally mild side effect of lemborexant. This is an expected effect of this drug given its mechanism of action. Participants will be monitored in the Clinical Translational Research Unit prior to discharge and will be assessed for level of alertness with a Stanford Sleepiness Scale testing and Psychomotor Vigilance Testing performed upon admission and at the time of discharge. The principal investigator will evaluate the participant and review these measures prior to approving discharge.

- Additional mild and common reactions to lemborexant include: headache, and abnormal dreams.
- Abnormal sleep behaviors, such as sleep walking, are rare with lemborexant (2 episodes in the registration program). All participants will take lemborexant on the Clinical Translational Research Unit at Washington University that has 24 hours a day nursing coverage monitoring the participants.
- Additional uncommon or rare reactions to lemborexant include heart palpitations, tachycardia, sleep paralysis (the inability to move or speak for up to several minutes upon sleep-wake transitions), hypnagogic/hypnopompic hallucinations (visualizations upon sleeping and awakening), and mild cataplexy-like symptoms (muscle weakness lasting from seconds to a few minutes but no confirmed cataplexy).
- Lemborexant may interact with multiple medications including alcohol, antifungals (e.g., itraconazole, fluconazole), antibiotics (e.g., clarithromycin, rifampin), antiretrovirals (e.g., efavirenz, etravirine), verapamil, carbamazepine, St. John's wort, bosentan, and modafinil.
- The principal investigator will evaluate participants for potentially interacting medications and participants will be excluded from the study if the interacting drug(s) cannot be discontinued.
- Very rare: For all individuals, but primarily in depressed participants treated with hypnotics, worsening of depression and suicidal tendencies have been reported.

9.4 Polysomnogram (PSG):

- Irritation of skin from electrodes used during study is rare and mild. Skin needs to be cleaned before adhesive is applied for the electrodes. Some individuals with sensitive skin may have irritation of their skin.
- Worse sleep quality is common and mild. People usually do not sleep as well in an unfamiliar environment. Therefore, participants may be more tired than usual the next day.

9.5 Questionnaires:

- Participants may experience emotional discomfort when answering some questions in the questionnaires. If any particular question makes the participant uncomfortable, the participant may discuss it importance and the need to answer it with the specially-trained interviewer. Participants have the right to refuse to answer any question for any reason.

9.6 Bedrest:

- There is risk of feeling dizzy, lightheaded, or woozy upon standing after 60 hours of bed rest. This is common and mild.

9.7 Privacy/confidentiality:

- Breach of privacy/confidentiality (very rare, severity varies): A risk in any research study is that confidential information about participants may be accidentally disclosed.

10. Adequacy of Protection Against Risks

Recruitment and Informed Consent

10.1 Recruitment:

Human subjects will be recruited from the ADRC, the ongoing SEABIRD study, or Volunteers for Health, a community-based registry of >10,000 individuals. We developed advertisements targeting poor sleep quality to be sent to this registry. Potential participants can indicate their interest in this study through an online system, in which case their name and contact information is sent to the principal investigator, or they can call the phone number that is on the advertising materials. Referral sources may provide additional information to the study team at the time of the referral, including name, age, date of birth. CDR scores, imaging data, CSF and plasma biomarker data (including amyloid status). Additional data, biofluid markers, and genetic information may be obtained from the referral sources after study enrollment.

10.2 Consent:

Two separate consent processes are required, one for an initial phone screen to assess if participants are likely to meet inclusion/exclusion criteria for the study, and a second formal written consent. No waivers of elements of consent will be sought.

- Phone screen consent: Because the initial phone screen contains questions that involve protected health information, verbal consent is obtained prior to going through the screen. The Washington University HRPO will review and approve a phone script for verbal consent, which includes (1) A statement that the phone screen is for research, (2) purpose of the phone screen, (3) expected duration of phone screen, (4) description of the phone screen, (5) identification of any procedures which are experimental, (6) description of risks and benefits, (7) disclosure of alternatives (*i.e.* not doing phone screen), (8) how confidentiality of records will be maintained, (9) a statement that participation is voluntary and not participating will involve no penalty or loss of benefits to which the person is otherwise entitled.
- Consent for enrollment in study: Potential subjects are mailed a copy of the consent document to review and discuss with other individuals (if desired) prior to an in-person consent process. Consent will take place in a private exam room at the Washington University Sleep Medicine Center or the Neurology Clinical Research Unit (NCRU). Only members of the research team who have had training and are approved by the Washington University HRPO (including PI) may participate in the consent process. Information in the consent document includes: (1) A statement that the study is for research, (2) purpose of the research, (3) expected duration of study participation, (4) description of the research procedures, (5) identification of any procedures which are experimental, (6) description of risks and benefits, (7) disclosure of alternatives, (8) how confidentiality of records will be maintained, (9) a statement that participation is voluntary and not participating will involve no penalty or loss of benefits to which the person is otherwise entitled. Potential participants may ask questions either beforehand by phone or during this face-to-face visit, before deciding to enroll in the study. After all questions have been answered, and if the person wants to enroll in the study, the consent process will be finalized by both participant and research team member signing the consent document. Every participant is provided with a copy of the signed consent document.

10.3 Lumbar catheter:

- Discomfort during the procedure: To minimize discomfort, participants will sit at the edge of the bed with supports and pillows to keep them in an ergonomic position. Lidocaine will be used for local anesthesia. Only physicians experienced in performing lumbar catheters will perform this procedure.
- Back soreness: Participants are provided with information sheets on using heat, stretching, and acetaminophen if back soreness occurs.
- Feeling faint: If a participant feels faint, s/he will be assisted in lying down in the bed until the feeling has passed. The lumbar catheter can be performed with participants lying down on their side, in this case.
- Post-lumbar catheter headache: This risk will be minimized by having the participants lie in bed with their heads down (the Trendelenburg position) for one hour after the catheter is removed. Participants will remain in bed for 6-8 hours after the catheter is removed. Participants are also informed they can minimize this risk by having caffeine after the lumbar catheter is removed, and by drinking plenty of water throughout the study while the catheter is in place. If a post-lumbar catheter headache does occur, participants will be offered treatment with a "blood patch."
- Bleeding: Individuals with this risk (blood clotting problems or taking anticoagulants) are excluded from participation in the study.
- Infection: Proper sterile technique will be used during all lumbar catheter placements. At the end of the study immediately prior to the removal of the catheter, an additional 2 ml of CSF will be drawn, when available, and sent to the lab to check for any signs of infection. Further, participants are given a 24/7 phone number for a member of the research team after discharge from the Clinical Translational Research Unit in case of complications.

10.4 Intravenous catheter:

- Discomfort during the procedure: Only physicians and nurses experienced in placing intravenous catheters will perform the procedure.
- Bruising at phlebotomy site: Discomfort from bruising can be alleviated by moist heat pack, which can be provided in the Clinical Translational Research Unit.
- Feeling faint: If a participant feels faint, s/he will be assisted in lying down in the bed until the feeling has passed. Participants will be encouraged to drink plenty of water during the study, to minimize the chance of feeling faint.

10.5 Lemborexant:

All participants will take lemborexant on the Clinical Translational Research Unit at Washington University which has 24 hours a day nursing coverage monitoring the participants. Participants will only take lemborexant in this monitored setting. Nurses will be available to assist all participants if they develop these symptoms. Further, the participants will be under constant video monitoring while in the Clinical Translational Research Unit; this video is not recorded and is only viewable by the nursing staff. Lemborexant will be discontinued if reactions are distressing to the participant or severe. Participants will be monitored in the Clinical Translational Research Unit prior to discharge and will be assessed for level of alertness with a Stanford Sleepiness Scale and Psychomotor

Vigilance Testing performed upon admission and at the time of discharge. The principal investigator will evaluate the participant and review these measures prior to approving discharge. Potential adverse events from lemborexant include:

- Somnolence: Somnolence may be severe in participants with compromised respiratory function; therefore, obstructive sleep apnea and chronic obstructive pulmonary disease are exclusion criteria. Lemborexant will be discontinued if somnolence is distressing to the participant or severe.
- Next day drowsiness
- Additional mild and common reactions to lemborexant include headache, and abnormal dreams
- Abnormal sleep behaviors
- Additional uncommon and rare reactions include heart palpitations, tachycardia, sleep paralysis, hypnagogic/hypnopompic hallucinations, and mild cataplexy-like symptoms.
- Lemborexant may interact with multiple medications: Participants taking medications that interact with lemborexant will be evaluated by the principal investigator. If the potentially interacting medications cannot be discontinued, then the participants will be excluded from the study.
- Depressed mood and/or suicidal ideations: All participants will be screened for suicidal ideations and psychiatric disorders. All participants with psychiatric disease requiring medications will be excluded both to control this risk and also because psychiatric medications may interfere with sleep. Participants with suicidal ideations will be excluded. This risk is also controlled because participants will only take lemborexant for 2 nights in the monitored setting of the CTRU with 24 hours a day nursing coverage.

10.6 Polysomnogram (PSG):

- Irritation of skin from electrodes used during study: When possible, an alternative cleaning agent or adhesive will be used. If irritation is more than mild, that electrode will not be used for the study.

11. Definitions

For this study, the following standard definitions for reporting will be used:

11.1 Adverse Event:
Adverse Event (AE) is any untoward medical occurrence associated with the use of the study drug in humans, whether or not considered related to the drug. An AE (also referred to as an adverse experience) may include any unfavorable and unintended sign (i.e., abnormal lab test finding), symptom, or disease temporally associated with the use of the drug, and does not imply any judgement about causality.

11.2 Suspected Adverse Reaction:
A suspected adverse reaction is any adverse event that is determined for which there is a reasonable possibility that the study drug caused the adverse event. For IND safety reporting,

'reasonable possibility' means there is evidence to suggest a causal relationship (reasonable possibility) between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

11.3 Adverse Reaction:
Adverse Reaction means any adverse event caused by a study drug. Adverse reactions are a subset of all suspected adverse reactions where there is a reason to conclude that the drug caused the event.

11.4. Serious:
An adverse event or suspected adverse reaction is considered serious if the event or reaction, in the view of the PI, results in any of the following serious outcomes:

- Death;
- A life-threatening adverse event as defined as placing the participant at immediate risk of death from the AE as it occurred;
- Initial or prolonged inpatient hospitalization (hospital admission defined as ≥ 24 hours or prolongation of a hospital stay due to adverse event);
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect.

11.5 Life-threatening:
An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of the PI, its occurrence places the participant at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

11.6. Important Medical Events (IMEs):
IMEs are not immediately life-threatening, events that places a participant at immediate risk of death or require hospitalization may be considered serious when, based upon appropriate medical judgment that jeopardizes the participant *and* may require medical or surgical intervention to prevent one of the above serious outcomes. Examples of such medical events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

11.7 Unexpected:
An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the Investigator's Brochure (IB) if one exists or is available or is not listed at the specificity or severity that is currently observed, or the event is not consistent with the risk information described in the protocol, informed consent form, general investigational plan or elsewhere in the current IND application when an IB is not required or available. The PI will determine if an adverse event is unexpected.

Expected adverse reactions are adverse events that are known to occur for the investigational drug and should be collected in a standard, systematic format using a severity grading scale. Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study drug. For risk related to this IND protocol, see "Expected Risk" section below.

11.8 Causality and Severity Assessment:
A clinical determination will be made, as to the likelihood that an AE is related to the administration of the study drug or research visit procedure. The PI or designee will determine the criteria below for the severity/intensity of the AE and its relationship to the administration of the investigational study drug. Other etiologies such as pre-existing conditions, concomitant therapy, study-related procedures, accidents that occur while on study, and other external factors will be considered during the evaluation of relatedness. The adverse event assessment report will be submitted with any reportable adverse event.

11.9 Severity/Intensity:
An adverse event will be classified as one of the following to describe the severity (intensity) and any necessary action taken in an attempt to resolve the event:

- Mild: A mild AE is usually transient in nature and generally does not interfere with normal activities. Participants are asymptomatic or have mild symptoms; intervention is not indicated.
- Moderate: A moderate AE is sufficiently discomforting to interfere with normal daily activities. Participants may need minimal, local, or noninvasive intervention;
- Severe: Also referred to as *medically significant* but not immediately life-threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting self-care activities of daily living (ADL). Note that a severe event is not necessarily a serious event.
- Life Threatening: Any AE that places the participant, in the view of either the PI or designee, at immediate risk of death from the AE as it occurred. It does *not* include an AE that, had it occurred in a more severe form, might have caused death.

11.10 Attribution:
The degree of certainty about causality will be graded using the following relationship categories:

- Unrelated: An adverse event is *clearly not related* to the drug or procedure.
- Unlikely Related: An adverse event is *doubtfully related* to the drug procedure.
- Possibly Related: An adverse event is *may be related* to the drug or procedure.
- Probably Related: An adverse event is *likely related* to the drug or procedure.
- Definitely Related: An adverse event is *clearly related* to the drug or procedure.
- For the purposes of regulatory reporting, a causality assessment of unlikely related will be managed as "unrelated". An adverse event that is definitely, probably, or possibly related will be managed as "related" and considered a "reasonable

possibility" that the drug caused the adverse event and, therefore, meets the definition of a suspected adverse reaction.

11.11 Documenting Adverse Events:
All serious and non-serious adverse events as well as abnormal test results/findings, regardless of suspected causal relationship will be documented in the subjects' research record. Adverse events will be reported to the IRB per university guidelines.

Adverse events that are classified as reportable will be followed until resolution or stabilization as evaluated by a physician. Clinically significant changes from the subjects' baseline will be considered noteworthy. All clinically significant changes, relevant medical history and concomitant medications and action taken will be documented in the subjects' case report forms (source document forms). Events that which do not meet the requirements of SAE reporting will be reviewed and confirmed by the Investigator and reported with the IRB annual continuing review, Data Safety and Monitoring report and the FDA annual report.

11.12 Pre-existing Conditions:
Pre-existing conditions that are present at the time of informed consent will be considered participant baseline. All baseline conditions will be recorded on the medical history in the source document forms. Worsening of the severity or frequency of a pre-existing condition in a research participant which does not necessarily have a causal relationship with the investigational drug is a reportable adverse event. Any changes, i.e., deterioration or worsening in these condition(s) will be documented and evaluated by the PI or designee. AEs with signs and symptoms that are believed to be due to the pre-existing condition(s) will not be reported as an AE unless there is an increase in frequency and severity. The investigator's clinical determination of any adverse event will be recorded on the AE form in the source document forms and maintained in the subjects' research record. All AEs will be documented on the adverse event log and maintained in the regulatory binder.

12. Food and Drug Administration Mandatory Reporting

12.1 Initial Safety Reporting:
The Sponsor-Investigator must report to the Food and Drug Administration (FDA) observed or voluntarily reported adverse events that meet criteria according to the following timeframe:

- 7-day IND Safety Report" – Unexpected Fatal or Life-threatening
  Report any unexpected fatal or life-threatening adverse reactions associated with the use of the study drug. Reporting will occur as soon as possible but no later than seven, (7) calendar days following the Sponsor-Investigator's initial receipt of the information. Preferably by facsimile or secure email addressed to the Regulatory Project Manager followed by a formal official submission.
  Any study event that is:
  - associated with the use of the study drug
  - serious (fatal or life-threatening)

- unexpected

- "IND Safety Report" – 15-day Written Reports
  Written IND safety reports may be submitted electronically on an FDA Form 3500A (MedWatch) or in a narrative format in accordance with 21 CFR 312.32. Report any of the following study events to the FDA as soon as possible but no later than 15 calendar days.
  Any study event that is:
  - associated with the use of the study drug
  - serious, but not fatal or non-life-threatening
  - AND unexpected

  Any previous or increased rate of events:
  - A previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting. The event must be reported within 15 calendar days from when the event was deemed reportable.
  - Findings from other clinical, animal, or in-vitro studies, including reports of mutagenicity, teratogenicity, or carcinogenicity that suggest significant risk to human subjects.
  - A clinically important increase in the rate of any serious suspected adverse reaction. The Sponsor-Investigator will identify previously reported similar adverse events in IND safety reports and analyze the significance of the current event in light of the previous reports.

  Any of the following adverse events as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event.
  - A single occurrence of an uncommon event and known to be strongly associated with the drug.
  - One or more occurrences of an event that is not commonly associated with the drug, but is otherwise uncommon in the population being studied.

12.2 Follow-up Reporting:

The Sponsor-Investigator will report any additional information that pertains to an IND safety report previously submitted or a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting.

- "Follow-up IND Safety Report"– Follow-up Information

Any relevant follow-up information that pertains to an IND safety report previously submitted will be submitted within 15 calendar days from day zero, date of the 7-day report.

A previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting must be submitted as a "Follow-up IND Safety Report". Reports should be submitted without delay, as soon as the information is available but no later than 15 calendar days after the Sponsor-Investigator receives the information.

12.3 Annual Report:
A summary of all IND safety reports, including the most frequent and most serious AEs, will be submitted each year within 60 days of the anniversary of the date that the IND became active, the date clinical studies were permitted to begin.

12.4 FDA Reporting Process - Written and Facsimile:
The FDA prefers IND "7-day" safety reports be submitted via secure email or by facsimile. Submit IND "15-day" written and follow-up safety reports and all other reports to the FDA.

12.5 Eisai (IND holder of Lemborexant):
The study team shall notify Eisai within twenty-four (24) hours of receiving notification of any serious adverse events experienced by a participant that could be reasonably related to lemborexant. For the purpose of this requirement, "serious" means: (1) death; (2) in-patient hospitalization or prolonged hospitalization; (3) life-threatening; (4) persistent or significant disability or incapacity; (5) congenital anomaly or birth defect; (6) other serious events that may jeopardize the patient and may require medical or surgical intervention to prevent one of the other five listed outcomes. Serious adverse events attributed to lemborexant will be reported using the Eisai serious adverse event form. Serious adverse events may be reported via one of the following:

Fax: 732-791-1111
Email: ESI_Safety@eisai.com

12.6 Institutional Review Board Mandatory Reporting:
The PI or designee will report, to the Institutional Review Board (IRB) at Washington University (WU), the Human Research Protection Office (HRPO) in accordance with WU IRB policies and procedures. The PI or designee will report immediately (within 10 working days unless it results in death), to the IRB, any observed or volunteered self-reported adverse event that meets the unanticipated problem (UP) criteria or if the Investigator believes the information meets the exception criteria as described below:

12.7 Unanticipated Problem (UP) Criteria:
The PI or designee will report, to the IRB, any observed or volunteered self-reported adverse event that is determined to meet all three criteria listed below:

- Must be unexpected in terms of (nature, severity, and/or frequency) given the research procedures that are described in the protocol and consent form and the characteristics the subject population being studied;
- Must be reasonably related to the research; and
- Must place participants or others at a greater risk of harm than previously known or recognized or approved by the IRB.

12.8 Exception Criteria:
The Investigator believes the information *identifies* a change to risks or potential benefits of the study or if the Investigator believes information indicates a change to the risks or benefits of the study.

12.9 IRB process of reporting events:
Events that do *not* meet the unanticipated problem (UP) criteria or the exception criteria, should be reported with the IRB annual continuing review, i.e., IND external safety reports. Federal regulations do not require immediate reporting to the IRB of IND/outside safety reports for events that do not meet the criteria outlined in 21 CFR 312.32(c). Thus, IND Safety reports that do not meet the regulatory requirement for immediate reporting may only be reported in summary format at the time of IRB continuing review.

Adverse events that meet the UP or exception criteria will be reported as follows:

- **1-day Reportable Events**
  Report the death of a research participant to the IRB within one (1) working day of the occurrence of the event or notification of the event to the PI, or to a member of the research team.
- **10-day Reportable Events**
  Report any unanticipated problems (UP) involving risks to participants or others which occur at WU or that impacts participants or conduct of the study, noncompliance, or receipt of new information that may impact the willingness of subjects to participate or continue participation in the research study, i.e., an updated package insert with a change to the risks or benefits resulting in a modification to the informed consent form and/or protocol. The occurrence of the event or notification of the event to the Investigator, or to a member of the research team must be reported to the IRB within ten (10) working days.

12.10 Data Safety Monitoring (DSM) Reporting:
In compliance with the Washington University School of Medicine Institutional Data Safety Monitoring (DSM) Plan, the PI will provide a DSM report to the IRB annually. The PI, research coordinator and members of the research team will review all study data as it becomes available. Investigators not involved with the study will independently review the safety data and state if the study should be continued or not. The report will include the protocol title, IRB protocol number, IND number, the activation date of the study, the number of subjects enrolled to date, the date of first and most recent subject enrollment, a summary of all adverse events regardless of severity or relationship to subject dropouts and corresponding reason for dropout, the investigational plan for the coming year, any new information related to the investigational drug or a summary of any recent literature that may affect the safety and ethics of the study.

12.11 Other Reportable Events:
Major deviations or series of minor deviations having the potential to adversely impact the health, safety, or welfare of participants or others or adversely impact the study's ability to produce scientifically valid results must be reported to the IRB within 10 working days of the occurrence of the event or notification to the Principal Investigator of the event. Major deviations resulting in death of a participant must be reported within one (1) working day of the occurrence of the event or the notification to the Investigator of the event. Any non-

compliance and protocol exceptions or deviations will be documented and maintained in the regulatory binder and participant's research chart.

12.11.1. Non-Compliance and Serious Non-Compliance:

- Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB. Noncompliance is defined as failure to follow any applicable regulation or institutional policies that govern human subject's research or failure to follow the determinations of the IRB.
- Serious noncompliance is defined as noncompliance that materially increases risks that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

12.11.2. Protocol Exceptions and Deviations:

- Protocol exceptions apply only to a single participant or a singular situation. Pre-approval of any protocol exceptions must be obtained by the IRB prior to the event. In the event a situation occurs which requires deviation from the protocol, the PI will make the final judgement on whether or not a participant study is prematurely completed.
- Protocol deviations such as less than expected tracer production can be accounted for during data analysis and will not necessarily result in cancellation of the scan. In the event of any deviations from the investigational plan/protocol that affects the life or physical well-being of a participant in any emergency, the PI will promptly notify the FDA and IRB as required.
- HRPO definitions of minor and major deviations
    - A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.
    - A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

12.11.3. Timeframe for Reporting Required Events

Table 1. Reporting Events

The table below summarizes the timeframe for reporting required events after initial receipt of the information by the PI or member of the research team as described above.

Serious, fatal life-threatening adverse events	IRB	FDA reporting for lemborexant
Any death and immediately life-threatening events, whether related or unrelated and any adverse event that meets the definition of IRB unanticipated	Within one (1) working day to the IRB	As soon as possible but no later than 7 calendar days

problem or FDA unexpected and serious suspected adverse reaction.		
Non-fatal/Non-life-threatening adverse events		
Any unexpected and serious suspected adverse reaction, findings from other clinical or animal studies (internal or external) and clinically important increases in severely suspected adverse reactions.	Within 10 working days to the IRB	As soon as possible but no later than 15 calendar days
Annual reportable adverse events		
Adverse events that do not meet the definition of unanticipated problem or serious and unexpected suspected adverse reaction should be submitted cumulatively.	IRB Annual Continuing Review; DSM report	FDA Annual Report
Other reportable adverse events		
New Information that may impact the willingness of subjects to participate in the research study – interim analysis, safety monitoring report, published paper, change in labeling, revised Investigator's Brochure.	Within 10 working days to the IRB	As soon as possible but no later than 15 calendar days
Noncompliance and Protocol Deviations		
A serious noncompliance, major protocol deviation or series of minor deviations which do not meet the above criteria.	Within 10 working days to the IRB	FDA Annual Report.
Non-serious, noncompliance issue	IRB Annual Continuing Review	None unless requested by IRB

13. Privacy/confidentiality

13.1 Breach of privacy/confidentiality:

- All data will be labeled with a random number rather than with any identifiable information. All physical materials (such as paper, data storage disks, and biospecimens) will be stored in locked cabinets/freezers in a locked research area, accessible only to the research team. All electronic information will require a password and will be accessible only to the research team. Any paper or electronic information that could link a participant to the study (such as consent documents) will be stored separately from all other study materials, also in a secure manner that is accessible only to the research team. If a report or article is written about this study or the study data are shared with other researchers, it will be done in such a way that participants cannot be identified.
- For individuals who give verbal consent for the Phone screen, and then do not "pass" the screening questions, their phone screen will be shredded, and *no* information is

kept about them.

Protections for research involving vulnerable populations: This project does not involve vulnerable populations.

13.2 Plan for Adverse Events (AE):
Response to an adverse event or risk will differ based on the type and severity of the event. A Data Safety Monitoring Committee (DSMC) will serve to mitigate these risks and ensure a formal process for dealing with adverse events. A DSMC consisting of experts in the study procedures and the effects of sleep disorders on cardiac and psychiatric morbidities has been established for the proposed study.

  - Qualified individuals monitoring the study: The DSMC members have substantial experience and expertise in this area of research and in clinical research, and are qualified to monitor the study.
  - The DSMC will meet prior to enrollment of any participants, to review study procedures and potential adverse events, and then provide guidance on HRPO application and consent/informational materials.

- *Web-Based Instruction on Conducting Human Research*: In accordance with the NIH policy effective October 1, 2000, this is to certify that Washington University key personnel involved in the design and conduct of the human subjects research aspect of this proposal have been educated on the protection of human research participants. An interactive web-based program that provides information on conducting human research and the informed consent process is organized into five modules. These modules include detailed information, examples and exercises related to basic principles, history, consent form process, and after approval requirements such as continuing review and adverse events.
- *Review of adverse events*: All personnel who will be in contact with participants and/or data are prepared to identify adverse events and have been instructed to report their occurrence immediately to the principal investigator.
- *Definition*: An adverse event will be defined as any negative change in health related to study-related procedures.
- *Classification of Events*: A Safety Documentation form will assess for details of any adverse events, severity, and relatedness to study procedures.
- *Data Collection Procedures for Adverse Events:* At each visit and with each interaction/phone call, adverse events will be screened for and recorded. If any adverse events have occurred, these will be logged in the Safety Documentation form.
- *Reporting Procedures:* The Safety Documentation form will be discussed during the DSMC meetings. The DSMC will meet according to participant completion goals (e.g., 5 participants complete the study, 10 participants complete the study, 15 participants complete the study, etc.). If a serious adverse event (SAE) occurs, the DSMC will meet as soon as possible to determine the course of action. If the DSMC deems changes to the study are necessary for safety reasons, the study will halt immediately until the changes have been approved through the Washington University HRPO.

- *Safety-related criteria for stopping the study*: The trial will be stopped if there is an unexpected serious adverse event (SAE). Otherwise, circumstances that would warrant stopping the study are not anticipated. However, should any circumstances arise that compromise the safety of the participants, they will be reported to the DSMC who will suspend research until appropriate safeguards allow continuation of the study.
- *Adverse Event Reporting Period:* Serious adverse events and Adverse events, regardless of the causal assessment to the study interventions (lemborexant, lumbar catheter, etc.), are collected from the time the subject signs the study informed consent through 28 days after the participant's last dose of lemborexant. Serious adverse events will reported directly to Eisai.
    - Expected adverse events (*i.e.,* are listed above and on consent forms) will be discussed at DSMC meetings. Also, they will be reported to the HRPO during annual renewals.
    - Unexpected serious adverse events will be reported within 10 days, per HRPO requirements.

Anticipated adverse events that would require medical intervention are:

- A post-lumbar catheter headache that does not resolve with conservative measures such as rest, hydration, and caffeine intake. This will be treated with "blood patch" in the Clinical Translational Research Unit, at no cost to the participant.
- Side effects to lemborexant such as somnolence and confusion upon waking. Risks from lemborexant are minimized with the inclusion/exclusion criteria and that participants will only receive the drug for 2 nights in the monitored setting of the Clinical Translational Research Unit at Washington University. If any severe adverse events occur after the first dose of lemborexant, then the drug will be discontinued and the participant monitored closely.

14. Potential Benefits of the Proposed Research to Human Subjects and Others

14.1 Potential benefits to participants:
The only direct benefit to the participant is that they will be screened for sleep apnea with the STOP-Bang questionnaire and other sleep disorders by the history and examination of the principal investigator. This may result in a diagnosis that may have otherwise been undetected. Participants who screen fail due to concerns for a sleep disorder like sleep apnea will be informed of the reason for exclusion, withdrawn from the study, and encouraged to follow-up with their primary care physician.

14.2 Potential benefits to others:
Potential benefits to society will be knowledge of whether or not sleep modification can change CSF Aβ production and concentrations. If CSF Aβ production and concentrations can be modified by improving sleep efficiency, then this opens up a new potential treatment option for the prevention or treatment of Alzheimer's disease from which they, or future AD patients, may benefit.

14.3 Risks vs. benefits:
The benefit of the knowledge to the gained in the study, as well as the personal benefit to participants, outweighs the risks in the study. Risks have been minimized to the greatest possible extent, while still achieving the scientific aims of the research study.

15. Importance of the Knowledge to be Gained

The knowledge to be gained in this study is very important because it is the first study directly assessing the relationship of poor sleep efficiency and Aβ in a relevant human population. If this study is successful, it may lead to treatments targeting sleep for possibly delaying or reducing risk of AD. AD is a devastating neurodegenerative disorder characterized by progressive cognitive decline. The public health impact of dementia is enormous with the number of individuals with AD projected to increase from 5 million Americans in 2010 to 13.5 million Americans in 2050. Even a modest reduction in the risk of AD would have a tremendous public health impact. Demonstrating that sleep alters Aβ may suggest innovative AD treatment approaches that involve sleep therapies.

16. Data and Safety Monitoring Plan

This study is a clinical trial, however it is not an NIH-defined phase III clinical trial. Lemborexant is FDA-approved for the treatment of insomnia. This study will use lemborexant 25 mg, which is higher than the FDA approved dose. Comparisons are being made between poorly-sleeping older adults treated (lemborexant) and untreated individuals (placebo). There is a Data Safety Monitoring Committee and monitoring plan as described in section "*2.B Protections Against Risk*" above. In compliance with the Washington University School of Medicine Institutional Data Safety Monitoring (DSM) Plan. The PI, research coordinator and members of the research team will review all study data as it becomes available. Investigators not involved with the study will independently review the safety data after 5 participants have completed the study and state if the study should be continued or not. The report will include the protocol title, IRB protocol number, IND number, the activation date of the study, the number of subjects enrolled to date, the date of first and most recent subject enrollment, a summary of all adverse events regardless of severity or relationship to subject dropouts and corresponding reason for dropout, the investigational plan for the coming year, any new information related to the investigational drug or a summary of any recent literature that may affect the safety and ethics of the study.

Given that the study requires significant involvement from the participants, we have taken steps to make the study less taxing for participants. These steps include having nurses available in the CTRU for assisting the research participants at all times and providing 24 hour/7 day/week access to study staff and physicians.

All participants will be identified by a de-identified study number to protect patient confidentiality. Participants may be linked to the "Lemborexant Sleep" study in Epic if they already have a record in the Epic system. Spreadsheets listing the participants' name and corresponding study number will be managed by the study coordinator, and will be stored in a password protected file behind the Neurology firewall. Only the research team will have

access to this spreadsheet. Data monitoring and analysis will be performed by the principle investigator. Data will also be collected on any adverse events related to the study procedures.

All data from mass spectrometry analyses are electronically uploaded and locked in the Bateman laboratory's database (Firebird 2.1.4) blinded to clinical status and other variables. Future studies may involve a collaboration with Dr. Donald Elbert, an Aβ stable isotope labeling kinetics (SILK) expert in modeling at Washington University, the Aβ SILK data will be analyzed with the compartmental model using the SAAM II modeling program blinded to clinical status and other variables. All statistical analyses will be conducted in collaboration with Dr. Chengjie Xiong, an expert in statistics at Washington University, or the ICTS biostatistics core. All data is compared in a pre-specified fashion.

17. ClinicalTrials.gov Requirements

This study will be registered at ClinicalTrials.gov and the final study results will be reported on the website within 12 months of study completion. This will enable registration of primary and secondary aims and allow for broadest publication in journals.

18. APPENDICES

18.1 Appendix 1: Lemborexant 25 mg Safety Data

Introduction

Lemborexant (LEM) is a dual orexin receptor antagonist approved in the US, Japan, Canada, Australia, and other Asian countries for the treatment of adult patients with insomnia. Doses of 2.5 (LEM 2.5; Japan only), 5 mg (LEM5) and 10 mg (LEM10) are currently approved for these patients. Across the clinical program, a total of 150 subjects in 7 studies were exposed to lemborexant (E2006) 25 mg (LEM25). Multiple doses of LEM25 were evaluated in three multiple dose studies, E2006-A001-002, E2006-A001-003, and E2006-G000-201, which are described in more detail below. Other studies not included in this overview employed single dose paradigms.

Study E2006-A001-002 (NCT01673451)

Study Design

This was a single-center, randomized, double-blind, placebo-controlled, sequential, multiple-dose study. The study was conducted in 2 parts: Part A (6 cohorts of healthy adults receiving evening dosing) and Part B (1 cohort of elderly adults receiving evening dosing).

In Part A, it was planned for a total of 48 healthy adult subjects (18 to 55 years) to be enrolled into 1 of 6 cohorts sequentially in a gradual dose escalation manner, randomized to receive either LEM or matching placebo in the evening 30 minutes before habitual bedtime, and after 3 hours fasting, each night for 14 days. Each cohort was comprised of 6 LEM- and 2 placebo-treated subjects. Blood samples were collected for PK analysis at prespecified timepoints, and PD assessments were conducted (not reported here).

During Part B, one cohort of 8 elderly subjects (≥65 to 80 years) were randomized and treated for 14 days in the evening with LEM25 or matching placebo 30 minutes before habitual bedtime, and after 3 hours fasting. This cohort comprised 6 LEM- and 2 placebo-treated subjects. Blood samples were collected at prespecified timepoints, and PD assessments were conducted.

Overall, 55 subjects were randomized, and all received study drug (placebo, lemborexant 2.5 mg, 5 mg, 10 mg, 25 mg, 50 mg, 75 mg). A total of 11 subjects (6 adult, 5 elderly) were randomized to LEM25.

Summary of Adverse Events

There appeared to be more treatment-related TEAEs with increasing doses of LEM, and the overall incidence of TEAEs was higher in LEM (all doses) (92.7%) compared to placebo (78.6%). There were no serious TEAEs. In the LEM groups, 1 subject (2.4%) in the LEM25 group had a TEAE leading to study drug dose adjustment and withdrawal from the study.

This subject discontinued study drug due to an AE (pyrexia) that was not considered to be related to study drug and was withdrawn from the study (Table 1).

Table 1 Overview of Treatment-Emergent Adverse Events – Safety Analysis Set, Study E2006-A001-002

Category	Placebo (N=14)	E2006							
		2.5mg (N=6)	5mg (N=6)	10mg (N=6)	25mg (N=6)	25mg Elderly (N=5)	50mg (N=6)	75mg (N=6)	E2006 Total (N=41)
TEAEs	11 (78.6)	5 (83.3)	5 (83.3)	6 (100)	5 (83.3)	5 (100)	6 (100)	6 (100)	38 (92.7)
Treatment-related TEAEs[a]	6 (42.9)	3 (50.0)	3 (50.0)	4 (66.7)	4 (66.7)	5 (100)	6 (100)	6 (100)	31 (75.6)
Severe TEAEs	0	0	0	0	0	0	0	0	0
Serious TEAEs	0	0	0	0	0	0	0	0	0
Deaths	0	0	0	0	0	0	0	0	0
Other SAEs	0	0	0	0	0	0	0	0	0
Life Threatening	0	0	0	0	0	0	0	0	0
Requires inpatient hospitalization or prolongation of existing hospitalization	0	0	0	0	0	0	0	0	0
Persistent or significant disability or incapacity	0	0	0	0	0	0	0	0	0
Congenital anomaly / birth defect	0	0	0	0	0	0	0	0	0
Important medical events	0	0	0	0	0	0	0	0	0
TEAEs leading to study drug dose adjustment	0	0	0	0	1 (16.7)	0	0	0	1 (2.4)
TEAEs leading to study treatment withdrawal	0	0	0	0	1 (16.7)	0	0	0	1 (2.4)

Table 1 Overview of Treatment-Emergent Adverse Events – Safety Analysis Set, Study E2006-A001-002

Category	Placebo (N=14)	E2006							
		2.5mg (N=6)	5mg (N=6)	10mg (N=6)	25mg (N=6)	25mg Elderly (N=5)	50mg (N=6)	75mg (N=6)	E2006 Total (N=41)

Placebo group includes placebo subjects pooled from all cohorts in the study. Percentages are based on the total number of subjects in relevant treatment group.
For each row category, a subject with 2 or more adverse events in that category is counted only once. MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event, SAE = serious adverse event.
a: Includes TEAEs considered by the investigator to be possibly or probably related to study drug or TEAEs with missing causality.
MedDRA Version 16.0.

Source: Table 14.3.1.2.

Serious Adverse Events – Safety Analysis Set

No TEAEs in any treatment group were severe. The adult LEM groups that reported only mild TEAEs were 2.5, 5, 10, and 25 mg. In the elderly LEM25 group, there was one TEAE of somnolence and one TEAE of hypoxia, both of which were rated moderate.

Overall Safety Conclusions

LEM was safe and well-tolerated following dosing in adult subjects up to 75 mg per day for 14 days, and in elderly subjects receiving 25 mg per day for 14 days. There was a higher incidence of treatment-related TEAEs among LEM subjects (all doses) (31/41, 75.6%) compared to placebo subjects (6/14, 42.9%). The incidence of treatment-related TEAEs appeared to be dose-related with higher rates occurring at higher doses, the highest incidence (100%) occurring in the LEM25 elderly, LEM50, and LEM75 dose groups. However, most TEAEs were mild in severity. No SAEs were reported during the study.

Study E2006-A001-003 (NCT02039089)

Study Design

This was a single-center, multiple-dose, randomized, double-blind, placebo-controlled, parallel-group study in healthy male and female subjects. The study consisted of 2 parts: Part A (3 cohorts of healthy Japanese subjects dosed in the evening) and Part B (1 cohort of healthy white subjects dosed in the evening). The cohorts were conducted sequentially. Part A was started first with the 2.5-mg dose cohort, followed by the 10-mg dose cohort, and then the 25-mg dose cohort. Part B was conducted in parallel with the 10-mg cohort of Part A, with overlap. Subjects received treatment with study drug for 14 days.

Summary of Adverse Events

The number of subjects with TEAEs and the incidence of TEAEs were low and similar across dose groups. No trends with dose or race were seen. Most TEAEs were judged by the investigator to be treatment related (i.e., related or not related as described in the clinical study protocol to study drug). All TEAEs were mild. There were no severe TEAEs, serious TEAEs, TEAEs leading to withdrawal, or deaths during the study. All TEAEs resolved by the end of the study.

Somnolence and abnormal dreams were the most frequently reported treatment-related TEAEs, with 4 Japanese subjects (1 each in the placebo and 2.5-mg groups, and 2 in the 25-mg group) and 1 white subject (10-mg group) reporting somnolence and 1 Japanese subject (25-mg group) and 2 white subjects (10-mg group) reporting abnormal dreams (Table 2).

Table 2 Summary of Treatment-emergent Adverse Events (Safety Analysis Set, Study E2006-A001-003)

	Japanese					**White**	
Characteristic	**Placebo N = 6 n (%) E**	**2.5 mg N = 6 n (%) E**	**10 mg N = 6 n (%) E**	**25 mg N = 6 n (%) E**	**Overall Japanese N = 18 n (%) E**	**Placebo N = 2 n (%) E**	**10 mg N = 6 n (%) E**
TEAEs	2 (33.3%) 3	1 (16.7%) 2	0	2 (33.3%) 4	3 (16.7%) 6	1 (50.0%) 1	3 (50.0%) 3
Serious TEAEs	0	—0	0	0	0	0	0
Severe TEAEs	0	0	0	0	0	0	0
Related TEAEs	2 (33.3%) 2	1 (16.7%) 2	0	2 (33.3%) 3	3 (16.7%) 5	0	3 (50.0%) 3
TEAEs Leading to Withdrawal	0	0	0	0	0	0	0
TEAEs Leading to Death	0	0	0	0	0	0	0

Overall Safety Conclusions – Study 003

Multiple ascending doses of LEM were safe and well tolerated in healthy Japanese and white subjects in this study. The incidence of TEAEs were low and similar across dose groups, and the most common TEAE was somnolence. There were no significant patterns in laboratory parameters to suggest a relationship to dose group. No TEAEs were related to laboratory or hematology measurements. Urinalysis test results were mostly negative or normal; no abnormal test results were considered to be of clinical significance. Vital signs, ECG parameters, and high-precision QT analyses were unremarkable, and an effect on the placebo-corrected change-from-baseline QTcI (ΔΔQTcI) exceeding 10 msec could be excluded at the observed peak plasma level after multiple dosing up to 25 mg/day for 14 days.

Study E2006-G000-201 (NCT01995838)

Study Design

E2006-G000-201 was a multicenter, multiple dose, randomized, double-blind, placebo-controlled, parallel-group, Bayesian adaptive, dose-response study in subjects with chronic insomnia. Subjects were randomized to 1 of 6 doses of LEM (1 mg, 2.5 mg, 5 mg, 10 mg, 15 mg, or 25 mg) or placebo according to the randomization scheme.

The study had 2 phases: Prerandomization and Randomization. The Prerandomization Phase lasted up to 21 days and consisted of a Screening Period (Days –21 to –2) and a Baseline Period (Day –1). After the Baseline Period, all eligible subjects were randomized in a double-blind manner to receive LEM or placebo for 15 nights during the Treatment Period (Days 1 to 15). All subjects then received placebo in a single-blind manner, for 2 nights (Days 16 to 17) during the Rebound Insomnia Assessment Period (Days 16 to 18). Subjects did not receive study drug during the Follow-up Period (Days 19 to 30).

Summary of Adverse Events

The overall incidence of treatment-emergent adverse events (TEAEs) was higher in subjects who received LEM (all doses except 1 mg) (50.2%), as compared with placebo (37.5%). One subject (2.0%) (Subject 10081004) in the LEM25 group had a severe and serious TEAE (generalized tonic-clonic seizure; see Appendix for further details), and one subject (1.8%) in the placebo group (Subject 10021003) had a serious TEAE (hyperkalemia). Both subjects recovered without sequelae. With LEM25, 3 subjects had TEAEs leading to study drug dose interruption, and 1 subject had a TEAE leading to study drug dose discontinuation (Table 3). There were no study drug dose interruptions or discontinuations due to TEAEs in any of the other dose groups.

Table 3 Overview of Treatment-Emergent Adverse Events – Safety Analysis Set, Study E2006-G000-201

Category	Placebo (N=56) n(%)	Lemborexant					
		1 mg (N=32) n(%)	2.5 mg (N=27) n(%)	5 mg (N=38) n(%)	10 mg (N=32) n(%)	15 mg (N=56) n(%)	25 mg (N=50) n(%)
TEAEs	21 (37.5)	11 (34.4)	11 (40.7)	16 (42.1)	19 (59.4)	31 (55.4)	30 (60.0)
Treatment-related TEAEs[a]	11 (19.6)	8 (25.0)	9 (33.3)	12 (31.6)	15 (46.9)	24 (42.9)	24 (48.0)
Serious TEAEs	1 (1.8)	0	0	0	0	0	1 (2.0)
Deaths	0	0	0	0	0	0	0
Other SAEs	1 (1.8)	0	0	0	0	0	1 (2.0)
Important medical events	1 (1.8)	0	0	0	0	0	1 (2.0)
Severe TEAEs	0	0	0	0	0	0	1 (2.0)
TEAEs leading to study drug dose adjustment	0	0	0	0	0	0	4 (8.0)
TEAEs leading to study drug dose withdrawal	0	0	0	0	0	0	1 (2.0)
TEAEs leading to study drug dose interruption	0	0	0	0	0	0	3 (6.0)
TEAEs of Special Interest	0	0	0	1 (2.6)	3 (9.4)	5 (8.9)	2 (4.0)
Sleep paralysis	0	0	0	1 (2.6)	3 (9.4)	4 (7.1)	2 (4.0)
Cataplexy	0	0	0	0	0	1 (1.8)	0

MedDRA = Medical Dictionary for Regulatory Activities, SAE = serious adverse event, TEAE = treatment-emergent adverse event.
Includes TEAEs considered by the investigator to be possibly or probably related to study drug or TEAEs with missing causality.
MedDRA Version 16.1. Source: Table 14.3.1.2

Adverse Events that Resulted in Discontinuation of Study Drug

There was 1 SAE of seizure leading to discontinuation of study drug (Subject 10081004), and the subject was withdrawn from the study (see CIOMS form, attached).

Adverse Events that Required Study Drug Dose Adjustment or Interruption

Study drug treatment was interrupted in 3 subjects who received LEM25, as follows:

Subject 10061004: On Study Day 15, the subject had a mild fever, considered not related to study drug by the investigator. This fever lasted less than 1 day. Study drug was interrupted on Study Day 15 and resumed on Study Day 16.

Subject 10111043: A blood sample was drawn on Study Day 3 and reported on Study Day 4 to have an incidental finding of a low white blood cell count. This TEAE was considered to be of mild severity and probably related to LEM by the investigator. Study drug was interrupted for 1 day (Study Day 5) because of this TEAE. An unscheduled blood sample was taken on Study Day 5 for hematology and results available on Study Day 6 indicated that this TEAE had resolved.

Subject 10191018: On Study Day 3, increases in levels of ALT and AST were observed, considered to be of moderate severity and possibly related to LEM by the investigator. Study drug was interrupted for 3 nights, then was resumed after AST levels started to decline.

Overall Safety Conclusions – Study 201

On the basis of the reported safety data from the Phase 2 study, doses of 1 mg to LEM25 were considered to be well tolerated. There were no clinically important differences between LEM treatment and placebo on blood chemistry, vital signs, weight, or ECG. Rates of AEs showed some evidence of dose response in the LEM groups compared to placebo subjects, particularly for somnolence. There were no deaths. There were 2 SAEs, 1 each in the placebo and LEM25 group.

During the Phase 3 clinical development program for lemborexant, an independent adjudication committee was established to evaluate events potentially concerning for seizures and/or cataplexy, and to determine whether these potential events represented true seizure, cataplexy, or neither. No events of potential seizure were referred to the adjudication committee, although events of potential cataplexy were evaluated. None of the latter was adjudicated as cataplexy.

There have been postmarketing reports of seizure with lemborexant. A review of these reports indicates that there is no single report where there is a clear causal relationship of the reported event to lemborexant therapy. Thus, evaluation of the overall available data does not support an association between lemborexant therapy and an increased risk of seizures.

Summary of All Clinical Studies with Exposure to Lemborexant 25 mg

LEM25 has been evaluated in 4 additional studies (Table 4 below). No new safety signals at the 25 mg dose were identified through these studies, and the most common AEs and TEAEs were consistent with the known safety profile of lemborexant. Therefore, the language proposed in the consent form with respect to potential safety risks is consistent with current US prescribing information with additional information about rare events.

Table 4 Clinical Studies with Exposure to LEM25

Study Number NCT #	Study Design/Population	No. Subjects Exposed to LEM25
E2006-A001-001 NCT01463098	Randomized, Double-blind, Placebo- and Active- controlled, Single Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of E2006 in Healthy Subjects and Otherwise Healthy Subjects with Primary Insomnia	18
E2006-A001-002 NCT01673451	Double-blind, PBO-controlled safety, tolerability, and PK study of MAD of LEM in healthy nonelderly and elderly subjects	11
E2006-A001-003 NCT02039089	Double-blind, PBO-controlled safety, tolerability, PK, and PD MAD study of LEM in healthy Japanese and White subjects	6
E2006-A001-005 NCT01673880	Open-label, crossover, bioavailability study of LEM capsule versus tablet formulations in healthy subjects	16
E2006-A001-102 NCT03471871	Double-blind, PBO-controlled, crossover study of respiratory safety of LEM10 and LEM25 in adult and elderly healthy subjects	49
E2006-G000-201 NCT01995838	Multicenter, randomized, double-blind, PBO-controlled, parallel group, Bayesian adaptive randomization design, dose- response study in subjects with insomnia disorder	50

LEM: lemborexant, LEM10: lemborexant 10 mg, LEM25: lemborexant 25 mg, MAD: multiple ascending dose, NCT: National Clinical Trial, PBO: placebo, PD: pharmacodynamic, PK: pharmacokineticL

18.2 Appendix 2: Schedule of Events

Visits	Pre-Screen	Visit 1	Visit 2	Visit 3	Visit 4			
					Admission	48-Hour Sampling	8-12 Hour Observation	Discharge
Informed Consent		X						
Demographics		X						
Medical History		X						
MMSE		X						
C-SSRS		X						
MEQ		X						
PSQI[a]	X	X						
STOP-BANG	X							
Amyloid-Beta Blood Test[b]		X						
Physical Exam		X						X
Neurological Exam		X						
Concomitant Medication		X		X	X			X
Vital Signs		X	X		X	X	X	
Height		X						
Weight		X		X	X			
12-Lead ECG		X						
Blood Collection (CBC w Diff & Comprehensive Metabolic Panel)		X						
Blood Collection (Coagulation Panel)				X				
Blood Draw (APOE)		X						
Attended PSG			X					
Unattended PSG					X	X	X	
Lumbar Catheter Placement					X	X		
Leucine Infusion					X			

Visits	Pre-Screen	Visit 1	Visit 2	Visit 3	Visit 4			
					Admission	48-Hour Sampling	8-12 Hour Observation	Discharge
Study Drug Administration (2 nights)						X		
CSF/Blood Catheter Sampling						X		
PVT					X		X	
SSS					X		X	
CSF Safety Labs						X		
Assess for AE's		X	X	X	X	X	X	X
Blood Patch								PRN
24-Hour Phone Follow-Up								X
48-Hour Phone Follow-Up								X

[a] May be obtained during phone screen or Visit 1
[b] Not required if referred by the SEABIRD study

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