STUDY PROTOCOL AND ANALYSIS PLAN

Is Obstructive Sleep Apnea Important in the Development of Alzheimer's Disease?

ClinicalTrials.gov ID NCT0509427

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04 / 24 / 2025

UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
The headings on this set of instructions correspond to the headings of the Research Plan.
General Instructions: Enter a response for all topic headings.
Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.

Version date: 9/30/2013

1. PROJECT TITLE

200228, Is Obstructive Sleep Apnea Important in the Development of Alzheimer's Disease?

2. PRINCIPAL INVESTIGATOR

Atul Malhotra, MD

3. FACILITIES

UCSD Chancellor Park, 4520 Executive Drive, San Diego, CA

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California Protons Cancer Therapy Center, 9730 Summers Ridge Road, San Diego, CA 92121

Diagnomics, 5795 Kearny Villa Rd, San Diego, CA 92123 (DNA Analysis, subject do not visit site)

4. ESTIMATED DURATION OF THE STUDY

5 years

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

Obstructive sleep apnea (OSA) is common in older adults and has recently been implicated in pathogenesis of Alzheimer's disease (AD). Research has shown that sleep disruptions have caused memory impairment. Sleep apnea is a form of sleep disruption. We would like to examine how obstructive sleep apnea may contribute to the progression of Alzheimer's disease.

6. SPECIFIC AIMS

Aim 1: We will assess the endotypes (mechanisms) underlying OSA in elderly individuals known to be high risk for AD (vs. non-OSA matched controls) using novel recently validated simplified techniques which do not require burdensome complex overnight experiments to assess endotypes (primary outcome loop gain). We will further assess the predicted response to O2 therapy in terms of respiratory outcomes among elderly OSA patients with varying levels of loop gain and pharyngeal collapsibility.

Hypothesis 1: A substantial proportion of high AD risk patients with OSA should be O2 responsive as predicted using pathophysiological assessments.

Aim 2: We will perform an overnight mechanistic study of oxygen therapy vs. room air in high AD risk patients with OSA (recruited from Aim 1 and others if necessary). Given the frequent intolerance of PAP in elderly patients, we anticipate that oxygen therapy may be a viable therapeutic approach in this fragile population. We will focus on respiratory outcomes (primary outcome: apnea hypopnea index) but also assess sleep dependent memory consolidation on word pairs task given the major impact in the elderly.

Hypothesis 2: O2, compared to room air, will improve OSA and neurocognitive outcomes in select elderly OSA patients at risk of AD.

Aim 3: Preclinical AD with OSA and non-OSA controls, from Aim 1 will have structural and molecular brain imaging focusing on hippocampal atrophy as a predictor of memory consolidation. We will also assess amyloid and tau in the medial temporal region as function of OSA severity and as a predictor of neurocognitive function. This aim will lay the groundwork for designing a robust clinical trial using neuroimaging outcomes.

Hypothesis 3: Impairment in memory consolidation is a function of hippocampal size in OSA patients at risk of AD.

Aim 4: We will perform a pilot randomized trial of oxygen vs. PAP therapy in OSA patients with preclinical AD.

Hypothesis 4: In preclinical AD with OSA, oxygen will be a viable therapeutic strategy to improve memory.

7. BACKGROUND AND SIGNIFICANCE

Several lines of evidence suggest a potential causal link between sleep disruption and Alzheimer's disease (AD). First, a large prospective observational study showed an increased incidence of AD among patients with untreated obstructive sleep apnea (OSA) Second, a prominent paper in Science recently showed accumulation of tau with sleep disturbance in mice as well as humans. The authors further observed increased tau pathology spreading with chronic sleep disturbance. Third, common genetic links have been observed between OSA and AD e.g. ApoE related. Fourth, we and others have observed substantial memory impairment with sleep fragmentation which is amenable to OSA therapy. Of note, the recent identification of markers of those at risk for AD including the polygenic hazard score (PHS) have made early interventional studies (or prevention) more feasible i.e. to bend the curve of AD progression by delaying or reducing cognitive decline, rather than trying to prevent fully established disease. Advances in human brain imaging also allow assessment of tau and amyloid which are thought to be pathogenic in AD, and can now be identified prior to symptomatic onset, i.e., in the preclinical phase. Thus, the investigation of sleep disorders as a therapeutic target to change AD trajectory and to improve well-being in those afflicted is clearly required.

Importance of OSA: OSA is an increasingly common condition with well established neurocognitive and cardiometabolic sequelae. Major risk factors for OSA such as aging and obesity are also on the rise contributing to the high prevalence of OSA. While debate continues regarding the impact of OSA, definitive data have now established major sequelae of OSA independent of known confounders. Regarding neurocognitive outcomes, we and others have shown that OSA leads to impaired memory consolidation, particularly that which occurs overnight with sleep (i.e., sleep dependent memory consolidation). Studies have suggested a link of OSA to AD although definitive data are still lacking.

Despite these recognized consequences, treatment of OSA remains unsatisfactory. Nasal PAP (positive airway pressure) is the treatment of choice for OSA based on randomized trials; its impact can be transformative for some patients. However, many patients have poor adherence to PAP or experience variable efficacy with alternative therapies such as oral appliances and upper airway surgery. Many patients avoid the diagnosis of OSA or are lost to follow-up prior to start of therapy emphasizing the need for improved OSA treatment. We have recently reported predictors of oxygen responsiveness using a cross-validated pathophysiological model, but require further data (e.g. further validation, assessment of generalizability to older cohorts) before implementation. This proposal will provide that further validation.

Importance of Aging in Sleep Apnea: The importance of aging in OSA is established. First, aging is a major risk factor for OSA. OSA has been shown to increase in prevalence with aging, in part as a function of increasing body weight. In women, menopause is also an important risk factor. Thus, aging of the population is

yielding an increasing burden of OSA. Second, some epidemiological data have suggested that the impact of OSA may be reduced with aging. That is for a given severity of OSA, the consequences of OSA may be less in older vs. younger people. However, this aging-related protection has been debated, and the purported survivor effects in OSA are likely overstated. Third, both data and clinical experience support the notion that elderly often struggle with PAP, emphasizing the need for new therapeutic approaches.

Importance of Memory Consolidation: Sleep dependent memory consolidation occurs whereby a given fact or event is consolidated into memory following a period of restful sleep. Up to 80% of the variance in overnight memory consolidation can be explained by quality of sleep, emphasizing the importance of sleep over other variables such as age, sex, race, education, IQ, socioeconomic status etc. We have recently observed impaired memory consolidation in mild OSA using a motor sequence learning task (MST). The MST is a well-established test which relies on various brain structures including the medial prefrontal cortex but is not primarily reliant on hippocampus. However, animal studies of severe hypoxia suggest that the hippocampus may be particularly vulnerable to the effects of intermittent hypoxia. Such studies may mimic severe OSA, but perhaps more modest hypoxia may affect other brain regions preferentially. Thus, uncertainty persists regarding the importance of hippocampal dysfunction in human OSA. The verbal paired associates test (VPA) is thought to be hippocampal dependent with our preliminary data showing considerable abnormality in human OSA. Of note, we recognize that memory impairment does not necessarily equate with AD. We have chosen to focus on the reversibility of hippocampal memory impairment with OSA therapy given the potential impact on countless afflicted patients. We doubt patients with advanced AD reverse with OSA therapy, but older at risk adults without major impairment may benefit from early intervention. This study will examine older OSA patients at high risk of AD to test whether OSA treatment improves memory consolidation and perhaps delays AD.

Importance of OSA Pathogenesis: Recent data have supported the concept that not all OSA occurs for the same reason. Traditionally, OSA was felt to be a disease of anatomical compromise with protective pharyngeal reflexes present during wakefulness but not sleep, leading to collapse of the pharyngeal airway with the loss of pharyngeal dilator motor tone at sleep onset in those anatomically predisposed. Recent studies have suggested that some patients develop OSA primarily for anatomical reasons, while others have dysfunction of upper airway dilator muscles as their main predisposing cause. Instability in ventilatory control (i.e. high loop gain) is also an important factor in a subset of OSA patients. The importance of these varying mechanisms is highlighted by the concept that personalized treatment of the underlying cause may lead to greater amelioration of OSA in selected patients, e.g. palatal surgery may be highly effective therapy for those patients who develop OSA from anatomical compromise at the level of the velopharynx. Similarly agents to increase hypoglossal output such as hypoglossal nerve stimulation and/or pharmacological therapies to increase hypoglossal motor output may work well in patients with pharyngeal dilator dysfunction. Patients may have many abnormalities, leading to the concept of tailored combination therapy to eliminate OSA in individuals.

Importance of Loop Gain: Loop gain is an engineering term used to define the stability of a negative feedback control system. A system with a high loop gain is prone to instability whereas a system with low loop gain is intrinsically stable. The loop gain concept has been applied to control of breathing such that certain factors can lead to fluctuations in CO2 levels such as robust chemosensitivity, high efficiency of CO2 excretion etc. Cheyne Stokes breathing common in heart failure is the hallmark of high loop gain. Interventions like oxygen and acetazolamide can lower loop gain and lead to improvements in breathing. Thus, elevated loop gain may be a therapeutic target at least for a subset of OSA patients.

We have recently published analyses and validation to predict the response to oxygen therapy in OSA based on underlying pathophysiological variables. Of note, loop gain was not a significant univariate predictor of responders/non-responders to supplemental oxygen. Using cross-validation, we developed a logistic regression model using loop gain as well as pharyngeal collapsibility and upper airway dilator muscle compensation

ability which showed 83% accuracy (89% before cross validation). Oxygen therapy was particularly effective in patients with high loop gain with concomitant effective upper airway dilator muscles in the context of minor pharyngeal collapsibility. Our model predicted both the responders and non-responders from the standpoint of apnea hypopnea index (AHI) but also blood pressure response and self-reported sleep quality. We can clearly identify a subset of elderly OSA who should be amenable to oxygen. However, additional studies are clearly required to validate our model further, to extend the observations to older participants with preclinical AD and to assess brain structure and tau load in this context.

OSA in the Elderly May be a Distinct Disease: We and others have suggested the mechanisms underlying OSA in the elderly may be different from matched younger individuals. While many OSA patients have major anatomical predisposition to OSA, i.e. elevated positive Pcrit about one third of individuals with OSA have loop gain abnormalities as a major contributing factor to their disease. Esophageal pressure measurements have shown a lower value in elderly OSA patients during respiratory events compared to younger OSA patients, suggesting an aging effect on major pathophysiological variables such as control of breathing141, airway mechanics or the arousal threshold. Also, central apneas increase with aging, again suggesting an underlying component of instability in ventilatory control. Sleep typically becomes more fragmented with aging, a finding of relevance to the type of unstable breathing which occurs with state transitions (i.e. CO2 fluctuations from sleep to wake to sleep). However, other studies, including our own have not shown a major impact of aging on loop gain (or low values in older vs. younger OSA patients), emphasizing the need for further study. Because studies show loop gain elevation in some elderly (e.g. using a pressure drop or pressure support technique) whereas other studies show no major elevation of loop gain in the elderly (using a proportional assist ventilation PAV technique), interventional studies will be required to bring clarity. Of note the PAV technique relies on stable sleep and stable upper airway two assumptions which are flawed in the elderly perhaps leading to an underestimate of the breathing control abnormalities in this age group. As stated, O2 responsiveness is not just a function of high loop gain but interacts with other OSA endotypes. Based on available data we estimate ~30% of elderly OSA patients should be O2 responsive.

These mechanistic observations regarding OSA in the elderly have three major implications. First, to resolve the discordant results and to determine whether elevated loop gain is causally important in OSA pathogenesis, interventional studies to lower loop gain will likely be required to assess whether breathing improves with O2. Indeed even the use of the word “high” or “elevated” in the description of loop gain is somewhat arbitrary, emphasizing the need to define what loop gain levels are amenable to therapeutic intervention116. Furthermore, the loop gain must clearly be interpreted in the context of other endotypes (e.g. pharyngeal collapsibility, upper airway dilator muscle function) since loop gain in isolation does not predict O2 responsiveness. Second, an individualized approach to OSA may involve providing O2 to selected elderly based on underlying endotypes. Patients with multiple pathophysiological abnormalities may also benefit from lowering loop gain, although apnea may not be eliminated without combination therapy in such individuals82. As such the optimal approach to improving neurocognitive performance in elderly OSA at risk of AD is also unclear without further study. Third, different causal pathways to disease may explain variability in disease consequences e.g. less negative intrathoracic pressure would be predicted to raise cardiac afterload to a lesser extent than a more negative intrathoracic pressure level. Similarly, a lower arousal threshold (i.e. wake up easily) may yield less hypoxemia for a given apnea in an elderly person compared to a younger person with a higher arousal threshold. The present study will define OSA endotypes in patients at risk of AD and how interventions may improve OSA and AD risk.

Importance of Brain Imaging: Considerable advances have been made in technology for structural and molecular imaging of the brain. The field of OSA has taken advantage of some of these techniques, leading to valuable insights. Data suggest important white matter abnormalities possibly as a result of disruption of myelin in people afflicted with OSA. Abnormalities in grey/white matter ratio as well as cerebellar structure have also

been observed. However, further advances are likely possible using the latest technologies particularly by combining structural and molecular imaging advances, by assessing neurocognitive outcomes concomitantly, by quantifying amyloid and tau deposition and by studying large sample sizes to provide adequate statistical power. We have previously made some efforts with brain imaging200, but now at UCSD have developed robust collaborations with imaging experts. These Co-Is are scientifically engaged and have provided us with major insights into the utility of various techniques but also for the underlying biology being assessed. We have only recently begun focusing on determining the biological basis underlying memory deficits in OSA, since we previously did not achieve adequate spatial resolution of the hippocampus and related brain structures. Newer imaging techniques have also been developed which allow the quantification of brain amyloid and tau with potential pathophysiological implications in AD. Indeed a recent (controversial) study has shown evidence of amyloid deposition in humans with a single night of sleep deprivation. Although some debate is ongoing whether amyloid is actually pathogenic or simply a marker of AD risk, genetic and other studies have provided compelling rationale to assess amyloid in the context of assessing AD risk. A recent major paper also pointed to the importance of tau accumulation in the context of rodent and human sleep disturbance7. We are not aware of prior large scale controlled studies to perform sophisticated brain imaging of elderly with known OSA plus careful neurocognitive assessments. This study will examine pre-clinical AD with OSA patients using brain imaging (structural MRI and amyloid/tau PET) to help answer these questions.

Deficiencies in the Literature: Despite considerable efforts in the OSA field, questions remain. First, can a mechanistic approach allow individualized therapy for OSA? By understanding the causal pathway(s) underlying OSA in a given patient, interventions could be targeted to alleviate the neurocognitive impact of OSA without the need for PAP therapy. Second, do some elderly OSA patients have underlying endotypes which are amenable to pharmacotherapy? Such a finding might also obviate the need for PAP in some individuals. Third, given the reported association of OSA on impaired memory consolidation, the question arises whether stabilization of breathing pattern and alleviation of hypoxemia can improve memory. Improvement in memory would be particularly crucial for the elderly who can be cognitively fragile and in whom AD risk has been reported related to OSA. Fourth, the application of cutting edge imaging techniques with neurocognitive assessments to OSA patients may yield insights in sleep-dependent memory consolidation. We will test whether personalized OSA therapy can improve memory and AD risk in some pre-clinical patients.

8. PROGRESS REPORT

N/A

9. RESEARCH DESIGN AND METHODS

After written informed consent has been obtained, the following procedures will be undertaken:

Daytime Visit 1

Participants will be asked to complete the following:

1. *Polygenic hazard score (PHS)*—a genetic assessment that predicts the age onset of Alzheimer's Disease through a saliva sample. The saliva sample kit will be provided to a Clinical Laboratory Improvement Amendments (CLIA) certified lab to obtain the PHS. The PHS is derived from an analysis of a 31 single-nucleotide polymorphism panel (including ApoE). After samples are processed by the lab, they will not be retained. Samples will not be shared with other researchers or institutions, other than the lab, which is processing and destroying the sample after results have been obtained.
2. *Montreal Cognitive Assessment (MoCA)*—a screening tool for mild cognitive impairment and early stages of Alzheimer's.
3. *Anthropomorphic assessment:* A basic exam, including height, weight, blood pressure, and oxygen saturation will be obtained (from clinical chart, if available).

4. *Questionnaires:* Standard sleep questionnaires will be completed Epworth Sleepiness Scale (ESS), Pittsburg Sleepiness Questionnaire Inventory (PSQI), Insomnia Sleepiness Index (ISI), and Morningness-Eveningness questionnaire (MEQ). In addition, participants will complete the Geriatric Depression Scale (GDS) to identify depression. If action needs to be taken in response to suicidality, a plan of action will be described in Item 15.
5.
6. Home Sleep Test: Due to the COVID-19 pandemic, we will allow subjects to opt out of the overnight sleep study and instead complete a Home Sleep Test (HST). Before these subjects leave, they will be given a HST device. A HST has the following components: 1) a chest belt which is secured around the chest to detect respiratory motion, 2) a nasal flow cannula inserted in the nostrils to detect nasal airflow, and 3) a pulse oximeter probe clipped to the finger for continuous oxygen saturation monitoring. Apneas and hypopneas will be defined using the recently published American Academy of Sleep Medicine (AASM) guidelines for syndrome definition and scoring techniques. Subjects will be asked to remain in the supine position as much as possible. After completing the HST, subjects will return the device in person or be given mailing supplies so that the device can be returned to our site. Preclinical Alzheimer's Cognitive Composite (PACC): This assessment is composed of 4 neurocognitive tests: a) Free and Cued Selective Reminding Test (FCSRT), b) Logical Memory from the Wechsler Memory Scale, c) Digit Symbol-Coding from the Wechsler Intelligence Scale and d) Mini-Mental Status Examination (MMSE). Before participants complete the PACC, we will obtain additional consent to obtain an audio recording of their verbal responses, which is conventionally done to allow researchers to score the assessment after completion. We anticipate that live scoring while administering the assessment may be too arduous and result in errors. Participants will be allowed to complete the assessment without recording if they choose.

<u>Overnight Visit 1</u>
Subjects will arrive to the research sleep laboratory at approximately 8PM and undergo the following procedures:

1. *Neurocognitive function assessments:* Participants will complete motor sequence testing and averbal word pairs task. Participants will also be asked to complete neurocognitive testing in the NIH toolbox. The NIH Toolbox battery has been nationally normed in a sample of 4,859 participants, English or Spanish speaking, ages 3-85 years, representative of the U.S. population based on gender, race/ethnicity, and socioeconomic status. Based on these data, demographically-corrected T-scores are available to adjust for age, gender, education, language and race/ethnicity. Additionally, this assessment tool is the result of an NIH initiative and is used in many studies, which allows for harmonization of findings. It can be completed in approximately 30 minutes.

2. *Psychomotor Vigilance Test:* Objective assessment of brain function will be done using 10-minute psychomotor vigilance test (PVT).

3. *Polysomnography:* A standard polysomnography sleep will be completed to determine sleep apnea severity. Monitoring for standard clinical polysomnography study will be applied to the subject, as follows: The subject will have EEG, EMG, EOG, and ECG electrodes, an adhesive body position sensor placed in standard locations. Pulse oximetry sensor will be attached either to a finger or ear lobe and secured by tape. The following parameters will be measured during sleep: electroencephalogram, eye movement, electrocardiogram, electromyogram, leg movement, snoring sounds, nasal pressure, and nasal-oral airflow by thermistor, respiratory effort and body position by piezo-electric bands of the thorax and abdomen or magnetometers, position sensors, and pulse oximetry. This equipment is standard for diagnostic polysomnography and should not be uncomfortable.

a. Once all of this equipment has been comfortably and securely fastened, the subject will be allowed to fall asleep and data recording will begin. Subjects will be asked to remain in the supine position as much as possible. All data will be acquired on a 1401 plus interface and Spike 2 software. The study will end at approximately 6 AM, at which time the monitoring equipment will be removed, and the subject will undergo a blood draw in this fasting state.

Blood will be analyzed for c-reactive protein (CRP), interleukin-6 (IL6) and metabolic biomarkers.

b. The study will end at approximately 6AM. The equipment will be removed and given to the sleep technician. In the morning, the following will happen:

4. *Morning neurocognitive function assessments:* Participants will complete motor sequence testing and a verbal word pairs task.

5. *Psychomotor Vigilance Test:* Objective assessment of brain function will be done using 10-minute psychomotor vigilance test (PVT).

6. Karolinska Sleepiness Scale (KSS): A sleep questionnaire.

Participants with obstructive sleep apnea will return to the sleep laboratory for two additional overnights. Subjects will be randomized to receive oxygen or room air during overnight 2 and 3. An Imaging visit will be completed by both sleep apneics and non-sleep apneics. This imaging visit will happen after overnight 1 and before either overnight 2 or overnight 3.

<u>Imaging Visit 1 (MRI and PET Scan) (2hours)</u>

The MRI and PET scan of the brain will occur on a separate day or evening from the sleep studies at the Radiology Imaging Laboratory.

1. *Questionnaires.* Before the scan, participants will complete a MRI Recruitment Safety form. Subjects will also be given the Spielberger State Anxiety Scale, a 20 item assessment used to measure current anxiety; the scale will be used to determine if the subject is safe to enter the MRI scanner.

2. *Structural Clinical Interview* (CSM-IV Axis 1 Disorders (SCID)) — A structural interview to identify a history or current diagnosis, of Axis 1 mental health disorder, which will be given at intake into the study.

3. *MRI and PET Scans*. MRI will be conducted to provide a structural image suitable for coregistering the PET image and observing white matter integrity. The scan should take 35 minutes. These will be MPRAGE images collected using the standard ADNI protocol at the in-house 3T MRI scanner at the UCSD Altman Clinical and Translational Research Institute (ACTRI). The PET scan should take 70-90 minutes.

The proposed study evaluates PET results in subjects with cognitive memory impairment and in normal volunteers. Each subject will be asked to come to the MRI/PET center for a single visit lasting approximately two hours. [18F]MK-6240 will be used for amyloid assessment.

[18]MK-6240 is to further characterize the brain pathology by looking at the second potentially deleterious

protein of Alzheimer's, which is Tau protein.

[18]MK-6240
MK-6240 has been developed as a second-generation, non-invasive tool for precise spatial and temporal quantification of NFTs in human brain using radioligand PET, designed for improved imaging capabilities compared to other tracers. MK-6240 is a non-biological, small molecule, low molecular weight, high specific activity [18F]-radiolabeled PET tracer ([18F]MK-6240) that is classified by the Food and Drug Administration (FDA) as a Type 1 radiopharmaceutical and by the European Union (EU) as a Class IIb radiopharmaceutical. An intravenous (IV) formulation of [18F]MK-6240 will contain ≤ 20 µg MK-6240 in a sterile solution of up to 10% (v/v) ethanol, and 0.5% sodium ascorbate in saline (0.9% sodium chloride) not more than 10 mL total volume. Given the short half-life (t1/2) of 18F (~110 minutes), [18F]MK-6240 will be locally synthesized, purified, and formulated when required for administration to human subjects, according to pre-specified radiopharmaceutical doses and quality assurance procedures that conform to accepted standards in the field. It is shipped to the California Protons center in Sorrento Valley in a shielded container.

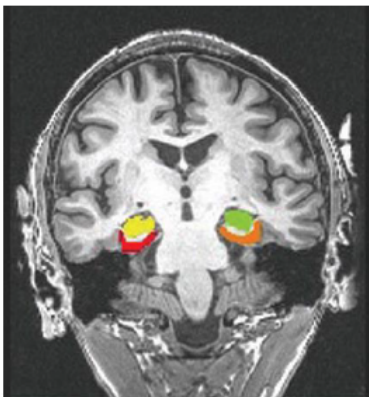

Figure 1. Coronal section displaying hippocampal and parahippocampal areas of interest.

The protocol will entail the injection of MK-6240 administered by intravenous bolus injection (over 45-60 seconds) followed by an uptake phase of 45-50 minutes during which time the subject will wait in a quiet room. The PET scan will begin at approximately 50 minutes (. Brain images will be acquired continuously for a period of 20 minutes as four 5-minute frames. The images will be immediately assessed for technical validity. If considered inadequate, the subject will have an additional 20 minutes of continuous imaging, collected as four 5-minute frames. A transmission scan will also be collected to allow for reconstruction of the images after the scan. As we have done to date in ADNI, we will use a common iterative reconstruction approach for all scans. The transmission scan is 6 minutes in length.

Vital signs will be taken immediately prior to administration of MK-6240(within 5 minutes prior to injection) and again at the end of the study visit, prior to discharge (approximately 70 minutes after MK-6240 administration). Subjects will be observed continuously for signs of adverse events or serious adverse events. Subjects who experience an adverse event will not be discharged until the event has been resolved or stabilized. The injection site will be observed for excessive inflammation or damage to the surrounding tissue. A board certified physician (Dr. Brewer) or physician's qualified designee (Alzheimer's Disease Research Center) will see the patient prior to discharge. The patient will be given the contact information of the ADRC, where phone calls can be directed to Dr. Brewer for any questions or concerns after the scan.

Rationale for Dosage

[18]MK-6240
Based on data obtained of a one single IV administration of [18F]MK-6240 no differences in dosimetry, biodistribution, pharmacokinetics or pharmacodynamics are anticipated between the different formulations. The average (± SD) value of effective dose (ED) was 29.4 ±0.6 µSv/MBq, which is in the typical range for 18F-

radiolabelled ligands. Based on this, the administration of 160 MBq (4.3 mCi) of [18F]MK-6240 for PET scanning (including CT scanning) is anticipated to result in a total human ED of about 4.8 mSv.

[18F]MK-6240 was generally well-tolerated. No clinical adverse events (AEs) were observed. No clinically significant abnormalities were noted in physical examinations, laboratory safety tests (chemistry, hematology, urinalysis), vital signs (VS), or electrocardiograms (ECGs). There were no serious adverse events (SAEs), events of clinical interest (ECIs), deaths, or discontinuations due to an AE.

Overnight Visit 2

Participants with obstructive sleep apnea will return to the sleep laboratory for an overnight sleep study and complete the following:

- *Neurocognitive function assessments:* Participants will complete motor sequence testing and a verbal word pairs task.

- *Psychomotor Vigilance Test:* Objective assessment of brain function will be done using 10-minute psychomotor vigilance test (PVT).

- *Research PSG:* Participants will return to our sleep research laboratory location to complete a **research PSG**. In preparation for this research PSG, subjects will be instrumented in a similar fashion to the standard PSG. However, they will also be randomized to receive either supplemental oxygen or room air during overnights 2 and 3. If subjects have been randomized to receive room air, subjects will have a nasal cannula with pressurized room air to avoid unblinding.

    - Speilberger State Anxiety Scale: Subjects will be given this assessment to measure their current anxiety before being placed on pressurized room air or supplemental oxygen. The assessment will be repeated in the morning.

    - Supplemental oxygen – Subjects will be instrumented with a nasal cannula to receive 2L/min supplemental oxygen. The oxygen will be kept at a fixed rate, however, the participant will be titrated to receive a max of 4 liters per min to maintain sats >90% based on oximetry readings.

The study will end at approximately 6AM. The equipment will be removed and given to the sleep technician. The following morning assessments will be completed:

1. *Endothelial function (EndoPAT)*: Endothelial vasodilator function will be assessed using the EndoPAT 2000 (Itamar Medical, Caesarea, Israel) device. The EndoPAT test has been shown to be highly predictive of traditional cardiovascular risk factors. This test takes approximately 15 minutes. EndoPAT measures digital pulse amplitude with the probes placed on the tip of each index finger. The EndoPAT probe comprises a pneumatic plethysmograph that applies uniform pressure to the surface of the distal finger, allowing measurement of pulse volume changes in the finger. Digital pulse amplitude will be measured during three stages from both right and left index fingers. Arterial flow of one arm will be occluded for 5 minutes by a blood pressure cuff placed on a proximal forearm at a pressure of 60 mm Hg above the systolic blood pressure or 300 mm Hg (whichever is higher). The digital pulse amplitude of the non-occluded arm will be measured as the control baseline. The three stages of the test will be: 1) Before occlusion (resting period) - digital pulse amplitude will be recorded for 5 min at rest; 2) During arterial flow occlusion- arterial flow occlusion will be applied in one arm and digital pulse amplitude will be recorded for another 5 min; 3) After occlusion- the cuff is rapidly deflated to reverse the

occlusion and digital pulse amplitude recorded for 5 minutes to assess peripheral vasodilator response to reactive hyperemia.

2. *Speilberger State Anxiety Scale*, *KSS Questionnaire* and a repeat of *Neurocognitive function assessments*, and a *10-minute PVT* will be completed in the morning.

Overnight Visit 3

Participants with obstructive sleep apnea will return to the sleep laboratory for an overnight sleep study and complete the following:

Within 1-2 weeks from their overnight visit 2, the participant will return to complete their research sleep study and receive either supplemental oxygen or room air (i.e. if the participant had room air during overnight visit 2, they will receive supplemental oxygen during overnight visit 3).

In the evening, before sleep, participants will complete PVT and neurocognitive function assessments.

The study will end at approximately 6AM. The equipment will be removed and given to the sleep technician.

In the morning after the sleep equipment have been removed, participants will repeat EndoPAT, KSS questionnaires, PVT, and neurocognitive function assessments.

3-Month Trial Therapy with Start and End of Treatment Sleep Studies

Participants with OSA (AHI >15 events/hour) and identified to benefit from nocturnal supplemental oxygen will be randomized to receive either O2 or PAP for treatment of OSA. At both the start and end of the treatment phase, participants will be scheduled for an evening visit followed by either an in-lab or at home overnight sleep study based on laboratory availability and participant preference. Over a 12-week period, participants will be contacted weekly to be asked about their adherence. Participants' adherence will also be monitored remotely through cloud-based monitoring. PAP and oxygen will be provided by a local DME company at no cost to our participants.

Start of Treatment Visit: Home or In-Lab Sleep Test

Participants will be scheduled for an evening visit where they will either receive positive airway pressure therapy (PAP) or therapeutic nightly oxygen to be used for 3 months (12 weeks) based on randomization. Participants will also complete questionnaires and evening/morning neurocognitive testing:

1. *Neurocognitive function assessments:* Participants will complete motor sequence testing, a verbal word pairs task, and the NIH toolbox picture-sequence memory test.

2. *Psychomotor Vigilance Test:* Objective assessment of brain function will be done using 10-minute psychomotor vigilance test (PVT).

3. *Questionnaires*: Participants will complete self-report questionnaires related to sleep and depressive symptoms.

Participants will then be asked to complete a baseline sleep study while wearing the sleep therapy device they were randomized to. Depending on availability, the sleep study will either be conducted at home or in-lab with

a WatchPat device (Home Sleep Test) to use for 6-8 hrs of sleep for 1 night. WatchPat is a wrist-worn device with a probe placed over a finger to detect changes in oxygen and autonomic nervous system activity (e.g., to enable sleep staging). Furthermore, participants will be given an electronic device (i.e. iPad or laptop) to complete brain function testing. In the morning, participants will complete the remaining neurocognitive testing independently, with support from a research team member as needed. The data from the disposable home sleep apnea test (HSAT) will be auto-scored and the data can be downloaded remotely. Once the data is retrieved, the participant can discard the home sleep test. If the morning testing was performed at home, participants will subsequently be scheduled to return the electronic device.

Mid-Treatment: Follow-Up Calls

Following the Start of Treatment Visit and sleep test, we will contact participants every week for a 3–5–minute phone call throughout the 3-month trial to ensure they are using therapy and to answer any questions. Staff will provide encouragement and help work through any issues that occur with the assigned therapy device. For the study, we ask that subjects use their therapy at least 4 hours every night during the 12-week period. Compensation will be given based on the number of weeks of successful treatment compliance (at least 4 hours/night every night) and number of weekly phone calls answered. If the assigned therapy is not used for at least 4 hours/night for a given week, subjects will not receive the compliance compensation for that particular week.

End of Treatment Visit: Home or In-Lab Sleep Test

At the end of the 3-month trial, participants will be scheduled for an evening visit where they will repeat activities from the Start of Treatment Visit. They will then complete a final sleep test while wearing their therapeutic device, either at home or in-lab with another WatchPat device. Participants will be provided with an electronic device to complete brain function testing and questionnaires. In the morning, participants will complete the remaining neurocognitive testing independently, with support from a research team member as needed. The data from the disposable home sleep apnea test (HSAT) will be auto-scored and the data can be downloaded remotely. Once the data is retrieved, the participant can discard the home sleep test. If the sleep study and morning testing was performed remotely, participants will subsequently be scheduled to return the electronic device. Participants will subsequently be scheduled to return the PAP therapy device or supplemental oxygen equipment they received for the trial, as well as the electronic device used for morning testing.

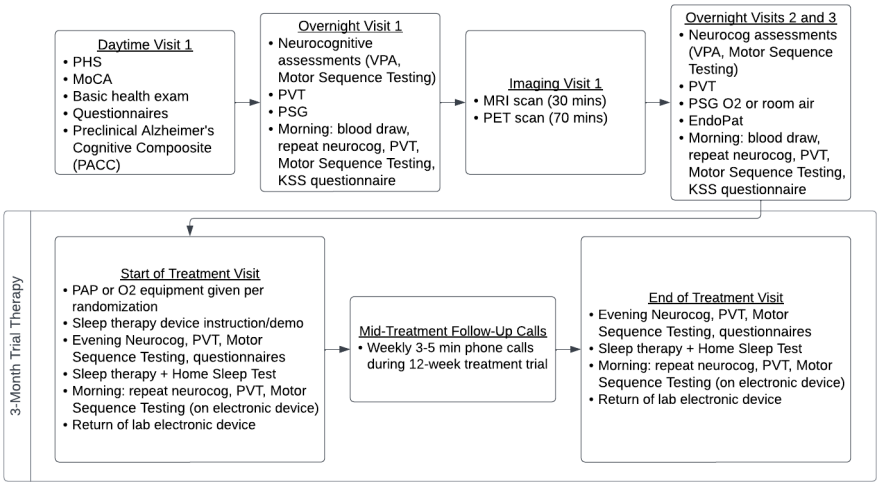

Figure 2. Summary of Study Activities.

Letter to Research Subject Participants

After their first overnight sleep study is completed, participants will be given a letter to summarize their AHI result. Subjects that score ≤ 26 will be asked to follow up with their primary care physician.

10. STATISTICAL ANALYSIS PLAN

Statistical analysis will be performed by tenured Prof. Sonia Jain in Biostatistics. Dr. Jain is involved in all statistical aspects of our lab including power calculations, secure data storage, data synthesis/ interpretation and guidance regarding analytical/quantitative techniques. In addition, we encourage all junior members of our laboratory to gain proficiency in analytical methods such that investigators emerge from the laboratory with both biological as well as statistical skills. Dr Jain is eager to help mentor our junior members to ensure that these goals are achieved. Dr. Jain has developed an interest in sleep science through our interactions and is a strong member of our team. In all cases we are adequately powered to assess the impact of sex as a biological variable which will be an important exploratory goal of our study, given known sex differences in sleep disorders, Alzheimer's, and tau. Moreover, we are careful to control for various covariates including age, gender, BMI and ApoE status (which is part of the PHS score). We recognize that ApoE status may be a risk factor for OSA as well as AD and may have complex interactions with OSA effects on memory, making careful analyses of interactions important.

Aim 1: Although all major pathophysiological traits will be assessed, our primary outcome is to compare the loop gain of older OSA patients (age 65-85 years) with the loop gain of carefully matched individuals without

OSA. We will assess our predictors of oxygen responsiveness to extend our recent publications[50]. We have powered this analysis using our preliminary data based on a mean loop gain difference of 2.1 with standard deviation 0.4 using an unpaired t test with alpha=0.05 and power>0.8. We will also analyze age as a continuous variable within the 20 year range. Our sample size will be adequate to adjust for any covariates (if matching is inadequate or other relevant covariates are imbalanced) and will provide participants for the other aims in which oxygen responsive OSA patients are required. We will also model the various pathophysiological traits (as previously described[260]) to determine how loop gain, upper airway gain, arousal threshold, and pharyngeal anatomy interact[135]. This aim will provide a rich data set for further analyses/signal processing[250,251,262,263] and characterizing thoroughly participants in the subsequent aims. For **Aim 2**, we will quantify the apnea hypopnea index (using criteria independent of desaturation) on 2 l/min supplemental O2 vs sham (room air) in patients with varying underlying endotypes. We have powered this analysis based on our preliminary data using a power>0.8 and alpha=0.05 with a change in AHI of 20/h (a clinically important difference) with standard deviation 5/h. We will also assess changes in neurocognitive function (Aim 2, VPA primary neurocognitive outcome).

The interpretation should be straightforward. If we confirm a major subset of OSA patients who are oxygen responsive, we will extend our recent findings that we can identify these patients based on underlying pathophysiology. If oxygen therapy lowers both loop gain and AHI then this finding would support our assertion that loop gain is causally important in a subset of OSA patients and that oxygen is a viable therapeutic approach.

If loop gain is not lowered with oxygen then we would conclude either the dose/duration of oxygen was inadequate or that the elevated loop gain is an intrinsic/irreversible property of OSA. If loop gain is lowered, but AHI is not improved, this finding might support the notion that elevated loop gain is either a consequence or an epiphenomenon in OSA but not causally important in these patients[311]. Clearly the changes in loop gain will be interpreted in the context of other endotypes, given that loop gain per se was not predictive of O2 response in our recent study. Rather, the combination of endotypes determined oxygen response with 83% accuracy in our pathophysiological model which we are working to validate further. Dr. Sarah Banks (Co-I), neuropsychologist will oversee cognitive assessment and impact of treatment on memory. We anticipate improvement in memory consolidation although we recognize a single night of therapy may be inadequate to see important changes. We have previously observed subjective without objective improvements in brain function with overnight therapy, leading us to speculate that the initial improvement with OSA therapy may be an “illusion”[312].

Aim 3: We have powered this aim based on the literature, our experience and that of our imaging collaborators. The techniques to acquire structural images of the brain are well established, but the sensitivity of these techniques to changes induced by alleviating hypoxemia/OSA is less clear. Although we have chosen hippocampal volume as our primary measure (outcome)[200], we will assess other brain regions for possible use in future studies. We will further quantify beta-amyloid neuritic plaque density and tau protein using PET scanning to assess the impact of OSA on predictors of AD. We will focus on the hippocampus and medial temporal lobe and other regions showing evidence of tau accumulation in the preclinical phase[313,314]. Given the multiple comparisons inherent in our exploratory aim assessing the entire brain, we are not powered for brain structures beyond our primary outcomes. Based on our prior work, with n=20 per group, we have power >80% with alpha=0.05 to find a hippocampal volume difference of 0.3 vs. 0.4 (as %ICV – intracranial cavity volume) with SD=0.03 which is a clinically important difference i.e. associated with measurable memory differences[200]. For amyloid PET imaging, with n=20 per group, we will have >80% power with alpha=0.05 assuming SUVr=1.53 (SD=0.18) to show a clinically important difference of 0.2 between OSA and matched non-OSA[315]. Further, we will use the preliminary data obtained from this aim to design further rigorous brain imaging studies in the future.

The interpretation of this aim will be based primarily on the hippocampal outcomes and the amyloid and tau quantification. Dr. Jim Brewer (Co-I) is a neuroimaging expert who will oversee these analyses with Sarah

Banks, PhD, who is an expert neuropsychologist. We anticipate that we will see smaller hippocampal volume in OSA vs. controls although this finding will depend to some extent on the magnitude of the memory impairment in Aim 2. Our use of amyloid and tau quantification will be of major interest given the novelty in OSA. We expect the extent of tau within the region of interest to be higher in participants with OSA. We will also explore how tau relates to improvement of learning with OSA therapy. We are also optimistic that beta-amyloid neuritic plaque density may be a robust outcome metric for future longitudinal studies. We can clearly determine how various neurocognitive abnormalities correspond with MR and PET findings[184,316,317].

Aim 4: Sonia Jain our Co-I Tenured Prof Biostats will oversee these analyses for our pilot clinical trial although the PI and the Co-Is (including Dr. Feldman for AD) have considerable clinical trial experience as well. We will compare the results of the word pairs test with 3 months of O2 vs. PAP therapy (n=60 total completing). We will use an intention to treat analysis such that all randomized patients will be included and any dropouts will be analyzed using last observation carry forward. We powered this analysis based on our preliminary data with a treatment related difference of 6.9% (a clinically relevant difference) and sd=2.2% assuming alpha=.05 and power>0.8. Although we will compare O2 to PAP, we will also assess within-subject changes with treatment. For treatment, we will perform an efficacy analysis since we anticipate that despite our best efforts adherence will be imperfect. We will analyze other neurocognitive variables as well in an exploratory manner, but are not powered for multiple comparisons. We will also compare preferences with therapy via structured exit interviews to understand how best to optimize a large multicenter comparative effectiveness trial if our results are indeed compelling[318,319]. We will adjust for covariates (eg. age, BMI, sex, PHS score) if imbalanced after randomization.

Several possibilities exist for our findings from this aim. Oxygen may be superior to PAP which would pave the way for multicenter comparative effectiveness trials which could change the current standard of care for selected patients[291]. Oxygen could be equivalent to PAP which could reflect reality or could imply that our studies are underpowered, or that the word pairs test is insufficiently sensitive to treatment differences, or perhaps the dose/duration of therapy was inadequate. Of note, we will be able to analyze the data examining various subsets based on underlying pathophysiology. Another possibility is that oxygen improves the AHI but not the corresponding tests of neurocognitive function. Such a finding might point to irreversible effects of apnea or perhaps inadequate statistical power. We will clearly be in a position to assess whether treatment is simply making the numbers look better or actually improving the health of the patient. Because we regard Aim 4 as a pilot study, we will also independently analyze the oxygen effect and the PAP effect to design future studies.

We work closely with Dilip Jeste (Co-I) who leads the IBM cohort and is very focused on healthy aging. One of the corollaries of identifying predictors of memory impairment will be to identify markers/predictors of healthy aging e.g. absence of OSA may identify healthy elderly people without neurocognitive impairment. Based on the substantial data acquired through the proposed studies, we will be in good position with our statistical team to create a multivariate model to assess predictors of healthy aging, of major clinical relevance.

11. HUMAN SUBJECTS

We will enroll 260 participants for the protocol.

Aim 1─ Overnight Visit #1

We anticipate that 130 subjects will complete Overnight visit #1.

Aim 2 ─ Overnight Visit #2 and #3

Of the 130 subjects that complete Aim 1, 65 subjects who have been identified to have obstructive sleep apnea will complete Overnight visit #2-#3.

Aim 3 ─ Imaging Visit #1

Of the 20 subjects that complete Aim 1 and Aim 2, the same participants will be invited to complete Imaging

Visit #1. We will attempt to enroll all 20 subjects. Additionally, 20 subjects without OSA, who completed overnight visit 1, will be invited to complete the imaging visit. In total, we will recruit 40 subjects for this aim.

Aim 4 – 3 month Trial of Supplemental O2 or PAP therapy

Of the 130 subjects that completed Aim 1, 60 obstructive sleep apnea oxygen sensitive subjects will be invited to complete a 3-month trial of supplemental oxygen or PAP therapy. Subjects that complete the trial will return for Morning Visit 1.

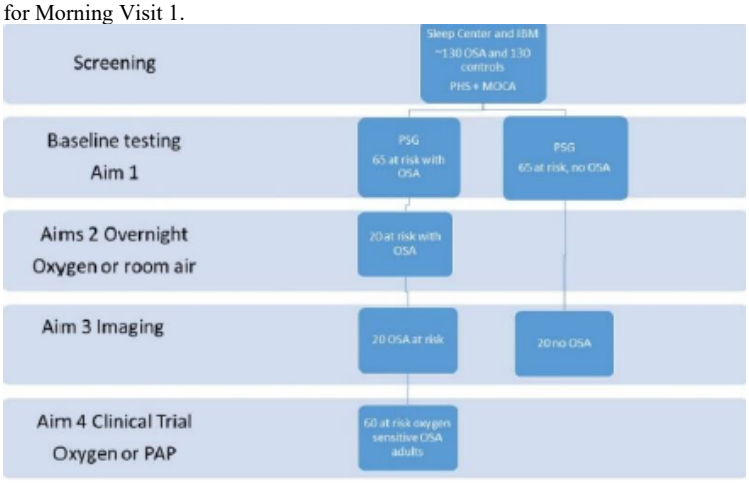

Figure 3. Schematic of study visits and participants to be enrolled per aim.

Inclusion:

1. Age 65-85 years
2. Gender: Men or Women
3. MOCA > 26
4. Independently living and able to drive
5. OSA (AHI ≥ 5/h) or no OSA
6. Subjects must consent to waiving their right to obtain their PHS score (since the score is not yet actionable and could lead to social stress and ethical dilemmas)

Exclusion:

1. Currently smoking
2. History of COPD or asthma
3. Heart Failure Class III or IV, unstable cardiovascular disease, or uncontrolled hypertension
4. Neuromuscular Disease
5. Drowsy Driving (ESS > 18/24)
6. Inability to complete study procedures, such as questionnaire that are only available/validated in English
7. Lack of decisional capacity to provide informed consent
8. Participants in whom magnetic resonance imaging Magnetic Resonance Imaging [MRI] is contraindicated including, but not limited to, those with a pacemaker, presence of metallic fragments

near the eyes or spinal cord, or cochlear implant
9. Presence of a brain tumor or lobar stroke
10. Current drug or alcohol abuse/dependence
11. Prisoners

12. RECRUITMENT AND PROCEDURES PREPARATORY TO RESEARCH

We seek a partial HIPAA waiver to identify patients with/without OSA, and age > 65 years. Once identified, their treating clinicians will be asked to obtain verbal permission to discuss the study with them. The patient will then be contacted (in person or by phone) by a member of the study team and provided with a brief summary of the research, and asked if they are interested in participating. If yes, the study team member will provide full details of the study, consent the participant, and obtain full authorization.

Some subjects may also be recruited in the UCSD Sleep Medicine Clinic, Brain Health Center, and other UCSD clinics as they will be made aware of ongoing research by non-study physicians. Subjects might also be recruited after they initiate contact in response to IRB approved standard posters, newspaper advertisements, and/or flyers posted within the Sleep Clinic. Interested potential subjects will be met in clinic by study personnel to discuss the study and provide written informed consent at that time. Those who initiate first contact to research staff via telephone will be followed up with a telephone screening to ensure they qualify for participation in the study. All participants will have an "opt-out" provision to avoid any possibility of coercion.

Advertisement for this study in the form of flyers and brochures will also be placed in UCSD Pulmonary and Sleep Medicine Clinic and other appropriate locations. If the text for advertisements, flyers, etc. is to be changed, each recruitment item will be submitted to the IRB for review and reapproval.
Slicer Dicer will be used to identify both Summary & Patient Level Requests:

Investigators plan to use UC San Diego Health's Epic SlicerDicer, a self-service cohort discovery tool. Investigators will have access to direct summary and patient level data. Using SlicerDicer, investigators will review the charts at a patient level to identify potential patients for recruitment based on the criteria outlined in Section 10 of this Research Plan.

UCSD Listserve, UCSD Hospital Bulletin Board, Facebook, Craigslist
IRB approved flyers will be digitally posted on the UCSD Listserv, Facebook, and Craigslist. The flyers will also be placed on UCSD Hospital Bulletin Boards by the Community Engagement Manager for the Clinical Transitional Research Institute (CTRI).

Facebook Page Welcome Message
We are requesting permission to have this message posted on a Facebook page) created for recruitment purposes.
Welcome to the UCSD Pulmonary and Sleep Medicine Research group's Facebook page. We have ongoing research studies, primarily investigating obstructive sleep apnea. If you or someone you know has obstructive sleep apnea, please see our IRB approved flyers below. Occasionally, we are looking for healthy individuals without sleep disorders. Please regularly check our page for flyers describing these research opportunities.

Research Match
ResearchMatch will be used to contact prospective subjects with an IRB approved recruitment message. The message will be submitted for approval in an amendment if it is not already included with the initial project submission. Prospective subjects will only be contacted three times via Research Match.

Research Group Website

The UCSD Pulmonary and Sleep Medicine Research group has a webpage hosted by UCSD health sciences. Wording from IRB approved flyers will be posted there.

Google Ad
A google ad will be posted with the following statements to recruit elderly people from the San Diego community. Each post includes two headlines and 1 description of the study.

Headline 1: Are you between the ages of 65-85?
Headline 2: UC San Diego (UCSD) Sleep Research Study

Alternative Headline 1: Are you interested in completing compensated sleep studies and brain imaging?
Alternative Headline 2: UC San Diego (UCSD) Research Study
Alternative Headline 1: UCSD Sleep Studies and Brain Imaging Research Study.
Alternative Headline 2: Compensation for time and travel provided up to $1,190.

Description 1.
The UCSD Sleep Medicine Research group is looking for individuals 65-85yrs to participate in a research study. For completion of 3 overnight sleep studies, 2 daytime visits, brain imaging (MRI/PET scan), and a 3-month trial of PAP therapy or nightly supplemental oxygen, you can receive up to $1,190. Parking will be covered at all study locations. If you are interested, please contact us at (858) 246-2154 to receive more information and see if you are eligible. We can also be reached by email at sleepresearch@health.ucsd.edu.

13. INFORMED CONSENT

A waiver of documented consent will be requested and once oral consent by the subject is obtained this consent will be used for the sole purpose of discussing the study with the subjects over the phone and performing the screening process as needed. The reason for this is because the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. In an effort to further protect the subject and their right to privacy, subjects will be screened for qualification prior to being asked to sign a written consent form to participate in the research project. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern. Additionally, the screening process presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required.

Once oral consent has been obtained, key personnel will screen the subjects over the telephone following the telephone script and screening questionnaires, including the University of California, San Diego Brief Assessment of Capacity to Consent (UBACC). The telephone screening will only proceed if the person is determined by the UBACC to have the ability to consent. Individuals interested in participating in this study will be given detailed explanation of the procedures, potential benefits, risks and discomforts of the study by the study researchers. If a person is determined to be eligible and agrees to participate, the subject will be sent the consent form over email to review and then will be given ample time to review the forms. Informed consent will be obtained, as discussed below. Participants will have either consent obtained remotely or in-person. DocuSign will be used to obtain informed e-consent in order to have subjects complete the Epworth Sleepiness Scale (ESS) remotely. Of note, the same IRB approved consent form will be used for remote and in-person consenting. Subjects that have an ESS score <18/24 will be scheduled to come in for the daytime visit; the other participants who have a ESS score >18/24 will be excluded Eligible subjects that do not have the ability to e-consent will be scheduled for a daytime visit and meet with research staff to obtain written informed consent before any data collection. Subjects who have e-consented will be sent a copy of their signed consent form. A copy of the e-consent will be kept in the subject’s research file in a locked cabinet.

When meeting in person, the presenting study team member will introduce and give a brief overview of the

study. The subject will then be given time to read the IC if necessary. The study team member will then carefully read/review the IC with the subject. Questions and concerns will be addressed at this time. The subject will be given as much time as required. If necessary the subject will be allowed to take the IC home to discuss with family members or their doctor. If they are still interested in participating they will be rescheduled for their study activities.

No study procedures will be performed until it is determined by the study team member that the subject fully understands the content of the IC and the IC is signed. The consent process will be clearly documented and copy of the signed IC and the California Experimental Subject's Bill of Rights will be placed in the subject's study record. The subject will be given a copy of the signed IC and California Experimental Subject's Bill of Rights.

Only subjects able to consent for study participation will be enrolled into this study. There is no surrogate consent process necessary.

13. ALTERNATIVES TO STUDY PARTICIPATION

Subjects may elect not to participate in the research.

15. POTENTIAL RISKS

Potential Risks to the Subjects, and Procedures to Minimize Risk:

1. Standard PSG: The nasal cannula, respiratory belts, electroencephalography, ECG and oximeter used to monitor sleep and breathing may be mildly uncomfortable and could interfere with normal sleep. Thousands of routine sleep studies are conducted every night across the country without incident.
2. Sleeping with PAP therapy: While generally well tolerated, subjects may experience temporary drying of the nose, mouth, or throat, nosebleed, bloating, ear or sinus discomfort, eye irritation, and skin rashes. Subjects who use PAP therapy nightly may experience sleepiness after the overnight is completed. Participants will be cautioned against drowsy driving and will be encouraged to be driven home by a responsible adult. They will be offered *ad libitum* sleep prior to leaving as needed.
3. Risk Associated with O2: While generally well tolerated, subjects who use Supplemental Oxygen may experience temporary drying of the nose, mouth, or throat, nosebleed, bloating, ear or sinus discomfort, eye irritation, and skin rashes.
4. Risks Associated with Venipuncture: Subjects may experience pain, discomfort, or infection from venipuncture.
5. Risks Associated with EndoPAT (non-invasive heart function test)/blood pressure test: Subjects may experience temporary pain or discomfort from the tightening of the blood pressure cuff.
6. Risks Associated with IV catheter: Subjects may experience infection, phlebitis (vein irritation), and infiltration/extravasation.

7. Psychological Risk: It should be noted that volunteers are often motivated by altruistic feelings and are usually cooperative with studies that serve to develop a better understanding of dementia. Some patients will seek to provide their raw imaging data to their physicians, and, if so, we will release the raw imaging data to the patient, which they can provide to their doctor. This will be true of MRI but not tau PET, as the latter involves an investigational drug. We do not feel that these studies pose any significant psychological risk for the patients.
   We will review responses of completed questionnaires before participants leave to assess for suicidal thoughts or wishes. If the subject answers yes to ≥10 of the Geriatric Depression Scale, the PI will be notified immediately. Dr. Malhotra is a board-certified pulmonologist and sleep medicine specialist. Dr. Malhotra is qualified to identify suicidal ideation. In the event, that a subject has suicidal ideation, Dr.

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Malhotra will determine if the subject poses an immediate risk to themselves. If the subject poses an immediate risk to themselves, emergency services will be called to have the subject taken to the ER. When the subject does not pose an immediate risk to themselves, we will request that they seek psychiatric services and report their condition and treatment plan to their primary physician within. If Dr. Malhotra is not at the study site, we will have him or other co-Is assess for suicidal ideation via teleconferencing and refer the patient to ER for assessment.
Genetic testing: buccal swabs will be used for genetic testing, which will be performed by Diagnomics. Participants will not be told the results of their genetic testing, as explained in the consent form. This is because we will not have the genetic testing performed in a CLIA-approved lab, and the polygenic hazard score is under investigation, and as such its performance as a diagnostic or prognostic biomarker is not fully understood.

8. MRI testing: There are no known long or short-term risks of MRI. However, precautions must be implemented in order to exclude subjects with absolute contraindications to MRI, which include a cardiac pacemaker, metal fragments in the eyes, skin, body; heart valve replacement, brain clips; being a sheet-metal worker or welder; aneurysm surgery, intracranial bypass, renal aortic clips; prosthetic devices such as middle ear, eye, joint or penile implants; joint replacements; hearing aid, neurostimulator, insulin pump; I.U.D., being pregnant or trying to become pregnant; shunts, stents, metal mesh/coil implants; metal plate, pins, screws or wires, or any other metal implants; permanent eyeliner, eyebrows. Any positive indication by the subject will result in exclusion. FDA guidelines will be strictly followed as to the maximal amount of radiofrequency energy that is deposited in tissues. Some subjects may feel claustrophobic and will be allowed to exit the scanner (with assistance from the investigators) whenever they wish. Subjects will be required to wear hearing protection to minimize the noise exposure from the scanner.
Incidental Findings: There is a risk of incidental findings that can be identified throughout the study. Examples include, but are not limited to, abnormal EKG observed during the sleep study, abnormal resolution in the MRI, abnormal EndoPAT, etc. If any significant, unknown findings are seen the PI will reach out to the subject to provide consultation. The subject will be given their results in a written report and asked to follow up with their primary care physician.

Fluorine (MK-6240) *PET imaging:*

Scanning with Fluorine labeled MK-6240 involves injection of 5 mCi of tracer. Participants may have discomfort, bruising, fainting, or infection from the venipuncture used to administer the tracer compound. Other risks of the test include discomfort if the participant is claustrophobic or uncomfortable with needles. It is also possible to have an allergic reaction to the tracers. Total radiation exposure due to imaging studies is as follows:

F-18MK-6240

Estimated exposure is 5.5 mSv for the F-18 MK-6240. The additional exposure from the low dose PET scan for attenuation correction is 1 mSv. Therefore total patient radiation exposure from the procedure is estimated to be 6.5 mSv. This amount is more than you would receive from one year of natural exposure in the San Diego area, which is approximately 1.6mSv. Cumulative exposure from radiation may increase your risk of developing certain types of cancer in the future.

The total exposure for the study will be 6.5 mSv.

Additional uncommon side effects reported were nausea, dysgeusia (bad taste in the mouth), flushing, pruritus, urticaria, and infusion site rash. Musculoskeletal pain in the neck, shoulder, and back, fatigue, anxiety, claustrophobia, insomnia, dizziness, chills/feeling cold, and hypertension were also reported. The PET imaging procedures could pose unknown risks to a fetus. Therefore, all women of childbearing potential will need to have a negative urine pregnancy test within 7 days of the PET imaging procedures. PET participants may have discomfort, bruising, fainting, or infection from the venipuncture used to administer the tracer compound.

9. Risks of an Incidental Finding: The MRI scan protocol has been designed for research purposes, not for clinical diagnostic purposes. All analysis will be done only by research staff for the purposes of this research study only. The scans will not be reviewed by a radiologist. However, it is possible that an abnormality may be identified. In that case, a written report of the abnormality will be shared with the subject and their doctor.

16. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

We will attempt to minimize the risk to subjects participating in these studies as follows:

All studies will be conducted by an experienced investigator knowledgeable in all aspects of the procedures utilized.

Subjects whose questionnaires suggest severe depression (GDS Score $\geq$12) will be informed of the result and this will also be relayed to their clinician.

Specifically, in case of a subject indicating suicidal ideation (verbally or as part of questionnaires) PI Malhotra (or his medically licensed co-Investigators) will undertake an immediate threat assessment with the following possible outcomes:

- if the subject can clearly contract to safety then information about psychiatric services will be provided and the subject's PCP will be informed within 1 business day;
- if the subject is unable to contract to safety, then depending on the subject's cooperation she/he will be either escorted to the Jacobs Medical Center ED (<150m from the lab) for further evaluation or 911 will be called for assistance.

After sleep studies, subjects will have the option of sleeping, without instrumentation, for as long as they would like before driving home. We will offer them a cab voucher to have a cab take them home. They will only drive home if they feel adequately rested to do so and is corroborated by an assessment by a health professional (MD, PhD, or equivalent) trained in sleep medicine.

Thus we believe that all possible safeguards are in place to minimize the risk. However, several steps will be taken to insure patient comfort and safety. This is not a clinical trial, but physiological research and a pilot clinical study. However, we have devised a safety monitoring plan to minimize risk and discomfort. This will include all the measures described above, and strict reporting any complications of our studies to the IRB according to the UCSD guidelines. The study coordinator will meet with the PI monthly (and as needed) to go over any complaints or problems. The PI will call the patients with problems directly to verify important issues. If problems are identified, the protocol will be adjusted as needed.

For the experimental portion of the studies (MRI), FDA guidelines will be strictly followed as to the maximal

amount of radiofrequency energy that is deposited in tissues. Each subject will be made as comfortable as possible within the scanner using blankets and pillows. Some subjects may feel claustrophobic and will be allowed to exit the scanner (with assistance from the investigators) whenever they wish. Subjects will be required to wear hearing protection to minimize the noise exposure from the scanner.

The risk associated with the amount of radiation exposure participants receive in this study is considered low and comparable to every day risks. No PET studies will be performed on pregnant or potentially pregnant women. Indeed, all women will be 65 or older, and hence postmenopausal. This will be confirmed with their reproductive history questionnaire.

Participants will be instructed to void two hours post injection to minimize bladder exposure.

Infusion of the PET radioligand will be done by trained personnel at the California Protons facility in Sorrento Valley. The PET scan technician will be starting the intravenous line and will be administering the MK6240 compound. As MK6240 exposure in humans is expected to be brief and to be given as a single administration, accumulation of drug/metabolites or prolonged exposure is not expected. Previous studies showed there were scattered changes in vital signs, laboratory and electrocardiogram (ECG) values, but no consistent and clinically significant changes were observed. A set of vital signs (blood pressure, pulse, respirations, temperature) will be obtained before and after the scan. PET participants may have discomfort, bruising, fainting, or infection from the venipuncture used to administer the tracer compound.

Patients must minimize movement during each PET procedure. The imaging system at the California Protons facility is designed, as are most systems, to reduce head motion and patient discomfort. The risk of immobilization will also be reduced by placing cushioned pads under the subject's head to increase comfort.

MK6240 will be manufactured and delivered by PETNET under contract with California Protons. The dose of MK6240 will be prepared the same day as the subject's appointment and delivered to the imaging center for immediate injection. The storage, handling, and quality assurance of MK6240 at the imaging center will follow the same practices that are used for handling The FDA has provided the IND number, 150441, for MK6240. FDA-approved F18 tracers used in standard PET scans.

17. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

There is always the risk of subject information being released accidentally. We will make every effort to maintain patient privacy and confidentiality both during and following the study. Electronic study data will be de-identified by substitution of codes for names and hospital identifiers and will be stored on a secure disk for access by Investigator and study staff only and any hard copies will be stored in a locked cabinet. In all correspondence and in internal study reports that require identification of individual subjects, subjects will be referred to and known by their ID number and character name code. All subject data forms (CRF's) will only list the ID number. All research staff are CITI certified and have had impressed upon them the importance of confidentiality. This study does not involve the collection of sensitive personal information from subjects. Data will be stored only at UCSD sites and its use will be confined to that specified in this protocol and its approved amendments.

Data generated as a result of this study will only be made available for inspection upon request of the UCSD Human Research Protection Program.

18. POTENTIAL BENEFITS

Benefits of this study are principally to science and society. We hope to understand how obstructive sleep apnea factors into the progression of Alzheimer's disease.
Only by achieving a more complete understanding of the relationship between the sleep and Alzheimer's

disease can better therapies be developed to treat AD.

19. RISK/BENEFIT RATIO

The investigators feel risks associated with these studies are outweighed by the benefits. We have greater than 10 years of experience doing this type of research and have never had a serious adverse event. We anticipate furthering of our knowledge on our understanding of sleep apnea as a result of this research.

20. EXPENSE TO PARTICIPANT

There will be no expense to the participants. Parking/transportation will be provided for the overnight visit and MRI/PET scan study as needed.

21. COMPENSATION FOR PARTICIPATION

Each subject will receive up to **$**1,190 for their participation in the study, and all parking expenses will be covered.

- Daytime Visit 1: $25
- Home Sleep Test: $25
- Overnight 1: $200
- Imaging Visit (MRI and PET): $100
- Overnight 2: $200
- Overnight 3: $200
- Start of Treatment Visit (with HSAT): $100
- Weekly Phone Call (12 weeks): $10 each week (total $120)
- Treatment Compliance (12 weeks): $10 each week (total $120)
- End of Treatment Visit (with HSAT): $100

As stated previously, the Home Sleep Test will be used as a substitute for the overnight sleep study when participants are unable to come on-site for polysomnography, however, participants will be allowed to complete overnight 1 if their availability changes. If an additional study night is needed, they will receive an additional $200. If an additional daytime visit/home sleep test is needed, subjects will receive an additional $25.00.

22. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

All physicians performing physical exams (PEs) and taking medical histories are medically licensed in the State of California. The personnel performing the sleep and upper airway collapsibility studies are experienced medical researchers and certified sleep technologists.

Atul Malhotra MD (PI has privileges at UCSD Med Center, and has very extensive clinical research experience in sleep disorders and is considered a world expert in upper airway mechanics and obstructive sleep apnea.

Robert Owens, MD (Co-Investigator) has privileges at the UCSD Med Center. He will be responsible for recruitment, consenting and answering questions with the study subjects, data collection, data analysis, and interpretation of study results. He has experience in all aspects of the proposed research.

Howard Feldman, MD (Co-Investigator) is a neurologist in the Department of Neuroscience at UCSD. He will provide expertise in Alzheimer's. Dr. Feldman will be responsible for answering questions with the study subjects, data collection, data analysis, and interpretation of study results.

Sonia Ancoli-Israel, MD (Co-Investigator) has privileges at the UCSD Med Center. She will be responsible for recruitment, consenting and answering questions with the study subjects, data collection, data analysis, and interpretation of study results. He has experience in all aspects of the proposed research.

Sara Banks, PhD (Co-Investigator) is an analyst in the Department of Neuroscience at UCSD. Dr. Banks will obtain the Polygenic hazard score (PHS) from participants. She will also be responsible for recruitment,

consenting and answering questions with the study subjects, data collection, data analysis, and interpretation of study results.

James Brewer, MD, PhD (Co-Investigator) is a neurologist and neuroscientist in the Department of Neuroscience at UCSD. Dr. Brewer will run the MRI and PET imaging scans. Additionally, he will be responsible for recruitment, consenting and answering questions with the study subjects, data collection, data analysis, and interpretation of study results.

Dilip Jeste, DPM, MBBS, MD, DPM (Co-Investigator) he will be responsible for recruitment, consenting and answering questions with the study subjects, data collection, data analysis, and interpretation of study results. Additionally, Dr. Jeste will be available for training and consultation to other key personnel, who have questions or concerns about using the UBACC tool to determine decisional capacity of prospective subjects.

Jeremy Orr, MD (Co-Investigator), **Christopher Schmickl** MD PhD, and **Brandon Nokes** MD have privileges at UCSD Med Center, and are Pulmonary physicians who will be assisting in data collection and analysis.

Sonia Jain PhD is a tenured Professor in Biostatistics and Bioinformatics who has helped design the studies, and who will provide support with statistical analysis on an as-needed basis.

Pamela DeYoung is a board Certified Polysomnography Technologists (RPSGT) and will be assisting in the sleep studies, scoring of the data, and serve as the IRB Contact for this study.

Dillon Gilbertson, MS, Jazmin Velazquez, BS, Stacie Moore, CRT-SDS, , **Janelle Fine, BS** and **Lana McGinnis** BA are research assistants and will be assisting with recruited subjects, equipment troubleshooting, and completing the sleep studies.

Naa-Oye A. Bosompra, B.A. is a research assistant, who will be recruiting subjects, assisting in data collection, and serve as the IRB Administrative Contact for this study.

All personnel are employed by UCSD and have completed the appropriate CITI training

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24. FUNDING SUPPORT FOR THIS STUDY

NIH RO1 (identifier pending)

25. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

N/A

26. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

N/A

27. IMPACT ON STAFF

There is no anticipated impact on nursing or ancillary staff.

28. CONFLICT OF INTEREST

The investigators and study staff have no relevant conflicts of interest for this study.

29. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

N/A

30. OTHER APPROVALS/REGULATED MATERIALS

N/A

31. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

Some subjects affected by Alzheimer's disease or other memory impairments may be reasonably expected to have decisional impairment during moderate to late stages of the disease. The University of California, San Diego Brief Assessment of Capacity to Consent (UBACC) tool will be used to demonstrate that all participants who have consented for participation have the capacity to consent for themselves.

To determine the potential subject's understanding of the key elements of the study and of his/her rights, a questionnaire to assess decisional capacity will be administered, based on a UBACC Competence Assessment Tool *

The subject will be asked questions that relate to information in the consent:
1) What is the purpose of the study that was just described to you?
2) What makes you want to consider participating in this study?
3) Do you believe this is primarily research or primarily treatment?
4) Do you have to be in this study if you do not want to participate?
5) If you withdraw from this study, will you still be able to receive regular treatment?
6) If you participate in this study, what are some of the things that you will be asked to do?
7) Please describe some of the risks or discomforts that people may experience if they participate in this study.

(Please describe the 2 serious risks associated with this study.)
8) Please describe some of the possible benefits of this study.
9) Is it possible that being in this study will not have any benefit to you?
10) Who will pay for your medical care if you are injured as a direct results of participating in this study?

They have to answer all questions correctly to meet a standard of being decisionally capable.

If the potential subject is not decisionally capable, they will not be enrolled in the study.

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