**PARTNERS HUMAN RESEARCH COMMITTEE**
**DETAILED PROTOCOL**

Version: April 11th, 2024

**Title:** A Novel Pharmacological Therapy for Obstructive Sleep Apnea

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## I. BACKGROUND AND SIGNIFICANCE

OSA is a highly prevalent disorder that has major consequences for cardiovascular health, neurocognitive function, risk of traffic accidents, daytime sleepiness and quality of life [1-17]. Unfortunately, the leading treatment, CPAP—which acts to pneumatically splint the pharynx open—is intolerable for many patients [18-20]. A pharmacological, non-mechanical therapy for OSA that is efficacious and tolerable remains elusive.

With the goal of identifying therapeutic targets, research in the last decade has established that a number of key pathophysiological *traits* contribute to the development of OSA [21-24]. These not only include a collapsible upper airway (1), but also a reduced dilator muscle responsiveness/effectiveness (2), a reduced arousal threshold (3), and an oversensitive ventilatory control system (4).

Recently, the loss of pharyngeal muscle tone during sleep has been largely attributed to the withdrawal of endogenous noradrenergic drive [25, 26] and active muscarinic inhibition [27] at the hypoglossal motor pool. Convincing data from animal studies led to our preliminary study in OSA patients of the noradrenergic *atomoxetine* in combination with an antimuscarinic *oxybutynin* for one night (N=20) which substantially reduced OSA severity; pilot data also suggest a sustained effect after 1 week [28]. Notably, these agents are not new medications: Atomoxetine is a selective norepinephrine reuptake inhibitor approved for the treatment of attention-deficit hyperactivity disorder in adults and children [29]. Oxybutynin is an antimuscarinic which has high affinity for all muscarinic receptors (M1-M5) and is approved for the treatment of overactive bladder [30]. Since these agents are already approved, their side-effects are well documented. Thus, the use of these two drugs for OSA is a highly-feasible and attractive prospect.

For the field to realize the goal of having a pharmacological therapy available for OSA, there are several key gaps in knowledge that must be filled. The current study will answer the following questions: Does ongoing, repeated-dose administration of atomoxetine-plus-oxybutynin (referred to as "**AtoOxy**") improve OSA severity, and do patients exhibit signs of symptomatic relief? Most importantly, which phenotypic subgroup of patients preferentially benefit from this intervention?

The proposed research is highly likely to be significant for several reasons:

- We will be the first to **demonstrate efficacy and tolerance** of a pharmacological treatment for OSA across multiple nights, if results are positive.
- We will identify the differences in pathophysiology underlying **individual responses to therapy**. Atomoxetine-plus-oxybutynin was remarkably efficacious in many patients but less so in others; by revealing the mechanisms leading to reduced efficaciousness in individuals (e.g. greater collapsibility) we hope to provide a practical means to identify these individuals (e.g. increased apnea vs. hypopnea frequency) and thereby build the necessary knowledge base for future precision sleep medicine.

The study will also offer constructive feedback to drive further animal and human-subjects research on methods for pharmacologically-manipulating hypoglossal motor output. Overall, these findings have the potential to improve quality of life and health outcomes, particularly in those unable to become established on other therapies such as CPAP.

*Review of Literature: Noradrenergic.* Loss of noradrenergic activity is now thought to play the key role in the sleep-related hypotonia of pharyngeal muscles, with serotoninergic mechanisms accounting for only about 10% [31]. Chan and colleagues [25] showed in rats that the noradrenergic antagonist terazosin administered at the hypoglossal motor pool substantially reduced genioglossus activity during wakefulness and produced REM-like atonia during non-REM sleep, illustrating the importance of noradrenergic mechanisms. Other studies [31, 32] also support the notion that progressive withdrawal of noradrenergic tone, from wakefulness to non-REM and REM sleep, is the major mechanism causing sleep-related pharyngeal hypotonia. Nevertheless, due to the only recent identification of this process, efforts to stimulate the pharyngeal muscles with noradrenergic drugs during sleep humans have only just begun. Our own study in humans showed that the administration of the noradrenergic tricyclic antidepressant desipramine [33] modestly improved genioglossus activity and OSA severity in a subgroup of patients [26].

*Review of Literature: Antimuscarinic.* It is also now clear that noradrenergic withdrawal is not the only mechanism involved in sleep-related loss of genioglossus activity. For example, Chan and colleagues [25] failed to reverse REM atonia with noradrenergic stimulation (alpha-1 receptor agonists) applied to the hypoglossal nucleus, indicating that an additional, potentially inhibitory, mechanism is at work. This inhibitory mechanism is likely to be predominantly muscarinic: Grace and colleagues delivered the muscarinic antagonist scopolamine to the hypoglossal motor nucleus in rats to demonstrate that there is progressive muscarinic inhibition of drive to the hypoglossal motor nucleus from wake to non-REM sleep and REM sleep [27, 34]. Muscarinic antagonism had a particularly strong restorative effect on genioglossus activity in REM sleep but also had important effects on respiratory-related muscle activity in non-REM. This new knowledge has not yet been applied to humans.

*Combination of atomoxetine and oxybutynin.* We chose representative noradrenergic and antimuscarinic agents *atomoxetine* and *oxybutynin* because they are already approved for other uses (see above) [29, 30]. Atomoxetine was the first non-stimulant drug approved for the treatment of ADHD and its use is allowed in children from 6 years old, given the relatively safe profile, especially because it is not associated with abuse or dependence. It is a selective norepinephrine reuptake inhibitor that has a high affinity for the norepinephrine transporter (Ki 5 nM), passes the blood brain barrier, has a half-life of ~4-19 hours, and the maximal plasma concentration (Cmax) is reached 1 or 2 hours after dosing. Oxybutynin is an antimuscarinic with high affinity for all muscarinic receptors (M1-M5, $K_i$ 5.9 to 14 nM), passes the blood brain barrier, has a half-life of ~2-5 hours and a C-max of ~1 h (note: the short half-life and bedtime administration is expected to limit daytime exposure to side-effects). Interestingly, it has been shown that the inhibitory receptor M2 which is antagonized by oxybutynin is one of the few G protein-coupled receptors differentially expressed in both the pre-motor (2.4-fold higher expression) and motor (3.6-fold higher expression) areas of the hypoglossal motor nucleus compared to the rest of the brain [35].

Both of the medications examined in the current proposal (atomoxetine, oxybutynin) are generic, have been approved for other indications, have been tested in patients with sleep apnea previously [28], but have not been approved to treat sleep apnea. Thus, administration of these medications will be off-label. The FDA will be asked to approve an IND exemption to study these medications.

This study will be funded by the NIH (NHLBI).

Per NIH request, a Data Safety Monitoring Board will be employed to independently monitor safety and quality assurance.

## II. SPECIFIC AIMS

### Aim 1 – Effect of AtoOxy on sleep apnea severity

In a randomized controlled double-blind crossover study, 48 patients with moderate-to-severe OSA will take atomoxetine-plus-oxybutynin ("AtoOxy") versus placebo nightly for 1 month, with a 2-week washout in between. We will test the hypothesis that AtoOxy reduces the following primary outcome measure:

- Apnea-hypopnea index (AHI)

and improves the following secondary outcome measures:

- Nocturnal oxygenation, per "hypoxic burden of sleep apnea" [36]
- Frequency of arousals from sleep (arousal index)
- Self-reported sleepiness (Epworth Sleepiness Scale)
- Disease-specific quality of life per Functional Outcomes of Sleep Questionnaire Short Form; FOSQ.
- Disease-specific quality of life per Sleep Apnea Quality of Life Index (Short Form; SAQLI).

Additional pre-specified exploratory outcome measures will be assessed, including Visual Analog Scales (Sleep Quality, Excessive Fatigue, Waking Unrefreshed, Low Energy, Treatment Satisfaction) and additional polysomnographic measures of sleep (Stage 1 sleep, %total sleep time).

Adherence and adverse events will also be carefully monitored to assess repeated-dose tolerance of the intervention.

### Aim 2 – Determine which patient phenotypes respond best to AtoOxy

Patients undertaking Aim 1 will also take part in an additional night before initiating study medication to measure the key mechanisms causing OSA. We will prospectively-test the hypothesis that greater pharyngeal collapsibility determines a reduced response to therapy (see Statistical Analysis Plan). We will also separately test the hypotheses that a reduced muscle responsiveness, reduced baseline muscle activation, a higher arousal threshold, and a lower loop gain will facilitate a greater response to therapy.

## III. SUBJECT SELECTION

In this study we anticipate an enrollment of N=134 patients (accounting for dropouts between consent and baseline ~40%, baseline screening failures ~25%, and a ~20% post-randomization attrition rate), yielding N=48 patients completing the protocol. The study will continue until 48 patients have completed the study.

To maintain equipoise, we will study subjects with OSA who are intolerant or non-accepting of CPAP. These participants will be identified through 1) self-report and 2) screening. Specifically, we will enroll subjects who are have previously been diagnosed with OSA and are not currently nor seeking PAP treatment for OSA (per patient self-report). However, as the vast majority of patients with OSA in the community (~84-93%) are currently undiagnosed [37], partly the consequence of an absence of treatments that are acceptable to patients, we will also enroll subjects at high risk of OSA (any clinical indication [e.g. snoring, witnessed apneas] / symptoms [excessive daytime sleepiness]; "clinically-suspected OSA"), who are also not interested in CPAP treatment. This will ensure that our study is of maximal relevance to the broader community of patients with OSA. For enrolled subjects with clinically-suspected OSA who we find to have OSA on baseline polysomnography: The lead study physician will 1) discuss the study findings with the participant, 2) explain that CPAP is the standard of care for OSA, 3) emphasize that our priority is for patients to be optimally treated over participation in the study, 4) provide information on drowsy driving/risks of hypersomnolence, and 5) facilitate clinical referral (BWHF Sleep Disorders clinic) as appropriate for interested participants. Further participation in the study can be delayed, or declined, per informed participant choice, to minimize the likelihood that study participation might delay appropriate clinical care.

Subjects will be otherwise healthy and will be on no medications that could affect respiration or muscle control. We will consider all applicants regardless of sex, race, color, creed, or national origin. We expect a 2:1 ratio of men:women based on the male predominance of OSA in the population.

Inclusion/exclusion criteria are summarized below:

Inclusion criteria:

- Ages 21–70 years
- Diagnosed OSA or clinically-suspected OSA
- Not using CPAP (>1 month).

Exclusion criteria:

- Any uncontrolled medical condition
- Current use of the medications under investigation
- Use of medications expected to stimulate or depress respiration (including opioids, barbiturates, doxapram, almitrine, theophylline, 4-hydroxybutanoic acid).

- Current use of SNRIs/SSRIs or anticholinergic medications.
- Conditions likely to affect obstructive sleep apnea physiology: neuromuscular disease or other major neurological disorder, heart failure (also below), or any other unstable major medical condition.
- Respiratory disorders other than sleep disordered breathing:
  chronic hypoventilation/hypoxemia (awake $SaO_2$ < 92% by oximetry) due to chronic obstructive pulmonary disease or other respiratory conditions.
- Other sleep disorders: periodic limb movements (periodic limb movement arousal index > 10/hr), narcolepsy, or parasomnias.
- Contraindications for atomoxetine and oxybutynin, including:
  - hypersensitivity to atomoxetine or oxybutynin (angioedema or urticaria)
  - pheochromocytoma
  - use of monoamine oxidase inhibitors
  - diagnosed benign prostatic hypertrophy, urinary retention
  - suspected benign prostatic hypertrophy / urinary retention based on a positive answer to either of the following questions:
    - "During the last month, when urinating, have you had the sensation of not emptying your bladder completely more often than 1 out of 5 times?"
    - "During the last month, have you had a weak urinary stream more often than 1 out of 5 times?
  - untreated narrow angle glaucoma
  - bipolar disorder, mania, psychosis
  - history of major depressive disorder (age<24).
  - history of attempted suicide or suicidal ideation within one year prior to screening
  - clinically significant constipation, gastric retention
  - pre-existing seizure disorders
  - clinically-significant kidney disorders (eGFR<60 ml/min/1.73$m^2$)
  - clinically-significant liver disorders
  - clinically-significant cardiovascular conditions
  - severe hypertension (SBP>180 mmHg or DBP>110 mmHg measured at baseline*)
  - cardiomyopathy (LVEF<50%) or heart failure
  - advanced atherosclerosis
  - history of cerebrovascular events
  - history of cardiac arrhythmias e.g., atrial fibrillation, QT prolongation
  - other serious cardiac conditions that would raise the consequences of an increase in blood pressure or heart rate
  - myasthenia gravis
  - pregnancy/breast-feeding
- Allergy to lidocaine (Aim 2 only)
- Claustrophobia
- Pregnancy or nursing

*Baseline blood pressure are measured supine prior to sleep in the evening and again after lights on in the morning. Measures are made in triplicate, and the average of measures 2 and 3 are taken. The average of evening and morning values will be used to determine eligibility. Development of new hypertension during a treatment period (per above exclusion criteria), e.g. that is recognized on the final day of study medications at an overnight study visit, will not be used as stopping criteria for discontinuing further outcomes data collection (see Stopping Criteria below).

## IV. SUBJECT ENROLLMENT

Subjects will be recruited from our clinical sleep laboratory at Brigham and Women's Faulkner Hospital, Neurocare, and from our existing database of previous subjects. Recruitment will also occur through notices on email, telephone, fliers, and social media such as *Facebook*, websites such as *Craigslist.org*.

At the Faulkner Clinic, the patient's physician will introduce the study to potential subjects that meet our inclusion criteria during the course of providing their medical care and obtain the patient's verbal permission to be contacted by study staff. It should be noted that all investigators will reinforce with their patients that participation is voluntary, that they do not have to participate, and the decision not to participate will not affect their care, now or in the future. If subjects agree to be contacted by our study staff, subjects will either meet with the study coordinator in person after their scheduled appointment (as we have a staff member that regularly attends clinic) or will be followed up with a telephone call or email.

At the Neurocare Clinic, one of the questions asks if they would like to be contacted regarding research studies. If they answer "yes", and if they indicate to their physician or a member of the physician's team that they are interested in participating in a research study at Brigham and Women's Hospital, then one of our study staff will contact them regarding our study.

Subjects who respond will be given a thorough review of the risks, discomforts, potential benefits to the study and their expected involvement using a prepared script approved by our Institutional Review Board. Subjects will be given a copy of the informed consent and allowed a minimum of 24 hours to review the information and make a decision on study participation. During this time, the subject will have the opportunity to discuss the research with his/her primary care physician or clinician. The study investigators will be available to answer any questions should any arise. Informed consent will be obtained by the lead clinical physician investigator (Suzie Bertisch, MD). An MD will be also be readily available overnight at the hospital. Any consent issues / problems will be reported to the PHRC in real time rather than waiting to report at the time of Continuing Review.

Inclusion and exclusion criteria will be carefully assessed prior to enrollment. Assuming subjects meet the inclusion criteria, they will begin the protocol by scheduling their consent visit and the overnight studies in the clinical/physiology laboratory. Subjects will be informed that they may withdraw from the study at any point, with no impact on their ongoing care. We have not previously had difficulty enrolling subjects into similar studies performed in our laboratory.

If the data collected will be considered insufficient by the PI or by the co-investigators, the subject may be asked to repeat the study components without signing a new informed consent form.

All email sent outside of the Partners firewall containing confidential information will be encrypted using "Send Secure" as per the Partners Policy. Subjects may opt out of encrypted email communications if they have been advised of the risks associated with unencrypted email, and they indicate a preference to receive unencrypted email despite the risks. Consent to receive unencrypted email will be documented per the Partners Policy in the subject's record. We will discourage subjects from communicating about medical issues by non-secure email.

All Zoom video calls will use the PARTNERS-approved Zoom application.

## V. STUDY PROCEDURES

***Protocol***

This study is a double-blinded, randomized crossover trial.

***1. Screening and Consent visit***

Subjects will attend a Screening and Consent visit to assess eligibility for enrollment.

On arrival, the study will be explained in detail and an assessment will be made by an investigator to ensure the study procedures can be performed and written consent will be obtained by a licensed physician investigator. If the subject enrolled is a premenopausal woman, we will perform a urine pregnancy test at this visit.

***COVID-19 Alternative: Consent Video Call***

If participants are unable to attend an in-person visit (i.e. to minimize risk of highly infectious diseases like COVID-19), participants will take part in a video call with the consenting doctor to obtain consent (Zoom). All procedures described above will take part during this video call. The subject will already have received a copy of the consent form and will sign and mail it back to the consenting doctor who will then sign it. A copy of the signed consent form will be sent back to the subject. If the subject is a premenopausal woman, a pregnancy test will also be sent prior to the video call, and we will ask her to perform the test and we will verify the results. All questionnaires will be sent to the subject to be completed in their home.

***2. Baseline Clinical Sleep Study***

A baseline clinical sleep study (see ***Measurements and Equipment—Clinical***) will also be performed to assess eligibility. The baseline study will used to determine if the subject has an apnea-hypopnea Index (AHI) greater than 15 events per hour. If their AHI is found to be less than

15 on the baseline visit, they will not be randomized and participation will be discontinued. If a subject has completed a Baseline Clinical Sleep Study with the same measurements and equipment used for this study within 3 months of enrollment, we will use the previous study and they will not be asked to do this repeat Baseline Clinical Sleep Study.

Subjects will also complete baseline questionnaires (see below).

***3. Baseline Physiological Sleep Study***

A baseline physiological sleep study (see ***Measurements and Equipment— Physiological***) will also be performed to assess the mechanisms causing sleep apnea. We may allow subjects who decline the physiological study (Aim 2) to take part in Aim 1.

***3. AtoOxy Treatment***

*Dosing and randomization.* After consent and baseline visits, eligible subjects will be randomized (allocation 1:1, blocks of 4 [AB and BA], randomization per BWH IDS) to receive the first period of study medication for 1 month (4 weeks). Allocations will be concealed from the subjects, investigators, physicians, and outcomes assessors. Patients will perform in random order the two study interventions:

A) atomoxetine 80 mg plus oxybutynin 5 mg (full doses described)
B) placebo

Half doses will be given on the first three nights (step-up dosing) per usual clinical use of atomoxetine. Placebos capsules will be given such that there are no differences in number or appearance of capsules taken between periods. There will be a 2-week washout between periods.

*Duration.* A 1-month duration is considered the minimum duration necessary for reliable assessment of short-term repeated-dose efficacy and effects on symptoms (e.g. Epworth Sleepiness scale assesses sleepiness over the last month) [20, 38-43]. Subjects will be treated for a 1-month period (30 days ± 7 days).

If unexpected rescheduling of the on-treatment sleep study is necessary: First, we will seek to extend treatment for up to an additional 7 days (study drugs for 37 nights will be provided at the outset). Second, if rescheduling within this time frame is not possible, treatment will be paused and restarted as soon as possible with the goal of providing at least 7 days of treatment before the final sleep study. Third, the entire treatment period may be restarted if necessary. Having the subject attend a study visit after some duration of treatment will be prioritized over obtaining no sleep information for a given treatment period.

If a subject experiences difficulties on the full dose but not half dose (on or after treatment day 4): The participant may be advised to continue on the half dose for a longer duration, based on discussions with the (blinded) lead study physician. Having the subject adhere to the half dose will be prioritized over treatment non-use for a given treatment period.

*Sleepiness and Disease-Specific Quality of Life.* Questionnaires after each study arm will assess: ● Epworth Sleepiness Scale (ESS). ● Functional Outcomes of Sleep Questionnaire (FOSQ) and Sleep Apnea Quality of Life Index (SAQLI) to assess disease-specific quality of life. ● Visual Analog Scales (VAASQ) and Treatment Satisfaction (VASTS, treatment only) ● Enthusiasm for continued therapy (ECT, treatment only; i.e. subjects will answer whether they would choose to continue using the treatment if given the opportunity)

*Adherence*. Nightly adherence will be monitored using electronic pill containers (MEMS cap) with real-time wireless monitoring capabilities that will keep the study staff informed of adherence throughout the study; direct feedback from technology (text message), and indirect feedback from study staff will serve to maximize adherence. We will also assess adherence through patient self-report and pill counts.

*Sleep study.* After 1 month of treatment at home, the subject will attend an overnight sleep study at the hospital (BWH, Tower 9A) and will perform a sleep study after taking the last dose of study medication for the treatment period (see ***Measurements and Equipment—Clinical***). On these treatment studies, once all the equipment has been secured, subjects will be given their medication to take (30 min before lights out).

*Safety Monitoring.* See Data and Safety Monitoring Plan for details. In brief:

- Incident adverse events (AE) and any serious adverse events will be monitored throughout the study period and for 2 weeks after the last dose. Patients will be contacted by telephone (days 1, 3, 7, 14, 21, 28 of intervention; 7, 14 days after completion) and in person at the 1-month visit (day 30), following a standardized framework, to ensure timely reporting and clinical assistance if required. Patients will be asked to describe the incidence and severity of common side effects by checklist and any other adverse events.
- At each study visit we will also measure
    - effects on sleep parameters (including total sleep time, proportion of REM sleep, proportion of slow wave sleep, proportion of stage 1 sleep, arousal frequency)
    - effects on sleepiness and quality of life (ESS, SAQLI, VASSQ, and FOSQ)
    - overnight heart rate and evening/morning supine blood pressure (triplicate),
    - Brief Psychiatric Rating Scale (BPRS) to monitor for the potential emergence of suicidal ideation or behavior
    - medication tolerance based on adherence

Any positive response to the suicidality item of the BPRS (very mild or higher) will lead to immediate assessment of the subject by the licensed Study Physician (Dr. Bertisch), who will determine whether the subject exhibits suicidal ideation (i.e. mild or higher on BPRS suicidality item) and will facilitate appropriate further clinical care. Those with suicidal ideation will be referred either to the local emergency room or timely and appropriate psychiatric care with a subject's existing physician, based on the best interests of the safety of the subject. Emergent suicidality that is determined to be related to study medication/participation will also lead to discontinuation of study participation.

***Measurements and Equipment—Clinical***

At arrival subjects will complete questionnaires (all: ESS, FOSQ, SAQLI, VASSQ; treatment visits: VASTS, ECT). For treatment visits, side effects will be assessed systematically from a list of the possible side effects of all drugs used in the trial.

Subjects will be instrumented with standard polysomnography (PSG) recording sensors. Sleep stage and arousals will be measured with electrodes pasted on to the scalp, face, chin and chest (EEG, EOG, EKG, chin EMG). Paste-on EMG electrodes will be placed over the anterior tibialis muscle to detect leg movements. Respiratory effort belts will be placed around the chest and abdomen to measure breathing movements. Oxygen saturation will be measured continuously with a pulse oximetry probe placed on either the fingertip or earlobe. Snoring will be detected with a small microphone positioned over the suprasternal notch. Body position will be recorded with a sensor taped to the thoracic belt. Each of these devices is standard for diagnostic PSG and should not be uncomfortable.

Nasal airflow will be monitored via a nasal cannula under the nose, secured around the ears and under the chin and secured in place with tape. In addition, we will measure expired carbon dioxide levels ($PCO_2$) using a calibrated infrared $CO_2$ analyzer (Capnograph/Oximeter Monitor), and if available, end-tidal oxygen levels using a calibrated $O_2$ analyzer.

Subjects will be asked to remain in the supine position for 50-to-75% percent of the night, and to maintain consistency across clinical studies.

The study will end at approximately 6:00-6:30 am, at which time the monitoring equipment will be removed.

The morning after the sleep study, supine blood pressure will be measured (triplicate), equipment will be removed, side effects will be assessed systematically from a list of the possible side effects of all drugs used in the trial. Five Visual Analog Scales will be performed (Sleep Quality, Excessive Fatigue, Waking Unrefreshed, Low Energy, and Treatment Satisfaction), and subjects will also be asked if they would continue taking the medication if given the opportunity.

***Measurements and Equipment—Physiological***

In addition to the clinical studies, subjects will undertake the following:

- An esophageal catheter will be inserted after one nostril is anesthetized with 4% lidocaine (~1 ml of used). The tip of the pressure catheter will be located in the stomach. Once in place, the catheter will be taped at the nose to ensure it does not move. The integrity of the signal will be checked through a maneuver whereby inspiratory force is generated against a temporarily-occluded airway.
- For the majority of the night, patients will breathe spontaneously. For approximately 1-2 hours towards the start of the night, a modified CPAP device (Pcrit 3000, Philips Respironics, Murrysville PA) will be used to measure (A) eupneic ventilation, by using a

therapeutic level that keeps the airway open, and (B) the passive and active collapsibility by lowering and raising CPAP to manipulate ventilation and ventilatory drive. The CPAP device has been modified to enable delivery of both positive and negative continuous airway pressure. When used, the device will be attached to the mask.

- Subjects will be asked to remain in the supine position for as long as possible during the night.

If any data collected are considered insufficient, the subject may be asked to repeat the whole study or a part of it.

***COVID-19 Alternative: Home Sleep Testing (for all nights EXCEPT the physiology night)***

If participants are unable to attend an in-lab sleep study (i.e. to minimize risk of highly infectious diseases like COVID-19), subjects will perform home sleep tests. If the subject is a premenopausal woman, a pregnancy test will be sent prior to the home sleep tests. We will ask her to perform the test and we will verify the results in a Zoom video call.

Subjects will be mailed a Nox Medical A1 home sleep test device along with the study medication. We will ask subjects to return unused medication after each treatment arm to be disposed of by the pharmacy. They will be given detailed instructions on how to self-apply standard polysomnography (PSG) recording sensors. Sleep stage and arousals will be measured with electrodes placed on to the face. Respiratory effort belts will be placed around the chest and abdomen to measure breathing movements and body position. Oxygen saturation will be measured continuously with a pulse oximetry probe placed on the fingertip. Snoring will be detected with a small microphone positioned over the suprasternal notch and plugged into a smart phone. Breathing will be measured using a nasal cannula. Each of these devices is standard for diagnostic PSG and should not be uncomfortable.

Prior to bed, subjects will video call study staff to confirm equipment has been correctly applied and take the study medication.

The morning after the sleep study, subjects will again attend a Zoom video call with study staff. On the Zoom video call, supine blood pressure will be measured, as above, by the subject using a blood pressure monitor (sent with devices with instructions). Equipment will be removed, side effects will be assessed systematically from a list of the possible side effects of all drugs used in the trial. Subjects will complete questionnaires (see above). Subjects will then mail back the devices and questionnaires so data can be downloaded and equipment can be properly cleaned and disinfected.

***Data analysis***

Apneas, hypopneas, sleep stages and arousals from sleep will be scored using current AASM guidelines (hypopneas defined by at least a 30% reduction in airflow in conjunction with either 3% desaturation or arousal) by a technician blinded to the study condition.

*Physiological measurements.* Gold standard phenotypic traits (collapsibility, pharyngeal compensation, arousal threshold, loop gain) will be calculated using established methods [44, 45] from data collected on the physiology night. Collapsibility is the value of ventilation (Vpassive, %eupnea) observed under passive/hypotonic conditions after a CPAP drop to 0 $cmH_2O$ (we will also measure Pcrit, the value of pressure at which the airway is closed). Compensation is obtained by calculating Vactive, the median overnight value of ventilation achieved under active conditions during supine non-REM sleep; compensation = Vactive minus Vpassive. Arousal threshold is based on the ventilatory drive swings (intraesophageal catheter) preceding arousal from sleep (presented as %eupnea). Loop gain is calculated as the increase in ventilatory drive (ventilatory overshoot) divided by the preceding reduction in ventilation (data fit to CPAP dial-ups) [22].

*Clinical estimates of physiological variables.* Values will be estimated from clinical polysomnography using recently-validated algorithms [46-49]. In brief, traits will be measured using scored sleep study data, with a focus on the ventilatory flow signal. First the sleep study is segmented into 7-min overlapping windows containing non-REM sleep. A "ventilatory drive" signal (the level of ventilation that would be observed if the airway was open, akin to neural output to the diaphragm muscle) is estimated using measured ventilation data (tidal volume × respiratory rate) and a chemoreflex model fit to ventilation data when the airway is considered open (between scored respiratory events). The model parameters are used to describe the chemoreflex "loop gain", namely the magnitude of the ventilatory drive increase in response to a prior reduction in ventilation. The arousal threshold is calculated as the value of ventilatory drive on the breath preceding each scored EEG arousal from sleep. Collapsibility is taken as the value of ventilation (sleep only) at normal ventilatory drive. Compensation is taken as the increase in ventilation that is achieved between the value at normal drive to that at maximal drive (at the arousal threshold). Parameters have been validated previously [46-49]. Median overnight values are used to represent each individual patient per treatment period.

***Reimbursement***

Subjects will receive $100/night for participation in the three overnight clinical studies (3 overnights = $300). Subjects will receive $200 for participation in the overnight physiology study. An additional $200 will be provided for successful completion of each study period (2 completed periods = $400). Thus, in total, a subject could receive up to $900 at study completion. If a baseline study is not needed, subject will not receive the $100 for the study not performed. If subjects repeat a part of the entire protocol because of insufficient data collection, they will be reimbursed $100 for any extra night. Reimbursement for parking expenses or taxi fares will be provided.

For any inpatient visits requiring a COVID-19 test prior to admission, subjects will receive an additional $50 for each test.

Home Sleep Tests Bonus: For home sleep tests, subjects will receive a $25 bonus for each home sleep test that is mailed back to study staff the day after the study. Therefore, they could

receive up to $100 for a timely return of the devices. If the subject does not send back equipment immediately, they will not receive this bonus.

## VI. BIOSTATISTICAL ANALYSIS

### *Aim 1*

*Statistical analysis plan.* We will perform intention-to-treat analysis, with values imputed for any study arm that was started but not complete (any dose taken) per multiple imputation with chained equations (see below). The quantitative primary outcome variable will be the percent change in apnea-hypopnea index (AHI) from baseline; the difference in this outcome variable between intervention and placebo will be evaluated (mixed model analysis, see below). Percent change from baseline will be used rather than absolute change because (1) statistical power in the single-night study was considerably greater when using the percent change versus absolute change, (2) the absolute but not percent change was strongly correlated with the baseline AHI, suggesting that the change in AHI with treatment is proportional to the baseline AHI, and (3) percent change is easy to interpret clinically. Standard linear mixed-effects model analysis for crossover studies [50-53] will evaluate the effect of intervention versus placebo on the outcome (fixed effect), independent of randomization sequence (AB or BA, i.e. carryover effects) and period (i.e. effect of time) as fixed effects, with each patient treated as a random effect; the approach also enables incorporation of incomplete data. We will also report the *response rate* i.e. percentage of patients who had a 50% percent reduction in AHI. Secondary outcomes will also be expressed as changes from baseline (percent change in arousal index; absolute change in nadir oxygen saturation, Epworth Sleepiness Scale, Functional Outcomes of Sleep Questionnaire) and assessed using the same approach. Per-protocol analysis will also be assessed to examine on-treatment effects (adherence≥80%). Variables will be transformed if necessary to provide a normal distribution before analysis (typically $x_{\text{transformed}} = x/(2-x)$) for AHI; 95% CI will be presented based on back-transformed results. $P<0.05$ will indicate statistical significance.

Imputation for missing data (multiple imputation using chained equations, [7]) will be performed for missing primary and secondary outcomes data. Variables used for imputation will include treatment allocation, randomization sequence, and period; all other primary and secondary outcomes variables, plus selected auxiliary variables designed to facilitate imputation of outcomes variables including baseline AHI, BMI, on-treatment side effects (where available), self-reported questionnaire outcome scores (where available), and any additional measures that are discovered to meaningfully improve model quality. For each imputed dataset, the routine will impute missing data using 50 iterations. Stochastic noise will be added to each imputed missing data result to match population heterogeneity. After each imputed dataset is generated, the primary model analysis will be repeated and recorded (similar approach for secondary analyses). In total, 20 imputed datasets will be generated; pooled model coefficients and 95%CI (Rubin's rules) will be used to generate a single imputed beta coefficient for the effect of treatment allocation on each outcome variable.

Interim analyses will not be performed (removed from plan per discussions with DSMB at study initiation).

*Power analysis.* Sample size is based on the secondary outcome with the smallest expected effect size: 48 patients will provide 80% power to detect a clinically-significant reduction in the Epworth Sleepiness Scale of 2 points (expected SD~=4.8) [20, 54, 55] (alpha=0.05). With 48 patients, the power to detect a clinically-significant change in the primary outcome variable (50% reduction in apnea-hypopnea index, expected SD~=19% from preliminary study) exceeds 99.9% (alpha=0.05). *Other secondary outcomes:* Power to detect A) an increase in nadir oxygen saturation by 5 percentage points (expected SD~=8%, preliminary data) is 98.9%; B) a reduction in arousal index of just 10% (expected SD~=21%, preliminary data) is 89.8%; C) an increase in the Functional Outcomes of Sleep Questionnaire score of +2 (expected SD~=3.1[55]) is 99.2%. The study will also be adequately powered to assess effects on the AHI separately in men (N=32; power>99%) and women (N=16; power>99%). By studying 48 patients (rather than a lower number), we expect results to be generalizable across groups by age, sex, race/ethnicity, obesity; we will also have the capacity to determine the physiological mechanisms determining responses to therapy, a major goal of this work (see Aim 2). Recruitment will continue until 48 patients have completed both arms (active and placebo) of the study; based on a ~20% dropout rate we expect to randomize 60 patients.

***Aim 2***

*Statistical analysis plan—Physiological determinants.* Tests are designed to illustrate that each trait *explains* individual differences in the *response to therapy* (defined below). We will prospectively-test the hypothesis that greater pharyngeal collapsibility determines a reduced response to therapy (linear regression, *response to therapy* versus Vpassive). We will also separately test the hypotheses that a reduced muscle responsiveness, reduced baseline muscle activation, a higher arousal threshold, and a lower loop gain will facilitate a greater response to therapy (linear regression). The primary outcome variable (*response to therapy*) will be calculated by assessing the percent reduction in AHI from baseline with intervention (at 1 month) minus the percent reduction in AHI from baseline with placebo, i.e. consistent with Aim 1. Per-protocol analysis will be used because the study aims to explain inter-individual physiological responses. All questions are of separate scientific relevance and will not be adjusted for multiple comparisons. Multivariable regression will confirm whether baseline physiological variables remain predictive after adjusting for baseline apnea-hypopnea index.

Commonly, knowledge of multiple traits *in combination* best predicts outcomes. We will therefore perform a multivariable linear regression analysis (with interactions and squared terms, as needed, to capture non-linear relationships when evident) to describe how the traits combine to determine responses to therapy. This approach has been used successfully previously [46] and has strong physiological rationale (i.e. in those with milder collapsibility, higher loop gain leads to therapeutic failure).

*Statistical analysis plan—Clinical predictors.* We will build a multivariable model to *predict* individual differences in the response to therapy (defined above) using 1) routine baseline polysomnographic variables (including ratio of apneas to total events), and 2) estimated polysomnographic phenotypic traits (described above). In both cases, as performed previously

[56], we will use multivariable linear regression (original terms, interactions, squared terms i.e. quadratic model). Non-contributory terms will be removed incrementally to provide a final regression model (backwards elimination with P>0.157, equivalent of AIC criteria [57-60]); this simple approach previously outperformed other approaches on out-of-sample data including use of looser criteria (e.g. P<0.05), stricter criteria (e.g. P<0.5), regularization methods e.g. LASSO, and nested cross-validation [46]. The predictive value of the model will be assessed using leave-one-out cross-validation to handle overfitting, with the entire backwards elimination process repeated for each loop in the cross-validation process. If results are promising, a separate test set will be studied in future for validation.

*Power analysis. Physiological determinants*: 36 patients will provide 88% power (alpha=0.05) to detect a physiologically-significant association ($R^2$=0.25) between each gold-standard phenotypic trait and the response to therapy (defined above). In previous studies, 14-20 patients were sufficient to show significant multivariable associations between the response to therapy and two variables (e.g. loop gain and collapsibility) [61] supporting the view that 36 patients will be sufficient for four gold-standard traits. 36 patients were also sufficient for multiple regression in a recent study [46] using multiple traits to predict outcomes. *Clinical predictors*: 48 patients will provide 81% power to detect modest independent associations ($R^2$=0.2) between each predictor and the response to therapy. We expect 36/48 patients taking part in Aim 1 to complete the Aim 2 study.

## VII. RISKS AND DISCOMFORTS

The study incorporates a trial of two established agents separately approved for other conditions, that have not yet been assessed in combination for OSA treatment for as long as 1 month. We consider the study to be more-than-minimal risk. Nonetheless, we feel the risks are reasonable given the potential benefits of providing a future treatment for OSA. Anticipated risks and discomforts are listed below:

Clinical Sleep Study: The equipment used for assessing sleep (paste on electrodes) is standard and poses no risk. The electrodes may be mildly uncomfortable and could cause some sleep interruption. Thus subjects may feel somewhat tired the day following this study.

Atomoxetine: **Common side effects include dry mouth, fatigue, irritability, nausea, decreased appetite, constipation, dizziness, sweating, insomnia, dysuria, dysmenorrhea, sexual problems, decreased libido, urinary retention or hesitancy, increased obsessive behavior, weight changes, palpitations, increases in heart rate and blood pressure.** Uncommon side effects (<1%) include tachycardia, rhythm disturbance, suicidal ideation, aggressive behavior, mood swings, serious allergic reaction. Rare side effects (<0.1%) include seizures, alopecia, hallucinations, liver injury.

Oxybutynin: **Common side effects include: urinary retention, dry mouth, dry eyes, dry skin, constipation, nausea, facial flushing, dizziness, headache, somnolence/fatigue, confusion, dyspepsia, diarrhea, blurred vision, vomiting.** Uncommon side effects (<1%) include drowsiness, light sensitivity, abdominal discomfort/pain, anorexia, decreased appetite,

dysphagia. Rare side effects (<0.1%) include restlessness, disorientation, concentration difficulties, phototoxicity, erectile dysfunction.

Physiological Sleep Study:

- Esophageal catheters: Ventilatory drive/effort will be monitored using an intraesophageal diaphragm EMG catheter. There is some discomfort passing the catheters through the nose and gagging can occur. The risk of aspiration must be considered in a subject where a mask is in place and a catheter has been inserted. Although this is a potential risk, we believe the likelihood to be quite small for several reasons. Once in place, the catheter causes little gagging. All subjects will have fasted for three hours prior to the procedure and thus their stomach content is minimal. Finally, the mask can be easily removed. For these reasons we believe aspiration to be a minimal risk.

- Lidocaine 4%: If the subjects have any history of lidocaine allergy, they will be excluded from Aim 2 of the study. Excessive use of lidocaine can cause seizures, but this is reported with much higher doses than will be utilized in this study. Note, lidocaine does taste unpleasant and the subjects will be informed of this.

- CPAP changes during sleep: The only real risk with the procedures for CPAP changes is sleep disruption. The ventilation device is not uncomfortable and actually facilitates ventilation. However, the procedures may awaken the subject leading to poor quality sleep.

**Stopping criteria:**

*For stopping a treatment period:* Any adverse event that in the judgment of the investigator necessitates the subject stopping to protect their safety. Additional criteria include: abuse, diversion or misuse of the agents; clinically significant hallucinations, amnesia, delusional thinking, delirium, manic symptoms, aggressive behavior, suicidality, homicidal behavior, agitation, confusion, or convulsions/seizures.

*For stopping catheter placement:* Vomiting, or more-than-mild gagging causing discomfort that in the judgement of the investigator necessitates stopping catheter placement. Data collection will continue in order to obtain other variables.

*For stopping the overnight physiological study:* Difficulty sleeping with study equipment in place such that by 3 am, or another time in the judgement of the investigator, the subject remains awake.

Thus, we believe that all possible safeguards are in place to minimize the risk.

## VIII. POTENTIAL BENEFITS

*Benefits to medical community.* This study provides a unique opportunity to gain insight into a combination pharmacological therapy for OSA, including factors involved in its successful

resolution. The results may, in the future, improve our understanding of sleep apnea and lead to improved strategies for therapies.

*Benefits to individual subjects.* Although it is unlikely that there will be any direct physical benefit to the subjects from participating in this study, we will make known to each subject, if requested, some of the information we have gathered from their baseline PSG. However, if previously unknown abnormalities of sleep and breathing are encountered, this information will be passed onto the subject. In order to maintain the subjects' privacy, results can be forwarded to the primary care physician or clinician only at the request of the subject.

## IX. MONITORING AND QUALITY ASSURANCE

### Data and Safety Monitoring

The Principal Investigator (Scott Sands, PhD) will be responsible for maintaining Quality Assurance, and will delegate tasks to study staff including Lauren Hess (i.e. IRB regulatory binder maintenance, source documentation completeness) and Nicole Calianese (i.e. database entry and monitoring). A licensed physician investigator study staff member (Suzie Bertisch, MD) will be designated as the lead study clinician and responsible provider for subjects participating in the study.

The current study comprises a single-center randomized-controlled clinical trial. Based on conversations with the NIH, in an abundance of caution, a formal Data and Safety Monitoring Board (DSMB) will be implemented. See the Data and Safety Monitoring Plan for details. The DSMB will independently monitor safety and quality assurance. The board will review all adverse events in order to classify them as serious adverse events, minor adverse events, and whether they are anticipated or unanticipated, and study related or unrelated as per our IRB rules and the NHLBI policy. The DSMB will strictly adhere to the definitions below (see ***Definitions***). Adverse events and severe adverse events will be reported to the PHRC and NIH according to respective guidelines.

Ongoing results, problems, and limitations of the study will also be presented on a regular basis to the investigators in the Division of Sleep Medicine.

### Adequacy of Protection Against Risks

#### *Training*

All of our laboratory personnel involved in the research of human subjects have completed the required institutional program for education in the protection of human research subjects and their confidentiality. The institutional educational program consists of the review of regulatory and informational documents pertaining to human-subject research, passing a test demonstrating knowledge of the ethical principles and regulations governing human-subject research and signing a statement of commitment to the protection of human subjects.

## *Informed Consent*

*Circumstances.* Subjects will be recruited from two sources: 1.) The Sleep Clinic at Brigham and Women's Hospital. Patients admitted to our clinic fill out a questionnaire. One of the questions asks if they would like to be contacted regarding research studies. If they answer "yes", then one of our study staff will contact them by phone regarding our particular study. 2.) The community: We frequently recruit subjects with obstructive sleep apnea by advertising in various newspapers, and thus we will use this as a source. Interested persons will be screened over the phone by a study staff member and given details about the study with a chance to ask questions.

*Obtaining consent.* Subjects will then be mailed, faxed or secure-emailed a copy of the consent form to read at their convenience (at least 24 hours before signing the consent; typically 1-4 weeks ahead of time). They will be explicitly informed that participation in the research study is completely voluntary, that their medical care will be not be adversely affected should they choose not to participate, and that they may stop the study at any time. The PI and/or senior co-investigator will interview each potential subject. Consent will be obtained by a licensed physician study investigator in-person prior to the first overnight study. At that time, the protocol will be explained in full detail, including the risks and benefits, and the subject will have a chance to ask more questions and discuss the study with a physician. Signature of the consent form by the subject and the investigator will be used for documentation.

## *Protection Against Risks*

We will attempt to minimize the risk to subjects as follows:

i. All subjects will be relatively healthy and without medically important disorders except for obstructive sleep apnea. History and physical will be assessed by a physician to confirm health and absence of exclusion criteria prior to administration of medications. A physician will also check on subjects while they are admitted to the Clinical Center of Investigation for overnight studies and will have ongoing overnight nursing coverage. A physician will be on-call throughout the stays. Should subjects need medical assistance, the nurse will notify the study physician and the PI.

ii. Identifiable data will be kept confidential by de-identifying (coding) subjects. All subject data are stripped of identifiable information, coded with a four-digit number, and kept online using a secure network service with a daily backup in accordance with Institutional policy. Paper forms with identifiable information (consent forms) will be stored in a locked file cabinet accessible only by study staff. Links between subject codes and identifiers are kept exclusively in a secure password-protected database. Only the study staff will have access to the subject identifying information.

iii. All studies will be conducted by an experienced investigator knowledgeable in all aspects of the procedures utilized. Breathing variables, including arterial oxygen saturation, and heart

rate will be monitored continuously in all studies. Blood pressure monitoring will be performed on arrival, before sleep, and after sleep at all study visits.

iv. Resuscitation equipment is readily available should the need arise. CPAP and 100% oxygen are available in the study rooms and can be quickly provided to the patient if needed. However, this seems unlikely.

v. While the clinical sleep studies in the laboratory are noninvasive, the physiological studies (with esophageal catheterization) are somewhat invasive and there is a risk that subjects may be sleepier than usual in the morning. Thus, subjects will have the opportunity to sleep without instrumentation for as long as they would like before driving home. We will also offer a cab voucher to those who did not drive.

vi. Women will have a urinary human chorionic gonadotropin test at each study visit to confirm absence of pregnancy.

vii. In the case of adverse effects, each subject will be given the responsible study clinician's (Dr. Bertisch) cell phone number, and the principal investigator's cell phone number to contact at any time (24/7) regarding questions or complications; subjects will be strongly encouraged to call the study team. Dr. Bertisch will facilitate referrals for further medical care when appropriate.

viii. During each period of the study, subjects will be contacted (e.g. telephone) for systematic safety assessment (adverse event checklist) according to a regular schedule (see above), including during the washout period after study completion. Subjects will additionally be asked to contact study staff if adverse events occur.

ix. When used clinically, the study medications are not tapered-down before discontinuation (stopping). We also emphasize that there is data demonstrating no significant rebound/withdrawal effects of atomoxetine (in contrast to other noradrenergic agents/antidepressants) [62]. Nonetheless, subjects will be asked to contact the lead study clinician to discuss any concerning rebound/withdrawal effects after stopping study medications, and will receive advice on appropriate actions to mitigate symptoms, including delaying further study periods or discontinuation (see criteria below) when appropriate.

x. Unanticipated problems involving risks to subjects or others including adverse events will be reported to the Human Research Committee as soon as possible and within 5 working days/7 calendar days for review according to the HRC adverse event reporting guidelines.

xi. Incidental findings are rare. Nonetheless, any findings will be communicated with the subjects by MD study staff. It is possible that subjects may discover a pregnancy. It is possible that study staff may witness a cardiac arrhythmia (e.g. atrial fibrillation). The lead study clinician will facilitate referrals for further medical care as appropriate.

xii. Our study seeks to recruit some individuals who 1) have "clinically-suspected" OSA, and 2) would refuse an offer of CPAP even if clinically recommended. In subjects who are discovered to have OSA on baseline polysomnography: The lead study clinician will 1) discuss the study findings with the participant, 2) explain that CPAP is the standard of care for OSA, 3) emphasize that our priority is for patients to be optimally treated over

participation in the study, 4) provide information on drowsy driving/risks of hypersomnolence, and 5) facilitate clinical referral (BWHF Sleep Disorders clinic) as appropriate for interested participants. Further participation in the study can be delayed, or declined, per informed participant choice, to minimize the likelihood that study participation might delay appropriate clinical care.

xiii. Questionnaires can contain sensitive personal information. For example, the Functional Outcomes of Sleep Questionnaire addresses information about sexual relationships and intimacy. Subjects will complete these in a private room and can choose not to answer any questions they feel uncomfortable answering.

To minimize discomfort due to esophageal catheterization, topical anesthesia (lidocaine) is used to minimize discomfort upon placement. Subjects will also fast for 3 hours prior to catheter placement to minimize the likelihood of vomiting. No patient has vomited from esophageal catheter placement under the PI's supervision despite hundreds of placements to date.

***Network Security for protecting the confidentiality of subject data***

All electronic data will be stored on Partners-approved secure network platforms (i.e. Secure File Area, Dropbox Enterprise) under password protection with no access allowed to individuals outside of our research team. All paper data will be stored under lock and key with access only given to the study staff.

***Definitions***

Definitions are per January 2007 OHRP *Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events, OHRP Guidance,* http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm

Adverse Event (AE): any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious adverse event (SAE): any adverse event that:

- Results in death
- Is life threatening, or places the subject at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects
- Is another condition which investigators judge to represent significant hazards

Unanticipated Problem (UP): any incident, experience, or outcome that meets all of the following criteria:

- unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;
- related or possibly related to participation in the research, in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse Events (FDA) versus Unanticipated Problems (OHRP)

- All adverse events are not necessarily unanticipated problems
- All unanticipated problems are not necessarily adverse events

Some events may be both

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