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Acute adenosine receptor antagonism in combination with acute intermittent hypoxia (AIH) to promote breathing plasticity in Amyotrophic lateral sclerosis: study protocol for a randomized, double-blinded, placebo-controlled trial

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Abstract

Background: Respiratory impairment is a major concern in amyotrophic lateral sclerosis (ALS), shortening survival and lowering quality of life. One therapy with promise to delay respiratory decline in ALS is acute intermittent hypoxia (AIH) consisting of alternating periods breathing mildly hypoxic (~9-12% O₂) and normoxic (21% O₂) gas. AIH stimulates spinal, serotonin-dependent neuroplasticity in rodent models, conferring functional benefits in diverse physiological systems without detectable pathology. However, in rodent models, AIH-induced neuroplasticity is constrained by distinct signaling cascades initiated by spinal adenosine.

Objective: We propose to investigate a therapeutic strategy to delay breathing compromise in those living with ALS by combining a selective adenosine 2A receptor (A2A) inhibitor (istradefylline) with AIH. The fundamental hypothesis guiding this proposal is that a single AIH trial after pretreatment with istradefylline (IST) enhances respiratory neuroplasticity versus AIH or sham intervention.

Methods: We propose to evaluate resting breathing, respiratory strength, and participant-reported symptoms, in adults living with ALS after combined istradefylline plus AIH. A mixed within and between subject study design incorporates 4 test sessions, separated by ~2 weeks (+/- 5 days). Testing conditions include single sessions of: AIH + IST, AIH + placebo, sham AIH (i.e., normoxia) + placebo, and sham AIH + IST.

Safety and feasibility will be characterized using the rate of adverse events, changes in vital signs, and participant-reported breathing sensations (Aim 1). Neuroplasticity of breathing and motor function will be evaluated as changes in resting breathing, voluntary respiratory strength, respiratory control, and maximal pinch force (Aim 2).

Results: As of January 2025, 10/16 subjects with ALS and 5/16 control subjects completed study procedures. Recruiting is ongoing, and the final subject will complete the study by December 2025. Publication of results is expected by the end of 2026.

Conclusions: These aims will provide crucial data regarding preliminary safety and feasibility of this paired intervention, and help optimize therapeutic AIH as a rehabilitation strategy, thereby guiding further research concerning this novel treatment for ALS. Clinical Trial: This trial was registered in the ClinicalTrials.gov database on 22 April, 2022 (registration number: NCT05377424).

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Original Manuscript

Background

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by motor neuron death in the primary motor cortex, brainstem, and spinal cord, leading to muscular weakness and atrophy.^{1,2} ALS can lead to progressive, variable weakness of the skeletal muscles involved in swallowing, speaking, coughing, limb movement, trunk control, and breathing. Respiratory muscle denervation causes diaphragm and accessory muscle weakness, hypoventilation, dyspnea, and orthopnea.^{3,4} Ineffective airway clearance and aspiration result from bulbar denervation. Respiratory and bulbar dysfunction lead to need for mechanical ventilation,⁵ shortened survival, and diminished the quality of life.⁶ Typical survival is 2-5 years post-diagnosis.⁷ Treatments that preserve or improve breathing would have considerable impact on the duration and quality of life.

Acute intermittent hypoxia (AIH) consists of short, repeated exposures to mild or moderate hypoxia (~9-12% O₂), separated by normoxic episodes (21% O₂). Hypoxic episodes activate the carotid body chemoreceptors, sending chemoafferent signals to stimulate medullary respiratory neurons⁸ as well as brainstem raphe neurons that contain the neurochemical serotonin. When this neural network is repeatedly stimulated during AIH,⁹ serotonin release from the raphe neurons triggers cell signaling pathways resulting in new synthesis of brain-derived neurotrophic factor (BDNF) protein. In turn, BDNF strengthens synapses onto respiratory (and non-respiratory) motor neurons,¹⁰⁻¹² increasing respiratory motor output. AIH-induced motor plasticity was first described as a persistent enhancement of phrenic neural activity lasting hours after AIH has ended, an effect known as phrenic long-term facilitation (pLTF); a similar effect occurs in hypoglossal motor neurons.^{13,14} pLTF is a form of activity-independent, neuromodulator-induced neuroplasticity that exhibits sensitivity to the pattern of hypoxic episodes and time of day.^{8,15,16} Repeated AIH appears to amplify and prolong pLTF without detectable pathology,¹⁷⁻²⁰ Thus, AIH may induced respiratory rehabilitation in ALS as well as other neurological conditions that compromise breathing.²¹

Our group recently completed the first clinical trial of AIH administration in patients with ALS compared with unaffected controls. In this study, AIH enhanced tidal volume, minute ventilation, and collective respiratory muscle activation of subjects living with ALS versus age/sex matched controls. Importantly, AIH was well-tolerated and free of treatment-related adverse effects; the participants typically could not distinguish between AIH versus sham AIH (*i.e.*, normoxia).²²

While the modest AIH used in rehabilitation typically elicits motor facilitation via a serotonin-dependent mechanism,^{8,14,23,24} an alternative AIH-induced signaling pathway results from adenosine,²⁵⁻²⁸ acting on adenosine 2A (A_{2A}) receptors.²⁹ Serotonin and adenosine signaling oppose one another through powerful cross-talk inhibition; in extreme cases, this “battle” cancels phrenic motor plasticity.^{28,30-32} Although both serotonin and adenosine receptors can be activated during mild hypoxia, the serotonergic Gq protein-coupled receptor signaling (*i.e.*, the “Q” pathway) predominates. However, multiple personal characteristics, disease states and treatment factors upregulate adenosinergic, Gs protein-coupled receptor signaling (*i.e.* the “S” pathway), sufficiently to inhibit plasticity (**Figure 1**), including aging,³³ low estrogen,³³⁻³⁵ inflammation,³⁶ active phase delivery,³⁷ genetic factors,³³ and traumatic or neurodegenerative diseases.³⁸

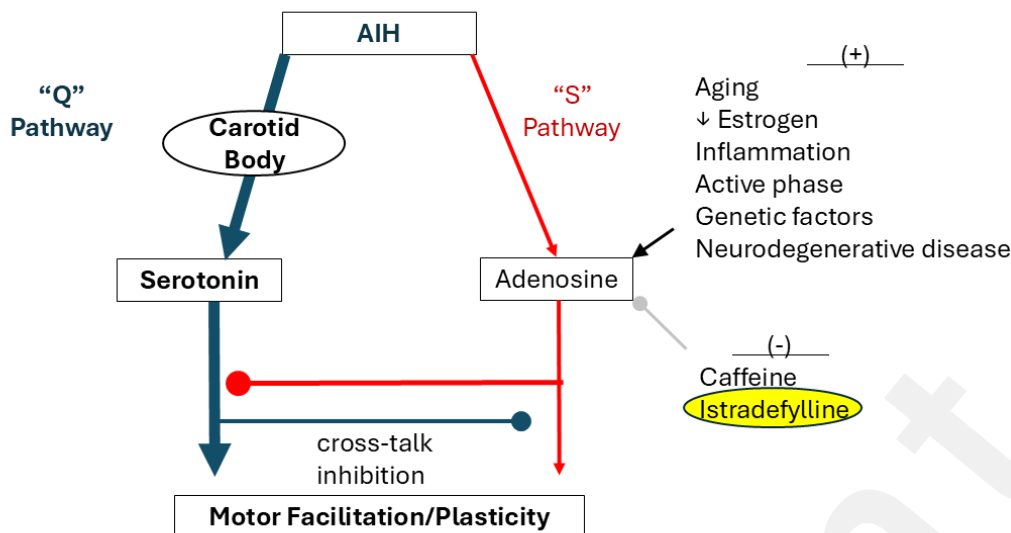


Figure 1. Competing serotonin- (Q) and adenosine-driven (S) pathways to phrenic motor facilitation. Listed on the right are factors that impact serotonin-dominant plasticity due to increased adenosinergic “S” pathway inhibition. By combining istradefylline with mild AIH, this protocol aims to minimize crosstalk inhibition by reducing S to Q pathway inhibition, allowing full expression of the “Q” pathway.

In rodent models of spinal cord injury (SCI), AIH paired with A2A receptor inhibition increases pLTF.³¹ In humans with chronic spinal cord injury, the non-selective adenosine receptor antagonist caffeine prolongs functional gains in walking walking ability.³⁹ Thus, combined AIH and A2A receptor antagonism may amplify respiratory motor plasticity.⁴⁰

Elevated spinal adenosine is characteristic of neurodegenerative diseases such as ALS.^{41,42} In the SOD1^{G93A} mouse model of ALS, A2A receptor signaling contributed to motor neuron degeneration, while A2A receptor antagonism delayed disease progression.⁴² A2A antagonism can also have neuroprotective and anti-inflammatory effects in motor neurons.⁴³ NOURIANZTM (istradefylline) is a highly selective adenosine A2A receptor antagonist currently approved for use in adults with Parkinson’s disease (PD) to offset inhibitory effects of upregulated A2A receptors on D2 dopamine receptors of the basal ganglia.⁴⁴ Istradefylline reduces daily “off” time in people with PD, improving periods of mobility, and is reported safe and well-tolerated.⁴⁵ Dyskinesia (5%) and hallucinations (3.4%) were the most common side effects associated with longer-term use,⁴⁶ but cognition, sedation, or risk of cardiovascular events were not impacted.⁴⁷

Considering the demonstrated safety of istradefylline and the potential to enhance AIH-induced plasticity with A2A receptor inhibition, we hypothesize that a single AIH trial paired with istradefylline will enhance respiratory neuroplasticity in patients with ALS *versus* AIH or istradefylline alone.

Aims

The objective of this study is to evaluate single and combined effects of selective A2A receptor antagonism and AIH on respiratory strength, resting breathing, and participant-reported symptoms in adults living with ALS and age-matched unaffected adults. Two aims are proposed to test the hypotheses that:

Aim 1: a single 20 mg istradefylline dose prior to AIH is safe. Dependent variables will include: rate of adverse events (primary outcome); acute changes in vital signs (heart rate, respiratory rate, blood pressure, etc.); patient-reported symptoms (dyskinesias, dizziness); and study completion.

Aim 2: a single 20 mg istradefylline dose prior AIH improves respiratory motor function when compared to either intervention alone. Dependent variables will include: resting tidal volume (primary outcome), breathing rate and minute ventilation; respiratory muscle assessments *via* multi-channel electromyography (EMG)); cough force; maximal inspiratory pressure; and maximal finger pinch force.

Methods

Study design, setting and recruitment

This is a repeated-measure, double-blinded, placebo-controlled and randomized trial, administered at a single site: The University of Florida (UF). This clinical trial was registered in the United States National Library of Medicine through ClinicalTrials.gov (NCT05377424).

Ethical approval was granted by the University of Florida Institutional Review Board (IRB) on May 16, 2022. The U.S. Food and Drug Administration provided clearance for off-label investigational use of istradefylline as described in the protocol. Informed consent will be obtained in writing from all subjects prior to enrollment by study team members. This work is supported by the ALS Association. Outside of the human research protection review, the funding sponsor does not have a direct role in directing the study design; collection, management, analysis, and interpretation of data; writing of the report; or the decision to submit the report for publication.

Patients living with ALS will be recruited from the UF ALS clinic, as well as flyers, word of mouth, and the study registry website. Patients who meet the study criteria will be identified by the study physicians through the UF adult neuromuscular clinic. Healthy age-matched controls will be recruited via advertising for the study on ClinicalTrials.gov, StudyConnect, social media for the UF Clinical and Translational Science Institute, and flyers throughout the local community. Additional potential healthy controls will be identified from the Pepper Center Registry maintained by the UF Institute on Aging, and IRB-approved flyers will be sent to their address. Study interventions will occur at a university-based Clinical Research Center located in Gainesville, FL.

Inclusion criteria

Eligible participants include non-smoking adults diagnosed with ALS or age-matched unaffected controls, aged 21-80 years. Eligible subjects will have El Escorial diagnostic classification of probable or definite ALS, vital capacity $\geq 60\%$ of predicted value, and ALS Functional Rating Scale – Revised bulbar and respiratory item scores of 2 or greater. Clinically prescribed ALS medications must be at a stable dose for 30 days.

Exclusion criteria

Participants (both affected and controls) are ineligible if they are pregnant, have an active respiratory infection, took antibiotics within 4 weeks of recruitment, are diagnosed with another neurodegenerative disease, have symptomatic cardiovascular disease or dysrhythmias, have a BMI $>35 \text{ kg/m}^2$, have a seizure disorder, use respiratory inhalers daily for airway disease, or require external respiratory support while awake and upright. In addition, the following conditions are exclusionary for the use of istradefylline: routine use of CYP3A4 inducers (e.g., carbamazepine, rifampin, phenytoin, St. John's Wort), history of moderate renal impairment or severe hepatic impairment, and history of hallucinations or psychosis.

Rationale for including age-matched controls

AIH has shown to improve cognitive and exercise performance by improving hemodynamics and work capacity in healthy older adults.^{48,49} While effects of AIH and istradefylline have been tested separately in healthy older adults, there is a lack of evidence regarding the additive effects of pairing istradefylline with AIH. Since older adults are the individuals who typically have the greatest need

for rehabilitation, we are interested to test whether combined istradefylline and AIH can augment respiratory motor plasticity in healthy older adults.

Healthy older adults have been previously included in clinical trials to evaluate the exposure-response relationship and pharmacokinetic disposition of istradefylline in PD patients.^{45,50} By including healthy older adults in our study, we can determine whether any observed differences with paired AIH and istradefylline among individuals with ALS are specific to ALS or are potentially more generalizable to the older adult population.

Study Procedures

The SPIRIT flow diagram with all study procedures can be found in **Table 2**.

Screening

Interested individuals who meet the basic study criteria, agree to participate, and sign the IRB-approved informed consent form will undergo further screening procedures prior to the first interventional visit. Prospective subjects will be informed of the eligibility criteria, study procedures, participation risks, privacy and safety precautions in place, alternatives to study participation, and the right to withdraw from the study at any time. The study coordinator will schedule visits and communicate with participants by phone and email. The study coordinator, or a designated investigator, will complete consenting and screening tests (See Figure 2).

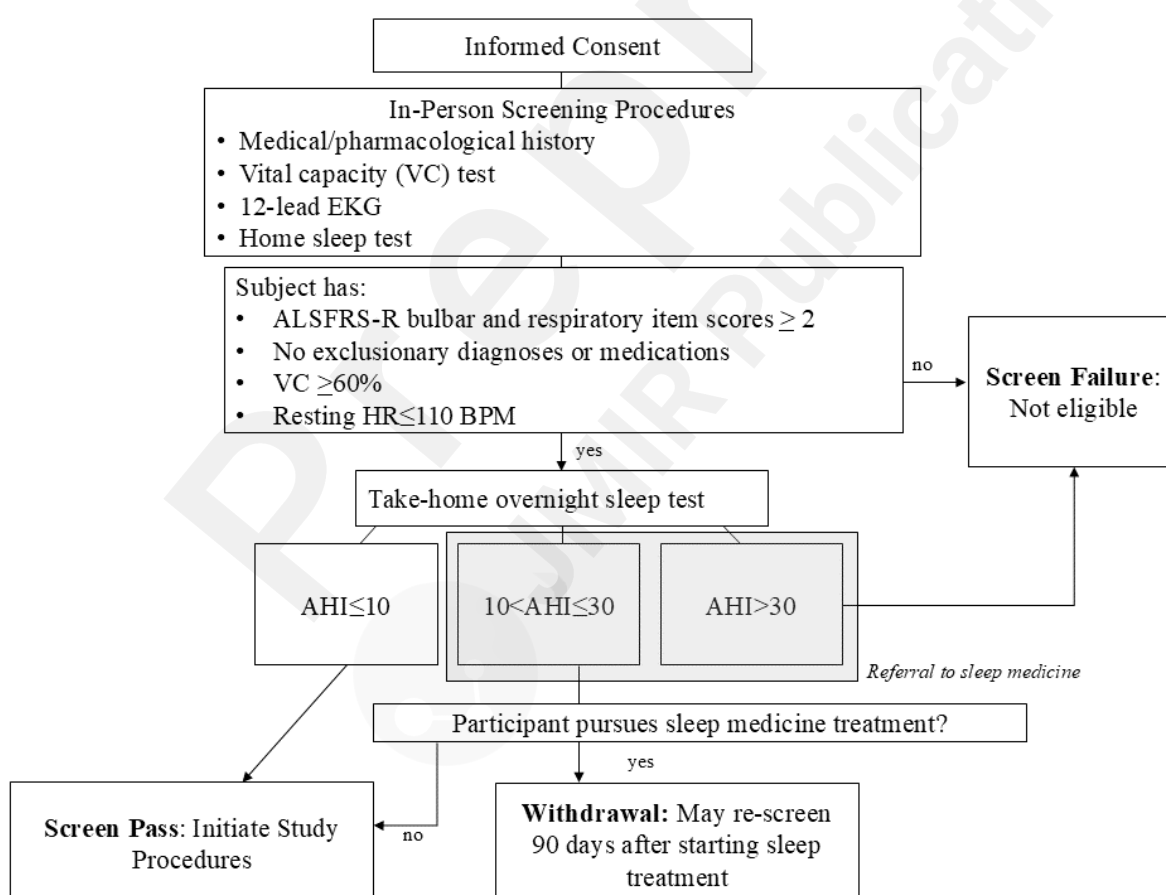


Figure 2. Screening procedures. ALSFRS-R: ALS Functional Rating Scale revised; AHI: apnea-hypopnea index.

Screening tests shall include:

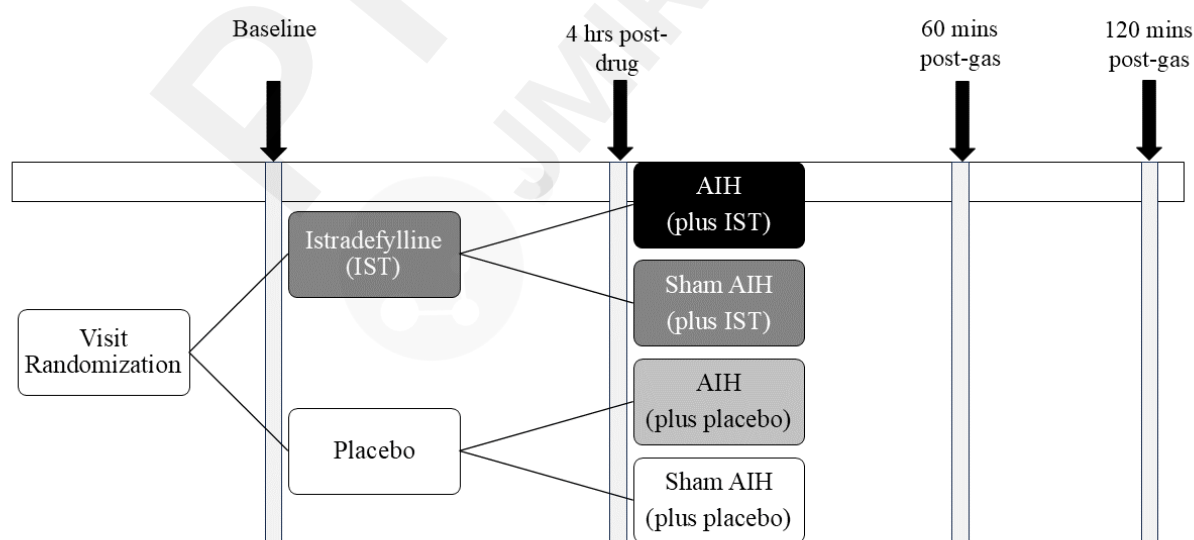
- Patient demographic information and medical history - sociodemographic (age, sex),

anthropometric (height and weight to calculate the body mass index), general clinical data (medical history, comorbidities, and medications).

- Vital capacity (VC) – the maximal volume of gas that can be exhaled, following full inspiration. Upright VC will be administered per American Thoracic Society and European Respiratory Society guidelines.^{51,52} Individuals with less than 60% of predicted VC will be ineligible for further participation in this study.
- 12-lead EKG – cardiac rate and rhythm will be evaluated. The presence of resting tachycardia (HR>110 bpm), atrial fibrillation, or other dysrhythmias will exclude subjects from participation.
- STOP-BANG questionnaire – questionnaire to estimate the risk of obstructive sleep apnea (OSA).⁵³ A meta-analysis found a STOP-BANG score of ≥ 3 had excellent sensitivity and high discriminatory power to exclude moderate-to-severe OSA.⁵⁴
- Overnight sleep monitor – subjects will wear an overnight sensor that non-invasively monitors arterial oximetry and breathing parameters (Nox T3S, Nox Medical), to estimate the severity of sleep-disordered breathing.⁵⁵ If clinically significant OSA [apnea-hypopnea index (AHI) >10 events/hour] is detected, subjects will be offered a referral to a clinical sleep specialist for further evaluation. If they choose to pursue clinical treatment for sleep apnea, they will be withdrawn and eligible to re-screen for participation after receiving 3 months of treatment. If they choose not to seek treatment, they will be included in the study. Subjects with severe OSA AHI >30 events/hour will be ineligible.

Study visits

After providing written consent and completing screening, participants will complete four individual study visits separated by 2 weeks (+/- 5 days). Visits will include (1) istradefylline + AIH, (2) placebo + AIH, (3) istradefylline + sham AIH, and (4) placebo + sham AIH (**Figure 3**), in randomized order. Participants will undergo single sessions of each treatment condition. They will be instructed to avoid exercise, caffeine and nicotine products prior to testing for a minimum of six hours and to avoid eating within one hour of sessions.



Fig

Figure 3. Study design. The gray bars with arrows represent the timing of data collection procedures.

Session order was determined by a random number table generated in advance of the enrollment of the first subject, and maintained by the investigational pharmacist. Participants and investigators will remain blinded to istradefylline vs placebo for study administration and data analysis. Participants as well as the investigator collecting test outcomes will be blinded to AIH vs sham AIH. Prior to each study visit, the investigational pharmacist will inform the study coordinator which gas to administer during intervention [i.e. hypoxic air for AIH vs normoxic air for sham AIH]. Study team members conducting gas treatment cannot be fully blinded to AIH vs sham AIH due to monitoring of SpO_2 , which is necessary for safety; thus, the investigator collecting testing outcomes will not be present during gas administration.

Subjects will sit upright in a chair with the head and trunk supported while breathing through a sealed face mask. Resting ventilation, respiratory muscle activation, maximal respiratory pressures, maximal pinch force, and blood draws will be completed at the start of each study visit, then subjects will receive study medication to be taken orally. After a 4-hour break, all outcome-measure testing will be repeated, followed by gas delivery. Post-intervention testing will occur post-intervention tests at 60- and 120-minutes following gas delivery. **Table 2** delineates outcome measures taken at each timepoint.

Study visits are expected to last ~7-8 hours, including equipment setup, repeated outcome measurements, oral administration of study medication, waiting periods, gas delivery, patient positioning and pressure relief, rest and snacks/meal breaks.

Independent Variable – Adenosine 2A antagonist versus placebo.

During each study visit, subjects will take a single, 20 mg NOURIANZ™ pill or an identical-appearing placebo. The study agent will be taken after the completion of baseline breathing tests. Study gas delivery will begin 4 hours later. During the 4-hour waiting period, Participants will have a private room with a bed and recliner; access to water, snacks, and a light meal; and bathroom facilities. Participants will also be free to move about the research unit.

Independent Variable - Acute Intermittent Hypoxia (AIH) vs Sham AIH

Following the 4-hour break and second round of testing, subjects will undergo a 45-minute session of either AIH (up to 1 minute of 9-10% O_2 with 2 minutes of 21% O_2 , 15 cycles, target SpO_2 nadir: 82-85%) or sham AIH (**Figure 4**). During sham AIH administration, the 1-minute periods of study gas will use a gas bag containing 21% O_2 , with 2-minute room-air periods, so that subjects are breathing normoxic air for the entire session. Outcome measures will be repeated 60- and 120-minutes after gas delivery.

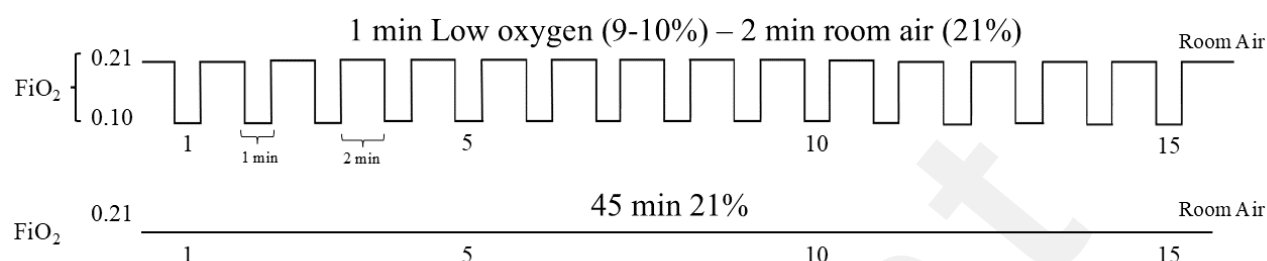


Figure 4. Schematic of AIH and Sham AIH interventions. AIH sessions will consist of 1-minute intervals of hypoxia (10% O₂) separated by up to 2-minute intervals of normoxic room air (21% O₂). Sham AIH will use identical equipment, but the gas bag will contain 21% O₂.

A schematic of the respiratory circuit is shown in **Figure 5**. Subjects will sit upright in a chair with the head and trunk supported while breathing through a sealed face mask (Hans Rudolph 7450). The face mask will be connected to a two-way non-rebreathing valve (HR 2700) that allows the exhaled breath to escape to room air. A pressure transducer (MKIS, GM Instruments) and gas analyzer (Gemini CO₂ & O₂ monitor, CWE, Inc) will be connected directly to the face mask connector. A pneumotachograph (Hans Rudolph 4813) will be connected to the inspiratory port of the non-rebreathing valve. Gas bags will be connected to a 3-way stopcock valve (Hans Rudolph 2100), and the direction of the valve will be manually alternated between the study gas (1-minute intervals) and normoxic room air (2-minute intervals). Respiratory data will be recorded during gas delivery, using a data acquisition system (PowerLab 16/35 and PowerLab C, ADInstruments).

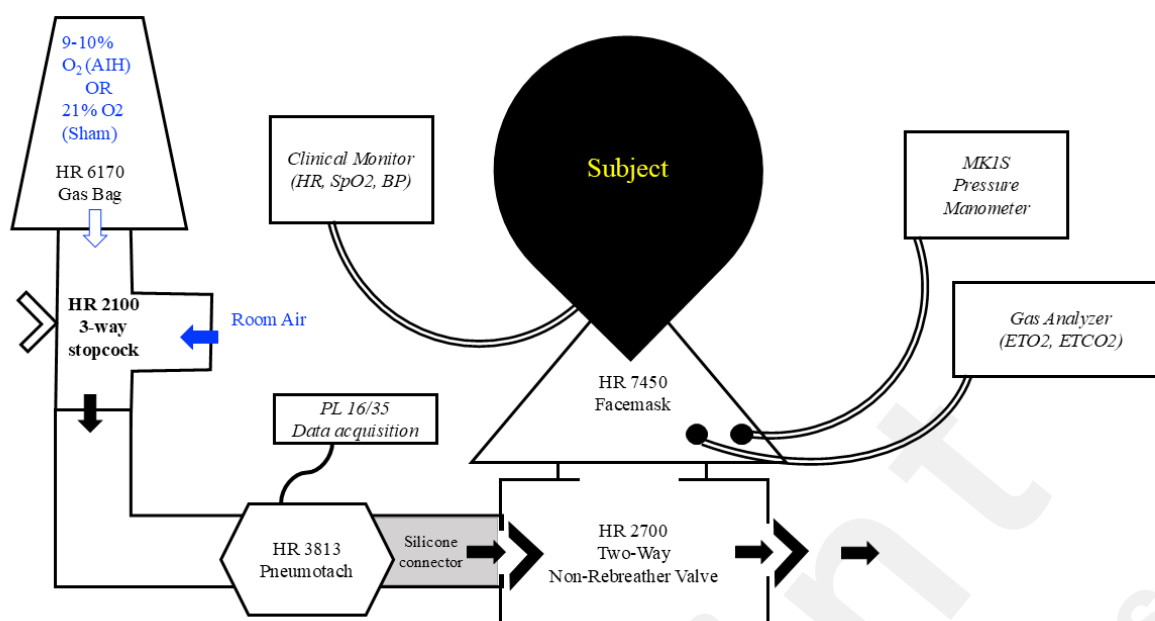


Figure 5. Schematic of the respiratory circuit for gas delivery.

Respiratory rate, targeted O_2 value, end-tidal CO_2 , heart rate/rhythm, and SpO_2 will be continuously monitored during the testing sessions. Multi-channel EMG (Trigno Centro, Delsys) will also be recorded from respiratory muscles (bilateral submental, scalene, sternocleidomastoid, 2nd parasternal and diaphragm) as well as abductor pollicis muscle during testing and captured in real time in LabChart (AD Instruments). At the end of each 1-minute hypoxic (or sham-hypoxic) presentation, subjects will report their rating of dyspnea (10-point modified Borg scale). For every fifth presentation, non-invasive BP will be taken. We will establish an individualized communication plan (e.g. hand signal, head tilt, call switch, etc) prior to study gas administration, to ensure that all participants can alert the investigator of any discomfort, shortness of breath, or requests to remove the face mask.

Safety Considerations

The target SpO_2 during AIH is 82-85% O_2 . If the SpO_2 reaches the lower limit of the target range, gas delivery will be switched to the normoxic recovery time. Subsequent hypoxia durations will also be reduced by 5 seconds. AIH will be stopped in the unlikely event the heart rate increases or decreases $>30\%$ from the resting value, and/or systolic blood pressure changes $>30\%$ from the resting value. The expected acute physiological response to hypoxia is a stable or decreased CO_2 due to increased ventilation. However, if $ETCO_2$ increases >3 mm Hg above baseline, we will shorten AIH bouts using the same process described above for SpO_2 . Additional stopping criteria include a respiratory rate >30 that fails to normalize within 1 minute of normoxia, $SpO_2 <82\%$ that fails to normalize within 1 minute of normoxia, or $SpO_2 <82\%$ occurring within 30 seconds of hypoxia initiation.

In the unlikely event that a participant is injured as a direct result of participation in this study, professional services rendered by any University of Florida Health Science Center healthcare provider will be provided without charge. These healthcare providers include physicians, physician assistants, nurse practitioners, dentists or psychologists. Any other expenses, including UF Health hospital expenses, will be billed to the participant or his or her insurance provider.

Safety oversight will occur internally through the local Institutional Review Board (IRB) and a data

safety monitoring board (DSMB). The DSMB was convened before enrollment of the first patient, after completion of the first three subjects, and at least annually thereafter. The DSMB includes three members - one expert in intermittent hypoxia, one clinical medicine expert who interprets and/or prescribes respiratory testing/treatment, and a rehabilitation specialist.

Testing and enrollment of participants will be paused, and DSMB convened for any of the following events: (1) any clinically significant respiratory event, (2) a clinically significant cardiovascular event, (3) a serious adverse event as defined by the UF IRB. Should any of the events occur, research staff shall inform the IRB and the DSMB. Any subsequent study activities would only be resumed when cleared or the protocol modified by the IRB and DSMB. As of January 2025, these serious events have not occurred, and the DSMB has only been convened for planned early and annual meetings.

Aim 1 Dependent Variables

- Adverse events – adverse events will be tracked and recorded per usual IRB requirements and reported as the Aim 1 primary safety outcome.
- Cardiopulmonary responses – Vital signs and cardiac rhythm will be monitored throughout administration of each test condition. Additionally, 1-minute averages of heart rate, blood pressure, end-tidal CO₂, and SpO₂ will be compared at baseline, 60- and 120-minutes following study gas delivery.
- Laboratory tests – Baseline labs will be drawn for each session and include routine clinical labs (complete blood count, chemistry) and uric acid. Blood samples will be drawn for plasma istradefylline levels at the baseline of each study visit, and 4- and 6- hours after ingestion of the study medication. Participants will be offered an IV to be placed at the start of the visit to avoid multiple venopunctures.
- Subject-reported symptoms - participants will complete 10-point visual analog scales to rate the intensity of symptoms (of dyspnea, dizziness, weakness, fatigue, nausea and involuntary movements) at the following time points: baseline, 4 hours after taking the drug/placebo, 60- and 120-minutes after gas delivery.
- Study completion rate – the primary feasibility outcome will be the proportion of subjects who complete all study visits. Additional feasibility metrics will include screening failures, proportions of interested subjects who are ineligible, and timeliness of study milestones.

Aim 2 Dependent Variables

- Quiet breathing assessment – the pneumotachograph and pressure transducer connected to the face mask will record breath-by-breath flow, tidal volume, airway opening pressure, and breathing rate. After achieving a stable tidal volume, minute ventilation will be recorded for five minutes. The primary outcome for Aim 2 is a change in tidal volume from baseline to post-intervention 120-minute follow-up. Minute ventilation will be measured at baseline, just prior to study gas delivery, and 60- and 120-minutes post-gas- intervention.
- Respiratory muscle assessment. Surface EMGs of the respiratory muscles and the abductor pollicis muscle (non-respiratory control muscle) will be recorded as described above. To identify the muscles active during quiet breathing assessment, the root mean square of each muscle signal will be averaged. Multi-muscle activation will be calculated using a validated, vector-based measure that indexes the magnitude and pattern of composite EMG activity.⁵⁶ The composite respiratory muscle activity from each ALS subject will be contrasted to the averaged composite magnitude and pattern of the pooled control sample.⁵⁷
- Maximal inspiratory pressures – Inspiratory force generation will be assessed as the maximal

voluntary pressure generated by the inspiratory muscles (MIP) against a closed valve, measured at the mouth.⁵⁸ Sniff nasal inspiratory pressure (SNIP) measures inspiratory force generation at the nose. Each test will be repeated until 3 measurements are obtained within 10% variability; a minimum 20-second rest will be provided between measurements. A recent systematic review reported a high level of evidence for concurrent validity for MIP; however, inter-rater reliability was low.⁵⁹ To minimize inter-rater reliability, we standardized instructions given to participants across sessions and used the same rater for each participant. MIP will be measured at baseline, immediately prior to study gas delivery (4 hours after drug administration), and 60 minutes post-gas. SNIP will be measured at baseline, immediately prior to study gas delivery, and 120 minutes post-gas.

- **Maximal airflow testing** –Peak airflows will be collected with a pneumotachograph attached to the face mask, during maximal inspiration [maximum inspiratory flow (MIF)] and voluntary coughing [peak cough flow (PCF)]. Tests will be repeated for 3-5 trials; at least 30 seconds rest will be provided between measurements, but subjects may rest as long as needed. MIF will be tested at baseline, just prior to study gas delivery, and 60 minutes post-intervention. PCF will be tested at baseline, just prior to study gas delivery, and 120 minutes post-intervention.
- **Maximal voluntary pinch force** – maximal static voluntary pinch force will be included as a non-respiratory control. Pinch has been recognized as a sensitive marker of ALS disease severity due to preferential thenar wasting.⁶⁰ A recent study found fair-to-good test-retest reliability for this measure.⁶¹ Pinch force will be evaluated in a seated position with the arm at the side in 90-degree elbow flexion, neutral forearm and wrist. The test will be repeated until 3 measurements are obtained with <10% variability, with a minimum 30-second rest between measurements. Pinch force will be measured at baseline, just prior to study gas delivery, and 120-minutes post-gas intervention.

Secondary outcome measures will be interpreted as exploratory in nature.

Sample size, data management and statistical analysis

The sample size calculation was based on pilot data from eight adults with ALS showing ventilation changes in AIH vs a sham condition. The preliminary data showed $10.3 \pm 23\%$ larger tidal volume and $10.0 \pm 29\%$ greater minute ventilation 60 minutes post-AIH, as compared to sham. Published work in rodent spinal cord injury models indicates that istradefylline paired with AIH greatly enhances improvement in diaphragm activity versus AIH or drug alone (effect size of combined approach: $d=1.92$).

Additional preliminary data using caffeine as a non-specific adenosine receptor antagonist conservatively suggested a capacity for a 10% additional gain in ventilation. With $n=16$ crossover participants, a repeated measures ANOVA comparing 4 conditions across 4 time points, with a pooled standard deviation of 15.3, will be sensitive to detect a difference of power >0.8 ($f=0.31$, $F(3,36) = 2.86$; $p = 0.5$, $\alpha = 0.05$). A target sample of 24 subjects will account for potential attrition. The design includes age-matched unaffected adults as a comparator group.

Descriptive statistics, including frequencies, means, standard deviations, and medians, will be used when appropriate. Continuous variables will be summarized with descriptive statistics and categorical variables will be summarized with counts and percentages. Change scores and percent change scores will be calculated from baseline for follow-ups at post-drug/placebo administration, 60- and 120-minutes after gas intervention. We will attempt to minimize missing data by planning the study procedures and proper biosignal acquisition, thoroughly documenting the study

administration and data collection, and collecting the essential information to complete the study aims. Should missing data points still occur, statistically valid analyses that appropriately address the missing values are maximum likelihood, expectation maximization, and multiple imputation.⁶² Any statistical code or algorithms developed to analyze acquired data will be available and shared upon request.

Health information will be collected, used, and shared with others (i.e. clinical and research staff, UF Research Participant Payment team) to determine if interested individuals are eligible to participate, and then as part of the participation in the study. The following information may be collected, used, and shared with others: medical history, including past conditions and surgeries, medications, and information related to diagnosis of ALS, social security number (for reimbursement purposes), demographic information, contact information, and the results of the research tests done for this study. The research team may collect this information from other healthcare providers, such as laboratories, which are a part of this research, as well as healthcare providers that are not part of this research (other doctors, hospitals, or clinics). Other professionals at the University of Florida or UF Health Hospital who provide study-related care, and the University of Florida Institutional Review Board (IRB), may also collect health information. Participants will be informed of the collection and protection of health information collected as part of the informed consent process. Health information and study data will be stored in locked filing cabinets or on computer servers with secure passwords, or encrypted electronic storage devices, as required by UF policy.

Results

Recruitment for this study began in June 2022. As of January 2025, 12 people with ALS and eight age-matched controls have completed informed consent. Of these, one person with ALS was withdrawn after the screening visit, and one was withdrawn after two study visits. Three of the consented potential control subjects were deemed ineligible following the screening visit. Thus, 10 subjects with ALS and five age-matched controls have completed all study procedures. Recruiting is ongoing, and the final subject will complete the study by December 2025. The publication of results is expected by the end of 2026.

Discussion

This clinical trial will evaluate the effects of istradefylline paired with AIH on breathing function in people with ALS and unaffected age-matched controls. Individuals living with ALS face progressive neuromuscular disease that leads to paralysis from motor neuron death. While recently approved ALS drugs offer more options to treat the disease, combinatorial strategies will likely be needed to preserve motor function and independent breathing for as long as possible. To our knowledge, this is the first clinical trial to assess the combined effects of A₂A receptor antagonism and AIH in ALS.

AIH is easy to apply and well-tolerated by most individuals, including people with ALS in a preliminary study conducted by our group.²² In several studies of humans with chronic spinal cord injury, similar AIH protocols did not lead to adverse events.^{18,63–68} We expect AIH will, likewise, be safe and well tolerated by people with ALS and age-matched controls in the current study.

NOURIANZTM (istradefylline) has been studied extensively both in subjects living with PD and in healthy adult subjects. Population pharmacokinetic studies were summarized previously and included both single and repeated, daily administration (up to 16 weeks) of istradefylline doses ranging between 10 and 200 mg.⁵⁰ Safety was established in subjects living with PD and unaffected elderly subjects, with few and minor side effects. In controlled trials, the most common adverse events with repeated daily treatment of istradefylline included: dyskinesias, dizziness, hallucinations, and insomnia. These adverse events occurred more frequently after treatment with 40 mg of istradefylline.⁵⁰ As the current study will use a treatment dose of 20 mg of istradefylline, we anticipate this therapy will be safe and well tolerated by participants with ALS as well as age-

matched controls.

Regarding efficacy in people with SCI, AIH also improved limb function, increased walking speed, and improved ankle strength.^{12,18,63,65,66,68,69} AIH combined with task-specific training enhanced hand and walking function.^{63,66} However, other studies reported variable responses, indicating that AIH efficacy can be dependent on other factors (i.e. time of day, gas composition, sex, age, and genetic mutations associated with variable plasticity/rehabilitation responses).^{33,70–72} These variables require further study to optimize rehabilitation strategies for treatment efficacy.

Serotonergic cellular signaling is crucial for AIH-induced pLTF. In SCI models, an increased pLTF was observed after the administration of paired AIH and the A_{2A}AR inhibitor istradefylline; combined with the fact that elevated A_{2A}AR levels have been found in humans with ALS and ALS animal models, this makes A_{2A}AR inhibition of particular clinical interest in the treatment of respiratory failure in neurodegenerative diseases.^{38,73}

We hypothesize that the combination of istradefylline and AIH, in ALS patients, will elicit greater gains in respiratory and non-respiratory motor function, than either intervention alone and that will contribute to improvements in breathing and motor function. In a rodent model of phrenic motor neuron death, significant A_{2A}AR upregulation preceded motor neuron loss, but the co-administration of istradefylline improved motor neuron survival and maintained diaphragm function.⁴³ Based on these previous studies, other groups studying the impact of combined AIH and caffeine, a non-selective adenosine antagonist, found the combined approach further improved walking speed and distance in people with chronic spinal injuries.³⁹ However, since caffeine is a non-selective adenosine antagonist that also inhibits phosphodiesterase action, off-target effects such as nausea and elevated heart rate could limit its use in the doses needed to augment AIH plasticity. This study utilizes istradefylline for its greater affinity of istradefylline for the A_{2A}AR and its tolerance in clinical studies of older adults and those with Parkinson's disease. The information gained in this investigation will help us optimize intervention approaches for future studies of repeated, daily AIH as a rehabilitation tool. Thus, the current study represents an important step in the iterative process of moving AIH protocols toward clinical practice.

We acknowledge multiple limitations of this study. ALS is a heterogeneous disease, and participants may have variable disease severity, chronicity of illness, limb and bulbar involvement, and requirement of ventilatory support. This limitation will be minimized by clarifying and emphasizing the inclusion/exclusion criteria. Additional considerations include inter-investigator variability in biosignal acquisition in sEMG and respiratory signals. To address this concern, we will train study staff to follow carefully designed and thorough standard operating procedures throughout data collection. Lastly, this study is not designed to test multiple repeated exposures of the treatment conditions and will not assess any long-term rehabilitative effects of AIH, istradefylline, or combined treatment. Rather, this study intends to inform design of optimized therapeutic approaches and guide future studies into rehabilitation of respiratory function in people with ALS.

Acknowledgments

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WLO was affiliated with the listed University of Florida departments at the time of protocol development and is currently affiliated with Appalachian State University. PSC was affiliated with the listed University of Florida departments at the time of protocol development and is currently affiliated with the University of Santa Catarina State,

Declarations

Ethics approval and consent to participate

The study protocol was approved by the University of Florida Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects (IRB202101568). This protocol has also been reviewed and approved by the U.S. Food and Drug Administration for the off-label use of isradefylline. All methods will be carried out in accordance with relevant guidelines and regulations set forth by the approved IRB protocol. Informed consent will be obtained from all subjects.

Availability of data and materials

Summary data will be submitted for publication in a peer-reviewed journal. Additional data and materials will be made available upon reasonable request to the corresponding author.

Authors' Contributions

BS, GM, NN, and JW developed the study protocol and secured funding. BS and PSC obtained regulatory approvals. BS, PC, MSH, WO, and WSL will complete study-related interventions. JPW will conduct medical oversight. BS, WO, GM, MSH, WSL and NN will contribute to data analysis and interpretation. All authors contributed original ideas, read and approved the manuscript, and did not use professional writers or generative AI in the preparation of this manuscript. Additional manuscripts generated by the authors using the data collected by this protocol will not employ professional writers.

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Conflicts of interest

The authors declare they have no conflicts of interest.

List of abbreviations

A₂A: adenosine 2A receptor

AIH: acute intermittent hypoxia

ALS: amyotrophic lateral sclerosis

BP: blood pressure

DSMB: data safety monitoring board

EMG: electromyography

HR: heart rate

IRB: Institutional Review Board

MIF: maximum inspiratory flow

MIP: maximal inspiratory pressure

OSA: obstructive sleep apnea

pAKT: phosphorylated protein kinase B

PD: Parkinson disease

PKC: protein kinase C

pLTF: phrenic long-term facilitation

SCI: spinal cord injury

SNIP: sniff nasal inspiratory pressure

SOD1: copper zinc superoxide dismutase 1

TrKB: tropomyosin-related kinase B

VC: vital capacity

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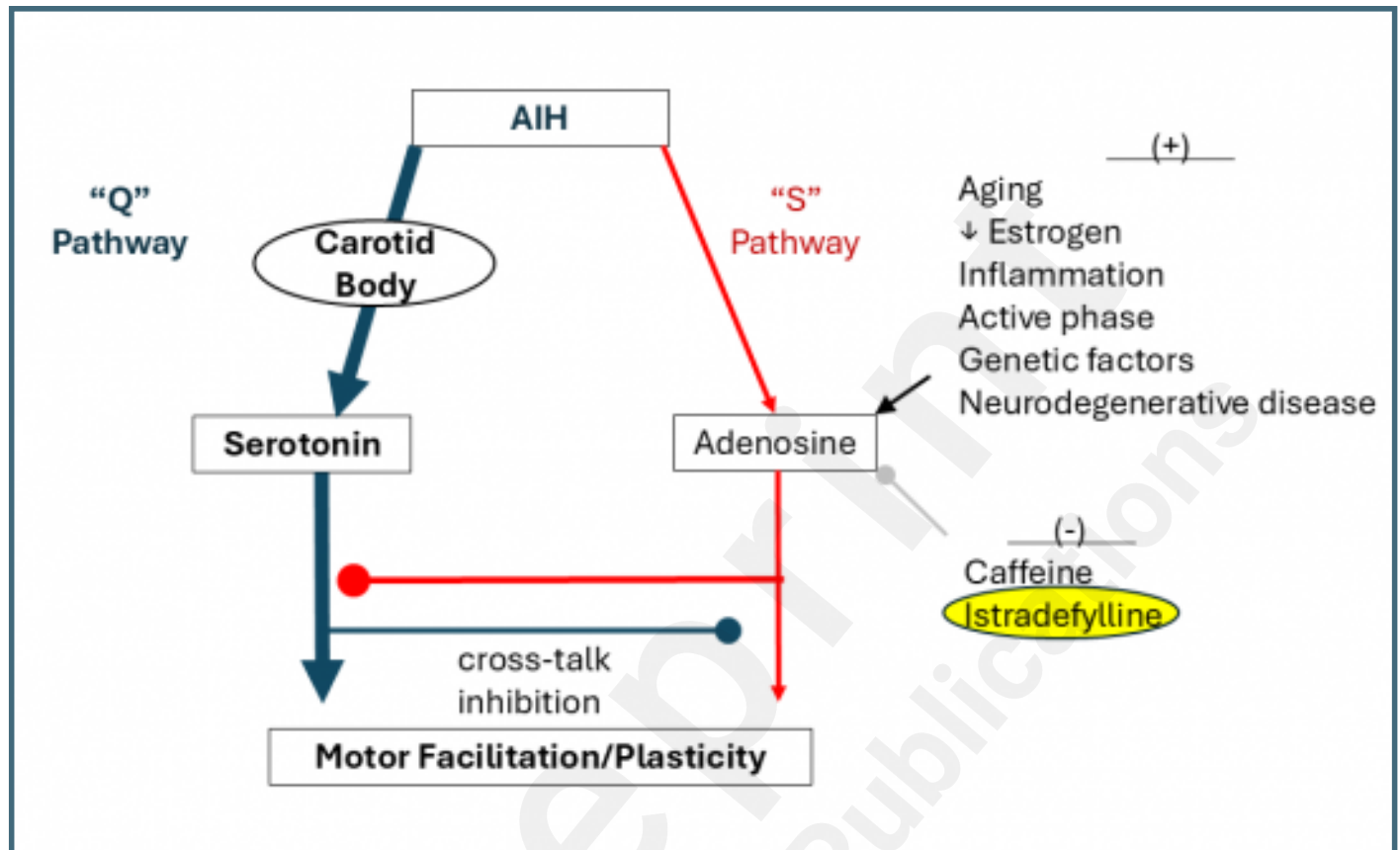
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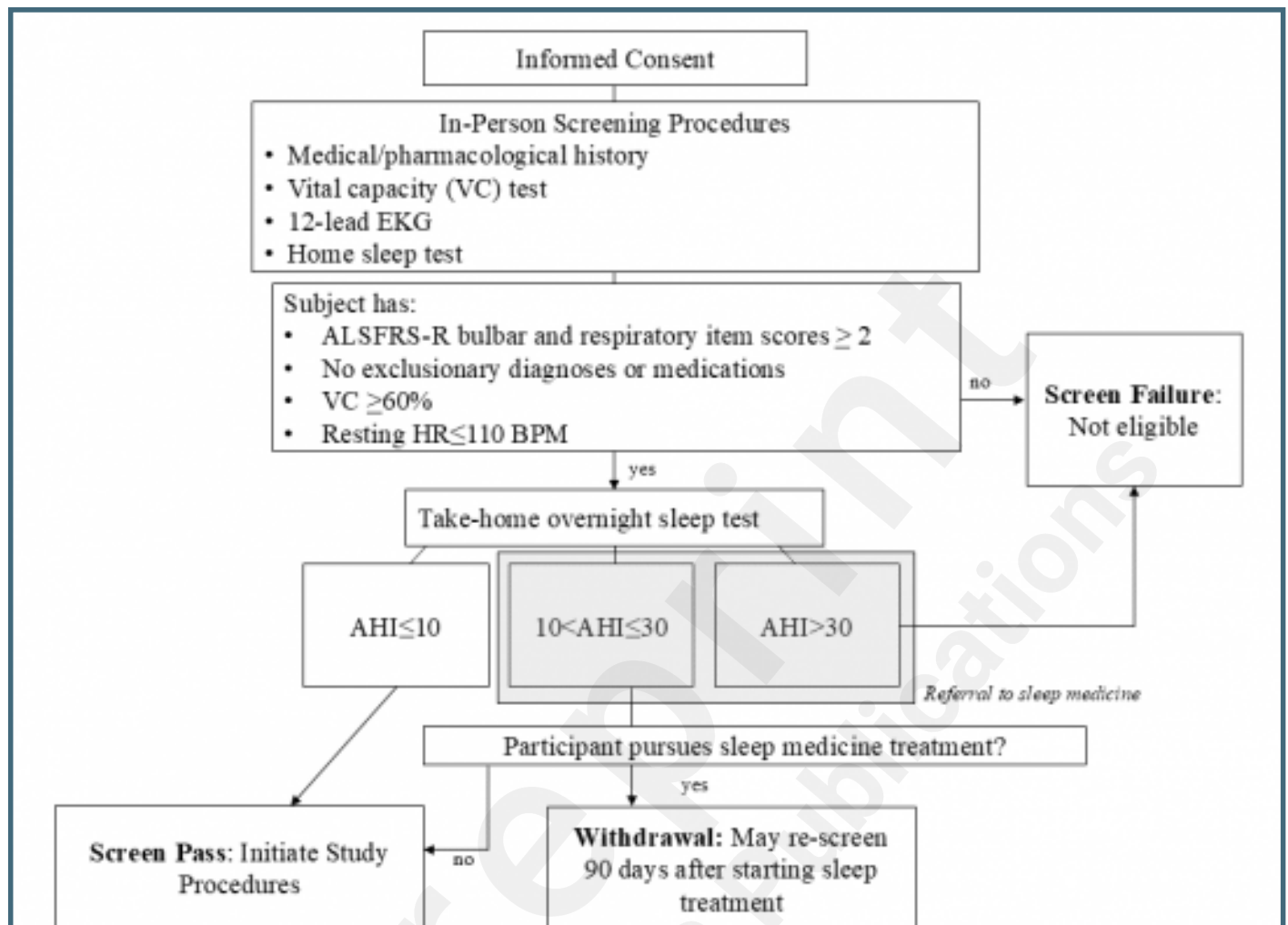
Supplementary Files

Figures

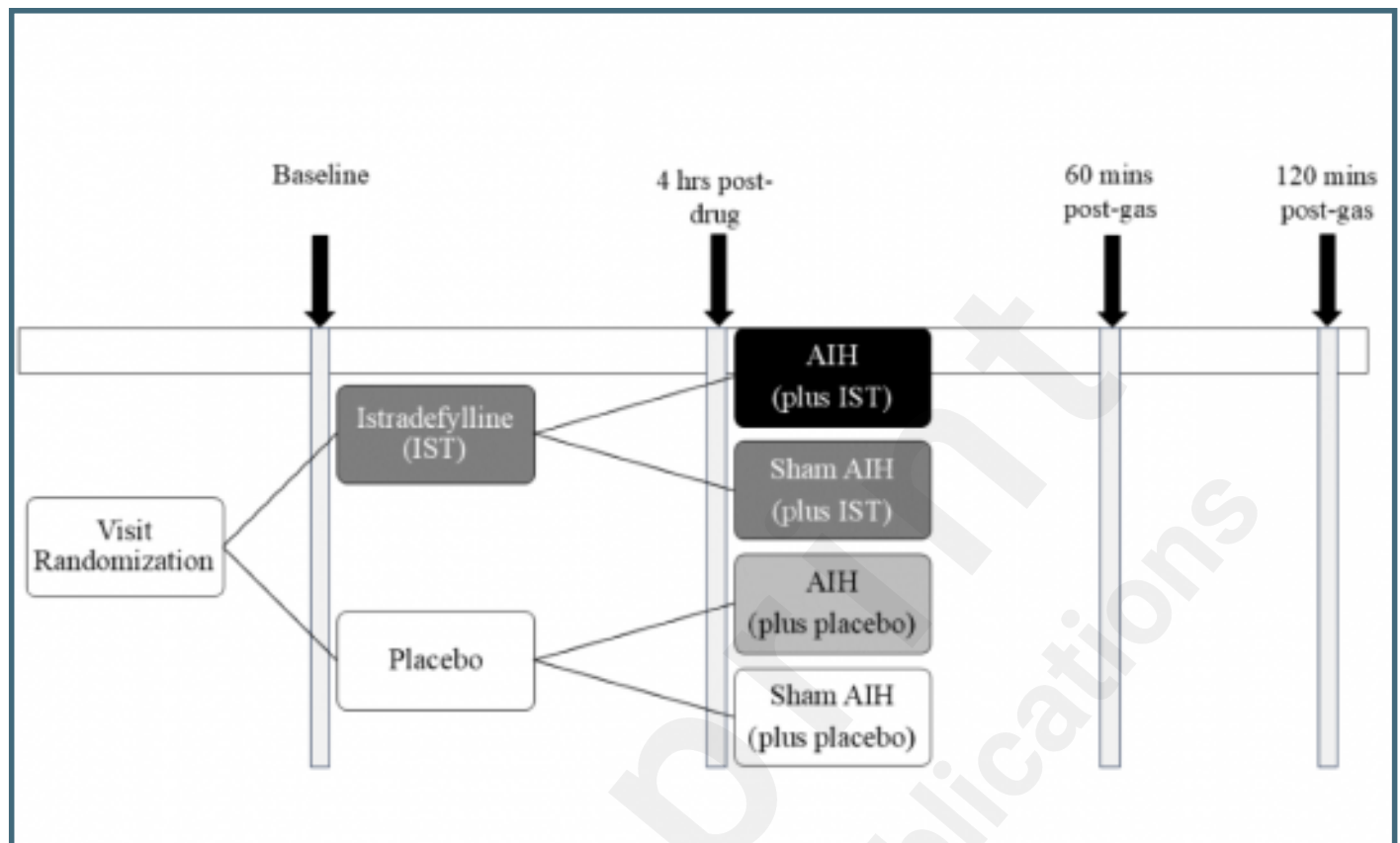
Competing serotonin- (Q) and adenosine-driven (S) pathways to phrenic motor facilitation. Listed on the right are factors that impact serotonin-dominant plasticity due to increased adenosinergic “S” pathway inhibition. By combining istradefylline with mild AIH, this protocol aims to minimize crosstalk inhibition by reducing S to Q pathway inhibition, allowing full expression of the “Q” pathway.



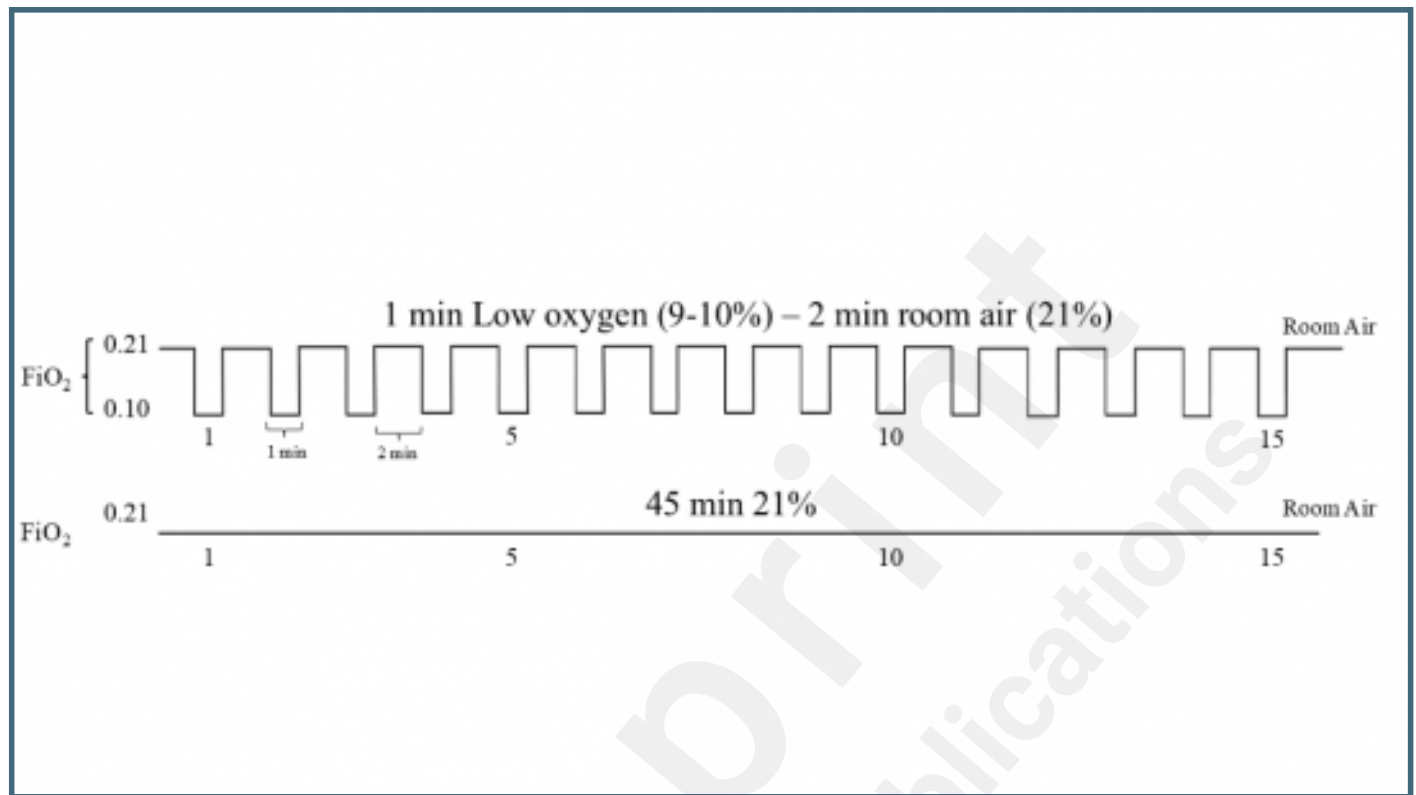
Screening procedures. ALSFRS-R: ALS Functional Rating Scale revised; AHI: apnea-hypopnea index.



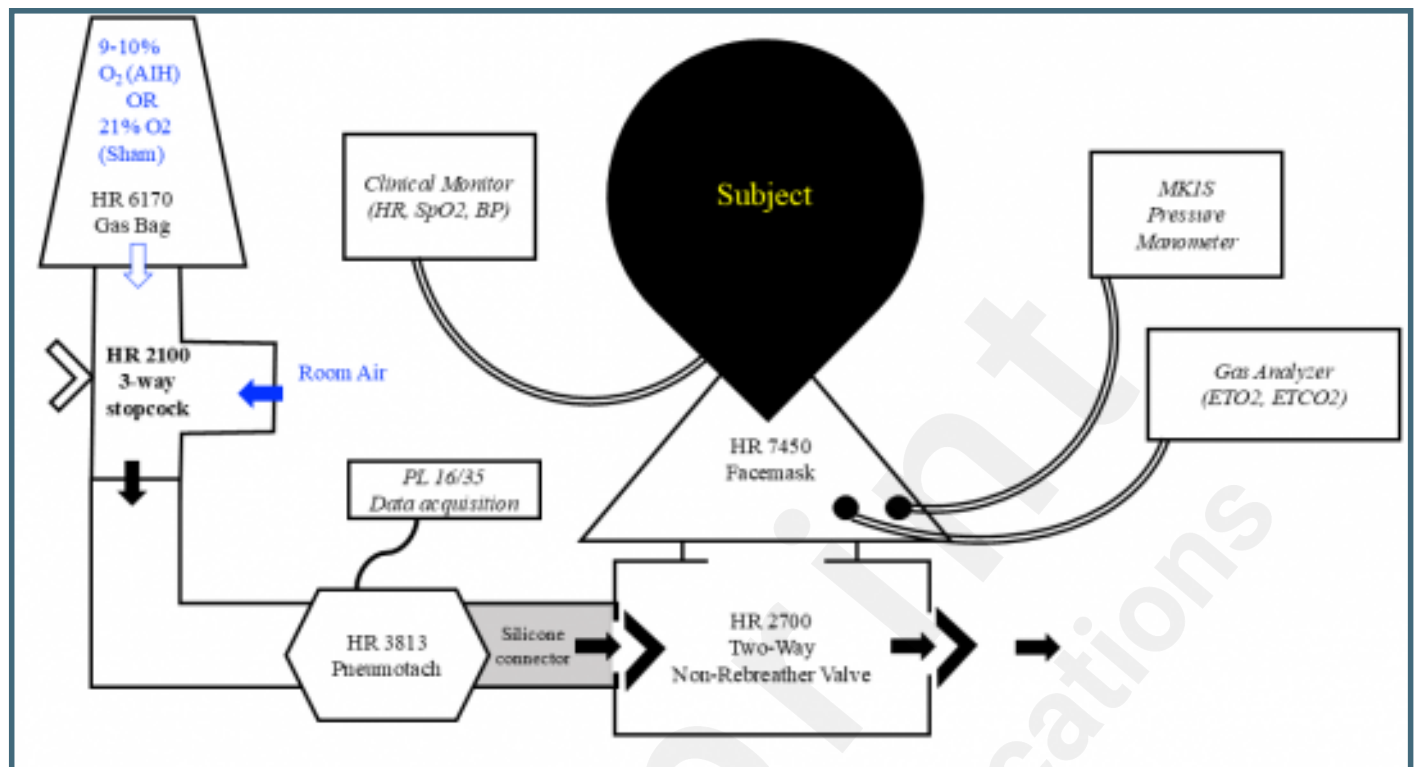
Study design. The gray bars with arrows represent the timing of data collection procedures.



Schematic of AIH and Sham AIH interventions. AIH sessions will consist of 1-minute intervals of hypoxia (10% O₂) separated by up to 2-minute intervals of normoxic room air (21% O₂). Sham AIH will use identical equipment, but the gas bag will contain 21% O₂.



Schematic of the respiratory circuit for gas delivery.



Multimedia Appendixes

Informed consent form for control participants.

URL: <http://asset.jmir.pub/assets/a03e76f1ae4451e239dca475f0513139.pdf>

Informed consent form for participants with ALS.

URL: <http://asset.jmir.pub/assets/a1936aaafa2d2f00cc0937bb689d30c7.pdf>



Existing Peer-Review Reports from Funding Agencies (for protocols/proposals only)s

Peer-review reports from ALSA.

URL: <http://asset.jmir.pub/assets/80531fbd3e16b4ae0f1a788de05f9993.pdf>