Mechanical Insufflation in the Philadelphia Amyotrophic Lateral Sclerosis Cohort (MI-PALS) Study Protocol

CLINICAL RESEARCH PROTOCOL

INVESTIGATIONAL PRODUCT(S):

Mechanical insufflation (MI) is a chest physiotherapy exercise that will be performed using a type of mechanical insufflator-exsufflator (MI-E) (also known as a cough assist device) known as the BiWaze Cough device (**Figure 1**). This device is manufactured by ABM Respiratory Care and is designed for use on patients unable to cough or clear secretions effectively due to reduced peak cough expiratory flow, resulting from disease such as neuromuscular deficits. The device connects to a tube that can interface with a patient using either a facemask, mouthpiece, or an adapter to a patient's endotracheal tube or tracheostomy tube. It is FDA approved for use in the home on adult patients and pediatric patients 3 years old and up.

Mechanical insufflation is a chest physiotherapy exercise that passively inflates the chest with positive pressure that is delivered in coordination with the patient's own inspiratory timing until maximal inflation capacity (MIC), determined by the patient or maximal chest rise on visual inspection. At MIC, the patient passively exhales, which completes one "cycle". Prior literature has used a "dose" of 5 sets of 5 cycles once or twice daily.[1–4] The maneuver

		is usually performed with assistance of a caregiver to hold the mask or mouthpiece in place.
STUDY NUMBER(S):	**IRB Number**	854981
	Other Protocol Identifiers	N/A
PROTOCOL(S) TITLE:		Mechanical Insufflation in the Philadelphia ALS Cohort (MI-PALS) Study
IND/IDE/CTA NUMBER:		N/A
REGULATORY SPONSOR:		Jason Ackrivo, MD, MSCE
FUNDING SPONSOR(S):		National Institutes of Health National Heart, Lung, and Blood Institute
MEDICAL DIRECTOR		Jason Ackrivo, MD, MSCE
ORIGINAL PROTOCOL DATE:		To be determined
VERSION NUMBER:		v1.1
VERSION DATE:		09.10.2025

PRINCIPAL INVESTIGATOR SIGNATURE

STUDY SPONSOR:	Jason Ackrivo, MD, MSCE Assistant Professor of Medicine Pulmonary, Allergy, and Critical Care Division Perelman School of Medicine at the University of Pennsylvania
STUDY TITLE:	Mechanical Insufflation in the Philadelphia Amyotrophic Lateral Sclerosis Cohort (MI-PALS) Study
STUDY ID	IRB protocol # 854981
PROTOCOL VERSION	v1.0

I have read the referenced protocol. I agree to conduct the study in accordance to this protocol, in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP), and all applicable regulatory requirements and guidelines.

Principal Investigator Name	Jason Ackrivo, MD, MSCE	Signature	
Affiliation:	University of Pennsylvania	Date	

Abbreviations

AE	Adverse Event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised
ALSSQOL-SF	Amyotrophic Lateral Sclerosis specific quality of life questionnaire – short form
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
MP	Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
DRE	Disease-Related Event
EMR	Electronic Medical Record
FDA	Food and Drug Administration
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IRB	Institutional Review Board
MI	Mechanical insufflation
MIC	Maximal insufflation capacity
MI-E	mechanical insufflation-exsufflation
MIP	Maximal Inspiratory Pressure
MOP	Manual of Procedures
NCT	National Clinical Trial
NIH	National Institutes of Health
NIV	Non-invasive ventilation
OHRP	Office for Human Research Protections
PANAS	Positive and Negative Affect Schedule

PCF	Peak Cough Flow
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
DSMC	Data Safety Monitoring Committee
SoA	Schedule of Activities
SOP	Standard Operating Procedure
SpO_2	Pulse oximetry oxygen saturation
TCO_2	Transcutaneous partial pressure of carbon dioxide
UADE	Unanticipated Adverse Device Effect
UP	Unanticipated Problem
US	United States

1 STUDY SUMMARY

1.1 Synopsis

Title:	Mechanical Insufflation in the Philadelphia ALS Cohort (MI-PALS) Study
Short Title:	MI-PALS
Study Description:	We will study how performance of mechanical insufflation (MI) using a mechanical insufflator-exsufflator (MI-E) affects respiratory mechanics in early ALS. This will be a single-center, single-arm feasibility pilot study of MI in 20 patients with ALS at Penn. Based on prior data,[5–12] we hypothesize that 6-months of MI will be associated with a reduced decline in peak cough flow (PCF) compared to matched controls who do not use MI. We will perform MI using a device designed for mechanical insufflation-exsufflation (MI-E) known as the BiWaze Cough system. Briefly, BiWaze Cough is a secretion clearance device that can sit on a bedside table. It is connected to corrugated tubing and mouthpiece (or anesthesia mask). The device will have preset pressure and timing settings. An assistant (or research subject) will operate the device via the touchscreen for 5 sets of 5 insufflations twice daily.
Objectives:	Supportive Care: MI will be evaluated for maximizing comfort, minimizing side effects, or mitigating against a decline in the participant's health or function.
Primary Endpoint:	Change in peak cough flow as measured by peak flow meter over a 6-month period.
Secondary Endpoints:	Forced vital capacity Maximum insufflation capacity

	Maximum insufflation capacity minus vital capacity
	Maximum insufflation capacity assisted peak cough flow
	Maximal inspiratory pressure
	Maximal expiratory pressure
	ALS Functional Rating Scale – Revised (ALSFRS-R) total score
	ALSFRS-R dyspnea score
	ALSFRS-R orthopnea score
	Transcutaneous carbon dioxide level (daytime)
	Pulse oximetry (daytime)
	Global rate of change score for peak cough flow
Tertiary Endpoints:	Time to start of non-invasive ventilation
	Tracheostomy-free survival time
Study Population:	Adults (18+ years of age) diagnosed with amyotrophic lateral sclerosis who are seen at the Penn Comprehensive ALS Clinic at the University of Pennsylvania. We will target individuals with ALS who have an able and willing caregiver for assistance with MI.
Phase:	Post market
Description of Sites/Facilities	Penn Comprehensive ALS Center Opened in 1999, this outpatient specialized Neurology center has been designed to provide comprehensive, multidisciplinary care for people with ALS and their families. This clinic has over 500 total patient visits annually. Lead by Director, Dr. Lauren Elman, MD, the multidisciplinary care in this clinic includes Neurology, Pulmonology, nursing, nurse

practitioner, dietary, physical and occupational therapy, respiratory therapy, speech and language pathology, and genetic counseling. In addition, this clinic also participates in several clinical research studies, including multiple Phase 2 and 3 clinical trials. They have research staff, including site-PIs, a research coordinator, and medical personnel formally trained in clinical trial data collection. The clinic also contains a modern clinical-research grade spirometer, peak flow meter for peak cough flow, and a transcutaneous carbon dioxide monitor.

Enrolling Sites: Penn Comprehensive ALS Center at the University of Pennsylvania.

The site is located at 330 S. 9th Street, 3rd Floor, Philadelphia, PA, 19107

Description of Study Intervention: We will perform mechanical insufflation (MI) using a type of mechanical insufflator-exsufflator (also known as a cough assist device) known as the BiWaze Cough device (**Figure 1**). This device manufactured by ABM Respiratory Care and is designed for use on patients unable to cough or clear secretions effectively due to reduced peak cough expiratory flow, resulting from disease such as neuromuscular deficits. The device connects to a tube that can interface with a patient using either a facemask, mouthpiece, or an adapter to a patient's endotracheal tube or tracheostomy tube. It is FDA approved for use in the home on adult patients and pediatric patients 3 years old and up.

Figure 1. BiWaze Cough device

MI is a chest physiotherapy exercise that passively inflates the chest using positive pressure breaths delivered in coordination with the patient's own inspiratory timing until maximal inflation capacity (MIC), determined by the patient or maximal chest rise on visual inspection. At MIC, the patient passively

exhales, which completes one “cycle”. Prior literature has used a “dose” of 5 sets of 5 cycles once or twice on prior data,[5–12] The maneuver is usually performed with assistance of a caregiver.

Study Duration: 18 months

Participant Duration: 6 months

1.2 Key Roles and Study Governance

Sponsor	**Medical Director**
Jason Ackrivo, MD, MSCE *Assistant Professor of Medicine* *Pulmonary, Allergy, and Critical Care Division* *Assistant Professor of Neurology* *Hospital of the University of Pennsylvania*	*Jason Ackrivo, MD, MSCE* *Assistant Professor of Medicine* *Pulmonary, Allergy, and Critical Care Division* *Assistant Professor of Neurology* *Hospital of the University of Pennsylvania*
Perelman School of Medicine at the University of Pennsylvania	*Perelman School of Medicine at the University of Pennsylvania*
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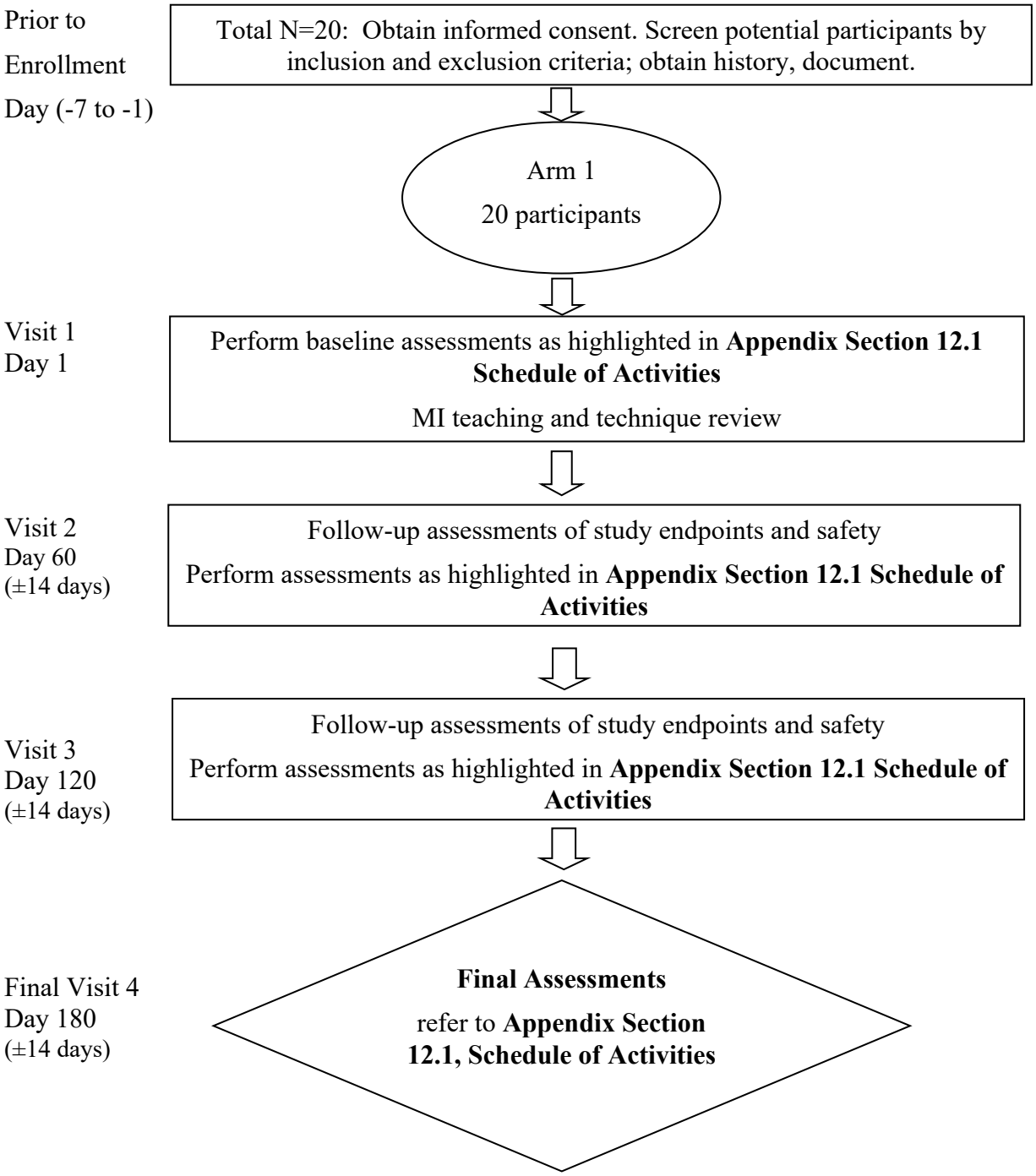
1.3 Schema


Prior to Enrollment Day (-7 to -1)
Total N=20: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, document.
Arm 1
20 participants
Visit 1 Day 1
Perform baseline assessments as highlighted in Appendix Section 12.1 Schedule of Activities
MI teaching and technique review
Visit 2 Day 60 (±14 days)
Follow-up assessments of study endpoints and safety
Perform assessments as highlighted in Appendix Section 12.1 Schedule of Activities
Visit 3 Day 120 (±14 days)
Follow-up assessments of study endpoints and safety
Perform assessments as highlighted in Appendix Section 12.1 Schedule of Activities
Final Visit 4 Day 180 (±14 days)
Final Assessments
refer to Appendix Section 12.1, Schedule of Activities

2 INTRODUCTION AND RATIONALE

2.1 Study Rationale

Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease that commonly leads to respiratory failure related to weakened breathing and coughing. Current United States guidelines for respiratory care for ALS do not recommend any respiratory interventions until the patient meets specific thresholds such as FVC <50% predicted normal, MIP< -60 cmH2O, or nocturnal oxygen saturation <88% for ≥5 minutes.[13] ALS has a heterogenous presentation and rate of progression, and thus the window between diagnosis and meeting criteria for respiratory interventions could span months or even years during which breathing and coughing are weakening. An early respiratory intervention during this "waiting period", when a person can still have weakened breathing and coughing, may offer an opportunity to slow progression of respiratory muscle weakness, strengthen coughing, and thus improve clinical outcomes.

Most patients with ALS develop some respiratory muscle weakness, including weakened cough, prior to meeting the aforementioned guideline recommended thresholds. A device that can assist with weakened cough includes a mechanical insufflator-exsufflator (MI-E). A MI-E device can provide mechanical insufflation (MI), which helps expand the chest wall through the use of positive pressure through a mouthpiece or mask. The increased volume in the chest helps provide increased elastic recoil, thus strengthening cough. Prior studies have described the use of MI-E to provide mechanical insufflation in neuromuscular diseases.[12,14] It remains unclear how early use of MI-E prior to guideline recommendations may help augment airway clearance and cough in ALS. Prior to performing a randomized trial, a feasibility study is necessary to estimate endpoint effect sizes, subject adherence, and adverse events.

2.2 Background

As ALS causes progressive respiratory muscle weakness, hypoventilation, atelectasis, and chest wall restriction occur. Decreased chest wall movement creates contractures of sternocostal and costovertebral joints, progressively worsening chest wall restriction. Chest wall contracture plus weakening of the muscles contributes to weakened cough and airway clearance in ALS, which often can occur well before guidelines recommend instituting respiratory therapies.

A major international expert panel for methods of airway clearance for neuromuscular disorders published a state-of-the-art review in 2018.[12] Within this review, they highlight that use of mechanical insufflation (MI) via a MI-E device can provide assisted inspiration to achieve insufflation capacity. A MI-E device is an airway clearance device which sits on a flat surface and is connected to corrugated tubing attached to a mouthpiece or mask (as per patient preference or for those with bulbar weakness)(**Figure 1**). MI will inflate the chest via mask or mouthpiece by using positive pressure in coordination with the patient's inspiratory timing until maximal inflation capacity (MIC), determined by the patient or maximal chest rise on visual inspection. At MIC, the patient removes the mask/mouthpiece and passively exhales, which completes one "cycle". Prior literature on insufflation of the chest has used a "dose" of 5 sets of 5 cycles once or twice daily.[5–11]

The maneuver is usually performed with assistance of a caregiver. This insufflation is believed to increase length of respiratory muscles, reduce chest wall restriction, and leverage the elastic recoil of the chest wall to increase cough peak flow and thus airway clearance. Multiple prior studies in neuromuscular disease (including ALS) has shown an improvement in cough efficacy, measured as peak cough flow, after using a MI-E device.[5–11]

This proposal aims to perform MI using a MI-E device. Contrary to respiratory muscle exercises that require active muscle contraction, MI is a passive maneuver that does not depend on strength and is thus ideal for ALS. MI may recruit atelectatic alveoli, improve chest wall range of motion, and slow decline of chest wall elasticity by preventing chest wall contractures. A critical knowledge gap includes whether several months of consistent MI use can improve respiratory outcomes in ALS. If proven effective, MI could serve as an early respiratory intervention which may delay respiratory decline, strengthen airway clearance ability, improve quality of life, and potentially prolong survival in ALS.

Given the paucity of data on MI in ALS, we propose a single arm feasibility trial of the use of MI in individuals with early-stage ALS who are not yet on alternative respiratory therapy such as non-invasive ventilation. Our results from this trial will inform our next study enrolling high-risk patients into a RCT investigating early MI or NIV with the goal of improving quality life, clinical outcomes, and survival in ALS.

2.2.1 Pharmacokinetics, Pharmacodynamics and Toxicology

Not applicable.

2.2.2 Assessment for Potential Study Products Drug-Drug, Drug-Device, Device-Device Interactions

All eligible participants will be devoid of respiratory therapies at enrollment. However, it is possible that during the 6-months of follow-up the subject may begin alternative respiratory therapies such as non-invasive ventilation (NIV) or require therapy assistance with cough strength MI-E. NIV would be used at separate time periods of the day and thus would not be used at the same time as the MI exercise. A cough that requires MI-E therapy to loosen secretions will be seen as an additional, augmented therapy in addition to the MI exercise described in this proposal. It is certainly possible that use of NIV or MI-E may attenuate the effects of MI alone; however, this has not borne out by the current literature.

2.2.3 Clinical Adverse Event Profile

Side-effects and adverse events, as assessed by participant self-report.

Potential side-effects associated with taking repeated deep breaths include discomfort or musculoskeletal soreness of the chest well, and light-headedness and/or transient fall in blood pressure.

Although extremely rare, a pneumothorax can occur at very high inspiratory pressures and would constitute a serious adverse event.

2.2.4 Dosing Rationale

The optimum "dose" of repetitions and sets to perform the MI maneuver is currently unknown. We will follow an MI protocol that has been published and replicated in multiple prior studies of individuals with various neuromuscular diseases.[12,14] Briefly, we will use a MI-E device, which is an airway clearance device which sits on a flat surface and is connected to corrugated tubing attached to a mouthpiece or mask (as per patient preference or for those with bulbar weakness)(**Figure 1**). To complete the MI maneuver, the BiWaze Cough will have preset positive inspiratory pressures and timing to coordinate inhalation prior to exhaling (see **section 6.1.3** on titration of pressure and timing). After creating a seal with the mouthpiece (or mask), an assistant will activate the BiWaze Cough to allow it to perform the preset positive pressure inhalation until the subject's chest is maximally inflated before the subject exhales. This

cycle is repeated 5 times consecutively, concluding the end of one "set". After a brief break (eg, 30-60 seconds), the MI maneuver is repeated for another 5 cycles at a time for a total of 5 sets. At the end of 5 sets, this concludes one MI session. Our protocol includes one MI session (5 sets of 5 inhalations) to be performed twice daily.

To determine optimum settings on the BiWaze Cough device for the patient, please refer to **Section 6.1.3**.

Risk/Benefit Assessment

2.2.5 Known Potential Risks

Prior studies on MI have demonstrated that it is well-tolerated without serious adverse events.[5–11] Performing the insufflation phase of an MI-E device involves inflating a person's lungs to the point of maximal inhalation prior to exhaling.

The most serious complication from using an MI-E device for applying positive pressure using to the lungs includes pneumothorax, or collapsing of a lung. The relative likelihood of this is felt to be extremely low, as the use of MI-E is widespread across the world in both the inpatient and outpatient setting, yet the occurrence of a pneumothorax related to MI-E is case-reportable.[15–18] In many of the described cases, patients had a comorbidity associated with increased likelihood of pneumothorax (eg, chronic obstructive pulmonary disease).

Accordingly, there are a few potential risks as listed below.

- Chest wall discomfort or soreness during inflation
- Increasing volume of air in the chest can slow blood flow to the heart, and thus may cause a temporary drop in blood pressure or light-headedness until the subject exhales.
- Very high inspiratory pressures may increase the likelihood of a pneumothorax (lung collapse).
- The long-term risks of MI are unknown. It is estimated that most of the risks are associated with the exercise performance, and thus most risks would be of the immediate risk category.

2.2.6 Known Potential Benefits

Given that MI is an exercise in inflating the lungs, it is theorized that mechanisms for benefit include[12]:

- recruitment of atelectatic alveoli (thus improving gas exchange and airway clearance)
- improve chest wall range of motion through the sternocostal and costovertebral joints (similar to how physical therapy improves joint range of motion)
- slowing decline of chest wall elasticity by preventing chest wall contractions

Specifically in ALS, volume recruitment of the lungs through techniques such as MI has shown short-term improvement in static respiratory measurements such as FVC, peak cough flow, volitional airway clearance, and lung compliance.[1,19–21]

In alternative neuromuscular diseases (such as Duchenne muscular dystrophy), volume recruitment, similar to MI, is believed to slow decline of vital capacity, stabilize lung compliance, and maintain effective assisted peak cough flow.[3,22]

2.2.7 Assessment of Potential Risks and Benefits

The known potential risks associated with MI (as described above) are felt to be low likelihood, as supported by multiple prior studies without significant events.[5–11] Multiple prior studies describe use of MI-E (with the additional use of exsufflation above the insufflation only function in this proposal) across a variety of patient populations without significant complications.[7–9,23–28]

We recognize that there are several potential risks with the use of an MI-E device for lung insufflation as described above. The most serious of risks includes a pneumothorax, which is felt to be extremely rare and a case-reportable event.[15–18]

We will mitigate risks through several means:

1) Individual titration of settings that are tolerated by the patient (see Secton 6.1.3)
2) MI-E settings allow you to precisely control the level of pressure in the chest, thus preventing unmonitored high intrathoracic pressure
3) Participants will be instructed to stop the insufflation maneuver immediately the moment they feel discomfort
4) We will provide in-person hands-on teaching as well as additional educational material via handouts and videos to guide patients in proper procedures
5) by excluding subjects with certain past medical history:

- cognitive dysfunction (based on Neurology attendings assessment) that would hinder a subject's ability to complete study procedures
- craniofacial abnormality will prevent incorrect performance of the MI maneuver
- the following conditions will lessen likelihood of pneumothorax, low blood pressure, incorrect MI performance, or other adverse events associated with transient increase in pressure in the chest:
    - Recent hemoptysis
    - Recent barotrauma
    - History or known susceptibility to pneumothorax

- History or known susceptibility to pneumomediastinum
- History of emphysema of any kind (including bullous emphysema)
- chronic obstructive pulmonary disease
- Smoking history of more than 19 pack years (defined as # average cigarettes per day while smoking multiplied by the number of years of smoking)
- Uncontrolled asthma (defined as recent exacerbation requiring corticosteroids in the previous 30 days)
- Symptomatic cardiomyopathy (heart failure) with left ventricular ejection fraction less than 50%
- History of right heart failure or pulmonary hypertension
- History of recent myocardial infarction or cardiac arrest in the last 90 days
- Systolic blood pressure less than 90 mmHg

In addition, we will discourage consented individuals from performing MI if any of the following scenarios occurring at any time:

- Immediately after a meal (less than 60 minutes)
- Symptoms of nausea and vomiting
- Experiencing chest pain, lightheadedness, or tightness in the chest
- After trauma to the chest or face (eg, surgery, accidents)

The potential benefits of MI include improved static respiratory measurements such as FVC, peak cough flow, volitional airway clearance, and lung compliance. Repeated use over time may slow respiratory muscle decline in a progressive neuromuscular disease such as ALS. The slowing of decline may prolong time to need for additional respiratory therapy (such as NIV), lower likelihood of a respiratory infection, and thus improve quality of life and potentially survival.

ALS is a rapidly progressive neurodegenerative disease without a cure, which commonly leads to death by respiratory complications. We are offering MI during a phase of the disease that subjects would otherwise receive no respiratory therapies but have already developed weakened breathing and coughing. Given that the risks of MI are low, it has been well-tolerated in prior studies,[5–11] and the risks are minimized by our risk mitigation strategies described above, we feel that the potential benefits of MI (and the information gained by the trial) outweigh the risks.

3 STUDY OBJECTIVES AND ENDPOINTS

Table 1. Study objectives and endpoints.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine how the use of twice daily MI, in addition to conventional treatment, affects decline from baseline in PCF (in L/min) over 6 months in individuals with early-stage ALS.	PCF in liters/minute as measured by use of a peak flow meter while the patient is in a seated upright position. An alternative device for measuring PCF can be the use of a handheld spirometer and using the measured peak expiratory flow by multiplying by 60 to convert from liters/second to liters/minute. The subject must be seated in an upright position and the interface must include a mouthpiece or an oronasal mask. Subjects are asked to perform a deep inhalation followed by a maximal cough.	MI is thought to reduce chest wall contractures and improve volitional airway clearance. Accordingly, we will measure strength of airway clearance through PCF.
Secondary		
To determine how the use of twice daily MI over 6 months, in addition to conventional treatment, affects respiratory strength decline, respiratory symptoms, and quality of life.	Forced vital capacity (FVC) o Defined as maximal volume, in liters, that a person can exhale forcefully after a complete inhalation. o Measured using a spirometer connected to a face mask or mouthpiece in the sitting and upright position. Maximal inspiratory pressure (MIP) o Defined as the maximum pressure, in cmH_2O, generated when the subject exhales as much air as possible and then immediately inhales as forcefully as possible.	MI is theorized to slow decline of respiratory muscle strength and respiratory symptoms. Accordingly, many of our secondary endpoints include static respiratory strength measurements (FVC, MIC, MIC-FVC, MIP, MEP), measure of ventilation adequacy (transcutaneous CO_2, oxygen saturation), as well as assessments of respiratory symptoms (ALSFRS-R dyspnea and orthopnea scores).

Table 1. Study objectives and endpoints.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	○ Measured using a hand-held manual or digital manometer connected to a mouthpiece or mask in the sitting and upright position. Maximal expiratory pressure (MEP) ○ Defined as the maximum pressure, in cmH_2O, generated when the subject inhales as much air as possible and then immediately exhales as forcefully as possible. ○ Measured using a hand-held manual or digital manometer connected to a mouthpiece or mask in the sitting and upright position. Maximum insufflation capacity (MIC) ○ Defined as exhaled volume, in liters, immediately following a MI maneuver to maximum insufflation capacity. ○ Measured using a spirometer connected to a face mask or mouthpiece, in the sitting and upright position. MIC-assisted PCF • Defined as the peak cough flow generated from MIC	

Table 1. Study objectives and endpoints.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	• Lungs are inflated to MIC using MI (as above) and before the subject exhales they insert a peak flow meter in their mouth and follow with a peak cough flow as described above. MIC – FVC Difference o defined by subtracting the FVC from the MIC Transcutaneous carbon dioxide (TCO_2) and oxygen saturation (SpO_2) o Defined as the average value in mmHg from a daytime in-clinic measurement over a 15-minute transcutaneous recording. o Measured using a Sentec transcutaneous digital monitoring system while the subject is sitting upright in a chair or wheelchair. The transcutaneous sensor can be placed on the subject forehead, cheek, or earlobe. o The Sentec transcutaneous digital monitor measures carbon dioxide, oxygen saturation, and pulse rate. It is standard of care in the Penn ALS clinic and would be offered to the patients as part of routine clinical care regardless of study enrollment. ALSFRS-R dyspnea and orthopnea score	

Table 1. Study objectives and endpoints.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	○ The ALSFRS-R is a 12-item standardized questionnaire to assess the motor function status of an individual with ALS. The ALSFRS-R is assessed by a research staff member who has been certified for performance of the ALSFRS-R. ○ The dyspnea score is one of the questions focusing on level of shortness of breath, scored on a scale of 0 to 4, with 4 being no symptoms at all and 0 being severely symptomatic with consideration of mechanical respiratory support. ○ The orthopnea score is one of the questions focused on breathing symptoms and difficulty sleeping while lying supine, scored on a scale of 0 to 4, with 4 being no symptoms at all and 0 being severely symptomatic with inability to sleep. Quality of life as measured by ALSSQOL-SF, a validated questionnaire to assess quality of life in terms applicable to a person with ALS. ○ The questionnaire includes 20 questions scored on a scale of 0-10. Global rate of change score for peak cough flow ○ The global rate of change score for the peak cough flow will ask patients how effective they think their cough is today by rating it on a Likert scale from -7 (extremely impaired) to +7 (extremely strong).	If MI attenuates decline of respiratory muscle strength and symptoms, then it may affect quality of life. Therefore, we are assessing quality of life through a standardized metric for quality of life that has been validated in the ALS population. The effect on MI may improve efficacy of cough. We are performing a global rate of change score for peak cough flow to compare the subjective versus objective change in PCF. This may help us determine a minimal clinically meaningful change in PCF.

Table 1. Study objectives and endpoints.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Tertiary		
To determine how the use of twice daily MI over 6 months, in addition to conventional treatment, can affect time to need for additional respiratory support or survival.	Time to start of NIV o Defined by the time period between enrollment for the MI trial and start of NIV. o NIV start date will be defined by patient-reported date or as documented in the electronic medical record. Tracheostomy free survival time o Defined by the time period between enrollment for the MI trial and death as long as the subject never had a tracheostomy placed. o Death date will be determined by family report, electronic medical record review, or publicly available obituaries. o Tracheostomy placement will be determined by patient or family report; or as documented in the electronic medical record.	If MI slows respiratory function decline, then it may slow time to need of additional respiratory support (such as NIV) or even improve survival given that respiratory failure is the most common cause of death in ALS.

4 STUDY PLAN

4.1 Study Design

This will be a single-center, single-arm interventional pilot study at Penn of MI in patients with early-stage ALS who are not yet on alternative respiratory therapy such as NIV or MI-E. We hypothesize that individuals who are adherent with MI will have a slower decline in PCF compared to matched controls.

To minimize bias, we will approach all eligible patients and adhere to strict research grade protocols for assessing our study endpoints.

Subject participation will last 6 months, but some endpoints could be collected for up to 6 months post last study visit.

4.2 Scientific Rationale for Study Design

Given the paucity of data on MI in ALS, a pilot study is necessary prior to implementing a RCT. We will compare results of our single arm study to 20 randomly selected matched controls from any of the sites of the NIH K23 Philadelphia ALS (PALS) Cohort study. We considered "sham MI" (placebo control), however this would be immediately evident to sham subjects and thus impossible to blind. A cross-over design is infeasible given the rapidly progressive nature of ALS.

The controls will be matched based on factors known to be associated with survival in ALS (eg, age, time since disease onset, FVC, bulbar vs spinal onset disease). However, using a historical control cannot account for all measured and unmeasured confounders which can affect a person's outcome in the same manner as a randomized controlled trial. As mentioned above, this pilot trial is meant to provide hypothesis generating data for a future RCT.

Figure 2. Performance of MI with BiWaze Cough device demonstration on simulated patient.

4.3 Justification for Dose

We will use a previously published method to perform MI (**Figure 2**).[12,14] The optimum "dose" of repetitions and sets to perform the MI maneuver is currently unknown. We will follow volume recruitment protocol that has been published and replicated in multiple prior studies of individuals with neuromuscular disease.[5–11] Briefly, we will use a MI-E device.

To complete the MI maneuver, BiWaze Cough will have preset positive inspiratory pressures and timing to coordinate a prolonged inhalation at a set pressure prior to the patient exhaling (see **Section 6.1.3**). After creating a seal with the mouthpiece (or mask), an assistant will activate the BiWaze Cough to allow it to perform a single positive pressure lung insufflation for 2-3 seconds until the subject's chest is maximally inflated before the subject exhales. The exhalation phase lasts for about 2 seconds before a brief pause and then another insufflation is repeated. The inhalation for 2-3 seconds and exhalation for 2 seconds is repeated for 5 consecutive cycles. This concludes the end of one "set". After a brief break (eg, 30-60 seconds), the 5-insufflation set is repeated again for a total of 5 sets. At the end of 5 sets, this concludes one MI session. Our protocol includes one MI session (5 sets of 5 insufflation) to be performed twice daily.

The duration of insufflation prior to maximum insufflation capacity (MIC) would be determined by the patient's tolerance of the pressure and length of inhalation (as outlined in **Section 6.1.3**). Alternative "doses" of MI, such as more sets or more sessions/day have not been studied. We hypothesize that fewer sets or sessions/day may be less likely to provide a therapeutic benefit. To our knowledge, no data have tested the dose-specific effect of performing more sets or sessions.

Adherence to the protocol will be tracked using usage data collected by the device. The data are uploaded to a cloud-based smartphone app known as "ARC Connect". Patients will be asked to bring in their smartphones with the Arc Connect app for data review at every visit.

4.4 End of Study Definition

A participant is considered to have completed the study if they have completed all phases of the study including the last visit or the last scheduled procedure shown in the **Schedule of Activities (SoA), Appendix Section 12.1.** We may collect follow-up data on secondary and tertiary clinical outcomes (eg, start of NIV or death) for up to 6 months after last study visit.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form.
2. Age ≥18 years.
3. Diagnosed with amyotrophic lateral sclerosis using the Gold Coast Criteria.
4. Have an able and willing caregiver to assist with MI on a daily basis.
5. Willingness and ability to participate in study procedures.

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Age <18 years old.
2. Inability to perform a cough peak flow or spirometry manuever
3. Current use of non-invasive ventilation (NIV), bi-level positive pressure ventilation, or "Bi-PAP" or physician prescribing NIV on day of potential enrollment.
4. Current use of MI-E (also known as a "cough assist device") for airway clearance. Please note that patients can start use of a MI-E device subsequent to enrollment while currently being followed for the study.
5. Active enrollment in hospice.
6. Current tracheostomy.
7. Presence of cognitive dysfunction that would impair ability to complete study procedures, as determined by neurology attending physician.
8. Absence of an able and willing caregiver to assist with MI twice daily as specified in the protocol.
9. Pregnancy
10. Medical history of:
    a) Recent hemoptysis
    b) Recent barotrauma
    c) History of emphysema of any kind (including bullous emphysema)
    d) History of or known susceptibility to pneumothorax

e) History of or known susceptibility to pneumomediastinum

f) Chronic obstructive pulmonary disease

g) Uncontrolled asthma (defined as recent exacerbation requiring corticosteroids in the previous 30 days)

h) Symptomatic cardiomyopathy (heart failure) with left ventricular ejection fraction less than 50%

i) History of right heart failure or pulmonary hypertension

11. Current smoker or tobacco use within the last 30 days.

5.3 Lifestyle Considerations

Not applicable.

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an erroneous history based on patient reporting may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 Strategies for Recruitment and Retention

The target study sample size for analysis is 20 patients. We anticipate an accrual rate of 50%, therefore we will approach up to 40 patients to obtain 20 analyzable subjects. To account for study dropouts and withdrawal, we may have to enroll up to 25 participants to obtain 20 analyzable subjects.

The source of our participants will include the Penn Comprehensive ALS Center at the University of Pennsylvania.

Potential participants will be identified upon chart review after they are officially diagnosed with ALS by one of the attending physician neurologists at the Penn Comprehensive ALS Center. Subjects will be screened and approached for interest in the study. Patients will be approached during 1 of 3 time periods:

1) At least 2 weeks after their diagnosis visit but in advance of an upcoming routine clinical visit with intention of performing a remote consent.
2) During a routine clinical visit to perform pen and paper consent.
3) Within 5-14 days after a routine clinical visit with the intention of performing remote consent.

To enhance participant retention over the 6 months of the study, we will collect contact information with multiple methods for contacting participants, provide visit reminders as necessary, and will provide patient stipends per visit completed throughout the study.

Inclusion of Children

ALS is extremely rare in children; therefore, the research topic is essentially irrelevant to children (one of the justifiable circumstances for excluding children from a research proposal). Age <18 years is an exclusion criterion for the study. Therefore, all individuals in our database will be above the age of 18 after enrollment. The proposed study involves minor risk regarding patient confidentiality and societal benefits are potentially significant. Because the risks are so small and the disease very rarely occurs in children, no specific additional protections for children are planned.

Inclusion of Women and Minorities

No participants will be excluded from study participation based on sex, race, or ethnicity. This is appropriate for the model under study, as ALS may impact both sexes as well as all races and ethnicities, and selective exclusion could result in bias. The distribution of subjects will likely reflect the epidemiology of ALS, which is predominantly a disease of older, white males (average age in mid to upper 50's). The etiology of ALS is currently unknown, and the leading theories for this demographic distribution includes genetic and/or environmental factors. The Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database is an international, multicenter, harmonized database of over 10,000 ALS clinical trial subjects. In PRO-ACT, sex is split by 38% females and 62% males, race is divided by about 95% white subjects, 2% black subjects, and 3% other races. However, these data could be affected by selective enrollment by race due to availability of access to study centers. We will attempt to reach more women and minorities in this study by approaching women and minorities whenever they are eligible. We also plan future studies of MI or NIV in early ALS at external academic referral centers in the Philadelphia area, which are likely to have significant demographic and socioeconomic diversity from one another. This study has no specific exclusions based on sex, race, or ethnicity.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

The BiWaze Cough device includes several components as shown in **Figure 3**:

1) BiWaze Cough System
2) BiWaze Cough Standard Breathing Circuit
    - 22 mm bacterial/viral filter
    - Corrugated tubing
    - Patient Interface (Anesthesia mask [small, medium or large] or Mouthpiece)
3) Nose clip
4) Carrying Bag

Figure 3. BiWaze Cough system (panel A) and ABM Respiratory carrying bag (panel B).

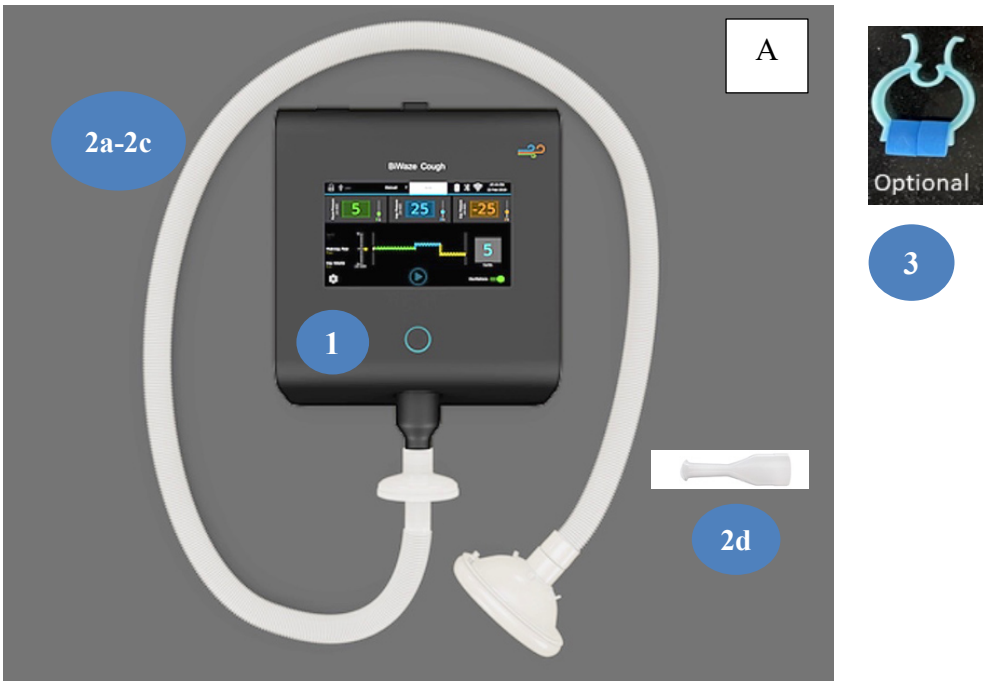


Table 2. The BiWaze Cough device components.

Item number in ***Figure 3***	*Name of Device*	*Manufacturer*	*Description*	*Marketing Status in the U.S.*	*FDA Device Classification (I, II, III)*
1	BiWaze Cough System BC20113	ABM Respiratory Care	Standard USA (includes control unit, internal battery, carrying bag, English user manual, power cord, filter adapter, single path circuit)	510k Clearance (K191912)	II
2a	BiWaze Cough Standard Circuit – Adult **Small** Face Mask BC21088	ABM Respiratory Care	Standard adult small patient circuit (includes single path breathing tube, adapter, bacterial/viral filter, adult small face mask)		None
2b	BiWaze Cough Standard Circuit – Adult	ABM Respiratory Care	Standard adult medium patient circuit (includes single path breathing tube, adapter, bacterial/viral filter, adult medium face mask)		None

	Medium Face Mask BC21089				
2c	BiWaze Cough Standard Circuit – Adult **Large** Face Mask BC21273	ABM Respiratory Care	Standard adult large patient circuit (includes single path breathing tube, adapter, bacterial/viral filter, adult large face mask)		None
2d	BiWaze Cough Standard Circuit – Mouthpiece BC21092	ABM Respiratory Care	Standard mouthpiece patient circuit (includes single path breathing tube, adapter, bacterial/viral filter, mouthpiece		None
3	Nose Clips w/Foam Pads	McKesson	Model: 16-MCKNC		None
4	BiWaze Carrying bag BC21083	ABM Respiratory Care	Soft sided carrying bag with ABM Respiratory Care logo		None

6.1.2 Dosing and Administration

We will use a previously published method to perform MI.[12,14] The optimum "dose" of repetitions and sets to perform the MI maneuver is currently unknown. According to prior studies, MI is performed as a **"therapy session"** according to the following "dose" for all patients throughout the study:

- Using an MI-E device, deliver MI as one prolonged inhalation until maximal lung expansion, after which the mask/mouthpiece if removed and the subject performs passive exhalation. This inhalation/exhalation cycle is repeated 5 times. This constitutes one "set".
- After each set, the subject takes a break for 30-60 seconds before repeating the MI maneuver.
- The MI maneuver and break is repeated as above for 5 sets.

The above described **"therapy session"** is repeated twice daily. Instructions for determination of settings are found below under **Section 6.1.3 "Instructions for initial set-up and determining optimum pressure, timing, and flow settings".**

The timing of the MI is ideally done on an empty stomach (at least 30 minutes prior to or 90 minutes after a meal) to allow for maximal chest expansion without incurring diaphragm restriction against a full stomach.

If the subject misses a dose, then the subject should attempt to perform an MI therapy session as soon as possible before or after their next meal (at least 30 minutes prior or 90 minutes after a meal). If the subject misses a therapy session at the end of a day, then the subject may perform a therapy session prior to bedtime.

If a subject realizes in hindsight that they missed a therapy session on the previous day, then performing a "make-up" session on the following day (ie, 3 sessions in one day) is not necessary. In that scenario, the subject is encouraged to continue performing MI twice daily as regularly scheduled.

6.1.3 Instructions for initial set-up and determining optimum pressure, timing, and flow settings:

To determine optimum insufflation pressure and program a MI setting for the patient, we will follow the following titration protocol with BiWaze Cough:

- Ensure the patient interface is secure
    - If using a mouthpiece, ensure patient can form a tight seal around the mouthpiece in a manner that is unlikely to leak with applied inhalation pressure
    - If using a face mask, ensure the face mask can create a tight seal on the patients face around the bridge of the nose and around the sides/bottom of the mouth. There are 3 face mask sizes available to ensure optimal fit.
- In manual mode, Titrate the inhalation pressure. Start with an inspiratory pressure of +20 cmH2O for timing of 2 seconds
- Set the exhalation pressure to 0 cmH2O for 0 seconds.
- Set the pause pressure of +5 cmH2O and timing for 2 seconds.
- Increase inhalation pressure in 5 cmH2O increments to visible maximal chest rise while avoiding patient discomfort based upon patient feedback. A patient's comfort level and patient interface will influence the final inhalation pressure.
- Do not increase the inhalation pressure beyond +50 cmH2O.
- The subject will receive an inhalation during the inhalation phase and will be taught to exhale during the pause phase before the next breath.

To determine optimum insufflation time duration:

- Once a comfortable inhalation pressure is identified, determine length of inhalation by re-trialing inhalation times by starting at 2.0 seconds and doing consecutive inhalation trials after increasing in 0.5 second increments to a maximum of 4.0 seconds.

- After determining inhalation time, determine pause timing by starting at 2.0 seconds and doing consecutive trials of inhalation/exhalation to assess for patient tolerance of exhalation time. Increase/decrease pause timing for exhalation in 0.5 second increments up to a minimum of 1.0 seconds and a maximum of 3.0 seconds.

To determine optimum insufflation flow setting:

- The rate of flow of air can be adjusted to patient comfort. The options are slow, medium, or fast.
- Start at a medium flow setting and assess for patient comfort with the speed of air entry by asking if the air is coming in too fast or too slow.
- Adjust the air flow to low or fast as necessary depending on patient answer above.
- Note that patients with significant bulbar weakness may require a low inhalation flow setting.

Setting the desired program into the device:

- Program Auto mode to deliver 5 consecutive cycles of insufflation/pause for exhalation breathes at the desired pressure and timing as determined above.
- Save this programmed therapy as a profile with a name "MI".
- The subject can use this Auto mode to do sets of the 5 cycle MI session with 30 – 60 second breaks between each set.

Final MI session will be performed as follows:

Using the MI preset in Auto mode after determining above pressure and timing titrated to patient comfort:

1. Insufflation breath at +20-50 cmH2O for 2-4 seconds followed by an exhalation phase for 1-3 seconds at +5 cmH2O
2. Insufflation breath at +20-50 cmH2O for 2-4 seconds followed by an exhalation phase for 1-3 seconds at +5 cmH2O
3. Insufflation breath at +20-50 cmH2O for 2-4 seconds followed by an exhalation phase for 1-3 seconds at +5 cmH2O
4. Insufflation breath at +20-50 cmH2O for 2-4 seconds followed by an exhalation phase for 1-3 seconds at +5 cmH2O
5. Insufflation breath at +20-50 cmH2O for 2-4 seconds followed by an exhalation phase for 1-3 seconds at +5 cmH2O

   Pause and then rest ~30 – 60 seconds and then repeat for a total of 5 sets.

   Perform above protocol twice daily.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

BiWaze Cough systems will be loaned for this study from ABM Respiratory Care, LLC:

ABM Respiratory Care, LLC
860 Blue Gentian Road
Suite 200
Eagan, MN, 55121-1567

The company contact is:
Leah Noaeill
VP of Marketing and Clinical Affairs
ABM Respiratory Care
Leah.Noaeill@abmrc.com
612.419.1071
www.abmrc.com

The BiWaze Cough will be mailed to the Penn Comprehensive ALS Clinic at:

Penn Neurologic Institute
330 S. 9th Street
Room 310
Philadelphia, PA 19107

Once the devices have been delivered, they will be distributed by the PI and/or research staff at the Penn Comprehensive ALS Clinic.

If any of the BiWaze Cough systems appear unusable for any reason (eg, damaged in shipment), then the devices will be rendered unusable until a replacement device or part has been obtained from ABMRC, LLC.

6.2.2 Formulation, Appearance, Packaging, and Labeling

There will be no modification of the appearance, packaging, or labeling of the BiWaze Cough prior to distribution to the study subject.

6.2.3 Product Storage and Stability

The BiWaze Cough is an FDA 510k cleared device made of medical grade materials. Whether it is in the Penn Comprehensive ALS Clinic or the patient's home the recommended storage location is in a temperature-controlled room so as to avoid extreme heat or cold.

BiWaze Cough does not expire unless one of the components is misplaced or becomes damaged.

Cleaning instructions:
Subjects will be instructed to clean the mouthpiece/mask and tubing least once per week. Subjects will be asked to clean the equipment more often if they have a cold or chest infection.

The instructions for cleaning will include:

- Disconnect the mouthpiece/mask and tubing from the BiWaze Cough.
- Separate the mouthpiece/mask from the tubing.
- Gently clean the tubing and mouthpiece/mask in mild soap and water. Rinse in warm water.
- Allow parts to completely dry before use. Do not use a hairdryer to dry the equipment.

6.2.4 Preparation

BiWaze Cough ships with all necessary components as listed in **Table 2**. The research subjects will go home with the BiWaze Cough in a travel bag included with the system. Note that patients will still have the option of exchanging the mouthpiece with the anesthesia mask if bulbar weakness prevents a proper seal on the mouthpiece.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is a single center, single arm study. We do not plan to implement any blinding or randomization.

We will minimize selection bias by approaching all eligible patients.

We will ensure measurement accuracy by implementing strict quality assurance/quality control procedures. The center involved in this study has significant prior experience with enrollment for clinical trials. The assessments and procedures used (eg, spirometry performance) will be performed according to ALS clinical trial grade standards.[29] Pulmonary function testing results will be calculated using American Thoracic Society guideline equations.[30]

6.4 Study Intervention Compliance

Adherence to MI therapy sessions will be recorded on the BiWaze Cough system in the subject's home. BiWaze Cough stores the data in an encrypted log file which can be sent via WiFi to an online, HIPPA complaint data repository platform called ARC Connect (ABM Respiratory Care, USA). The clinicians can access the usage data through a secure online ARC Connect portal and review the subject's compliance to the MI therapy.

Reports will include: date/time of use, number of inhalation/exhalation cycles, number of sets of insufflation/exhalation cycles, and pressure used.

ARC Connect's data privacy policy states that it does collect the following personal information:

- Name
- Email Address
- Phone Number

To help protect subject privacy: we will create subject accounts with altered contact information for each subject's profile in ARC Connect as follows:

Title	**Will be changed to:**
• Name ----------→	Subject ID number
• Email Address ----------→	An @pennmedicine account for research staff
• Phone Number ----------→	The Penn ALS clinic research staff mobile number

We will review usage reports with subjects at the research study visits.

6.5 Concomitant Therapy

For this protocol, subjects will be allowed to be on concomitant respiratory therapies as indicated by standard of care. Examples of concomitant respiratory therapies include the use of non-invasive ventilation (NIV), mechanical insufflation-exsufflation (MI-E) therapy, or airway clearance therapy with inhalers and/or inhaled nebulized medication (eg, albuterol).

We will assess for concomitant respiratory therapies at each visit (baseline and follow-up) throughout the study. We will record therapy start date as reported by the patient/caregiver or via review of electronic medical record. Usage of NIV or MI-E (with the additional exsufflation function) may also be confirmed via remote telemonitoring data if the patient is set-up for this service through the durable medical equipment (DME) company.

It is possible that concomitant use of respiratory therapies may confound the effect of MI. To account for this, we will ensure accurate recording of start dates (via means stated above), confirm usage of concomitant therapies by telemonitoring (if available), and ensure strict clinical-trial-grade measurements of our endpoints.

6.5.1 Rescue Medicine

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation from MI use does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- Subjects will be asked to come into the clinic as scheduled to perform assessments as described in the study protocol.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant MI non-compliance, as defined by failure to use MI for more than 4 weeks.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of MI
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation (eg, hospice enrollment)
- Participant unable to receive MI for 4 weeks or more

The reason for participant discontinuation or withdrawal from the study will be recorded on the MI-PALS Case Report Form (CRF). Subjects who sign the informed consent form and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced by another subject.

7.3 Lost To Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for more than 2 scheduled visits and is unable to be contacted by the study site staff for more than 60 days.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 4 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENT AND PROCEDURES

8.1 Efficacy Assessments

Primary endpoint:

- Peak cough flow (PCF)

Secondary endpoints:

- Forced vital capacity (FVC)
- Maximal inspiratory pressure (MIP)
- Maximal expiratory pressure (MEP)
- Maximum insufflation capacity (MIC)
- MIC-assisted PCF
- MIC-FVC difference
- Transcutaneous carbon dioxide (TCO_2) and pulse oximetry (SpO_2)
- ALSFRS-R
- Global rate of change score for PCF

Tertiary endpoints:

- Start date of non-invasive ventilation
- Tracheostomy free survival time

8.1.1 Peak cough flow (PCF)

PCF will be measured by use of a peak flow meter while the patient is in a seated upright position. The peak flow meter will be connected to a mouthpiece or anesthesia mask if the subject has too much bulbar weakness to create a sufficient mouth seal on the mouthpiece.

An alternative device for measuring PCF can be the use of a handheld spirometer and using the measured peak expiratory flow by multiplying by 60 to convert from liters/second to liters/minute.

8.1.2 Forced vital capacity (FVC)

Measured using a hand-held clinical-research grade spirometer connected to a mouthpiece in the sitting and upright position. An anesthesia mask may be used in place of a mouthpiece if the subject has too much bulbar weakness to create a sufficient mouth seal on the mouthpiece.

Defined as maximal volume, in liters, that a person can exhale forcefully after a complete inhalation.

8.1.3 Maximal inspiratory pressure (MIP)

Measured using a hand-held manual or digital manometer connected to a mouthpiece or mask in the sitting and upright position.

Defined as the maximum pressure, in cmH2O, generated when the subject exhales as much air as possible and then immediately inhales as forcefully as possible.

8.1.4 Maximal expiratory pressure (MEP)

Measured using a hand-held manual or digital manometer connected to a mouthpiece or mask in the sitting and upright position.

Defined as the maximum pressure, in cmH2O, generated when the subject inhales as much air as possible and then immediately exhales as forcefully as possible.

8.1.5 Maximum insufflation capacity (MIC)

Measured using a hand-held clinical-research grade spirometer connected to a mouthpiece in the sitting and upright position. An anesthesia mask may be used in place of a mouthpiece if the subject has too much bulbar weakness to create a sufficient mouth seal on the mouthpiece.

Defined as exhaled volume, in liters, immediately following a MI maneuver to maximum insufflation capacity.

8.1.6 MIC-assisted Peak Cough Flow

MIC-FVC difference will be obtained by asking the subject to perform a peak cough flow (as described above) from the MIC.

8.1.7 MIC-FVC difference

MIC-FVC difference will be obtained by subtracting the FVC from the MIC.

8.1.8 Transcutaneous carbon dioxide (TCO_2) and oxygen saturation (SpO_2)

Measured using a Sentec transcutaneous digital monitoring system while the subject is sitting upright in a chair or wheelchair. The transcutaneous sensor can be placed on the subject forehead, cheek, or earlobe.

Defined as the average value in mmHg (TCO_2) or in percentage of saturated hemoglobin (SpO_2) from a daytime in-clinic measurement over a 15-minute transcutaneous recording.

The Sentec transcutaneous digital monitor measures carbon dioxide, oxygen saturation, and pulse rate. It is standard of care in the Penn ALS clinic and would be offered to the patients as part of routine clinical care regardless of study enrollment.

8.1.9 Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R) dyspnea and orthopnea scores

The ALSFRS-R is a 12-item standardized questionnaire to assess the motor function status of an individual with ALS. It is a validated assessment used as part of standard of care and all clinical trials for ALS. The ALSFRS-R is assessed by a research staff member who has been certified for performance of the ALSFRS-R.

Specifically, the dyspnea score is one of the questions focusing on level of shortness of breath, scored on a scale of 0 to 4, with 4 being no symptoms at all and 0 being severely symptomatic with consideration of mechanical respiratory support.

The orthopnea score is one of the questions focused on breathing symptoms and difficulty sleeping while lying supine, scored on a scale of 0 to 4, with 4 being no symptoms at all and 0 being severely symptomatic with inability to sleep.

8.1.10 Global rate of change score for cough peak flow

The global rate of change score for cough peak flow will obtain a participant's subjective sense of efficacy of their cough. The global rate of change score for the cough peak flow will ask patients how effective they think their cough is today by rating it on a Likert scale from -7 (extremely impaired) to +7 (extremely strong).

8.1.11 Start date of non-invasive ventilation (NIV)

NIV start date will be defined by patient-reported date or as documented in the electronic medical record.

Defined by the time period between enrollment for the MI trial and start of NIV.

8.1.12 Tracheostomy-free survival time and death date

Death date will be determined by family report, electronic medical record review, or publicly available obituaries. To determine tracheostomy-free survival time, tracheostomy placement will be determined by patient or family report; or as documented in the electronic medical record.

8.1.13 ARC Connect data collection

Each subject will have daily usage data collected from the ARC Connect smartphone app. The usage data will be collected via reports from the app which will be downloaded at each clinical visit. The usage reports will include device usage data such as date/time of use, number of inhalation cycles, number of sets of inhalation cycles, and pressure used.

8.2 Safety and Other Assessments

8.2.1 Consent

Consent will be obtained for enrollment from participants. For each consent process, study personnel will discuss the details of the study, the risks and benefits, and the subject's rights and responsibilities if they choose to participate in the trial and their right to refuse to participate. It will be made clear that their clinical care will not be affected by their decision.

8.2.2 Institutional Review Board process

Study staff will obtain IRB approval before any study procedures are initiated.

8.2.3 Screening and enrollment

Study staff will screen participants who are seen at the study center and have a diagnosis of amyotrophic lateral sclerosis based on Gold Coast Criteria.[31]

Screening must occur within 3 months of enrollment.

The screening process will involve review of electronic medical records to review inclusion and exclusion criteria.

Eligible subjects will be approached either via telephone or during a routine clinic visit to the study center. When approaching patients, study staff will again confirm inclusion and exclusion criteria. Study staff will discuss a brief overview of the study. If the potential subject is interested, then the informed consent process will begin (see **Section 10.1.1 "Informed Consent Process"**).

8.2.4 Medical history

Medical history will be obtained through both a) speaking to a consenting subject and b) review of electronic medical records.

8.2.5 Physical exam

A physical exam will be performed by an attending physician. For the purposes of this study, it will either be a board-certified neurologist or pulmonologist.

8.2.6 Vital signs

Routine vital signs will be obtained by trained medical personnel.

8.2.7 Study assessments

Study assessments will be performed during study visits as described in **Section 12.1 Schedule of Activities**. Electronic medical record review and questionnaires will be performed by study staff who have Collaborative Institutional Training Initiative (CITI) training in human subjects research. Pulmonary function testing will be performed by qualified personnel trained to perform testing in accordance with clinical trial standards.[29] Results of all study assessments will be discussed with subjects.

8.2.8 Education of study intervention

Consenting subjects will be provided with a BiWaze cough device. Study staff will provide in-person hands-on training in performance of MI using the BiWaze cough device. Subjects will also receive additional education via MI performance both in writing and videos. Study staff will call subjects periodically to address any concerns or questions.

8.2.9 Assessment of study intervention adherence

Adherence to MI therapy sessions will be recorded on the BiWaze Cough system in the subject's home. BiWaze Cough stores the data in an encrypted log file which can be sent via WIFI to an online, HIPPA compliant data repository platform called Arc Connect (ABM Respiratory Care, USA). The clinicians can access the usage data through a secure online Arc Connect portal and review the subject's compliance to the MI therapy.

Reports will include: date/time of use, number of inhalation/exhalation cycles, number of sets of inhalation/exhalation cycles, and pressure used.

8.2.10 Assessment of adverse events

The use of MI in neuromuscular disease has been well tolerated without significant adverse event across multiple prior studies.[5–11]

We will avoid discontinuing MI for clinical events not thought to be serious MI-related AEs. For example, a hospitalization for clinical worsening will not result in cessation of trial participation. Such events could result in missing data for primary and secondary endpoints, comprising the integrity of the analysis. As the trial does not prohibit any therapies which are the standard of care in ALS, there is no ethical or safety reason to stop trial participation under such circumstances. Even if subjects are withdrawn from MI, outcome assessments will continue, allowing analysis by intent-to-treat.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

An adverse event (AE) is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related. Intercurrent illnesses or injuries should be regarded as adverse events.

A pre-existing condition should be recorded as an adverse event if the frequency, intensity or the character of the condition changes.

8.3.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Events (SAE)

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that, in the view of either the investigator or the sponsor, is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- an important medical event when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.
- required intervention to prevent permanent impairment or damage (for devices only)

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to

prevent one of the other serious outcomes noted above. For example, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious. All AEs that do not meet any of the criteria for serious should be regarded as **non-serious AEs**.

8.3.3 Unanticipated Adverse Device Effect (UADE)

Unanticipated Adverse Device Effect (UADE)

A UADE is any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.3.4 Classification of an Adverse Event

8.3.4.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.4.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to MI assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be considered.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to MI administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of MI (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of MI, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to MI administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of MI) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Unrelated** – The AE is completely independent of MI administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.4.3 Expectedness

The medical monitor will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for MI.

8.3.5 Time Period and Frequency for Event Assessment and Follow-Up

Safety will be assessed by monitoring and recording potential adverse effects using the grading system described above for adverse events (Definitely, probably, possibly, unlikely, or unrelated), at each study visit. Participants will be monitored by medical histories, physical examinations, and chart review. If the above-mentioned grading system does not apply for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator will seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events will be recorded in the source documentation. To the extent possible, adverse events will

be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event.

As much as possible, each adverse event or follow-up information will be evaluated to determine:

1. Severity grade (CTCAE Grade 1-5)
2. Duration (start and end dates)
3. Relationship to the study treatment or process – [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected) - is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment or process – [Unexpected – if the event severity and/or frequency is not described in the investigator brochure (if applicable) or protocol].
5. Action taken with respect to study or investigational treatment or process (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether the event is serious

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Unsolicited adverse events will be captured within case report forms for each study visit. Information collected on unsolicited adverse events will be collected as described above for adverse events.

Solicited adverse events will include targeted questions at each visit regarding the use of MI:

- Chest discomfort
- Dizziness
- Lightheadedness
- Sudden worsening shortness of breath

Data on solicited adverse events will also be captured within the case report form.

We will plan our data reporting system to avoid double capture of unsolicited and solicited adverse events.

Solicited and unsolicited adverse events will be assessed throughout study duration and follow-up after initiating the intervention (ie, days 0 through 180 for 6 months of follow-up).

8.3.6 Adverse Event Reporting

Reporting Period

Adverse events will be reported from the time of informed consent until study completion.

Investigator Reporting: Notifying the Study Sponsor

Every non-fatal SAE, regardless of suspected causality (e.g., relationship to study product(s) or study procedure(s) or disease progression) must be reported to the sponsor within 15 calendar days of learning of its occurrence.

Fatal or life-threatening SAEs which are probably or definitely related to death must be reported to the IRB and study sponsor (Jason Ackrivo) within 3 calendar days.

Serious and unexpected AEs which are fatal or life-threatening must be reported within 7 days to the NIH NHLBI.

Recurrent episodes, complications, or progression of the initial SAE must be reported to the Sponsor as a follow-up to the original episode within 10 business days of the investigator receiving the follow-up information. A SAE considered completely unrelated to a previously reported one should be reported separately as a new event.

Send the SAE report to the local IRB and NIH NHLBI.

New information regarding the SAE will be reported as it becomes available and in the same manner that the initial SAE (i.e. SAE form). The investigator must follow the event to resolution or until the event is deemed and documented irreversible, whichever is longer.

Disease-related events which would be expected in ALS include:

- Pneumonia
- Worsening shortness of breath
- Mucus plugging
- Weakness
- Fatigue

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the institution required form or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

The Primary Investigator is expected to provide as much of the following information as is available:

- Protocol name and number
- Subject identifiers
- Demographic data
- Nature of the event
- Severity of the event
- Probable relationship (causality) of AE to study procedure
- Date and time of AE onset Date and time of AE resolution, if available
- Concomitant medications that the participant was taking for an underlying medical condition or disease and the therapeutic agents used for the treatment of the adverse event Clinical assessment of participant conducted at time of SAE/AE
- Results of any laboratory and/or diagnostic procedures, and treatment
- Follow-up plan
- Outcome
- Autopsy findings (if appropriate)

The Principal Investigator and research coordinator will provide details about the AE as they become available. If additional information cannot be obtained for whatever reason, this will be documented.

The Principal Investigator should promptly determine an assessment of causality.

The Principal Investigator/designee should keep originals or photocopies of all relevant documentation, including facsimile confirmations and email exchanges, and file them in the participant's file.

The Principal Investigator/designee should file copies of all correspondence with the IRB in the appropriate section of the Regulatory Master File or site study file.

Investigator Reporting: Local Reporting Requirements

The investigator will report AEs and SAEs to the IRB/EC of record and other local regulatory groups per the local requirements.

8.3.7 Serious Adverse Event Reporting

The study clinician will immediately report to the sponsor any serious adverse event, whether or not considered MI related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that MI caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between MI and the event (e.g., death from pneumothorax). In that case, the investigator must immediately report the event to the sponsor.

New information regarding the SAE will be reported as it becomes available and in the same manner that the initial SAE (i.e. SAE form). All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

The SAE reports to the Sponsor should be sent to: Jason Ackrivo, MD, MSCE at the contact information listed in **Section 1.2 Key Roles and Study Governance**.

The study sponsor will be responsible for notifying the NIH and IRB of any unexpected fatal or life-threatening suspected adverse reaction per applicable regulations. In addition, the sponsor must notify NIH and all participating investigators of potential serious risks, from clinical trials or any other source, as per the applicable regulation.

The study investigator shall complete an Unanticipated Adverse Device Effect Form and submit to the study sponsor and to the reviewing Institutional Review Board (IRB) within 10 working days. The study sponsor is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the IRB, NIH, and Food and Drug Administration (FDA), (as applicable) and to all reviewing IRBs and participating investigators per the applicable regulation.

8.3.8 Reporting Events to Participants

Any AEs related to MI will likely occur at the time of or shortly after use of MI. Therefore, participants will likely be aware of the AE and will most likely be the primary source of AEs.

8.3.9 Events of Special Interest

Any device malfunction would likely be related to the integrity of the components in the BiWaze cough device. Participants may report a device issue (eg, malfunctioning device, crack in the corrugated tubing) to the research staff. If the BiWaze cough device or its accessory components

(tubing, mask, etc) are deemed to be defective beyond simple repair, then the participant will be provided with a replacement device or accessory component within 2 weeks.

8.3.10 Reporting of Pregnancy

Pregnancy, in and of itself, is not regarded as an AE. When a pregnancy has been confirmed in a subject, and the fetus is exposed to the physiologic effects of MI, then the research staff will withdrawal the subject from the study. Any data collected up to and including the day of pregnancy notification can still be used in the final analysis. The subject may continue to use MI at the discretion of the attending physician.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("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); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 Unanticipated Problem Reporting

Unanticipated problems (UPs) such as:

- Post-marketing withdrawal of a drug, device, or biologic used in a research protocol due to safety concerns.
- FDA ban of a drug, device, or biologic used in a research protocol due to safety concerns.
- Complaint of a participant when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team
- Breach of confidentiality
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study

- Premature closure of a study (e.g., due safety, lack of efficacy, feasibility, financial reasons, etc.)

should be reported by the investigator to the Sponsor, reviewing Institutional Review Board (IRB) and to the lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported as any other SAE.
- UPs that are not SAEs must be reported to the NHLBI, the IRB, and/or DCC within 14 days of the investigator becoming aware of the problem. All UPs that are not SAEs must be reported within 30 days to OHRP by the Penn IRB.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 30 days of the IRB's receipt of the report of the problem from the investigator.

8.4.3 Reporting Unanticipated Problems To Participants

Any UPs related to MI will likely occur at the time of or shortly after use of MI. Therefore, participants will likely be aware of the AE and will most likely be the primary source of AEs.

8.5 Device Reporting

Safety reporting for the device(s) will be according to 21 CFR 812.150.]

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

Our null hypothesis is in individuals with ALS there is no difference in peak cough flow between those who use MI versus those who do not use MI over 6 months.

Our primary endpoint is change in peak cough flow over 6 months compared to baseline.

Our Secondary Efficacy Endpoint(s) will be change in the following endpoints over 6 months:

- Forced vital capacity
- Maximal inspiratory pressure
- Maximal expiratory pressure
- Maximum insufflation capacity
- Maximum insufflation capacity minus vital capacity
- Maximum insufflation capacity assisted peak cough flow
- ALSFRS-R total score
- ALSFRS-R dyspnea score
- ALSFRS-R orthopnea score
- Partial pressure of transcutaneous carbon dioxide
- Oxygen saturation
- Global rate of change score for peak cough flow

Our tertiary endpoints include:

- Time to start of non-invasive ventilation
- Tracheostomy-free survival time

9.2 Sample Size Determination

Our outcome measure use for calculations is the change in peak cough flow (PCF).

We will base our calculations on results from the following study:

Kaminska M, Browman F, Trojan DA, Genge A, Benedetti A, Petrof BJ. Feasibility of Lung Volume Recruitment in Early Neuromuscular Weakness: A Comparison Between Amyotrophic Lateral Sclerosis, Myotonic Dystrophy, and Postpolio Syndrome. *PM&R* 2015;7:677–684.

Primary endpoint:
PCF:

In the study listed above, the ALS group had a change in PCF of (mean, 95% CI): -25.0 (-58.4, 8.4) L/min. This corresponds to a mean, standard deviation (SD) of -25 ± 26.9 L/min.

A mean ± SD of -25 ± 26.9 L/min can determine an effect size of 0.669 with a change in the mean by -18 to -43 L/min with the same SD of 26.9.

Assuming the effect size of 0.669, we estimate that 20 subjects will provide 80% power to detect a difference in PCF of at least 18 L/min with a SD of 26.9.

Secondary endpoints (selected):
FVC:
Assuming an effect size of 0.413, we estimate that 20 subjects will provide 42% power to detect a difference in FVC of 0.16 with SD of 0.387.

MIC:
Assuming an effect size of 0.451, we estimate that 20 subjects will provide 48% power to detect a difference in MIC of 0.188 with SD 0.417 using a two-tail t-test.

To obtain 20 subjects, assuming a 50% consent rate, we will approach 40 eligible patients. To attain 40 eligible patients, we will screen 60 total patients with incident diagnoses of ALS.

While there is adequate power to detect clinically important differences, the main purpose of this study is to obtain effect estimates in order to assist with the design of future larger studies.

9.3 Populations for Analyses

This is a single arm study of MI. We will screen, approach, and enroll subjects seen for regularly-scheduled clinic visits at the University of Pennsylvania Penn Comprehensive ALS center.

We will compare our findings to 20 matched control subjects who do not use MI who are enrolled for longitudinal data collection portion of the Philadelphia ALS (PALS) Cohort study. The PALS cohort study routinely collects peak cough flow (our primary endpoint) and select secondary/tertiary endpoints such as: FVC, ALSFRS-R scores, ALSSQOL-SF scores, time to start of non-invasive ventilation, and tracheostomy-free survival time.

9.4 Statistical Analyses

9.4.1 General Approach

Descriptive statistics will include mean ± standard deviation for parametric distributed variables and median (interquartile range for 25th, 75th percentile) for non-parametric distributed variables.

Hypothesis testing will use a two-sided $\alpha = 0.05$ without correction for multiplicity. We will summarize demographics along with baseline and follow-up primary and secondary endpoints.

We will characterize subjects with regard to baseline and follow-up primary and secondary endpoints. We will summarize demographics and other predictors of clinical status. Continuous variables will be summarized by the mean, median, standard deviation, and range, as appropriate. We will use contingency tables for discrete and dichotomous variables.

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

Our primary endpoint is decline from baseline in peak cough flow (PCF) over 6 months in individuals with early-stage ALS who have yet to start respiratory therapies. PCF is a continuous variable.

PCF is measured in in liters/minute. It is measured via use of one of 2 devices:

1) a peak flow meter while the patient is in a seated upright position. The patient is asked to inhale as deep as possible, then create a tight seal on the flow meter with their mouth, and then cough as quickly and forcefully as possible through the flow meter.
2) a handheld spirometer using the measured peak expiratory flow output. The technique is similar as described above for the peak flow meter. After completion of the maneuver, the peak expiratory flow is typically reported in L/seconds, and can be converted to peak cough flow units by multiplying by 60 to convert from liters/second to liters/minute.

To compare our intervention arm to the matched controls, we will compare means with unpaired two-tailed t-tests or Wilcoxon rank sum tests.

9.4.2.1 Missing data

We will attempt to minimize missing data, however we have planned for its occurrence. For subjects lost to follow-up, we will use all of the information available until the end of follow-up.

This protocol will continue to follow and perform test procedures as described even if a subject drops-out from the therapeutic portion of the study. That is, if a subject decides that he/she does not wish to continue with MI, the subject will stop the investigational treatment, but will still be strongly encouraged to continue to follow-up with the study personnel for all scheduled study procedures (e.g., peak cough flow, spirometry, etc.), so that missing data (and assumptions regarding these data) will be minimized.

9.4.3 Analysis of the Secondary Endpoint(s)

All secondary endpoints will be measured as described in Section 3, Study Objectives and Endpoints.

- Forced vital capacity is a continuous variable in Liters or as a percent of predicted normal compared to age, sex, and height-matched controls.
- Maximal inspiratory compacity – vital capacity difference is a continuous variable in Liters.
- Maximal inspiratory pressure is a continuous variable in cmH_2O.
- Maximal expiratory pressure is a continuous variable in cmH_2O.
- ALSFRS-R total score is a continuous variable reported as an integer.
- ALSFRS-R dyspnea score is an ordinal variable on a scale of 0, 1, 2, 3, or 4.
- ALSFRS-R orthopnea score is an ordinal variable on a scale of 0, 1, 2, 3, or 4.
- Global rate of change in peak cough flow is a Likert scale from -7 to +7.
- Partial pressure of transcutaneous carbon dioxide is a continuous variable measured in mmHg.
- Oxygen saturation is a continuous variable measured in % saturation of hemoglobin.
- Time to start of non-invasive ventilation is a continuous variable measured in days.
- Tracheostomy-free survival time is a continuous variable measured in days.

Exploratory multivariate analyses will be performed incorporating all of the available endpoint assessments (baseline, 2 months, 4 months, and six months) in an ANCOVA model with active treatment/control status as the independent variable.

9.4.4 Safety Analyses

All subjects will be assessed in the safety analysis. This analysis will include summary statistics of any adverse events. Safety interim analyses will be performed and reported to the medical monitor after subjects 5, 10, and 15 have completed study participation.

Subjects will be evaluated for SAEs.

Serious adverse events or events leading to premature discontinuation of study participation will be in the form of a table as follows:

Subject ID	Adverse event	Start date	Stop date	Duration	Severity	Relationship	Expectedness	Outcome

9.4.5 Baseline Descriptive Statistics

Baseline description variables will be separated by intervention arm (MI) and control (no MI) groups.

Variables will include:

- Age
- Sex at birth
- Race (patient-reported)
- Body mass index
- Diagnosis delay time between symptom onset and date of diagnosis
- Symptom onset site
- Forced vital capacity % predicted
- ALS Functional Rating Scale – Revised total score
- ALS Functional Rating Scale – Revised dyspnea score
- ALS Functional Rating Scale – Revised orthopnea score
- Smoking history
- Comorbidities

9.4.6 Planned Interim Analyses

Not applicable.

9.4.7 Sub-Group Analyses

Analysis of the primary and secondary endpoints will also be analyzed by the following subgroups:

- Symptom onset site (limb versus bulbar)

9.4.8 Exploratory Analyses

Not applicable.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 Consent/Assent and Other Informational Documents Provided To Participants

Consent forms describing MI in detail, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting MI. The following consent materials are submitted with this protocol:

- Informed consent document
- Telephone script for remote consenting
- One-page flyer advertising trial

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures

being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Consent will be obtained for enrollment from participants. Each study center will have the option of obtaining informed consent either in-person (pen and paper hard-copy signed consent) or via remote consent.

Remote consent

Remote consenting will be performed in advance of an eligible subject's regularly scheduled clinical visit and prior to any data collection.

At the University of Pennsylvania Penn Comprehensive ALS clinic, remote consent will be performed using REDCap. Eligible subjects will receive a phone call from the research coordinator in advance of their regularly scheduled clinic visit. The research coordinator will use a remote consent script to introduce themselves, describe the reason for the call, introduce the research, and ask whether the eligible subject is interested in hearing more about the study. If the eligible subject is interested in the study, then the research coordinator will send the subject a survey link via e-mail/text message that leads to a REDCap survey which contains an electronic copy of the latest IRB-approved and stamped informed consent form. While the eligible subject views the electronic consent, the research coordinator will read the consent to the patient over the telephone. If an eligible subject consents to participation, their consent will be documented via electronic signature within the REDCap survey e-consent. After signing, REDCap offers subjects the opportunity to download an electronic PDF version of the signed consent. Alternatively, the research coordinator can offer to provide the subject a hard copy of the signed consent by either a) mailing to the subject's home or b) providing a signed copy during a clinical visit.

As a reference, remote consenting procedures will mimic the procedures outlined in the Penn IRB guidance for remote consenting document located at the following link:

https://irb.upenn.edu/wp-content/uploads/2023/02/PM-Guidance-on-Remote-Consent-Procedures.pdf

For each consent process, study personnel will discuss the details of the study, the risks and benefits, and the subject's rights and responsibilities if they choose to participate in the study and their right to refuse to participate. It will be made clear that their clinical care will not be affected by their decision.

Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Only if the subject voluntarily agrees to sign the ICF and has done so, may they enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The subject will receive a copy of the signed and dated form for the main study (clinical data collection).

The signed informed consent statement (and verbal consent documentation to the semi-structured interview) are to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

The ICF and any other written information provided will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and/or the written ICF. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm his/her willingness to continue participation. Any revised written ICF and written information must receive the IRB's approval / favorable opinion in advance of use.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated by the Sponsor or the PI at any site if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the study participants, investigator, NIH NHLBI, and the IRB. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or are not evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and/or IRB.

In terminating the study, the Sponsor and the Principal Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored within REDCap. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by University of Pennsylvania Penn Comprehensive ALS Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived within REDCap and stored at the Penn Comprehensive ALS Center at the University of Pennsylvania.

<u>Telemonitoring protection of privacy:</u>

Adherence to MI therapy sessions will be recorded on the BiWaze Cough system in the subject's home. BiWaze Cough stores the data in an encrypted log file which can be sent via WIFI to an online, HIPPA compliant data repository platform called Arc Connect (ABM Respiratory Care, USA). The clinicians can access the usage data through a secure online Arc Connect portal and review the subject's compliance to the MI therapy.

Reports will include: date/time of use, number of inhalation/exhalation cycles, number of sets of inhalation/exhalation cycles, and pressure used.

ARC Connect's data privacy policy states that it does collect the following personal information:

- Name
- Email Address
- Phone Number

To help protect subject privacy: we will create subject accounts with altered contact information for each subject's profile in ARC Connect as follows:

Title	**Will be changed to:**
• Name ----------→	Subject ID number
• Email Address ----------→	An @pennmedicine account for research staff
• Phone Number ----------→	Penn ALS clinic research staff mobile number

Certificate of Confidentiality (if applicable)

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored within REDCap and at the Penn Comprehensive ALS Center at the University of Pennsylvania. After the study is completed, the de-identified, archived data will be transmitted to and stored within REDCap and at the Penn Comprehensive ALS Center at the University of Pennsylvania, for use by other researchers including those outside of the study. Permission to transmit data to REDCap will be included in the informed consent.

When the study is completed, access to study data and/or samples will be provided through REDCap.

10.1.5 Safety Oversight

Safety oversight will be under the direction of a Medical Monitor who is an individual with the appropriate expertise, including pulmonary care of individuals with chronic respiratory failure.

The Medical Monitor should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The Medical Monitor will meet at least semiannually to assess safety and efficacy data on each arm of the study. The Medical Monitor will operate under the rules of an approved charter. At this time, each data element that the Medical Monitor needs to assess will be clearly defined. The Medical Monitor will provide its input to the study sponsor, Dr. Jason Ackrivo, MD, MSCE.

10.1.6 Clinical Monitoring

Site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the PI and a Medical Monitor.
- On-site monitoring will occur monthly by the PI and semi-annually by the Medical Monitor in a targeted fashion.
- A Medical Monitor will be provided copies of monitoring reports on a semi-annual basis.
- Details of clinical site monitoring are documented in a Data Safety Monitoring Plan (DSMP). The DSMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits or compliance reviews may be conducted by a Medical Monitor semi-annually to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the DSMP.

10.1.7 Quality Assurance and Quality Control

All monitoring and audits are to be performed according to ICH GCP E6(R2).

The Penn Comprehensive ALS Center at the University of Pennsylvania will be the only clinical site for this study. The Penn Comprehensive ALS Center will perform internal quality management of study conduct, data collection, documentation, and completion. An individualized quality management plan will be developed to describe a site's quality management.

There will be a Investigator Initiation meeting prior to any subject enrollment to review study procedures, measurements, data collection, record keeping, and data entry expectations. The PI

will train the appropriate research staff (eg, research coordinator, nurse practitioner, physicians) on proper MI technique and methods for teaching patients/caregivers. Staff training will be tracked using Staff Training Log and Delegation of Responsibilities Log.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. The PI will perform QA and QC checks by reviewing case report forms, REDCap data, and clinical records such as EMR clinic notes and spirometry reports. PI review of data quality will occur at least every 3 months, or more frequently, if necessary. Any missing data or data anomalies will be communicated to the research coordinators and other research staff (as applicable) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The Penn Comprehensive ALS Center will provide direct access to source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.8 Data Handling and Record Keeping

10.1.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the Penn Comprehensive ALS Center under the supervision of the PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data and follow ALCOAC standards (attributable, legible, contemporaneous, original, accurate, and complete).

Hardcopies of the study visit worksheets (case report forms) will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded into REDCap derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) will be entered into REDCap, a 21 CFR Part 11-compliant data capture system provided by the University of Pennsylvania. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.8.2 Study Records Retention

Study documents should be retained for at least 2 years have elapsed since the formal discontinuation of MI. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.9 Protocol Deviations

Protocol deviations are not allowed. The PI will review data entry within REDCap at least every 3 months to monitor for protocol deviations. The PI and the study team should document all scenarios where the protocol is not followed and provide, in particular:

- Who deviated from the protocol
- What was the deviation
- When did the deviation occur
- How did the deviation happen
- What is the impact of the deviation
- A root cause analysis of why the deviation occurred

If the assessment results in a determination that any of the following are potentially affected, the deviation would be considered of significant impact:

- having the potential to adversely affect subject safety; OR
- increases risks to participants; OR
- adversely affects the integrity of the data; OR
- violates the rights and welfare of participants, OR
- affects the subject's willingness to participate in research.
- there is a potential for an overall impact on the research that should be shared with the IRB for consideration and development of next best steps to address it.

10.1.10 Publication and Data Sharing Policy

Full details of a publication policy will be described in the study MOP. The Penn Comprehensive ALS Center at the University of Pennsylvania is the only site in the study, therefore a data use agreement is unnecessary.

10.1.11 Conflict of Interest Policy

As of this writing, no study staff involved in this study have an active conflict of interest with BiWaze or any other respiratory device company. BiWaze is providing these devices in-kind as a loan for the duration of the study. The independence of this study from any actual or perceived influence, such as by respiratory device companies, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 Additional Considerations

Not applicable.

10.3 Protocol Amendment History

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by the sponsor, Dr. Jason Ackrivo. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB/IEC is notified within 5 days.

Any permanent change to the protocol must be handled as a protocol amendment. The written amendment must be submitted to the IRB/IEC and the investigator must await approval before implementing the changes. The sponsor, Dr. Jason Ackrivo, will submit protocol amendments to the appropriate regulatory authorities.

If in the judgment of the sponsor (Dr. Jason Ackrivo) the IRB/IEC, and/or the investigator, the amendment to the protocol substantially changes the study design and/or increases the potential risk to the subject and/or has an impact on the subject's involvement as a study participant, the currently approved written informed consent form will require similar modification. In such cases, informed consent will be renewed for subjects enrolled in the study before continued participation.

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale.

Version	Date	Description of Change	Brief Rationale
1	11.27.23	Initial submission	

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12 APPENDIX

12.1 Schedule of Activities (SoA)

Procedures	**Screening Day -7 to -1**	**Enrollment/ Baseline**	**Study Visit 2 Day 60 +/-14**	**Study Visit 3 Day 120 +/- 14 days**	**Final Study Visit 4 Day 180 +/-14**
Informed consent	X				
Demographics	X				
Contact information	X	X			
Medical history	X	X			
Social history	X	X			
Respiratory history		X			
MI teaching and technique review		X	X	X	X
Review MI usage data on ARC Connect app		X	X	X	X
Concomitant medication review	<~~~~~~~~~~~~~~~~~~X~~~~~~~~~~~~~~~~~~>				
Physical exam (including height and weight)		X	X	X	X
Vital signs		X	X	X	X
Height	X				
Weight		X	X	X	X
ALS symptoms and history		X			
Peak cough flow (PCF)		X	X	X	X
Forced vital capacity (FVC)		X	X	X	X
Maximal inspiratory pressure		X	X	X	X
Maximal expiratory pressure		X	X	X	X
Maximal insufflation capacity (MIC)		X	X	X	X
MIC-assisted PCF		X	X	X	X
MIC – FVC Difference		X	X	X	X
Transcutaneous carbon dioxide and oxygen saturation		X	X	X	X
ALS Functional Rating Scale-Revised scores		X	X	X	X
Global rate of change score for peak cough flow		X	X	X	X
Outcomes assessment (tertiary endpoints)			X	X	X
Adverse event review and evaluation		<~~~~~~~~~~~~~X~~~~~~~~~~~~~>			
Complete Case Report Forms (CRFs)	X	X	X	X	X

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