Protocol Title: Reducing Lung Cancer Survivors’ Anxiety (RELAX)

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Amendment/Update # & Date: Amendment 2: 01/12/15
Amendment 3: 03/05/15
Amendment 4: withdrawn
Amendment 5: 07/10/15 (staff change)
Amendment 6: 08/31/15
RELAX - SCHEMA

Pre-Screening
Score of ≥8 on the anxiety subscale of the HADS, Hemoglobin > 9.5

Randomization

Stratification
75 participants will be stratified by site and baseline self-reported dyspnea and randomized to 3 groups
25 participants per Group:

- Group A: The low-dose group will use the RESPeRATE device for 15 minutes once per day, at least 5 days per week for 12 weeks
- Group B: The high-dose group will use the RESPeRATE device for 15-minute twice per day, at least 5 days per week for 12 weeks.
- Group C: The Usual Breathing control group will use the RESPeRATE device (with chimes timed to the rate at which they are already breathing) for 15 minutes once per day, at least 5 days per week over 12 weeks.

Intervention
RESPeRATE device constantly synchronizes and automatically adjusts the melody/tones to the person’s breathing pattern to reduce breaths per minute by prolonging exhalation. It is the size of a portable compact disc player with a respiration sensor. Throughout the intervention, participants rate symptom intensity before and after using the devices.

Baseline Data Collection
Participants will be seen in clinic to receive their RESPeRATE device. The site coordinator will provide a demonstration on use of the device, assist participant with first use of the device and obtain baseline breathing data. The following forms will be completed: Self-Reported Dyspnea, Pulmonary Function Testing, Functional Fitness Testing, Anxiety/Depression, Fatigue, and Cough Questionnaires. Blood draw and salivary cortisol collection.

Weeks 1 – 11 Data Collection
Site coordinators will contact participants on a weekly basis by phone to assess use of the device, answer questions and collect breathing data for the prior 7 days stored on the device.

Week 6 (mid-intervention) Data Collection
In addition to the device breathing data, the following forms will be completed by phone and/or mail. Brief questionnaires to include self-Reported Dyspnea, Anxiety/Depression, Fatigue, Cough.

Week 12 Data Collection
Participants will be seen in the clinic for collection of final breathing data and to complete forms: Self-Reported Dyspnea, Pulmonary Function Testing, Functional Fitness Testing, Anxiety/Depression, Fatigue, and Cough Questionnaires. Blood draw, salivary cortisol collection. Participants will return their RESPeRATE device to the site coordinator.
Endpoints
Feasibility (accrual, participation, adherence, retention), Anxiety, Self-Reported Dyspnea, Pulmonary Function, Functional Fitness, Epigenetic and Gene Expression Changes, Salivary cortisol changes (for those who are eligible related to Supplemental Objective 1.5)

Stratification: Site; self-reported dyspnea
Study Sample: 75 participants (25 per group)
Study Duration: 12 weeks
Brief Eligibility Criteria:

- Histologic or cytologic diagnosis of primary non-small cell Stage 0-II lung cancer
- 2-12 months post-completion of surgery, radiation therapy and/or chemotherapy
- no further planned treatment during the 12-week study
- Score of ≥8 on the anxiety subscale of the HADS\(^1\)
- Eastern Cooperative Oncology Group performance status 0-2
- Hemoglobin ≥9.5

*Note: Whole blood will be collected for DNA (for epigenetic analysis) and RNA (for gene expression analysis) isolation (at baseline and Week 12). These data will be used as preliminary data to indicate whether DNA methylation and/or gene expression variation correlates with changes in anxiety. This added genomics piece is a supplemental study objective and will capitalize on the larger parent RELAX trial. Twelve participants from each group will be required for analysis.
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OBJECTIVES

1.1. To assess feasibility (accrual, participation, adherence, retention) of a randomized study of device-guided breathing in 75 post-treatment early-stage lung cancer survivors with significant anxiety.

1.2. To obtain preliminary data on the variability and efficacy of two doses of a device-guided breathing intervention versus a usual breathing control group for reducing anxiety (primary outcome) and for improving self-reported dyspnea and respiratory functioning (secondary outcomes) in post-treatment lung cancer survivors.

1.3 To select the optimal dose of the device-guided breathing intervention (15 minutes once/day or twice/day) for subsequent randomized study.

1.4 Supplemental Objective: To determine if changes in anxiety attributed to device-guided breathing are correlated with epigenetic (DNA methylation) and/or gene expression changes.

1.5 Supplemental Objective: To obtain preliminary data on changes in salivary cortisol (diurnal slope, cortisol awakening response, area under the curve) in each intervention group and associations between salivary cortisol and anxiety.

2. BACKGROUND

2.1a Early-Stage Lung Cancer & Anxiety

The number of early-stage lung cancer (ESLC) patients with improved prognosis who could benefit from symptom management interventions is likely to increase substantially with improved screening methods demonstrating a 20% reduction in mortality. New clinical practice guidelines stemming from the National Lung Cancer Screening Trial (NLST) promoting lung cancer screening among high-risk groups will likely increase lung cancer screening and result in more ESLC survivors with improved survival after earlier diagnosis. If clinical patterns reflect trial data, we expect 57% (versus 15% currently) of those diagnosed after screening to have localized disease. An estimated 8.7 million adults in the U.S. will be eligible for low-dose computed tomography (LDCT) screening according to NLST eligibility criteria, suggesting that the number of ESLC patients will increase dramatically.

Anxiety is a significant problem for lung cancer survivors. Anxiety symptoms are common in post-treatment cancer survivors, significantly more so than pure depressive symptoms. Compared with other cancer types, lung cancer patients have the highest rates of psychological distress and report a higher number of unmet psychological needs, including fears of recurrence, physical disability or deterioration, difficulty making long-term plans, feeling dependent and helpless, and preoccupation with being ill. Anxiety may increase in the year after lung cancer surgery, and recent studies have reported rates of clinically significant anxiety among lung cancer patients and post-treatment survivors ranging from 20-30%.

Anxiety commonly co-occurs with distressing physical symptoms in patients with lung cancer. The correlation between self-reported anxiety and dyspnea is estimated at 0.3. This relationship is bidirectional, reflecting affective and physiological processes. The number of lung cancer patients reporting dyspnea at some stage in their illness ranges from 55-90%.
ESLC survivors range from 40-60%. Thus, dyspnea commonly occurs with anxiety after lung cancer treatment and is associated with worse functioning in multiple domains, greater distress, and lower quality of life.

Breathing interventions have been effective at reducing anxiety/distress in cancer patients. Interventions focused on slow, deep breathing have decreased anxiety, stress symptoms, and physiological arousal in clinical populations and healthy adults. Relaxation training is effective for relieving anxiety in cancer patients. Breathing interventions have been incorporated into interventions to address dyspnea and related distress in lung cancer patients. Few studies have examined concomitant changes in anxiety/distress, and anxiety level has not been a criterion for study entry. Such multi-component interventions have yielded promising results for anxiety and distress related to breathlessness in post-treatment lung cancer survivors.

2.1b Background for Supplemental Objective: Epigenetic Changes & Anxiety

A number of recent studies have reported epigenetic changes associated with mental health and mood disorders. These have included post-traumatic stress disorder, depression, and suicide, and provide support for our hypothesis that epigenetic variation also contributes to changes in anxiety levels. Taken together, epigenetic variation in a number of individual genes and biological pathways has been identified for these conditions, and include inflammatory and immune related genes, as well as genes involved in the hypothalamic-pituitary-adrenal (HPA) axis.

2.1c Background for Supplemental Objective: Cortisol Changes & Anxiety

Cortisol is a glucocorticoid and a biomarker of stress that is released from the hypothalamic pituitary adrenal (HPA) axis. Studies within the psychoneuro-endocrinology literature have demonstrated a relationship between psychological distress and cortisol response. For example, studies have demonstrated an association between cortisol and depressive symptoms, post-traumatic stress disorder symptoms, and anxiety. Additionally, other similar psychosocial interventions have demonstrated changes in cortisol response. Collectively, these studies support our hypothesis that changes in anxiety, attributed to the device-guided breathing, will be associated with changes in cortisol response.

Participants with endocrine disorders (e.g., diabetes and thyroid disorders) or on steroid-based medications are excluded from the cortisol portion of the study.

2.2 Study Intervention

The RESPeRATE device constantly synchronizes and automatically adjusts the melody/tones to the person’s breathing pattern to reduce breaths per minute by prolonging exhalation. It is the size of a portable compact disc player with a respiration sensor (that attaches to an elastic belt worn over clothing) and a pair of headphones. The device is easy to use, and pilot participants have found it appealing. In our pilot work and other studies, attrition rates have been low, and adherence to the recommended rate of use has been high.

For the low-dose group, participants will use the RESPeRATE device for 15 minutes once a day, at least 5 days a week for 12 weeks based on previous research that found this dose to be effective in reducing blood pressure. For the high-dose
group, participants will use the RESPeRATE machine in 15-minute increments twice a day, at least 5 days a week for 12 weeks similar to the dose used in previous research conducted with other clinical populations with significant respiratory symptoms. An effective rate of slow breathing is considered to be <10 respirations/minute. The device automatically records use (i.e., accumulated duration, number of times used, average duration), initial breathing rate, and final breathing rate. This information will be used to measure intervention adherence.

2.3 Rationale for Intervention & Control Conditions

Device-guided breathing has not been studied in lung cancer survivors, yet it shows promising results in patients with chronic medical conditions and anxiety disorders. We propose use of a low-cost biofeedback device called RESPeRATE that can be used at home and guides patients to reduce their respiration rate and increase exhalation time. RESPeRATE has FDA approval as an adjunctive treatment for hypertension. Positive outcomes, including improved cardiorespiratory fitness and quality of life have been observed in heart failure patients, particularly in groups receiving longer treatment and in participants who were successful at increasing their inhalation/exhalation ratio. These studies did not assess anxiety or other psychological outcomes. A similar biofeedback device used in a single-arm pilot study for outpatients with anxiety disorders yielded significant reductions in anxiety and increased levels of relaxation, suggesting that this approach is promising for reducing clinically significant anxiety symptoms.

Despite high rates of psychological distress, no behavioral interventions identified in a recent meta-analysis specifically targeted lung cancer survivors, and very few (8%) were conducted with any post-treatment survivors. Research has identified the time after lung cancer treatment as particularly challenging, with reports of increased anxiety and uncertainty about the future. Intervening with post-treatment ESLC survivors with significant anxiety is important because psychological symptoms may receive less attention when contact with providers is less frequent. A recent review of psychosocial oncology interventions reported that only 5% of psychosocial oncology intervention studies addressed patients with significant distress, even though high distress patients are most likely to benefit.

The device-guided breathing intervention can be easily implemented at home with minimal instruction. This intervention could make behavioral treatments for anxiety more accessible to rural cancer survivors who experience mental health issues (including anxiety) at disproportionately higher rates than non-rural survivors and often lack access to mental health professionals. It could also be broadly applied in community-based cancer survivorship settings.

The proposed device-guided breathing intervention works by gradually slowing the respiration rate. Slow, deep breathing enhances parasympathetic tone, decreases acute and chronic sympathetic arousal, and reduces excitatory nervous activity, likely through cellular signaling activated by stretch receptors in the lungs.

The relaxation response occurs acutely, but indicators of chronic sympathetic over-activation such as resting hypertension also decrease. Deep breathing improves respiratory and cardiovascular function, decreases effects of stress, and improves mental health. Disordered respiration is thought to be a key aspect of anxiety and
stress-related disorders, thus slow breathing also reduces anxiety and potentially dyspnea. 

Participants in the usual breathing control group will use an identical-looking device but only the RESPeRATE intervention is targeted at reducing respiration rate. The chimes for the control device will be timed to the breathing rate at which the participants start their session, but will not gradually slow to reduce respiration rate.

3. SUMMARY OF STUDY PLAN

The RESPeRATE device constantly synchronizes and automatically adjusts the melody/tones to the person’s breathing pattern to reduce breaths per minute by prolonging exhalation. It is the size of a portable compact disc player with a respiration sensor.

This pilot study will have three groups: RESPeRATE device-guided breathing group (low-dose) (n=25), RESPeRATE device-guided breathing group (high-dose) (n=25), and a Usual Breathing control group (n=25).

The low-dose group will use the RESPeRATE device for 15 minutes once per day, at least 5 days per week for 12 weeks.

The high-dose group will use the RESPeRATE device for 15-minutes twice per day, at least 5 days per week for 12 weeks.

The Usual Breathing control group will use the RESPeRATE device (with chimes timed to the rate at which they are already breathing) for 15 minutes once per day, at least 5 days per week over 12 weeks.

After randomization, participants will be introduced to their assigned device and receive instructions and a demonstration on use. A baseline breathing assessment will be obtained. Participants will use the device at least 5 days per week for the assigned amount of time over 12 weeks.

All participants will complete pre- and post-intervention questionnaires, pulmonary function/functional fitness tests (at baseline and Week 12), telephone contact a weekly to provide breathing data for the prior 7 days, brief mid-intervention questionnaires (at Week 6), and rate symptom intensity daily while using the devices. Eligible participants will collect salivary cortisol samples for three days following the baseline and Week 12 follow-up. Participants will have a blood draw at the baseline and 12 weeks visits to determine if DNA methylation and/or gene expression variation correlates with anxiety changes. The duration of the study is 12 weeks.

Participants will be stratified by site and by baseline self-reported dyspnea score and assigned with equal probability to either intervention group or the control group using variable length permuted block randomization. Block sizes will be chosen randomly to ensure that future assignments cannot be inferred from previous ones.

Drs. Danhauer and Weaver will meet with designated staff members from each NCORP site for a training session to review the protocol and provide intervention information. This training will take place at the annual meeting of the WF NCORP RB and/or at individual sites, as needed.
4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Histologic or cytologic diagnosis of primary non-small cell Stage 0-II lung cancer
4.1.2 2-12 months post-completion of surgery, radiation therapy and/or chemotherapy
with no further planned treatment during the 12-week study
4.1.3 Score of ≥8 on the anxiety subscale of the HADS-Anxiety/Depression Scale \(^1\)
4.1.4 Eastern Cooperative Oncology Group performance status 0-2
4.1.5 Hemoglobin ≥9.5 (within 60 days prior to registration)
4.1.6 Smoking status anticipated to be stable during the study
4.1.7 Patients with mild to moderate COPD are allowed
4.1.8 Willing/able to attend brief introductory session and use assigned device for the
assigned period of time (15 minutes once or twice per day), at least 5 days per
week for 12 weeks
4.1.9 Age ≥18 years
4.1.10 Must have telephone

4.2 Exclusion Criteria

4.2.1 Patient does not understand English
4.2.2 Active lung infection
4.2.3 Radiation pneumonitis currently being treated with oral steroids
4.2.4 Unstable angina
4.2.5 Pulmonary embolism in past six months
4.2.6 Progressive cancer (must be considered no evidence of disease or stable)
4.2.7 Any change in psychotropic medications in past month
4.2.8 Hearing loss that would preclude participating in interventions. Adequate hearing
to participate will be determined via: (1) Response of “no” to the question [“Do you
have a hearing problem now?”] Participants with hearing aids will be allowed to
enroll as long as their hearing is adequate to hear the sounds on the study devices.
If necessary, potential study participants will receive a brief test trial with the
RESPeRATE device. If they indicate inability to hear the guiding tones, they will not
be enrolled in the study.

Cortisol Exclusion
4.2.9 Participants with endocrine disorders (e.g., diabetes and thyroid disorders) or on
steroid-based medications are excluded from the cortisol portion of the study.
4.3 Inclusion of Women and Minorities

*Both men and women (as applicable)* and members of all races and ethnic groups are eligible for this trial.

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4.4 Recruitment and Retention Plan

We plan to recruit 75 post-treatment lung cancer survivors through the WF NCORP RB. The CCCWF sees 328 lung cancer patients per year. Most patients complete treatment within 3-12 months of diagnosis; patients diagnosed in the past 24 months will be potentially eligible. We anticipate that approximately 280 survivors will be eligible for screening for this study (140 patients*2 years). Based on data from other studies, we anticipate that 25% will screen positive for anxiety (HADS-Anxiety/Depression Scale score ≥8), and 20% will meet all study criteria, leading to a potential 56 participants at WF NCORP RB alone. We will recruit participants over 15 months, with ≥5 participants/month as a highly achievable target accrual goal.

NCORP sites will accrue to this trial through the National Cancer Institute NCORP program. The WF NCORP RB will sponsor and administer this trial. The WF NCORP RB has infrastructure in place to conduct the trial, including a web-based, interactive database for online participant registration and data management.

Potential participants may be recruited via (1) in-clinic screening during medical appointments; (2) identifying ESLC survivors through the site cancer registry; (3) screening clinic charts; and (4) patient recruitment posters and recruitment letters.

Survivors will be approached in-person or by telephone by NCORP staff to ascertain interest and initial eligibility. We will track numbers of ESLC survivors approached and screened, reasons for nonparticipation, and number randomized.

We have targeted a racial/ethnic minority recruitment goal per the table above (consistent with national rates of ESLC). To reach this goal we will use several strategies: (1) We will work directly with oncology nurse navigators at each site to ensure that our clinic referrals include eligible minority patients and to enlist assistance in recruiting these patients. Their trusted relationships with our lung cancer patients will lend credibility to our participation request and commitment to protect confidentiality; and (2) We will attempt to recruit all potentially eligible minority lung cancer survivors identified through institutional cancer registries, but whom we cannot recruit in person or via another medical center provider, by direct mail or phone. We will review our mailed
recruitment materials to ensure that they are at an appropriate literacy level and are appealing to minority participants.

5. AGENT ADMINISTRATION

See Section 8. Protocol Specific Training Requirements

5.1 Adherence/Compliance

5.1.1 Adherence will be calculated as the actual amount of time the device is used divided by the prescribed time. Successful adherence will be defined as use of the device ≥75% of the time assigned. Note that all participants will be included in the primary analyses, regardless of adherence. A secondary analysis will include only those participants who were at least 75% adherent.

5.1.2 To determine intervention adherence, the RESPeRATE device tracks the number and duration of device uses and initial and final respiration rates.

6. DEVICE INFORMATION

6.1 Availability

The RESPeRATE devices are easily available and do not require any sort of prescription or permission to purchase.

A finger type pulse oximeter will be provided to the sites to measure oxygen saturation levels at the beginning and end of the six-minute walk tests. A measuring wheel will be provided to measure and record the distance participants walk during the six-minute walk.

6.2 Device Request and Distribution for Sites

Devices will be distributed to the sites from Wake Forest when study is IRB approved at their site. Upon completion of the last participant, the site will return the RESPeRATE, pulse oximeter, and measuring wheel devices to Wake Forest. See Section 6.6 for more information.

6.3 Device Accountability

Not applicable. The intervention is a simple and publicly available device. It is not a drug agent.
6.4 Registration/Randomization

6.4.1 Registration Process

A form 310 or IRB letter of approval and an IRB approved consent form must be received by the Research Base Protocol Information Office – Attn: Site Coordinator prior to patient registration. Fax: (336) 716-6275

Fill out Appendix 2, ‘Eligibility Checklist / Registration Form’. Use this to complete the on-line registration.

Online Registration

NCORP site staff will register their study participants into the WF NCORP RB database by following the instructions below:

Log on to the WF NCORP Research Base registration web site at <https://ccrbis.phs.wakehealth.edu>. Enter your user name and password (which may be obtained by contacting June Fletcher-Steede at jsteede@wakehealth.edu.) In the ‘Patient Registration and Protocol Information’ table, click the ‘Register Patient/Patient Info’, with the corresponding protocol number found in the drop down box to the right. Fill in the eligibility criteria forms using the drop down boxes. If further information is needed by Data Management, they will contact you. Once the patient information has been entered online print a copy of the eligibility checklist/registration form for your records. Press the submit button, a confirmation page will appear. Print this confirmation sheet for your records. The WF NCORP Research Base On-line Protocol Registration/Eligibility form, initial flow sheet, signed consent, histology reports, scan reports and lab reports (as required in protocol) should be faxed to (336) 713-6476 or mailed to Data Management:

Wake Forest School of Medicine
WF NCORP Research Base
Data Management Center
2000 West First Street, Suite 101
Winston-Salem, NC 27104

These forms should be retained in the patient’s study file. These forms will be evaluated during an institutional WF NCORP Research Base site member audit.

If you have questions related to the registration process or require assistance with registration, please contact the WF NCORP Research Base DMC between 8:30am and 4:00pm EST, Monday through Friday at (336) 713-3172 or 713-6507.

6.4.2 Randomization Process

Patients will be stratified by site and by baseline dyspnea score (Cancer Dyspnea Scale score ≥8 v. <8; see Table 7.8) and assigned with equal probability to either RESPeRATE intervention group or the control group using variable length permuted block randomization. Block sizes will be chosen.
randomly to ensure that future assignments cannot be inferred from previous ones. Patients need to report a minimum level of clinically significant anxiety symptoms (HADS-Anxiety/Depression Scale anxiety subscale ≥8) to enter the study.

6.5 Unblinding Methods

Not applicable.

6.6 Device Return

Study participants will return any study devices to the site that issued them. All sites must return study devices to the study PI at Wake Forest to:

Suzanne Danhauer, Ph.D.,
Wake Forest School of Medicine,
Department of Social Sciences & Health Policy
Division of Public Health Sciences
Medical Center Boulevard
Winston-Salem, NC 27157

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Participants will be randomized to one of three groups: RESPeRATE device-guided breathing group (low-dose) (n=25), RESPeRATE device-guided breathing group (high-dose) (n=25), and a Usual Breathing control group (n=25).

The low-dose group will use the RESPeRATE device for 15 minutes once a day, at least 5 days per week for 12 weeks.

The high-dose group will use the RESPeRATE device for 15-minutes twice a day, at least 5 days per week for 12 weeks.

The Usual Breathing control group will use the RESPeRATE device (with chimes timed to the rate at which they are already breathing) for 15 minutes once per day, at least 5 days per week over 12 weeks.

After randomization, participants will be introduced to their assigned device and receive instructions and a demonstration on use. A baseline breathing assessment will be obtained. Participants will use it at least 5 days per week for the assigned amount of time over 12 weeks. All participants will complete pre- and post-intervention questionnaires, pulmonary function/functional fitness tests (at baseline and Week 12), telephone contact weekly to provide breathing data for the prior 7 days, brief mid-intervention questionnaires by mail and/or telephone contact at Week 6, and rate symptom intensity daily while using the devices. Eligible participants will collect salivary cortisol samples for three days following baseline and the Week 12 follow-up. Participants with endocrine disorders (e.g., diabetes and thyroid disorders) or on steroid-based medications are excluded from the cortisol portion of the study. Participants will have a blood draw at the baseline and 12 week visits, to determine if DNA methylation...
and/or gene expression variation correlates with anxiety changes. The duration of the study is 12 weeks.

The NCORP staff member will instruct each participant in use of the RESPeRATE device at baseline. Participants will be told that RESPeRATE analyzes a person’s breathing pattern and plays 2 distinct tones which guide the person to inhale and exhale. The RESPeRATE control device looks and sounds identical but is not programmed to gradually slow breathing rate as in the active intervention arm. All participants will be provided with written instructions for the assigned device and will be observed using the device during the visit. A baseline breathing assessment will be obtained. To enhance adherence, we will have a NCORP staff member call participants weekly to assess experience with the device, troubleshoot difficulties that may have arisen and obtain breathing data for the prior 7 days. Participants who have not been using the device at the recommended frequency will trouble-shoot ways of overcoming barriers to adherence, as needed.

Self-reported dyspnea and pulmonary function/functional fitness measures include questionnaires, pulmonary function tests (PFTs), and a 6-minute walk. A heart rate and oxygen saturation levels using a finger type pulse oximeter (provided) will be obtained at the beginning and end of the six-minute walk. A measuring wheel will be provided to measure and record the distance participants walk during the six minute walk.

These measures will be administered in-person at the NCORP site during baseline and post-intervention (Week 12) assessments. A shorter battery will be administered mid-intervention via mailed paper questionnaire with a stamped return envelope enclosed (Week 6). Any adverse event related to the intervention or the blood draw will be reported to the WF NCORP RB at the time they become known to the NCORP site. (See Section 10.0)

7.2 Pre-Screening Evaluation/ Baseline Testing

Pre-screening testing:
Score of ≥8 on the anxiety subscale of the HADS-Anxiety/Depression Scale is required for eligibility. The full HADS (including the depression subscale) will be administered either over the phone or in person. Scores will be used as the baseline value to minimize burden by administering the measure again within a short time interval.

- The HADS-Anxiety/Depression Scale is a 14-item self-report measure that is scored by assigning a numeric value to each of the 14 items (0, 1, 2, or 3), and then totaling item scores. The minimum score is 0 and the maximum score is 42. Anxiety and depression subscales each consist of the sum of 7 questions (range 0-21 for each subscale). A score > 8 on the anxiety subscale indicates significant anxiety. Some items may be left blank. In this case, scores will be calculated as long as more than half the items are answered. The scores will be calculated as the mean of the responses times the number of items in the scale (7 for the subscales and 14 for the overall scale).
- Eastern Cooperative Oncology Group performance status 0-2
- Hemoglobin ≥9.5 within 60 days prior to registration. If a routine hemoglobin is available in the participants medical record this is acceptable. If a routine hemoglobin is not available, a blood sample of approximately 1 teaspoon will be taken from a vein in the arm/central line for lab analysis.
Baseline testing:
- Hospital Anxiety/Depression Scale (HADS) (use form completed for screening)
- Cancer Dyspnea Scale (CDS) **will be needed for registration**
- PROMIS Fatigue
- Manchester Cough in Lung Cancer Scale
- Activities form
- Six-Minute Walk (Heart rate and O₂ Sat pre and post walk)
- Current medications
- Participant expectations rating
- Pulmonary function testing [forced expiratory volume in one second (FEV1)]
- Visual Analogue Scales (VAS) for distress, anxiety and dyspnea - This form should be completed. Pre- and post- each practice session (each use of the device) ≥ 5 days/week from Weeks 1-12
- Blood draw - a blood sample of approximately 2 teaspoons will be taken from a vein in the arm/central line for DNA methylation levels and RNA.
- Salivary cortisol collection- saliva samples will be collected by participants at time of awakening, 30 minutes post-awakening, and at bedtime for three consecutive days (9 samples per participant).

7.3 Evaluations during Study Intervention at Weeks 1 - 11

Weeks 1 - 11:
- Weekly Breathing Assessment Form via telephone interview
  The participant will be contacted by phone to assess his/her experience with the device and troubleshoot difficulties that may have arisen and obtain breathing data for the prior 7 days. Participants who have not been using the device at the recommended frequency will troubleshoot ways of overcoming barriers to adherence, as needed.

Week 6:
- In addition to phone contact, participants are to complete the following questionnaires (may be done via mail):
  - Hospital Anxiety/Depression Scale (HADS)
  - Cancer Dyspnea Scale
  - PROMIS Fatigue
  - Manchester Cough in Lung Cancer Scale
  - Activities form
  - Weekly Breathing Assessment forms (via telephone)

7.4 Evaluations at Completion of Study Intervention

Week 12:
- Hospital Anxiety/Depression Scale (HADS)
- Cancer Dyspnea Scale (CDS)
- PROMIS Fatigue
- Manchester Cough in Lung Cancer Scale
- Activities form
- Six-Minute Walk (Heart rate and O₂ Sat pre and post walk)
- Current medications
- Collect Weekly Check-In Call/Data Capture from Device forms
7.5 Methods for blood draw and transport

- For detection of DNA methylation levels:

  Genomic DNA will be isolated from whole blood (one 8 ml yellow-top, ACD tube) from 12 participants in each intervention group, at baseline and at the Week 12 follow-up visit. Once collected, Dr. Timothy Howard’s lab will be notified by phone at (336) 713-7509. For participants from the Wake Forest site, the blood will be stored at room temperature until personnel from the Center for Genomics & Personalized Medicine Research can transport the sample to the laboratory. For participants from other sites, blood samples need to be sent via overnight shipping packed in several ice packs. DNA will be isolated using the AutoPure LS (Qiagen, Inc.), and then bisulfite-converted using the EZ DNA Methylation Gold kit (Zymo, Irvine, CA). To determine the proportion of DNA methylation at each of over 485,000 CpG sites, we will use the HumanMethylation450 BeadChip (Illumina, Inc.) along with the iScan Reader (Illumina, Inc.).

- RNA:

  RNA will be isolated from whole blood collected in one 4 ml tube using the PAXgene Blood RNA System (Qiagen), following the manufacturer’s instructions. The PAXgene system stabilizes the RNA and minimizes degradation, a process that can lead to false results in subsequent analysis. As with the DNA, the whole blood for RNA will be stored with the tube upright at room temperature until transported to the laboratory. RNA will be isolated with the PAXgene Blood RNA System (Qiagen), following the manufacturer’s instructions. Once isolated, RNA will be evaluated for quality and quantity using the RNA 6000 Nano chips and analyzed on the Agilent 2100 Bioanalyzer (Agilent Technology, Inc). Expression profiles will be generated with HumanHT-12 v4 Expression BeadChips (Illumina, Inc.), which assay over 47,000 probes spanning approximately 30,000 genes. BeadChips will be scanned with the iScan Reader (Illumina, Inc.), and preliminary analysis will be performed with Genome Studio (Illumina, Inc.).

- Methods for cortisol collection, assays, and mailing

  Participants with endocrine disorders (e.g., diabetes and thyroid disorders) or on steroid-based medications are excluded from the cortisol portion of the study.

  Materials for saliva collection will be distributed to participants in person at baseline. Participants will collect saliva samples three times a day (at awakening, 30 minutes post-awakening, and bedtime) for three consecutive days following baseline and three consecutive days following the Week 12 follow-up visit. Saliva
samples are collected by placing a cotton roll under the tongue for approximately 1-2 minutes which is subsequently stored in a plastic tube and refrigerated. Participants will be instructed to refrain from eating, drinking, smoking, brushing their teeth, using mouthwash, or engaging in exercise or similar physical activity for 30 minutes prior to saliva collection. Participants will be provided with saliva collection diaries to record compliance to these behaviors as well as the time in which their saliva samples were collected. After all nine samples have been collected at each time point (baseline, Week 12 follow-up), participants will use pre-paid postage to return their samples and saliva collection diaries to the Wake Forest study site. Once received at the study site, all samples will be refrigerated (-80°C) until assayed.

Samples will be assayed in duplicate at Wake Forest using The Salimetrics® Cortisol Enzyme Immunoassay Kit (State College, PA). At no time in the testing process are samples identified by name of subject or any information that would link the sample directly to an individual. On the day of analysis, samples are thawed at room temperature (20 to 22°C), centrifuged (1500 x g) for 15 minutes and assayed. The test used 25 μL of saliva per determination, has a lower limit of sensitivity of 0.003 μg/dL, standard curve range from 0.012 μg/dL to 3.0 μg/dL, an average intra-assay coefficient of variation of 3.5% and an average inter-assay coefficient of variation of 5.1%. Method accuracy determined by spike and recovery averaged 100.8% and linearity determined by serial dilution averaged 91.7%. Values from matched serum and saliva samples show the expected strong linear relationship, r (47) = 0.91, p < 0.0001.

Samples are returned to the freezer upon completion of pipetting. Assay data are reviewed by the supervisor and samples needing to be retested are identified. Samples needing retesting are again thawed, analyzed and refrozen. After assays are complete, samples will be stored for up to 60 days and then disposed of per applicable waste handling regulations.

7.6 Post-intervention Follow-up Period

Not applicable.

7.7 Methods for Clinical Procedures

Not applicable.
### 7.8 Study Parameter Table

<table>
<thead>
<tr>
<th></th>
<th>Pre-Screen</th>
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<td>Manchester Cough in Lung Cancer Scale</td>
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<td>Activities Form</td>
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<td>Early Withdrawal Form</td>
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<td>If patient withdraws prior to completion of study.</td>
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<td>RNA/DNA Labs (WF Only)</td>
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<td>Salivary cortisol collection*</td>
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<td>** Visual Analogue Scales for Distress, Anxiety, and Dyspnea</td>
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</tbody>
</table>

*Participants with endocrine disorders (e.g., diabetes and thyroid disorders) or on steroid-based medications are excluded from the salivary cortisol portion of the study.
7.9 Off Treatment Criteria
Participants may stop using the device for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate device functioning, noncompliance, medical contraindication.

Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. Participants will not be replaced for discontinuing the device.

7.10 Off Study Criteria
Participants may go ‘off-study’ for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death.

We anticipate an approximate 20% attrition rate, and the total sample size has been adjusted accordingly.

8. Protocol Specific Training Requirements

8.1 Specific Training Procedures or Certification Procedure
Several methods will be used in training sites. A training session will be held at the annual Research Base meeting and/or other meetings as designated by Drs. Danhauer and Weaver.

9. SPECIMEN MANAGEMENT
Not applicable.

10 Reportable Adverse Events/Serious Adverse Events

Only report unexpected serious adverse events that are related to the use of the breathing device used in this study. Report only unexpected hospitalizations, grades 4 and 5 that are related or not related to the use of the breathing device.

- Adverse Event/Serious Adverse Event reporting begins after the informed consent is signed. Serious Adverse Events occurring within 30 days of study completion must be reported via FDA Form 3500 (MedWatch).
11. STUDY MONITORING

11.1 Data Management Schedule

The Eligibility checklist/Registration Form should be completed on-line prior to placing the patient on study. Data forms will be submitted to the WF NCORP Research Base. See Section 6.4.1 for mailing address, or fax to (336) 713-6476.

<table>
<thead>
<tr>
<th>Form</th>
<th>Submission Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Form</td>
<td>Pre-Screen or Baseline, Week 6, Week 12</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>Baseline</td>
</tr>
<tr>
<td>Cancer Dyspnea Scale (CDS)</td>
<td>Baseline, Week 6, Week 12</td>
</tr>
<tr>
<td>Flow Sheet/Addenda</td>
<td>Baseline, Week 6, Week 12</td>
</tr>
<tr>
<td>Current Medications</td>
<td>Baseline, Week 12</td>
</tr>
<tr>
<td>Pulmonary Function Tests (FEV1)</td>
<td>Baseline, Week 12</td>
</tr>
<tr>
<td>Minute Walk (Heart rate and O₂ Sat pre and post walk)</td>
<td>Baseline, Week 12</td>
</tr>
<tr>
<td>Participant Expectations</td>
<td>Baseline</td>
</tr>
<tr>
<td>PROMIS Fatigue</td>
<td>Baseline, Week 6, Week 12</td>
</tr>
<tr>
<td>Manchester Cough in Lung Cancer Scale</td>
<td>Baseline, Week 6, Week 12</td>
</tr>
<tr>
<td>Activities Form</td>
<td>Baseline, Week 6, Week 12</td>
</tr>
<tr>
<td>Weekly Check-In/Call Data Capture from Device</td>
<td>Baseline, Weeks 1 - 12</td>
</tr>
<tr>
<td>Visual Analog Scales</td>
<td>Week 6, Week 12</td>
</tr>
<tr>
<td>RNA/DNA Blood Draw</td>
<td>Baseline, Week 12</td>
</tr>
<tr>
<td>Intervention Feedback</td>
<td>Week 12</td>
</tr>
<tr>
<td>Salivary Cortisol Collection</td>
<td>Baseline, Week 12</td>
</tr>
<tr>
<td>Early Withdrawal Form</td>
<td>Upon withdrawal from active treatment or consent</td>
</tr>
<tr>
<td>Decline Form (Eligible and Ineligible participants)</td>
<td>Monthly</td>
</tr>
</tbody>
</table>

11.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF).

11.3 Source Documents

Source documents are the original signed and dated records of participant information (e.g., the medical record, shadow chart) which may include electronic documents containing all the information related to a participant’s protocol participation. Source documents are used to verify the integrity of the study data, to verify participant eligibility, and to verify that mandatory protocol procedures were followed. An investigator and other designated staff are required to prepare and maintain adequate and accurate documentation that records all observations and other data pertinent to the investigation for each individual participating in the study. All data recorded in the research record (including data recorded on CRFs) must originate in the participant’s medical record, study record, or other official document sources.

Source documents substantiate CRF information. All participant case records (e.g., flow sheets, clinical records, physician notes, correspondence) must adhere to the following standards:
Clearly labeled in accordance with HIPAA practices so that they can be associated with a particular participant or PID;

Legibly written in ink;

Signed and dated in a real time basis by health care practitioner evaluating or treating the participant; and

Correction liquid or tape must not be used in source documents or on CRFs.

Corrections are made by drawing a single line through the error. Do not obliterate the original entry. Insert the correct information, initial, and date the entry.

All laboratory reports, pathology reports, x-rays, imaging study and scans must have:

Complete identifying information (name and address of the organization performing, analyzing, and/or reporting the results of the test); and

Range of normal values for each result listed.

11.4 Data and Safety Monitoring Board

The Data Safety Monitoring Board meets every six months to review all phase II and phase III protocols. The Board includes members demonstrating experience and expertise in oncology, biological sciences and ethics. The DSMB report is generated by the statistician and includes a summary of accrual, adherence, and retention, descriptive statistics for baseline characteristics and patient status, descriptions of all adverse events and toxicities, estimates of data completeness, a summary of the primary and secondary outcome measures.

11.5 Record retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with HIPAA, OHRP, FDA regulations and guidance, and NCI/DCP requirements unless the standard at the site is more stringent.

Record retention should be 5 years after the study is discontinued for studies without an IND (21 CFR 312.62).

11.6 CDUS Reporting

The CCCWFU NCORP Research Base Data Management Center will submit quarterly reports to DCP/CTEP by electronic means using the Clinical Data Update System (CDUS).
12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

**Objectives:** The objectives of this pilot study are:

1) To evaluate the feasibility of conducting a randomized phase III study to test the efficacy of device-guided breathing in post-treatment ESLC survivors with significant anxiety. Feasibility will be assessed using measures of accrual, participation, adherence, and retention.

2) To collect preliminary data on the variability and efficacy of two doses of a device-guided breathing intervention versus a usual breathing control group for reducing anxiety (primary outcome) and for improving self-reported dyspnea and respiratory functioning (secondary outcomes) in post-treatment lung cancer survivors.

3) To select the optimal dose of the device-guided breathing intervention (15 minutes once/day or twice/day) for a subsequent randomized study. The optimal dose will be defined as the one that results in the greatest improvement in anxiety scores at 12 weeks.

A parallel, randomized pilot study will be conducted to address these objectives. Eligible patients will be stratified by site and initial dyspnea level and randomized with equal probability to high dose device-guided breathing, low-dose device-guided breathing, or a usual breathing control. Study endpoints include anxiety (primary outcome: anxiety subscale of the HADS-Anxiety/Depression Scale), dyspnea, and respiratory function. These constructs will be measured at baseline and 6 and 12 weeks post-randomization. Estimates of treatment efficacy will be obtained using the 'intent to treat' approach. That is, all randomized patients will be included in the primary analyses whether or not they were treated according to protocol.

4) **Supplemental Objective:** To determine if changes in anxiety attributed to device-guided breathing are correlated with epigenetic (DNA methylation) and/or gene expression changes.

5) **Supplemental Objective:** To obtain preliminary data on changes in salivary cortisol (diurnal slope, cortisol awakening response, area under the curve) in each intervention group and associations between salivary cortisol and anxiety.

12.2 Sample Size/Accrual Rate

The sample size for this trial will be based on statistical selection theory criterion as described by Simon et al. For a selection trial, one simply chooses the regimen that results in the ‘best’ response. While Simon used this idea in the context of choosing the regimen with the best tumor response, the idea is applicable to outcomes with other distributions (in addition to the binomial). In our trial, the response is self-reported anxiety, which we will assume is normally distributed, so the ‘best’ regimen will be the one with the lowest mean anxiety score. We will proceed to a phase III trial if one of the RESPeRATE intervention arms results in the least anxiety, and the mean anxiety score in that group is at least 0.5 standard deviations (SD) less than mean anxiety score in the control group.
The sample size is chosen to ensure a high probability of selecting the ‘best’ arm, assuming that the mean anxiety score in the low dose arm is 0.5 SD less than the mean in the control group and the mean anxiety score in the high dose arm is 1.0 SD less than the mean in the control arm. Under these assumptions, we used simulations to determine the sample size needed in each group to ensure that the probability of selecting the best regimen was 90%. We randomly selected n independent observations from the normal distribution with an SD = 1, calculated the mean anxiety scores for each group, and selected the group with the least observed mean. This was repeated 200,000 times. The probability that the ‘best’ regimen was chosen is calculated as the proportion of times (of the 200,000 repetitions) that the group with the true lowest mean was selected. In addition to selecting the ‘best’ regimen, we will only pursue a subsequent phase III trial if there is some evidence of effect. Thus, we will require that the mean in the ‘best’ regimen is at least 0.5 SD lower than the mean for the control group. Again using simulations, we find that 20 patients per group provides 90% probability that the ‘best’ regimen will be chosen and the mean in that group be at least 0.5 SD lower than the mean in the control arm. Assuming a 20% dropout, we will accrue a total of 75 participants to this study (20x3/.8). Assuming no effect of the RESPeRATE device, we have 90% probability of stopping with the phase II trial.

12.3 Randomization and Stratification

Patients will be stratified by site and by baseline dyspnea (0-7 versus 8+ for CDS total score) and assigned with equal probability to either RESPeRATE group or the control group using variable length permuted block randomization. Block sizes will be chosen randomly to ensure that future assignments cannot easily be inferred from previous ones. There will be no interim analyses for this pilot study, and analyses will not be done separately for each stratum.

12.4 Primary Endpoint(s)

The primary objective of this study is to collect data that will allow us to assess the feasibility of conducting a phase III randomized study of device-guided breathing in ESLC survivors with significant anxiety. These endpoints include: participation, accrual, adherence, and retention.

The participation rate will be calculated as the proportion of eligible patients who agree to participate.

The accrual rate will be calculated as the number of patients accrued to the study divided by the number of months of accrual.

The actual amount of time the participant used the device will be retrieved from each device. Adherence will be calculated as the actual amount of time the device is used divided by the prescribed time. Successful adherence will be defined as use of the device ≥75% of the time assigned. We will also estimate the proportion of patients who use the device more than prescribed. (If “low-dose” participants use the device more than prescribed, this may limit our ability to detect a dose effect.)

Retention will be calculated as the number of participants who complete the final assessment divided by number randomized.
Confidence intervals for the binomial proportions will be calculated using methods described by Wilson.\textsuperscript{78} Approximate 95% confidence intervals based on the normal distribution will be calculated for the continuous measures.

The clinical endpoints are anxiety (primary outcome: anxiety subscale of the HADS-Anxiety/Depression Scale), dyspnea, and pulmonary function at baseline and 6 and 12 weeks post-randomization. Estimates of treatment efficacy will be obtained using the ‘intent to treat’ approach. Descriptive statistics (means, standard deviations, frequencies, etc.) will be presented for each outcome measure at baseline and follow-up points stratified by treatment arm. Repeated measures (RM) ANCOVA models (fitted using PROC MIXED in SAS) will estimate the treatment effect for each outcome, test for treatment differences, and obtain adjusted post-treatment estimates of the variability of these outcomes. Estimates of outcome measure variability will be used to determine sample size for the subsequent trial. The primary RM ANCOVA model will include time, treatment arm, and the stratification factors. An unstructured covariance matrix will be used to account for the within-patient correlation over time. In secondary models, additional covariance structures (Toepplitz, Autoregressive, Compound Symmetry) will be assessed and the optimal structure will be chosen based on likelihood ratio tests for nested structures and the BIC statistic for non-nested structures. Least squares means and 95% confidence intervals will be provided for each outcome, stratified by treatment arm, and for the difference between treatment arms. Regression diagnostics and residual plots will be used to find appropriate transformations for variables in the model. Subsequent RM ANCOVA models will include additional covariates (e.g., age, sex, stage) to correct for chance imbalances in important prognostic variables and account for variability in outcome measures due to covariates. These latter models will inform us of possible stratification factors for the subsequent trial. Limited exploratory analyses will be done to determine if the treatment effect differs for different levels of covariates (e.g., by including treatment by covariate interactions in the model) to see if the subsequent trial should be done in particular subsets of ESLC survivors. It is likely that some data will be missing. In addition to the mixed models above, we will use multiple imputation pattern-mixture models under various assumptions (e.g., participants in the active arms who drop out would have subsequent patterns similar to participants in the control arm) to assess the sensitivity of our modeling assumptions.

The optimal dose will be defined as the one that results in the greatest improvement in anxiety scores at 12 weeks (based on estimates from the RM ANCOVA model). This dose will be used in the subsequent trial, assuming that it is one of the RESPeRATE intervention groups.

Assuming the 95% confidence interval for the treatment effect includes a clinically meaningful difference, the sample size for the subsequent phase III trial will be determined based on detecting a clinically meaningful difference with a high power using estimates of variability obtained in this study. The estimates of retention and adherence observed in this study will be used to refine that sample size estimate. Subsequent trial feasibility will be assessed based on our ability to recruit the required number of participants. For that, we will use the accrual and participation rates observed in the participating sites. For sites that did not participate in this pilot study, accrual will be estimated by multiplying the estimated number of eligible patients times the participation rate across the participating sites.
12.5 **Secondary Endpoint(s)**

**Epigenetic and Gene Expression Analyses.**
To characterize the DNA methylation and gene expression patterns between the different groups, mixed effects models will be used. Changes of methylation or expression levels will be treated as the dependent variables and the intervention groups will be the independent variable of interest. Baseline methylation or expression levels, age, chip, chip position, and other covariates will be adjusted in the model. After comparing the fit of these data at a set of 20 random CpG markers (for the methylation analysis only), the alternative paired difference between baseline and twelve weeks (percent change dividing the difference by baseline value) will be examined to see if it provides a better fit to these data.

To determine if changes in anxiety are associated with methylation or gene expression patterns, mixed effects models will be used, and changes in anxiety variables will be treated as the dependent variables. Baseline methylation or expression, change of methylation or expression, intervention group, and interaction between intervention group and change of methylation or expression will be included in the model, along with the appropriate covariates. If the interaction effect is significant, it means that the association between the change in anxiety and change in methylation or expression proportion is not the same between the two exposure groups. Multiple comparisons will be adjusted using Bonferroni correction as before.

**Salivary Cortisol Analyses.** Participants with endocrine disorders (e.g., diabetes and thyroid disorders) or on steroid-based medications are excluded from the cortisol portion of the study. Cortisol levels will be assessed upon awakening, 30 minutes post-awakening, and at bedtime for three consecutive days at baseline and three consecutive days at 12 weeks. Several measures will be derived from the three daily cortisol values, including the mean levels at each time, the mean cortisol awakening response (CAR – change in cortisol from awakening to 30 minutes), the area under the curve (AUC), and the diurnal slope (change in cortisol between awakening and bedtime). Scatterplots and correlations will be used to quantitate the associations between these measures, and anxiety at each time (baseline and 12 weeks) as well as association between the change in cortisol measures and the change in anxiety. Additionally, mixed effects models, as described above, will be used to assess the association between baseline cortisol and changes in anxiety over time. To assess group differences in cortisol parameters, each of these measures at 12 weeks will be used as dependent variables in analysis of covariance models, using appropriate transformations as needed. Baseline levels of the measures will be used as covariates along with treatment group.

12.6 **Reporting and Exclusions**

An objective of this study is to estimate adherence and retention as defined above in Section 12.4. Note that all participants will be included in all primary analyses, regardless of adherence or retention using all data that have been collected. Secondary analyses will be conducted using those participants who were at least 75% adherent to see if the ‘per protocol analysis’ gives similar results as the ‘intent-to-treat analysis.’
12.7 Evaluation of Toxicity

All participants will be evaluable for adverse events and toxicity from the time of their randomization. Toxicities will be evaluated using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Fisher exact tests will be used to assess differences in toxicity rates between the three arms.

12.8 Evaluation of Response

Tumor response is not an outcome. Response for this study will be the change in anxiety from baseline to 12 weeks, and this outcome will be assessed in all participants. Analysis of this outcome is described above in Section 12.4.

12.9 Interim Analysis

There will be no formal stopping rules based on interim analyses for this pilot study. However, all Research Base studies are reviewed by the DSMB every six months so the data from this study will be reviewed on that schedule. The report completed for the DSMB includes a summary of accrual, adherence, and retention, descriptive statistics for baseline characteristics and patient status, descriptions of all adverse events and toxicities, estimates of data completeness, a summary of the primary and secondary outcome measures.
Reference List


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