American Lung Association
Airways Clinical Research Centers

Pilot of Zinc Acetate to Improve Chronic Cough (ZICO)

Protocol

Version 1.5
NCT number NCT03135522

22 Apr 2019

Document history

Version 0, draft (September 2016)

Version 1.0 (17 March 2017)

Version 1.1 (26 May 2017)

Version 1.2 (11 Oct 2017)

Version 1.3 (21 Feb 2018)

Version 1.4 (04 Jan 2019)

Version 1.5 (22 Apr 2019)

Abstract

Chronic refractory cough in adults is defined as a cough lasting more than 8 weeks that does not resolve with treatment for asthma/eosinophilic airway disease, gastroesophageal reflux disease (GERD), or rhinosinusitis/post-nasal drip; and is not caused by smoking, ACE inhibitors, or parenchymal lung disease. It is one of the most common conditions leading to specialty referral, accounting for about 20% of new pulmonary consultations. Chronic refractory cough can lead to severe impairment of quality of life and social isolation as well as sleep deprivation and chronic fatigue. The few available treatments have limited benefit and substantial side effects or abuse potential. While there are validated tools to measure the health-impact of chronic cough which can provide feasible clinical trial outcome measures, there have been no academic multi-center trials of chronic cough, and guidelines for treatment continue to rely largely on opinion rather than evidence. The Pilot of Zinc Acetate to Improve Chronic Cough (ZICO) is a pilot study for a larger randomized clinical trial to establish the safety and tolerability of zinc acetate to treat cough in this population. The pilot trial will enroll 36 participants, 18 in each group, at 3 clinical centers. Participants will be 18 years or older, with chronic cough lasting at least 3 months, which has been unresponsive to treatments for asthma, GERD or other upper airway disease. Individuals that are current smokers, use an ACE inhibitor, currently take zinc supplements (or multivitamins with zinc), or whose medical history includes primary parenchymal lung disease, congestive heart failure, chronic kidney disease, pancreatitis, or another medical condition that could interfere with the study or are pregnant or breast-feeding will be excluded. Participants will be randomized to receive 6 weeks of treatment with either zinc acetate or placebo. Follow-up assessments will occur at 1, 3, 6 and 8 weeks after randomization; the final assessment, after a two week washout, will help establish the duration of treatment effect. The primary objective of the trial is to evaluate the feasibility of a full scale trial including estimating the statistical properties of the Cough Specific Quality of Life Questionnaire (CQLQ) and other measures of cough severity and overall health.

Contents

1. Introduction

1.1. Title

Pilot of Zinc Acetate to Improve Chronic Cough

1.2. Sponsor

NHLBI

1.3. Investigators and study centers

Network Chair

Bailey, William	University of Alabama, Birmingham

Main Study Principal Investigators

Wise, Robert	Johns Hopkins University
Canning, Brendan	Johns Hopkins University

Participating Centers

Que Loretta	Duke University Medical Center
Katial, Rohit	National Jewish Health
Rogers, Linda	Mt. Sinai Medical Center

Data Coordinating Center

Holbrook, Janet	Johns Hopkins University
Wise, Robert	

1.4. Background and significance

Chronic cough, defined as a cough lasting 8 weeks or longer, is cited as the most frequent condition for referral to pulmonary specialty clinics and occurs in 9-12% of the general population.[1, 2, 3] In 50-90% of these patients the underlying cause of cough can be established and the cough is responsive to specific therapies for asthma/eosinophilic airway disease, gastroesophageal reflux, or post-nasal drip/sinusitis syndrome or cessation of ACE-inhibitors or smoking.[4, 5] In the remaining patients, the cough persists, often associated with noxious upper airway sensations. There is no universally accepted terminology of this syndrome, but terms such as upper airway cough syndrome, neuropathic cough, idiopathic refractory cough, treatment-resistant cough, and cough hypersensitivity syndrome have been applied to this well-described group of patients.[6, 7, 8] There are many conditions that may trigger this condition, but it is generally accepted that hypersensitivity of the airways to physical and chemical stimuli is the final common pathway.[9, 10] Accordingly, the recommended approach to treatment of chronic refractory cough involves treatment of aggravating conditions as well as antitussive treatment that increases cough threshold. (Figure 1)

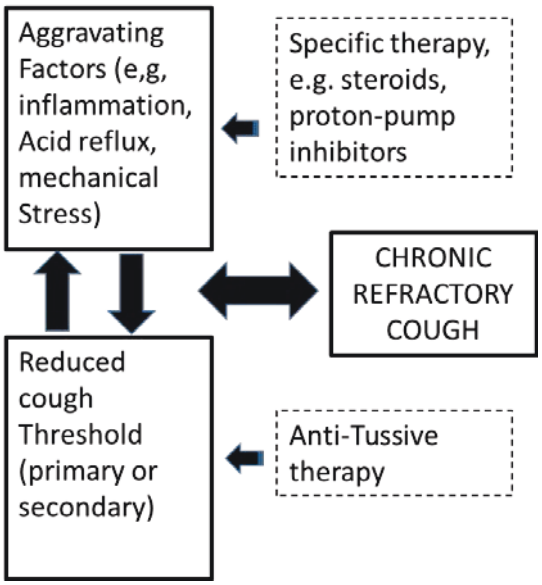


Figure 1 Pathogenesis of chronic refractory cough. Chronic refractory cough is thought to result from a primary or secondary increase in cough sensitivity induced or aggravated by external stimuli. Treatment requires intervention targeting both pathways – elimination of aggravating factors and suppression of cough hypersensitivity.

Patients with chronic cough often suffer severe psychosocial isolation and physical impairment due to chronic fatigue.[11] These patients report being unable to attend social events, public gatherings, or travel because of embarrassment for coughing in public.[12, 13]

The repetitive maximal muscle contractions that accompany cough and the associated sleep deprivation leads to musculoskeletal pain syndromes, chronic fatigue and depression. Some studies have shown that the impairment of quality of life is comparable to patients with COPD.[11, 12, 13] Resolution of the cough is associated with marked improvement in their quality of life and well-being.[12] However, because chronic refractory cough is frustrating for clinicians to diagnose and

treat, the impact of the condition is often underestimated and patients are told that they need to accept their condition.

Despite advances in our understanding of the neurobiology of cough, there has been a paucity of rigorous clinical research on this condition. For example, ClinicalTrials.gov lists only 3 clinical trials for chronic refractory cough, none sponsored by NIH. This lack of evidence is particularly notable in that the evidence-based guidelines for chronic cough treatment must rely more on expert opinion than rigorous evidence, leading to an "urgent" call for multicenter trials of potential cough therapies.[1, 14]

Usual OTC treatments with expectorants and antihistamines are not beneficial vs. placebo, although widely used at a cost of $5 Billion / year.[15] Treatments such as morphine, amitriptyline, gabapentin, and P2X3 receptor blockers have been effective in well-designed single-site trials, but the treatment effects are moderate and adverse effects are common.[16, 17, 18, 19, 20] In a 2013 meta-analysis, Yancey et al., found 49 comparative studies of antitussive treatments, 13 of which were considered good quality. They concluded that the only proven effective treatments were opioid narcotics and dextromethorphan and raised concerns about adverse effects and abuse potential, making them unsuited for chronic use.[15]

The lack of evidence regarding effective treatments for chronic cough is supported by a survey of opinion leaders in which 82% agreed with the statement: "There are no currently effective treatments for cough hypersensitivity syndrome." [21] Despite its high morbidity and prevalence, no approved treatments are available for chronic refractory cough. Indeed, the FDA has not approved a new treatment for cough since 1958 (benzonatate, Tesalon®).

This trial takes a novel approach to chronic refractory cough that specifically targets the recently discovered major mechano-receptor and chemo-receptor for cough supported by strong pre-clinical and highly supportive human data.

<u>Role of Na-K ATP-ase</u>

Considerable progress has been made by the Canning laboratory in defining the vagal afferent pathways regulating cough and this has facilitated the identification and evaluation of novel therapeutic strategies for cough suppression.[9, 22, 23, 24] At least 2 vagal afferent nerve subtypes essential to cough regulation have been identified (figure 2).[25, 26] Notably, the cough receptors express unique isozymes of Na+-K+ ATPase.[27] These sodium pump isozymes play an essential role in regulating cough receptor excitability. Cardiac glycosides (digoxin and ouabain) and other drugs known to interact with neuronal sodium pumps dose-dependently suppress cough in guinea pigs and in patients.[26, 27, 28, 29] This work strongly suggests that neuronal sodium pump inhibition may be an effective antitussive strategy, prompting clinical assessments by our consultants Jaclyn Smith and colleagues to evaluate the actions of digoxin in chronic cough patients and a study by Lorcan McGarvey and colleagues to measure cough reflex sensitivity in a group of patients with loss of function mutations in their neuronal sodium pumps.[28]

We found no evidence for neuronal isoform expression in structural cells of the human airways based on exhaustive microarray analysis of >40K genetic loci in human bronchi (n=5 lung donors), suggesting that neuronal selective inhibitors of this isoenzyme will have limited off target effects in the lung. Unfortunately, both digoxin and ouabain are relatively nonselective inhibitors of sodium pump function, and have narrow therapeutic windows, thus limiting their utility as antitussives. Thus, other strategies for inhibition of this isoenzyme are necessary. Zinc is a strong candidate for this role.

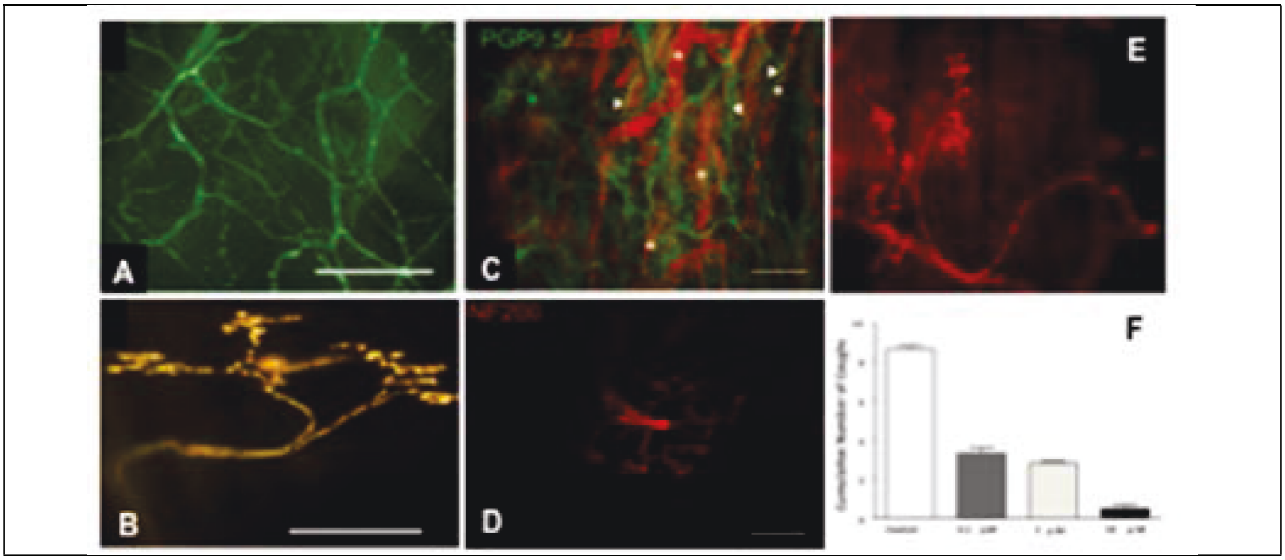


Figure 2. The subtypes of vagal afferent nerves regulating cough in guinea pigs are differentiated by their chemical and mechanical sensitivities. A) Capsaicin-sensitive C-fibers, and B) proton-sensitive, mechanically sensitive cough receptors, are also differentiated by the morphology of their terminals in the airway mucosa.[25, 26] C and D) We find comparable structures in mucosal biopsies taken from chronic cough patients who respond reliably to the same tussive agents (capsaicin, citric acid) used to evoke cough in guinea pigs.[29] E) The cough receptors uniquely express isozymes of Na+-K+ ATPase (immunohistochemical labeling of alpha-3 subunits shown). F) Sodium pump inhibition with digoxin and other agents, including Zinc, known to inhibit sodium pump function dose-dependently suppress cough (digoxin study performed in guinea pigs).

Zinc as a candidate antitussive treatment

Based on the work summarized above, the divalent cation zinc is a promising therapy. Zinc and other electrophilic metals (e.g. gold) inhibit Na+-K+ ATPase with some selectivity for neuronal isoforms.[30, 31] Zinc has other actions on neurons, including blockade of acid-sensing ion channels[32, 33], blockade of voltage gated ion channels[34], and modulation of NMDA-type glutamate receptor channels[35] that may also be relevant to cough suppression.[22] This electrophilic metal is also an anti-oxidant that prevents mucus secretion in the airways and inhibits inflammation.[36] These known actions of zinc and the evidence implicating sodium pumps in cough prompted us to investigate in the guinea pig model the efficacy of zinc as a potential antitussive treatment. We found that zinc, applied topically to the airways mucosa at concentrations as low as 10^{-3} mg/mL, reduced citric-acid induced coughing in guinea pigs. (Figure 3) Thus, combined with its effects on inflammation and mucus secretion we hypothesize that zinc will be a safe and effective antitussive treatment in humans.

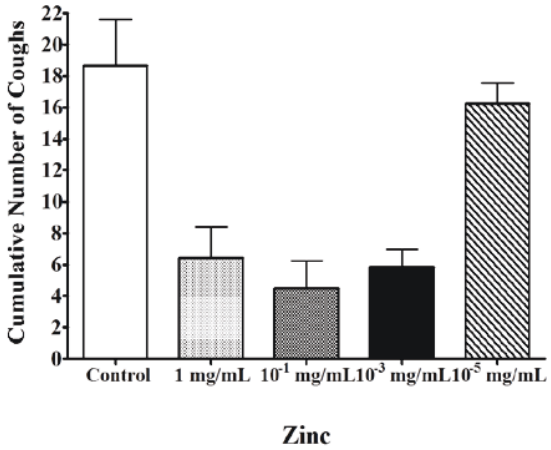


Figure 3 Zinc aerosol causes reduction in cough with citric acid challenge in guinea pig

In humans, zinc lozenges have been effective in reducing the duration of viral upper respiratory infections (URI). The theoretical, though unproven, mechanism has been to block adhesion of virus to epithelial cells via ICAM1.[37] Three controlled clinical trials of zinc acetate or zinc gluconate have demonstrated symptom reduction URI, particularly the duration of acute cough.[38, 39, 40] A meta-analysis showed a benefit with zinc therapy for URI. The summary statistics indicated that acute cough duration was reduced with zinc lozenges at a dose greater than 75 mg/day.[41, 42] Thus, there is sufficient evidence to support the notion that adequate doses of zinc can benefit humans who suffer from chronic cough.

Zinc is a widely available molecule with a long safety history as a nutritional supplement, treatment for colds, and for therapy of Wilson's disease that will be immediately available for patients should it be shown to be effective. Zinc may be administered long-term if necessary, unlike narcotic antitussives. We are proposing to use the FDA-approved formulation of zinc acetate (Galzin®) in the approved adult dose of 150mg/day for long-term use in Wilson's disease.

Previous studies of antitussives that have shown an effect have had treatment periods of 2 weeks to 12 weeks, with a treatment effect apparent at 2-4 weeks. The treatment duration of 6 weeks was selected as a reasonable period to determine whether there is a treatment effect and to assess for tolerability, adherence, steady-state serum zinc levels, potential adverse effects, and persistence of treatment effects. Longer treatment periods would not substantially augment the goals of this pilot/feasibility study.

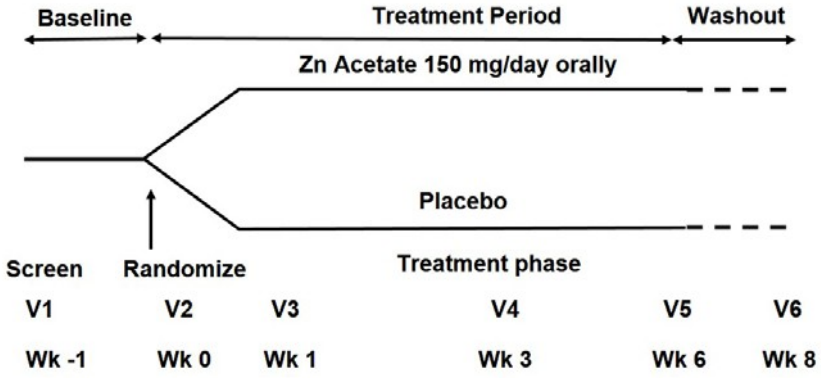
2. Study design

Randomized, two parallel arm, placebo-controlled clinical trial (n = 36, 18/arm).

The study aims are:

- To establish if treatment with zinc acetate is well tolerated
- To determine if the trial logistics are feasible
- To assess statistical properties of patient reported measures of cough

2.1. Trial schema



2.2. Eligibility criteria

Participants must affirm that they have multiple daily episodes of cough that have persisted for more than three months. Individuals with asthma/eosinophilic airway disease or gastroesophageal reflux may be eligible if treatment with inhaled corticosteroids and/or bronchodilators, or H2 blockers and/or proton pump inhibitors, respectively, did not resolve cough. In addition, individuals with upper airway cough and/or rhinosinusitis whose cough did not respond to nasal steroids, antihistamines and/or expectorants/decongestants may be eligible to participate.

Individuals on treatments for cough (e.g. inhaled corticosteroids, H2 blockers, nasal steroids) should remain on their background treatment during the trial.

Individuals cannot be current smokers (not smoked for at least six months), cannot be using an ACE inhibitor, and cannot have any parenchymal lung disease as all of these can cause cough.

Inclusion criteria:

- Men or women age 18 or older
- Chronic cough for more than 3 months
- No upper or lower respiratory infection within 4 weeks
- Either
    - Negative evaluation for:
        - Asthma; no symptoms of disease or no evidence of asthma based on spirometry and/or methacholine challenge test
        - GERD: no symptoms of acid reflux disease or negative pH probe
        - Rhinosinusitis/upper airway cough

  Or

    - Cough persists despite treatment for the following potential causes:
        - Asthma –treated for at least 8 weeks with at least medium dose inhaled corticosteroids or with oral corticosteroids
        - GERD – treated for at least 8 weeks with either a proton pump inhibitor (PPI) or H_2 blocker
        - Upper airway disease, postnasal drip or sinusitis – treated for at least 8 weeks with nasal steroids, antihistamines or both.

- Non-smoker; defined as
    - no smoking of any substance (e.g., tobacco, e-cigarette, marijuana) in the past 6 months, and
    - less than 20 pack-year smoking history
- Chest x-ray or CT scan in the past 24 months and after the onset of cough; negative for parenchymal lung diseases (such as interstitial lung disease, idiopathic pulmonary fibrosis, pneumonia, or TB) and negative for lung cancer
- Overall Cough VAS score of 30 or higher at V1
- Willing to halt use of zinc supplements or multivitamins containing zinc for the duration of the study
- Provide written consent

Exclusion criteria:

- Marijuana use (smoking or ingestion of marijuana) in the past 6 months
- Use of ACE inhibitor currently or within the past 6 weeks
- Use of zinc supplements or multivitamins containing zinc currently or within the past 6 weeks
- Occupational exposure to dust or chemicals that may cause cough, as determined by study physician
- Diagnosis or evidence of COPD ($FEV_1/FVC < 0.70$ and FEV_1% predicted < 80%)
- History of:
    - Bronchiectasis
    - Interstitial lung disease
    - Sarcoidosis
    - Pneumoconiosis
    - Asbestosis

    - Chronic mycobacterial infection
    - Lung cancer
    - Pancreatitis
- Congestive heart failure
- Chronic kidney disease (creatinine clearance < 30mL/min)
- Pregnant or breast-feeding
- Other medical conditions that would interfere with participation in study

2.3. Recruitment

Patients with chronic refractory cough will be identified according to methods that have been successful at each participating ALA ACRC center. Methods include screening patients regularly seen at the participating clinical center, review of existing patient registries, response to advertisements approved by the local Institutional Review Board (IRB), or referral by other health care providers. Patients will be asked information about their cough, demographic characteristics, and their interest in the study. Pre-screening interviews can be conducted in person or by telephone.

2.4. Summary of visits

Visit 1 Screening Visit (week -1, 2 hours)

- Consent
- Eligibility assessment
    - Chest x-ray, if participant does not have chest x-ray or CT from within the past 2 years
- Cough Visual Analog Scales (C-VAS)
- Baseline medical history
- Spirometry (pre- and post-bronchodilator)
- Pregnancy test for women of child-bearing potential
- Blood draw for creatinine level, serum zinc and copper levels, and biospecimens
- Explain and distribute cough diary

Visit 2 Baseline / Randomization (week 0, 2 hours)

- Interval medical history
- Collect and review cough diary
- Cough questionnaire completion
    - Cough Visual Analog Scales (C-VAS)
    - Cough Quality of Life Questionnaire (CQLQ)
    - Leicester Cough Questionnaire (LCQ)
    - Global Assessment of Change in Cough (GACC)
    - Cough-related Impact on Quality of Life Scale (Cough IQOLS)
    - EQ-5D
- Physical examination
- Eligibility review and randomization
- Dispense study treatment

Visit 3 Phone follow-up visit (week 1, 10 minutes)

- Review study procedures
- Assess for adverse events
- Review and troubleshoot adherence

Visit 4 On-treatment follow-up visit (week 3, 1.5 hours)

- Interval medical history
- Assess for adverse events
- Collect and review cough diary
- Cough questionnaire completion

  - Cough Visual Analog Scales (C-VAS)
  - Cough Quality of Life Questionnaire (CQLQ)
  - Leicester Cough Questionnaire (LCQ)
  - Global Assessment of Change in Cough (GACC)
  - Cough-related Impact on Quality of Life Scale (Cough IQOLS)
  - EQ-5D

Visit 5 On-treatment final follow-up visit (week 6, 2 hours)

- Interval medical history
- Assess for adverse events
- Collect and review cough diary
- Spirometry (pre- and post-bronchodilator)
- Cough questionnaire completion
  - Cough Visual Analog Scales (C-VAS)
  - Cough Quality of Life Questionnaire (CQLQ)
  - Leicester Cough Questionnaire (LCQ)
  - Global Assessment of Change in Cough (GACC)
  - Cough-related Impact on Quality of Life Scale (Cough IQOLS)
  - EQ-5D
- Drug return
- Blood draw for serum zinc and copper levels

Visit 6 Off-treatment washout visit (week 8, 2 hours)

- Interval medical history
- Assess for adverse events
- Collect and review cough diary
- Spirometry (pre- and post-bronchodilator)
- Cough questionnaire completion
  - Cough Visual Analog Scales (C-VAS)
  - Cough Quality of Life Questionnaire (CQLQ)
  - Leicester Cough Questionnaire (LCQ)
  - Global Assessment of Change in Cough (GACC)
  - Cough-related Impact on Quality of Life Scale (Cough IQOLS)
  - EQ-5D
- Exit interview
- Provide information on treatment assignment in sealed envelope

2.5. Study data collection schedule

Visit	V1	V2	V3 (phone)	V4	V5	V6
Target (weeks)	**-1**	**0**	**1**	**3**	**6**	**8**
Consent, eligibility evaluation	●					
Baseline medical history	●					
Interval health history/ adverse events		●	●	●	●	●
Return cough diary		●		●	●	●
Spirometry (pre-post BD)	●				●	●
C-VAS	●	●		●	●	●
CQLQ, LCQ, GACC, Cough IQOLS, EQ-5D		●		●	●	●
Drug return					●	
Exit interview/provided treatment assignment envelope						●
Physical examination		●				
Pregnancy test	●					
Randomization / drug dispensing		●				
Blood for creatinine level	●					
Blood for zinc and copper levels	●				●	
Blood for optional biospecimens	●					
Chest x-ray*	●					

Key: (V#) Visit Number, (BD) Bronchodilator, (C-VAS) Cough visual analog scales, (CQLQ) Cough specific Quality of Life, (LCQ) Leicester cough questionnaire, (GACC) global assessment of change in cough, (EQ-5D) EuroQOL quality of life instrument, (Cough IQOLS) Cough-related Impact on Quality of Life Scale

*If needed to determine eligibility

2.6. Outcome measures

2.6.1. Patient Reported Outcomes

Cough specific Quality of Life (CQLQ): This is a 28 item self-completed questionnaire with six subscales with excellent psychometric properties (Cronbach alpha = 0.92, ICC = 0.89) that is responsive to treatment.[13, 43] The estimates of the standard deviation are consistent (12-15) across studies. A minimum clinically important difference of 5 has been proposed, which is based upon a small single center study of idiopathic pulmonary fibrosis and has not been validated in a larger, multicenter cohort.[44]

Leicester cough questionnaire (LCQ): The LCQ is a patient reported cough outcome measure which has been validated and will be used to assess convergent validity for the CQLQ. The LCQ has similar properties as the CQLQ with 19 items and 3 domains, with a total possible score ranging from 3 to 21.[45, 46] The minimal important difference of the LCQ for patients with chronic cough is 1.3. [47]

Cough visual analog scales, overall, daytime, nighttime, urge to cough (C-VAS): The C-VAS is a patient reported cough outcome measure that provides a measure of the severity, intensity and noxious qualities of cough that are not as well represented on the health impact scales. Each element of the VAS has a possible score of 0 to 100.[16]

EuroQol EQ-5D- 5L: The EQ-5D-5L is an overall measure of health related quality of life that that provides a measure of utility for calculation of Quality Adjusted Life Years (QALYs). The EQ-5D measures 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression graded from level 1 (i.e., no problems) to level 5 (i.e., extreme problems). Use of the 5-levels (EQ-5D-5L) rather than the standard 3 level EQ-5D (EQ-5D-3L) decreases the ceiling effect, improves discriminative ability, and potentially has more power to detect differences between groups.[48]

Cough-related Impact on Quality of Life Scale (Cough IQOLS): This is a health related quality of life measure that includes the impact of cough adapted from Sandra Wilson's Asthma Impact on Quality of Life Survey (AQOLIS).

Global assessment of change in cough (GACC): Global rating of change scales are widely used to measure change in self-assessed patient condition and determine the MID for other health related quality of life questionnaires.[49, 50] The Global Assessment of Change in Cough (GACC) asks patients to assess changes in their quality of life related to their cough on a 7 point scale on four domains (activity limitation, symptoms, emotions, and overall quality of life).

Cough Diary: The study will use the Cough Severity Diary to track cough symptoms. The diary is a simple, seven-item daily diary designed to assess patient reported outcomes regarding frequency and severity of cough.[51])

2.6.2. Pulmonary function

Spirometry (pre- and post-bronchodilator): Spirometry is a common office test of lung function; both pre and post-bronchodilator tests will be performed. Post-bronchodilator spirometry will be performed after 2 inhalations of albuterol. This will be done to exclude patients with significant

airflow obstruction or restriction that may indicate underlying parenchymal lung disease or COPD and to evaluate the association of lung function with cough.

2.6.3. Other measures

Demographic data collected includes age, gender, race, marital status and educational level.

Clinical data includes height, weight, body mass index, smoking status and history of smoking in pack years. Adverse events, changes in symptoms, medication use and utilization of medical care with also be assessed.

2.7. Specimen collection, storage and shipping

Serum will be collected for measurement of serum creatinine and subsequent calculation of creatinine clearance to determine if the participant meets the eligibility criteria.

Biospecimens collected for measurement of serum zinc and copper levels will be processed at the clinical sites and sent to Quest Diagnostic laboratories, a CLIA approved central laboratory, for zinc and copper assessment. Zinc assessment will be measured to assess whether serum zinc levels are associated with cough and response to treatment, and whether supplementation increases serum zinc levels. Copper assessment will be performed to check for signs of potential copper deficiency. Participants should fast for at least 4 hours prior to collection of blood for zinc and copper analysis. Specimens for serum zinc and copper analysis must be processed within 2 hours to ensure accurate assessment of zinc and copper levels.

Additional blood will be collected and stored for future use. The ALA-ACRC maintains a biorepository of specimens from our studies to conduct ancillary studies of the relationship of biomarkers to airway and related diseases. Permission to draw and store biospecimens for future ancillary studies and/or DNA testing is optional and is not a requirement for participation in this study. The results of future genetic testing will not be provided to study participants. Blood specimens for future use are stored at -70° or -80° C at the local site and shipped periodically in batches on dry ice for storage at the ACRC biospecimen repository at Nemours Children's Center (NCC). The DCC supplies blood drawing tubes, bar-code labels, shipping labels, containers, and transmittal slips. All sites are required to be certified in biosafety for handling biological specimens in accordance with local hospital / university policy and applicable law.

3. Study treatment

3.1. Description of study treatment

Participants will be randomly assigned to receive a daily dose of 150 mg of zinc acetate or matched placebo taken in three doses of 50 mg each or placebo. For this trial 50 mg zinc acetate capsules will be over-encapsulated and a matched placebo will be created. Zinc will be taken with the morning or evening meal. Participants will be instructed to titrate up the dose of study drug starting with one capsule (50 mg) in the morning on day 1, increasing to two capsules (100 mg) on day 4, and reaching three capsules (150 mg) on day 8. Participants will be instructed to take the medication with food. If participants are not able to tolerate the full dose, they may split the dose

into two or three doses per day taken at meal-time (e.g. two capsules in AM and one capsule in PM, or one capsule with breakfast, lunch, and dinner). If a participant is not able to tolerate the full dose, then the dose should be titrated down to the highest tolerable dose, i.e., one or two capsules as tolerated.

The study will use the FDA-approved formulation of oral zinc, Galzin®. FDA has approved Galzin® 150mg/day for long-term use in adults with Wilson's disease. Zinc supplementation for food has been considered Generally Recognized as Safe (GRAS) by the FDA. NIH recommends up to 40 mg/day as a dietary supplement in adults and multivitamins used long-term for prevention of macular degeneration contain 80mg of elemental zinc. OTC lozenges (Cold-eez ®) contain 13.3 mg of zinc as zinc gluconate and are taken in doses of up to 80 mg/day for relief of URI symptoms. Zinc gluconate is available as unregulated nutritional supplements in 100mg capsules.

3.2. Randomization

Participants will be randomized to zinc acetate or placebo at V2. Treatment assignment will be randomly allocated in a 1:1 ratio with stratification by clinic with permuted block design will be used. Assignments will released from the DCC after evidence that an eligible participant has signed consent and completed all baseline evaluations.

Zinc acetate or placebo will be dispensed to the participant at the V2 visit. At randomization the participant will be assigned a unique bottle ID code and clinics will dispense that bottle to the participant. Each participant will receive a single bottle of 168 capsules for the 6 weeks of treatment. At the second to last study (V5) visit, the bottle with the unused capsules will be returned.

3.3. Side effects

The most common adverse effects of zinc supplementation are dyspepsia, nausea, and a metallic taste. These side effects can be mitigated by downward adjustment of the dose, taking the drug with meals, and/or spreading the dose out over the day (i.e. taking 1 capsule in the morning and 2 capsules at night, or taking 1 capsule each at breakfast, lunch, and dinner).

Elevations of liver and pancreatic enzymes have been reported in patients with Wilson's disease, treated with zinc acetate, but this has not been reported in other populations and the relationship with zinc supplementation is unclear. Participants with a history of pancreatitis will be excluded from the study. Study participants experiencing abdominal pain should be evaluated for possible pancreatitis. With zinc gluconate nasal spray some patients have noted loss of smell, but this has not been reported with oral zinc.

Zinc is listed as a class A drug for pregnancy indicating that it does not have substantial teratogenic potential. We are excluding pregnant and lactating women from this trial because pregnancy may have either beneficial or deleterious effects on chronic cough and zinc is transmitted in breast milk that may alter infant feeding behavior. Women of child bearing potential must be willing to use appropriate methods of pregnancy prevention during the duration of the study. Women of childbearing potential includes all female participants except those who are surgically sterile (both ovaries and/or uterus removed), postmenopausal (no menstrual period for longer than 12 consecutive months), or incapable of pregnancy.

The FDA-approved prescribing information for zinc acetate states the following regarding over-dosage:

"One fatality associated with over-dosage of zinc sulfate has

> been reported. The death of this adult woman followed the accidental ingestion of approximately 28 g of zinc sulfate. [Equivalent to 560 capsules of zinc acetate or 3 bottles of dispensed study drug]. Death occurred on the fifth day after ingestion and was attributed to renal failure. Hemorrhagic pancreatitis and hyperglycemic coma resulted from the overdose. The amount ingested was 500 mg/Kg of zinc sulfate, a value that is in the same order of magnitude as that found to be lethal in animals."

Drug interaction information contained in UpToDate indicates that absorption of the following medications might be impaired by zinc: cephalosporin antibiotics, quinolone antibiotics, tetracycline antibiotics, bisphosphonates, dolutegravir, deferiprone, and eltrombopag. If a participant is prescribed one of these medications during the study, the participant will be instructed to take zinc/placebo at least 1 hour prior to or 1 hour after taking that medication.

In theory, prolonged consumption of zinc could lead to copper deficiency which is manifest as a reversible anemia and neutropenia as well as neuropathy. Because copper is ubiquitous in the diet, and deficiency is rare in the absence of malabsorption syndromes or gastric bypass surgery, it is unlikely that the duration of treatment in this trial will lead to copper deficiency. However, in patients using long-term high-dose zinc, monitoring for hematologic abnormalities and peripheral neuropathy would be indicated.[52] We will measure serum copper levels prior to starting study drug and after completing study drug treatment to assess for potential signs of copper deficiency, as well as to assess the likelihood of side effects in more long-term use of zinc.

3.4. Unmasking

Unmasking of treatment assignment is rarely necessary. If adverse events occur that may be related to the study treatment, then the study treatment is stopped and the patient continues follow-up. Envelopes with treatment assignment are provided to the clinic in the case a treating physician feels it is important to know the treatment assignment. In addition, the DCC provides a phone number where authorized personnel can receive unmasking information. All participants are provided with a wallet card that provides information that they are participating in a clinical trial and gives the contact numbers of personnel at the clinic.

4. Analysis plan

4.1. Sample size

The primary goals of the pilot study are to establish the feasibility of performing a randomized trial and to obtain preliminary estimates of the relative efficacy of zinc versus placebo and the statistical properties of the patient reported outcome measures. A sample size of 36 (18/arm) has 80% power to detect a treatment effect of 0.56 standard deviations (SD) or greater, where SD is for the difference from baseline to 6 weeks in CQLQ score, based upon a two-sample t-test and a one-sided type 1 error rate of 0.20. We are conducting a contemporaneous cohort study to establish the minimal important difference (MID) and SD for change in CQLQ scores in 100 participants with chronic cough. This analysis will not necessarily provide information whether the changes are clinically important, but will provide a framework for establishing changes that are outside of the range of variability of symptoms and would be noticeable by the patient. Thus, in

this context, the MID would be analogous to the "Just Noticeable Difference" (JND) used in psychophysics of perception.

Primary Analysis

The primary analysis will focus on the tolerability of zinc acetate versus placebo. These analysis will rely on qualitative assessments of adverse drug effects as well as unadjusted comparisons of the frequency of events and adherence by treatment assignment.

The statistical analysis for efficacy will be an unadjusted comparison of 6-week change in CQLQ between treatment groups. A linear mixed effects model will be used to model change in CQLQ over time. A saturated means model with indicators for each time point, treatment and treatment by time interaction, will be specified and a random effect for individual will be used to account for the repeated measurements over time. The 6-week treatment by time interaction term represents the difference in change in CQLQ between the treatment groups, i.e. the primary outcome. If the data are non-normal, then transformations and non-parametric alternatives (e.g. Wilcoxon Rank-Sum test comparing the raw differences from baseline to 6-weeks between treatment groups) will be explored. Using the methods described by Cocks and Torgerson, we will test whether or not the upper boundary of the 1-sided 80% confidence interval of the treatment effect excludes the MID.[53] For example, a total sample size of 36 would be sufficient to exclude a MID of 5 or higher for a 1-sided 80% confidence interval if the standard deviation for the change in CQLQ was 15. Sensitivity analyses using alternate mean and covariance structures will also be performed. Analyses will be done based upon principles of intention to treat, i.e. all data from all randomized participants will be included in the analysis.

Secondary outcomes include, but are not limited to, LCQ, diary data, C-VAS, GACC, quality of life measures, and safety data. Continuous variables will be analyzed similarly to the primary outcome. Event outcomes will be analyzed in two ways: negative binomial regression for rates and Kaplan-Meier curves and Cox proportional hazards models for time to first event.

Assessment of futility for a definitive trial will not rest with any single measure but will depend on a synthesis of the overall study feasibility and treatment tolerability as well as magnitude and consistency of the observed treatment effect. However, in line with formal futility designs in neuroscience and oncology, we will not likely proceed with a definitive trial if the upper boundary of the one-sided 80% confidence interval of the treatment effect excludes the minimally important difference for the CQLQ, which we will validate in the concurrent cohort study.

Descriptive statistics will be computed for baseline clinical and demographic characteristics by treatment group. Results will be displayed as means with standard deviations, medians with 1st and 3rd quartiles, and proportions as appropriate.

5. Protection of human subjects

5.1. Risks to the participants

Study procedures present minor risks:

Spirometry: Participants will undergo spirometry at V1, V5 and V6. This procedure involves forceful respiratory maneuvers. Occasionally people develop light-headedness during the procedure or soreness of the chest for a few days afterward. In the Lung Health Study, in approximately 25,000 tests, one individual sustained a serious cervical injury from syncope during spirometry while standing. The risk of syncope in this study is minimized by having participants sit during the maneuvers.

Questionnaires and assessments: Participants will be asked to provide information about their psychological, physical, and medical functioning on questionnaires. While we will make every effort to protect privacy and keep data confidential, there is a risk that information can be disseminated in ways that can risk the privacy of a person. To minimize risk, we will use only study codes to identify data and study records, store study data and records in a secure place, and personal information such as names, addresses, and telephone numbers will not be in the central database.

Chest x-ray: Participants without a chest x-ray or CT scan from within the past 2 years will have a chest x-ray taken to determine eligibility. Chest x-ray is a standard medical procedure for imaging of the lung. Risks to the volunteers are minimal. Participants undergoing chest x-ray for this study will be exposed to a small amount of radiation as part of the study. The effective dose equivalent for a chest x-ray is 7.2 millirem (mrem), or 0.072 millsieverts (mSv). This compares to the average background radiation dose a U.S. resident receives yearly from natural sources of 310 mrem, or 3.1 mSv.

We are requiring that all x-rays taken as part of the study be interpreted by a certified radiologist on site. The scans will be inspected for image quality and inclusion of all parts of the chest. It may occur that other findings are found on these images that may represent clinically significant findings (e.g. lung nodules). If any incidental findings are discovered during the study, the clinical site study physician will be notified, the information will be given to the participant and, with their permission, the information will also be transmitted to the participant's usual caregiver for appropriate medical care or follow-up.

Specimens will be obtained for serum creatinine, serum zinc and copper levels, and future research. Risks of blood draw are minimized by limiting the amount drawn to no more than about 70 mL over the course of the study. Patients have venipuncture in the seated or lying position to minimize syncopal events and pressure is applied after the procedure.

The risks of genome and other ancillary analyses of specimens are primarily related to the possibility that an outside agency not approved by the participant might obtain and use the information in such a way that it would harm the participant. To minimize this risk, specimens will be identified only by a numeric identifier. The results will not be a part of a participant's general medical record. Genetic information or other information obtained from participants will not be supplied to any outside agency except as authorized by the consent statement. Genetic and biomarker data will not be linked to the participants' personal identifiers and will be shared

with other investigators in the network through study ID codes. Results may be presented in publications and meetings but individual names will not be identified.

5.2. Data Confidentiality

Data which includes identifiable protected health information (PHI) are collected at each of the clinical sites. PHI is stored at each of the clinical sites in accordance with HIPAA regulations and local university and hospital policies. This includes the storage of PHI in locked cabinets or rooms, limited access to secure data areas by certified participating study personnel, password protection for electronic medical records, and explanation of HIPAA regulations on the study consent form. Data such as lung function or laboratory tests that are collected as part of this study may be transmitted to the participants' treating physicians with the consent of the participant. Participants are informed in the consent that PHI may also be disclosed for auditing purposes by the FDA or other regulatory bodies and is subject to subpoena.
Date of birth and gender of participants must be shared with the central laboratory to determine zinc and copper reference values. The resulting data is shared only between the originating site, central laboratory, and DCC through a HIPAA-compliant results application run by the central laboratory. Personal identifiers are not otherwise transmitted to the DCC or central laboratory. All data are identified by study ID, and other identifying information is not entered into the central study database. Source records that are transmitted to the DCC for data quality audits have identifying information redacted, however on-site inspections may involve access by DCC personnel to medical records.

5.3. Recruitment and consent procedures

Participants will be recruited by each participating clinic using their own IRB approved methods. These methods may include local American Lung Association campaigns, solicitation in physician offices, clinics, workplaces, and public media advertisements. All public advertisements are subject to approval by the local Institutional Review Board (IRB) and must indicate that it is a research study. The DCC will help coordinate recruitment among clinics and promote sharing of effective recruitment strategies within the network.

The consent form will be subject to approval by the clinical center IRB. A copy of the consent form will be given to each participant, and the signed original will be kept in the participant's research chart.

6. Data and safety monitoring plan

A Data and Safety Monitoring Board (DSMB) is appointed and chartered by the American Lung Association to monitor ALA-ACRC trials. The DSMB will serves in a consultative capacity to ALA and will make recommendations regarding the initiation and continuation of ACRC studies. The NIH program officer is invited to attend review meetings, and recommendations and minutes will be forwarded to the NIH program officer and to all IRBs involved in the oversight of the study. The primary responsibility of the DSMB is to protect participants but they may also make recommendations regarding the scientific conduct of the study.[14] The DSMB will meet to review the protocol and consent prior to study initiation and then periodically throughout the study to review the progress of the trial. Typically the meetings take place twice a year. The investigators will present the DSMB with study performance data including screening and enrollment data, and measures of data quality and timeliness. Safety data will include reporting of adverse events, protocol deviations, and unexpected or unusual events that may affect the safety or scientific validity of the study. Because of the nature of this study, we are not proposing interim efficacy analyses or stopping guidelines. At the end of each DSMB meeting, the board will vote whether to continue the study as planned or whether to recommend changes to the study.

Dr. Wise serves as the medical monitor for the research group and reviews all serious adverse event or unusual event reports to determine whether any immediate local or study-wide actions are indicated. Because Dr. Wise reviews patient events, he will remain masked to the coded treatment assignments. Interim serious adverse events or significant protocol deviations are transmitted to the DSMB as they occur with concurrent notification of the clinical site IRBs and, if appropriate to the FDA. The DSMB is given the option of having an interim meeting to review the event.

The DSMB reports will be masked with respect to treatment assignments groups by using codes to identify the zinc acetate and placebo groups. The DSMB may elect to maintain the masking or they can be unmasked at any time. They may also decide to be unmasked for an individual case if there is a serious adverse event that may be related to a specific treatment.

ClinicalTrials.gov Registration

The trial will be registered with ClinicalTrials.gov under the Johns Hopkins University account.

7. Literature cited

1. Morice, A.H., L. McGarvey, I. Pavord, and G. British Thoracic Society Cough Guideline, *Recommendations for the management of cough in adults.* Thorax, 2006. **61 Suppl 1**: p. i1-24.

2. Irwin, R.S. and J.M. Madison, *The diagnosis and treatment of cough.* The New England journal of medicine, 2000. **343**(23): p. 1715-1721.

3. Ford, A.C., D. Forman, P. Moayyedi, and A.H. Morice, *Cough in the community: a cross sectional survey and the relationship to gastrointestinal symptoms.* Thorax, 2006. **61**(11): p. 975-979.

4. McGarvey, L.P., L.G. Heaney, J.T. Lawson, et al., *Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol.* Thorax, 1998. **53**(9): p. 738-743.

5. Pavord, I.D., *Chronic cough: a rational approach to investigation and management.* The European respiratory journal, 2005. **25**(2): p. 213-215.

6. Lee, J.K. and S. Mintz, *Chronic cough as a sign of laryngeal sensory neuropathy: diagnosis and treatment.* The Annals of Otology, Rhinology, and Laryngology, 2006. **115**(11): p. 871.

7. Morice, A.H., L.P. McGarvey, and P.V. Dicpinigaitis, *Cough hypersensitivity syndrome is an important clinical concept: a pro/con debate.* Lung, 2012. **190**(1): p. 3-9.

8. Chung, K.F., *Chronic 'cough hypersensitivity syndrome': a more precise label for chronic cough.* Pulmonary pharmacology & therapeutics, 2011. **24**(3): p. 267-271.

9. Canning, B.J., A.B. Chang, D.C. Bolser, et al., *Anatomy and neurophysiology of cough: CHEST Guideline and Expert Panel report.* Chest, 2014. **146**(6): p. 1633-1648.

10. Chung, K.F., *Approach to chronic cough: the neuropathic basis for cough hypersensitivity syndrome.* Journal of thoracic disease, 2014. **6**(Suppl 7): p. S699-707.

11. Young, E.C. and J.A. Smith, *Quality of life in patients with chronic cough.* Therapeutic advances in respiratory disease, 2010. **4**(1): p. 49-55.

12. French, C.L., R.S. Irwin, F.J. Curley, and C.J. Krikorian, *Impact of chronic cough on quality of life.* Archives of Internal Medicine, 1998. **158**(15): p. 1657-1661.

13. French, C.T., R.S. Irwin, K.E. Fletcher, and T.M. Adams, *Evaluation of a cough-specific quality-of-life questionnaire.* Chest, 2002. **121**(4): p. 1123-1131.

14. Irwin, R.S., C.T. French, S.Z. Lewis, et al., *Overview of the management of cough: CHEST Guideline and Expert Panel Report.* Chest, 2014. **146**(4): p. 885-889.

15. Yancy, W.S., Jr., D.C. McCrory, R.R. Coeytaux, et al., *Efficacy and tolerability of treatments for chronic cough: a systematic review and meta-analysis.* Chest, 2013. **144**(6): p. 1827-1838.

16. Morice, A.H., M.S. Menon, S.A. Mulrennan, et al., *Opiate therapy in chronic cough.* American journal of respiratory and critical care medicine, 2007. **175**(4): p. 312-315.

17. Ryan, N.M., S.S. Birring, and P.G. Gibson, *Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial.* Lancet (London, England), 2012. **380**(9853): p. 1583-1589.

18. Chung, K.F., *Gabapentin: a suppressant for refractory chronic cough.* Lancet (London, England), 2012. **380**(9853): p. 1540-1541.

19. Abdulqawi, R., R. Dockry, K. Holt, et al., *P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study.* Lancet (London, England), 2015. **385**(9974): p. 1198-1205.

20. Jeyakumar, A., T.M. Brickman, and M. Haben, *Effectiveness of amitriptyline versus cough suppressants in the treatment of chronic cough resulting from postviral vagal neuropathy.* The Laryngoscope, 2006. **116**(12): p. 2108-2112.

21. Morice, A.H., E. Millqvist, M.G. Belvisi, et al., *Expert opinion on the cough hypersensitivity syndrome in respiratory medicine.* The European respiratory journal, 2014. **44**(5): p. 1132-1148.

22. Dicpinigaitis, P.V., A.H. Morice, S.S. Birring, et al., *Antitussive drugs--past, present, and future.* Pharmacological reviews, 2014. **66**(2): p. 468-512.

23. Muroi, Y., F. Ru, Y.L. Chou, et al., *Selective inhibition of vagal afferent nerve pathways regulating cough using Nav 1.7 shRNA silencing in guinea pig nodose ganglia.* American journal of physiology.Regulatory, integrative and comparative physiology, 2013. **304**(11): p. R1017-23.

24. Canning, B.J., S.B. Mazzone, S.N. Meeker, et al., *Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs.* The Journal of physiology, 2004. **557**(Pt 2): p. 543-558.

25. Muroi, Y., F. Ru, M. Kollarik, et al., *Selective silencing of Na(V)1.7 decreases excitability and conduction in vagal sensory neurons.* The Journal of physiology, 2011. **589**(Pt 23): p. 5663-5676.

26. Canning, B.J. and D.G. Farmer, *Regulation of cough by neuronal Na(+)-K(+) ATPases.* Current opinion in pharmacology, 2015. **22**: p. 140-145.

27. Mazzone, S.B., S.M. Reynolds, N. Mori, et al., *Selective expression of a sodium pump isozyme by cough receptors and evidence for its essential role in regulating cough.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2009. **29**(43): p. 13662-13671.

28. de Carvalho Aguiar, P., K.J. Sweadner, J.T. Penniston, et al., *Mutations in the Na+/K+ -ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism.* Neuron, 2004. **43**(2): p. 169-175.

29. West, P.W., B.J. Canning, E. Merlo-Pich, A.A. Woodcock, and J.A. Smith, *Morphologic Characterization of Nerves in Whole-Mount Airway Biopsies.* American journal of respiratory and critical care medicine, 2015. **192**(1): p. 30-39.

30. Gettelfinger, D.M. and G.J. Siegel, *Irreversible zinc ion inhibition of (Na+ -K+)-adenosinetriphosphatase, Na+ -phosphorylation, and K+-p-nitrophenylphosphatase of Electrophorus electricus electroplax.* Journal of neurochemistry, 1978. **31**(5): p. 1231-1237.

31. Nechay, B.R., *Inhibition of adenosine triphosphatases by gold.* Arthritis and Rheumatism, 1980. **23**(4): p. 464-470.

32. Chu, X.P., J.A. Wemmie, W.Z. Wang, et al., *Subunit-dependent high-affinity zinc inhibition of acid-sensing ion channels.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2004. **24**(40): p. 8678-8689.

33. Jiang, Q., C.J. Papasian, J.Q. Wang, Z.G. Xiong, and X.P. Chu, *Inhibitory regulation of acid-sensing ion channel 3 by zinc.* Neuroscience, 2010. **169**(2): p. 574-583.

34. Gilly, W.F. and C.M. Armstrong, *Slowing of sodium channel opening kinetics in squid axon by extracellular zinc.* The Journal of general physiology, 1982. **79**(6): p. 935-964.

35. Anderson, C.T., R.J. Radford, M.L. Zastrow, et al., *Modulation of extrasynaptic NMDA receptors by synaptic and tonic zinc.* Proceedings of the National Academy of Sciences of the United States of America, 2015. **112**(20): p. E2705-14.

36. Zalewski, P.D., A.Q. Truong-Tran, D. Grosser, et al., *Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. A review.* Pharmacology & therapeutics, 2005. **105**(2): p. 127-149.

37. Eby, G.A., 3rd, *Zinc lozenges as cure for the common cold--a review and hypothesis.* Medical hypotheses, 2010. **74**(3): p. 482-492.

38. Prasad, A.S., J.T. Fitzgerald, B. Bao, F.W. Beck, and P.H. Chandrasekar, *Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate. A randomized, double-blind, placebo-controlled trial.* Annals of Internal Medicine, 2000. **133**(4): p. 245-252.

39. Mossad, S.B., M.L. Macknin, S.V. Medendorp, and P. Mason, *Zinc gluconate lozenges for treating the common cold. A randomized, double-blind, placebo-controlled study.* Annals of Internal Medicine, 1996. **125**(2): p. 81-88.

40. Eby, G.A., D.R. Davis, and W.W. Halcomb, *Reduction in duration of common colds by zinc gluconate lozenges in a double-blind study.* Antimicrobial Agents and Chemotherapy, 1984. **25**(1): p. 20-24.

41. Rev., C.D.S., 2013.

42. Das, R.R. and M. Singh, *Oral zinc for the common cold.* Jama, 2014. **311**(14): p. 1440-1441.

43. Lechtzin, N., M.E. Hilliard, and M.R. Horton, *Validation of the Cough Quality-of-Life Questionnaire in patients with idiopathic pulmonary fibrosis.* Chest, 2013. **143**(6): p. 1745-1749.

44. Wise, R.A., S.J. Bartlett, E.D. Brown, et al., *Randomized trial of the effect of drug presentation on asthma outcomes: the American Lung Association Asthma Clinical Research Centers.* The Journal of allergy and clinical immunology, 2009. **124**(3): p. 436-44, 444e1-8.

45. Berkhof, F.F., L.N. Boom, N.E. ten Hertog, et al., *The validity and precision of the Leicester Cough Questionnaire in COPD patients with chronic cough.* Health and quality of life outcomes, 2012. **10**: p. 4-7525-10-4.

46. Birring, S.S., B. Prudon, A.J. Carr, et al., *Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ).* Thorax, 2003. **58**(4): p. 339-343.

47. Raj, A.A., D.I. Pavord, and S.S. Birring, *Clinical cough IV:what is the minimal important difference for the Leicester Cough Questionnaire?* Handb Exp Pharmacol, 2009(187): p. 311-20.

48. Johnson, J.A., S.J. Coons, A. Ergo, and G. Szava-Kovats, *Valuation of EuroQOL (EQ-5D) health states in an adult US sample.* PharmacoEconomics, 1998. **13**(4): p. 421-433.

49. Yousaf, N., K.K. Lee, B. Jayaraman, I.D. Pavord, and S.S. Birring, *The assessment of quality of life in acute cough with the Leicester Cough Questionnaire (LCQ-acute).* Cough (London, England), 2011. **7**(1): p. 4-9974-7-4.

50. Juniper, E.F., G.H. Guyatt, A. Willan, and L.E. Griffith, *Determining a minimal important change in a disease-specific Quality of Life Questionnaire.* Journal of clinical epidemiology, 1994. **47**(1): p. 81-87.

51. Vernon, M., N. Kline Leidy, A. Nacson, and L. Nelsen, *Measuring cough severity: development and pilot testing of a new seven-item cough severity patient-reported outcome measure.* Ther Adv Respir Dis, 2010. **4**(4): p. 199-208.

52. Kumar, N., *Copper deficiency myelopathy (human swayback).* Mayo Clin Proc, 2006. **81**(10): p. 1371-84.

53. Cocks, K. and D.J. Torgerson, *Sample size calculations for pilot randomized trials: a confidence interval approach.* Journal of clinical epidemiology, 2013. **66**(2): p. 197-201.

Appendix