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The anti-inflammatory effect of prophylactic macrolides on children with chronic lung disease: a double blinded RCT

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ABSTRACT

Introduction: Recent studies suggest that the high mortality rate of respiratory viral infections is a result of a neutrophilic overactive inflammatory response. Macrolides have been found to have anti-inflammatory properties, including the ability to down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbations. In this study, we will test the hypothesis that the use of prophylactic macrolides will effectively reduce the severity of respiratory viral illness in children with chronic lung disease by preventing the full activation of the inflammatory cascade.

Methods and analysis: A randomized double-blind placebo controlled trail that will enroll 92 children to receive either azithromycin or placebo for a period of 3-6 months during two different RSV seasons (2015-2016 and 2016-2017). We expect a reduction of at least 20% in unscheduled face to face encounters in the treatment group as compared to placebo group. Standard frequentist and Bayesian analyses will be performed using an intent-to-treat approach.

Discussion: We predict that the prophylactic use of azithromycin will reduce the morbidity associated with respiratory viral infections during the winter season in patients with chronic lung disease as evidenced by a reduction in the total number of days with unscheduled face to face provider encounters.

Ethics and dissemination: This research study was approved by the Institutional Review Board of the University of Texas Health Science Center in Houston on October 9th, 2014. Upon completion, the results of the trial will be published.

Trial registration: clinicaltrials.gov identifier: NCT02544984

INTRODUCTION

For the past 3 years, the High Risk Children's Clinic (HRCC) at UTHealth has been providing a medical home for medically complex children. We have demonstrated major benefits (e.g., 50% fewer serious illnesses & 55% fewer hospital days) and savings (~$10K/child/year) from the comprehensive care provided in our enhanced medical home to high-risk chronically ill children including patients with chronic lung disease (CLD).[1] These benefits have not been previously shown for medical homes for patients of any kind or age.[2, 3] These benefits result primarily from 24/7 access by phone to dedicated and experienced caregivers directed by a pediatric pulmonologist. The clinic offers same day appointments and provides comprehensive coordination of care for this population. We now aim to further cut morbidity rates by developing specific outpatient interventions to augment comprehensive care for each major disorder that we treat.

A significant portion (44%) of our patients in the High Risk Children clinic are chronically ill children who have some form of CLD including patients with bronchopulmonary dysplasia (BPD).[1] Chronic lung disease, as defined by the ATS statement from 2002, is "a heterogeneous group of respiratory diseases of infancy that usually evolves from an acute respiratory disorder experienced by a newborn infant" [4] specifically, infants with bronchopulmonary dysplasia, defined as the need for supplemental oxygen therapy in children over 28 days old that were born before 32 weeks gestation.[4] These infants often incur long-term pulmonary function abnormalities, including oxygen dependency after discharge, recurrent respiratory infections, and other reactive airway diseases. From our data, we have learned that many of the hospital admissions in our group of patients were related to respiratory infections (37%) during the winter season. Despite vaccination rates of nearly 100%, administration of Palivizumab to all of those eligible patients, and access to our comprehensive care clinic, viral respiratory illnesses continue to cause considerable morbidity and high healthcare costs in this patient population. [1] Innovative new prophylactic treatments are needed.

Macrolides have received considerable attention for their anti-inflammatory and immunomodulatory actions beyond the antibacterial effect. Such properties may ensure some efficacy against a wide spectrum of respiratory viral infections.[5] Recent studies, including a study performed in our lab with elderly BALB/c mice infected with RSV, have shown that the high mortality rate of respiratory virus infections is a result of a neutrophilic overactive inflammatory response. [6, 7] A recently published study examined the inflammatory response in hospitalized infants with RSV and evaluated the predictive value of cytokines in nasopharyngeal aspirate in comparison to disease severity and found an increase in Th1 and Th2 cytokines.[8] Respiratory viral infections are characterized by the appearance of cytokine storms which are an extreme production and secretion of numerous pro-inflammatory cytokines. Severity of infection is closely related to virus-induced cytokine dysregulation, which is responsible for the development of fatal clinical symptoms, such as massive pulmonary edema, acute bronchopneumonia, alveolar hemorrhage, and acute respiratory distress syndrome.[5] Macrolides down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbation.[5]

Clinical trials have demonstrated controversial results in the effects of macrolides in respiratory viral infections.[9,10,11] To date, studies have only evaluated macrolide use as a treatment, not as a prophylactic therapy. Long-term therapy with the macrolide antibiotic erythromycin was shown to alter the clinical course of diffuse pan bronchiolitis in the late 1980s.[12] Since that time, macrolides have been found to have a large number of anti-inflammatory properties in addition to their antimicrobial effect. These observations provided the rationale for many studies performed over the last decade to assess the usefulness of macrolides in other inflammatory airways diseases including cystic fibrosis, asthma, COPD, and bronchiolitis obliterans syndrome.[13] One randomized controlled Trial (RCT) looked at the daily use of macrolides for up to six weeks to prevent bronchopulmonary dysplasia in premature infants in a NICU setting and found the neonates had better outcomes without an increase in adverse effects.[14] However, the chronic use of macrolides has not been studied for an ability to prevent respiratory infection complications in patients with CLD of infancy. We will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness by preventing the full activation of an inflammatory cascade.

METHODS AND ANALYSIS

Study Design:

A single-site double-blinded RCT that will enroll 92 children between the ages of 6 months to 6 years that have CLD secondary to bronchopulmonary dysplasia (BPD) during two seasons of peak-viral respiratory illness defined as October 1st to March 31st of each year (2015-2016 Season and 2016-2017 Season). At the conclusion of the first season, an interim analysis will be performed to justify the need for the second season. Clinic electronic health care records will be screened to determine eligibility.

Study intervention:

Patients that have parental consent will be given a baseline EKG, a nasal aspirate, an oscillometer reading (over 2 years of age only), and a three to six month supply of either the medication or the placebo at the initial study visit (which will be done during a regularly scheduled follow up clinic visit). Both medications will be taken once a day three days a week: Monday, Wednesday and Friday. The azithromycin medication will be dosed at 5 mg/kg/day. Any child that is eligible to receive Palivizumab (an injection that while not preventing RSV infections, can make it less severe in premature infants) will be given this every 28-30 days in clinic as per usual care. Patients will be monitored closely for adverse reactions over phone, in clinic during their regularly scheduled appointments, and/or during any necessary illness visits. Any children with adverse reactions will discontinue the medication, but will continue to be followed clinically. At any clinic visit in which a child presents with respiratory infections including pneumonia, upper respiratory illness, bronchiolitis, etc., he/she will have an additional nasal aspirate and/or tracheal aspirate (if applicable), and an oscillometer reading (only for children >2 years) performed. At the completion of the 3-6 months treatment phase, each child will have a final nasal aspirate and/or tracheal aspirate, and an oscillometer reading performed. Data will continue to be collected for the following 2 months (April 1st to May 31st), to monitor for respiratory illnesses and possible side effects.

Study Population:

High risk children, born before 37 weeks gestation with a current diagnosis of CLD secondary to BPD between the chronological age of 6 months and <6 years who attend either the HRCC or the High Risk Infant Clinic (HRIC) at UTHealth will be screened by the clinic providers. The high risk infant clinic follows premature infants born before 32 weeks gestation for their first 2 years of life; the high risk pediatric clinic follows medically complex children who have had at least 3 ED visits, 2 hospitalizations, and/or 1 PICU visit within the last year for a chronic health condition. We have chosen to exclusively recruit from these two clinics because ~90% of premature children with CLD from the UTHealth System are followed up at either one of these two clinics.

Inclusion/Exclusion Criteria:

All children who currently attend either the HRCC or the HRIC that are between 6 months and 6 years at the time of enrollment that meet the ATS definition of CLD secondary to BPD will be screened. Chronic lung disease, as defined by the ATS statement from 2002, is "a heterogeneous group of respiratory diseases of infancy that usually evolves from an acute respiratory disorder experienced by a newborn infant."[4] BPD is defined as either (1) for infants born less than 32 weeks, the need for supplemental oxygen for at least 28 days (2) For infants born between 32 weeks and 36 weeks, the need for supplemental oxygen for at least 56 days[4]. All screening will be done by clinic providers (Table 1).

Exclusion criteria include children with cystic fibrosis or bronchiectasis, [15] because the prophylactic use of macrolides has already demonstrated value and become usual care for these patients. Children with cardiac arrhythmias will be excluded, due to the potential increase in cardiovascular death that has been shown in the adult population.[16] Patients with known cyanotic heart disease will be excluded. Children with colitis or short bowel syndrome will also be excluded due to the potential effects to the gastrointestinal flora or malabsorption. In addition, any child with a known macrolide allergy or who is taking any medication that has a known interaction with macrolides, and any child with kidney or liver failure will also be excluded.

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• 6 months-6 years of age • Diagnosis of chronic lung disease (CLD) secondary to bronchopulmonary dysplasia (BPD) as defined by ATS. • Receive primary care at High Risk Infant Clinic or High Risk Children's Clinic	• Cystic Fibrosis or bronchiectasis • Cardiac arrhythmias • Cyanotic heart disease • Colitis • Known Macrolide allergy • Taking medications known to interact with macrolides • Short bowel syndrome • Cystic Fibrosis or bronchiectasis • Kidney or liver failure

Study Procedures:

After patients are screened as eligible, they will be approached during a routine office visit in the clinic. If interested, a baseline EKG will be conducted to ensure that patients enrolled do not have a prolonged QT or any other undiagnosed arrhythmias. If EKG is normal, written informed consent will be obtained in the clinic from the parent or legal guardian of each eligible child by any of the co-investigators or the research nurse at the time of enrollment. Either the research nurse or another clinical member of the HRCC team will then collect a nasal aspirate sample at the first study visit (description in Laboratory section). The nasal aspirate will be stored and

studied after the conclusion of the treatment phase for its levels of myleoperoxydase, cytokines, respiratory virology, and micro biome. In addition, all patients 2 and older who are able, will have a spirometry reading performed using a TremoFlo airway oscillometry system (AOS) manufactured by Thorasys. Patients will be recruited for this study on a rolling basis from October 1st to December 31st, and all participants will complete the intervention phase of the protocol on 31st March. Half of the patients will receive azithromycin at a dose of 5 mg/kg to be given once a day on Monday, Wednesday, and Friday. The other half, the control group, will be provided with a placebo medication of similar taste, color, texture, and consistency, also to be taken once a day on Monday, Wednesday, and Friday. Both the study medication and the placebo will have a fish-oil base to ensure a shelf life of more than six months, and flavored with citrus to improve palatability. Parents will be contacted monthly, either in clinic or by phone, to monitor for their progress and potential adverse reactions. If a significant adverse reaction occurs, the medication will be discontinued. If an allergic reaction (such as rash or shortness of breath) is noted, the blind will be broken by the statistician, who is not involved with patient care or data collection. This un-blinding will be done to note if it is an allergy to the medication.

After the initial appointment, at any face to face encounter (unscheduled sick visit or hospital or ED admission Monday through Friday) in which the patient presents with respiratory symptoms, the patient will be evaluated by the research nurse or one of the co-investigators. Specifically, if a patient presents with the following symptoms: cough, wheeze, tachypnea, rhinorrhea, increased respiratory secretions, hypoxemia, and/or an increased oxygen requirement, an additional nasal aspirate sample and, if applicable, a tracheal aspirate will be done. Oscillometer reading will also be performed for those above 2 years of age when the patients are in clinic during each sick clinic visit for respiratory illness or after the study. At the conclusion of the 3-6 months treatment phase, a final nasal aspirate and/or tracheal aspirate and an EKG will be collected. Additionally an Oscillometer reading will also be collected in those patient 2 years of age and older. There will be no expected/additional study visits and no compensation will be provided for parents or patients.

Risks:

Potential risks include an allergic reaction or adverse reaction to the medication or placebo. Examples of potential side effects include nausea, vomiting, diarrhea, and skin reactions. In addition, there may be an increased risk of diaper rash and/or oral thrush with the increased use of antibiotics. Other adverse outcomes associated with azithromycin include an increase in pyloric stenosis with both prenatal and infant exposure for the first 4 months of life;[17] however, our population for this project will be >6 months of age as approved by IRB. Each of these risks and any other unexpected outcomes will be monitored at any visits to the clinic, or with monthly phone calls. With the anti-inflammatory properties of the macrolide, we predict an overall reduction in the severity of respiratory illnesses during the study period. Additionally, we will continue to monitor patients for an 8 month period following the last azithromycin administration as the medication may lead to a more lasting reduction in the number of unscheduled office visits, emergency room visits, and hospital admissions. With less face to face provider encounters, there will be less opportunity for potential exposure to other viruses, as

well as less time away from home or work for the patients' parents and, potentially, decreased health care related costs.

Study Outcomes

1. In the treatment group, we expect a 20% total reduction in face to face encounters (defined as unscheduled sick visits, urgent care visits, emergency room visits, and hospital admissions) with the prophylactic use of azithromycin, as compared to the control group.
    a. In the treatment group, we expect to have a 20% decrease in the number of unscheduled sick clinic visits during the 3-6 month study period with the prophylactic use of azithromycin, as compared to the control group .
    b. In the treatment group, we expect to have a 20% decrease in the number of Emergency Room/Urgent Care visits during the 3-6 month study period with the prophylactic use of azithromycin, as compared to the control group.
    c. In the treatment group, we expect to have a 20% reduction in the number of hospital admissions during the 3-6 month study period with the prophylactic use of azithromycin, as compared to the control group.
2. In the treatment group, we expect a 20% total reduction in face to face encounters (defined as unscheduled sick visits, urgent care visit, emergency room visits, and hospital admissions) for acute respiratory illness with the prophylactic use of azithromycin, as compared to the control group.
3. We expect no significant difference in adverse side effects between the treatment group receiving the macrolide and the control group receiving the placebo during the 3-6 months intervention period.
4. We expect the intervention to be cost-effective from a healthcare system perspective. We will define cost-effectiveness as:
    a. Decreased days of care without increasing cost
    b. Decreased cost without increasing days of care
    c. Decreased days of care while decreasing cost
5. In the treatment group, we expect a 10% total reduction (as compared to baseline) in the level of myeloperoxidase in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter, as compared to the control group.
6. In the treatment group, we expect a 10% total reduction in the level of pro-inflammatory cytokines in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter, as compared to the control group.
7. In the treatment group, we expect a 10% reduction from baseline in the level of airway obstruction as measured by a tremoFlo Airwave Oscillometry System during the 3-6 month study period, respiratory illness and at the conclusion of the intervention phase.
8. In the treatment group, we expect a 10% total reduction in face to face encounters (defined as unscheduled sick visits, urgent care visit, emergency room visits, and hospital admissions) for respiratory related illness with the prophylactic use of

azithromycin in the 12 months after the intervention phase, as compared to the control group.

The percentage for study outcome (1) was based on our previous experience in the High Risk Children's Clinic. The other percentages were a priori.

Laboratory Evaluation

Microbiologic studies for exploratory outcomes will be conducted. The study involves collecting a minimum of 2 nasal aspirates from each patient. Nasal aspirate samples will be tested to measure the levels of myeloperoxidase and Lactate Dehydrogenase; we will also use ELISA tests to measure the levels of specific pro-inflammatory cytokines, including IL-8 and IL-6, and PCR, in order to screen for several major respiratory viral pathogens.

Nasal aspirate specimens will be collected at the first visit, the final visit, and during any episodic respiratory sick visits at clinic or at Children's Memorial Hermann Hospital during regular office hours. Once collected, the specimen will be immediately diluted (1:1 solution) with viral transport medium (15% glycerol in Iscove's media). The final solution (aspirated specimen mixed with viral transport medium) will contain a maximum of 9 mls (with a range of 3-9 mls). After dilution, the specimens will be immediately refrigerated at 37 degrees, until transportation to the freezers, where they will be stored at -80^{o}C. The samples will be frozen and store until the completion of the trial (table 2).

Laboratory Procedures*:*

The level of myeloperoxidase will be measured using reagent tests, and several cytokines that are markers of inflammation will be tested using ELISA tests, details of the primers and probes to be used are found in a 2005 article by Beckham et al.[18] In addition, RT-PCR assays will be performed using TaqMan-based primers and probes to detect the presence of 5-6 major respiratory viruses, and the remaining volume of aspirate will be stored for future studies of additional biomarkers. Nasal aspirate samples will be collected for assays of up to 4 cytokines and chemokines. Samples will be tested according to the manufacturer's instructions. Samples and serial dilutions of the cytokine standards will be incubated with anti-human cytokine-coated beads in a 96-well filtration plate.

Table 2: Study Schedule

	Study Period			
	Enrollment	Allocation	Post allocation	Close out
Timepoint	Oct-Dec	Oct-Dec	Oct-March	March-Oct
ENROLLMENT:				
Eligibility Screen	X			
Informed consent	X			
EKG	X			
Nasal Aspirate	X		X	
Oscillometer	X			
Allocation		X		
INTEVENTION:				
Take Medicine			X	
Take Placebo			X	
ASSESSMENT:				
Demographics	X			
Unscheduled Clinic visits			X	X
Unscheduled Hospital visits			X	X
ER visits			X	X
Evaluation of Nasal Aspirates				X
Analysis				X
Publication				X

Data Analysis Plan

Patients will be randomized to one of two different branches by the REDCap's database randomization program. Standard frequentist and Bayesian analyses will be performed using an intent-to-treat approach. Total hospital days (counting each hospitalization as an event, and length of hospital stay), total ER/Urgent care visits (counting one day for each ER visit), and unscheduled clinic visits (counting one day for each visit) will be analyzed and related to treatment group (Azithromycin vs Placebo), with logistic regression models and the treatment group as a covariate and random intercept to account for within patient correlation (due to multiple ED visits). To assess the probability of benefit, we will use Bayesian hierarchical models with interaction terms between treatment groups (Azithromycin vs Placebo) and predefined potential moderators. The groups will be stratified by the use of palivizumab and by current tracheostomy. The conservative Bayesian approach of Dixon and Simon allows us to shrink the subgroup estimates to the overall mean treatment effect. For all Bayesian analyses, prior distributions for main effects will be neutral ~N(0,1000), and Uniform (0,1000) for SD parameters. Neutral and skeptical priors will be used for interaction terms. Point estimates of

treatment effect and 95% credible intervals will be reported along with probability of treatment benefit.

Sample size and power

Based on data from our HRCC, we expect the control group to have 1.6 encounters per child-year (SD=1.66). Assuming a two-sided alpha = 0.05, a sample size of 92 (46/group) will have 80% power to detect a difference of 1 in the encounter rate between placebo and azithromycin groups (i.e., 1.6 vs 0.6 in encounter rate or 38% reduction). Power will be more limited for secondary outcomes but Bayesian analyses will provide an estimate of the probability of benefit in these outcomes. A reduction of 1 encounter per child-year in the HRCC and the HRIC is clinically significant and realistically achievable. Since our clinics have been proven to decrease the number of days of care given outside the home by providing comprehensive care, we believe that the reduction of face-to-face provider encounters could be more pronounced in usual care.

Ethics and Dissemination

This research study was approved by the Institutional Review Board (IRB) of the University of Texas Health Science Center in Houston on October 9th, 2014 (HSC-MS-14-0476) (Appendix A). Parental informed consent was obtained at time of enrollment by either the provider who approached the family or the research coordinator. Details concerning the enrollment process can be found in the SPIRIT checklist (Appendix B).

Results from this trial will be published upon completion in a peer reviewed scientific journal.

DISCUSSION

A substantial portion of our high risk chronically ill children have some form of chronic lung disease, including patients with tracheostomies, bronchopulmonary dysplasia, and chronic respiratory failure requiring mechanical ventilation. Most of the hospital admissions in this group of patients were related to respiratory infections in children less than 6 years of age (37% during the winter season for the previous 3 years). Despite vaccination rates of nearly 100% and 24/7 access to our clinic, viral respiratory illnesses continue to cause considerable morbidity and high healthcare costs. [1] Innovative new prophylactic treatments are needed.

With this proposal, we will determine if the prophylactic use of azithromycin will 1) reduce the number of days when unscheduled medical treatment health care related encounters was given outside the home, 2) reduce the number of emergency room/urgent care visits, hospitalizations and clinic visits due to respiratory illness, 3) reduce the level of myeloperoxydase and pro-inflammatory cytokines during viral illnesses requiring face to face physician interaction, 4) demonstrate a reduction in airway obstruction as measured by an oscillometer 5) have a similar safe profile compared to the placebo, and 6) demonstrate cost-effectiveness of macrolides use. Understanding the anti-inflammatory effects of azithromycin when used as a prophylactic drug will provide important insight into the prevention of more serious sequelae of respiratory infections. In

particular, this study will contribute to understanding disease in children ages 6 months to 6 years with chronic lung disease, a population that has a higher rate of hospitalizations for respiratory symptoms.

We predict that the prophylactic use of azithromycin will reduce the morbidity associated with respiratory viral infections during the winter season in patients with chronic lung disease as evidenced by a reduction in the days with unscheduled face to face provider encounters based on the preliminary results from our laboratory study that indicated that prophylactic azithromycin can effectively reduce airway inflammation and disease severity in a RSV-infected mouse model.[6,7] Recent studies have shown that the high morbidity rate of respiratory virus infections is a result of a neutrophilic overactive inflammatory. [5] Macrolides down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbation.[15]

TRIAL STATUS:
Actively screening and enrolling patients.

LIST OF ABBREVIATIONS:

HRCC: High Risk Children's Clinic
CLD: Chronic Lung Disease
BPD: bronchopulmonary dysplasia
RCT: Randomized controlled trial
HRIC: High Risk Infant Clinic

COMPETING INTERESTS:
All authors have no conflict of interest to report.

AUTHORS' CONTRIBUTIONS:

R. Mosquera, G. Colasurdo, C. Jon, K. Smith, J. Stark, A.Yadav, K. McBeth, T. Gonzales, E. Avritscher, C. Pedroza, C. Samuels, T. Harris, A. Gomez-Rubio, S. Wootton, P. Piedra, and J. Tyson, were involved in the Concept and design of the study.

R. Mosquera, C. Jon, A.Yadav, K. McBeth, T. Gonzales, C. Samuels, T. Harris, A. Gomez-Rubio were involved in Enrollment.

C. Samuels, T. Harris, A. Gomez-Rubio were involved in Daily research activities.

R. Mosquera, J. Stark, E. Avritscher, C. Pedroza, C. Samuels, A. Gomez-Rubio, S. Wootton, P. Piedra, and J. Tyson, were involved in the manuscript preparation and approved the final manuscript. R. Mosquera wrote the initial protocol. A. Gomez-Rubio helped with the editing of tables and figures and the refining of the protocol. J. Stark helped in editing the protocol and gave suggestions to improve the methodology of the protocol. C. Pedroza was involved in writing the statistical analysis section of methods. S. Wootton and P.Piedra were involved in the planning and writing of the microbiology/laboratory portion of the protocol. J. Tyson mentored and adviser the primary investigator and was involved in the revision of the protocol. C.

Samuels helped create, write, finalize, edit and refine the protocol from the rough draft to the final protocol.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	**The anti-inflammatory effect of prophylactic macrolides on children with chronic lung disease: a double blinded RCT**
Trial registration		NCT02544984
Protocol version	3	September 23, 2015
Funding	4	No funding available

Roles and responsibilities	5a	Ricardo A. Mosquera, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study, enrolment and the manuscript preparation and final approval. Tomika Harris, PNP. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrolment, daily research activities and the manuscript preparation and final approval. Ana M. Gomez-Rubio, MPH. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrolment, daily research activities and the manuscript preparation and final approval. Aravind Yadav, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study, enrolment, and the manuscript preparation and final approval. James Stark, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study and the manuscript preparation and final approval. Cindy Jon, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrolment, and the manuscript preparation and final approval. Katrina McBeth, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrolment, and the manuscript preparation and final approval. Traci Gonzales, PNP. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrolment, and the manuscript preparation and final approval. Elenir Avritscher, MD PhD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Claudia Pedroza, PhD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Keely Smith, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Giuseppe Colasurdo, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study. Susan Wootton, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Pedro Piedra, MD. Department of Virology and Microbiology, Baylor College of Medicine, Houston, Texas. He was involved in the concept and design of the study, laboratory procedures and the manuscript preparation and final approval. Jon E. Tyson, MD, MPH. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study and the manuscript preparation and final approval. Cheryl Samuels, PNP. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrolment, daily research activities and the manuscript preparation and final approval.

	5b	NA
	5c	NA
	5d	NA

Introduction

Background and rationale	6a	Macrolides have received considerable attention for their anti-inflammatory and immunomodulatory actions beyond the antibacterial effect. Such properties may ensure some efficacy against a wide spectrum of respiratory viral infections.[5] Recent studies, including a study performed in our lab with elderly BALB/ mice infected with RSV, have shown that the high mortality rate o respiratory virus infections is a result of a neutrophilic overactive inflammatory response.[6] A recently published study examined the inflammatory response in hospitalized infants with RSV and evaluated the predictive value of cytokines in nasopharyngeal aspirate in comparison to disease severity and found an increase Th1 and Th2 cytokines.[7] Respiratory viral infections are characterized by the appearance of cytokine storms which are an extreme production and secretion of numerous pro-inflammatory cytokines. Severity of infection is closely related to virus-induced cytokine dysregulation, which is responsible for the development fatal clinical symptoms, such as massive pulmonary edema, acute bronchopneumonia, alveolar hemorrhage, and acute respiratory distress syndrome.[5] Macrolides down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infection and may reduce virus-related exacerbation. Clinical trials have demonstrated controversial results in the effects of macrolides in respiratory viral infections.[8] To date, studies have only evaluated macrolide use as a treatment, not as a prophylactic therapy. Long term therapy with the macrolide antibiotic erythromycin was shown to alter the clinical course of diffuse pan bronchiolitis in the late 1980s.[9] Since that time, macrolides have been found to have a large number of anti-inflammatory properties in addition to their antimicrobial effect. These observations provided the rationale for many studies performed over the last decade to assess the usefulness of macrolides in other inflammatory airways diseases, such as cystic fibrosis, asthma, COPD, and bronchiolitis obliterans syndrome.[10] One Randomized Controlled Trial (RCT) looked at the daily use of macrolides for up to six weeks to prevent bronchopulmonary dysplasia in premature infants in a NICU setting and found the neonates to have better outcomes without an increase in adverse effects.[11] However, chronic use of macrolides has not been studied to prevent respiratory infection complications in patients with CLD of infancy. We will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness and that this is associated with the prevention of the full activation of an inflammatory cascade.
	6b	No other standard of care

Objectives	7	With this RCT, we will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness and that this is associated with the prevention of the full activation of an inflammatory cascade..
Trial design	8	With this RCT, we will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness and that this is associated with the prevention of the full activation of an inflammatory cascade.

Methods: Participants, interventions, and outcomes

Study setting	9	High risk children, born before 37 weeks gestation with a current diagnosis of CLD secondary to BPD between the chronological age of 6 months and <6 years who attend either the HRCC or the High Risk Infant Clinic (HRIC) at UTHealth will be screened by the research nurse, or by the clinic providers.
Eligibility criteria	10	All children who currently attend either the HRCC or the HRIC that are between 6 months and 6 years at the time of enrollment that meet the ATS definition of CLD secondary to BPD will be screened. Chronic lung disease, as defined by the ATS statement from 2002, is "a heterogeneous group of respiratory diseases of infancy that usually evolves from an acute respiratory disorder experienced by a newborn infant."[3] BPD is defined as either (1) for infants born less than 32 weeks, the need for supplemental oxygen for at least 28 days (2) For infants born between 32 weeks and 36 weeks, the need for supplemental oxygen for at least 56 days[3]. All screening will be done by clinic providers (Table 1). Exclusion criteria include children with cystic fibrosis or bronchiectasis, [7] because the prophylactic use of macrolides has already demonstrated value and become usual care for these patients. Children with cardiac arrhythmias will be excluded, due to the potential increase in cardiovascular death that has been shown in the adult population[8]. Patients with cyanotic heart disease will be excluded. Children with colitis or short bowel syndrome will also be excluded due to the potential effects to the gastrointestinal flora or malabsorption. In addition, any child with a known macrolide allergy or child who is taking any medication that has a known interaction with macrolides, and any child with kidney or liver failure will also be excluded.
Interventions	11a	Both medications will be taken once a day three days a week: Monday, Wednesday and Friday. The azithromycin medication will be dosed at 5 mg/kg/day.
	11b	Any children with adverse reactions will discontinue the medication, but will continue to be followed clinically.

	11c	NA
	11d	NA

	Outcomes	12	1. In the treatment group, we expect a 20% total reduction in face to face encounters (defined as unscheduled sick visits, urgent care visit, emergency room visits, and hospital admissions) with the prophylactic use of azithromycin, as compared to the control group. a. In the treatment group, we expect to have a 20% decrease in the number of unscheduled sick clinic visits during the 3-6 month study period with the prophylactic use of azithromycin, as compared to the control group . b. In the treatment group, we expect to have a 20% decrease in the number of Emergency Room visits during the 3-6 month study period with the prophylactic use of azithromycin, as compared to the control group. c. In the treatment group, we expect to have a 20% reduction in the number of hospital admissions during the 3-6 month study period with the prophylactic use of azithromycin, as compared to the control group. 2. In the treatment group, we expect a 20% total reduction in face to face encounters (defined as unscheduled sick visits, urgent care visit, emergency room visits, and hospital admissions) for acute respiratory illness with the prophylactic use of azithromycin, as compared to the control group. 3. We expect no significant difference in adverse side effects between the treatment group receiving the macrolide and the control group receiving the placebo during the 3-6 months intervention period. 4. We expect the intervention to be cost-effective from a healthcare system perspective. We will define cost-effectiveness as: a. Decrease days of care without increasing cost b. Decrease cost without increasing days of care c. Decrease days of care while decreasing cost 5. In the treatment group, we expect a 10% total reduction (as compared to baseline) in the level of myeloperoxidase in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter, as compared to the control group. 6. In the treatment group, we expect a 10% total reduction in the level of pro-inflammatory cytokines in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter, as compared to the control group. 7. In the treatment group, we expect a 10% reduction from baseline in the level of airway obstruction as measured by a tremoFlo Airwave Oscillometry System during the 3-6 month study period, respiratory illness and at the conclusion of the intervention phase. 8. In the treatment group, we expect a 10% total reduction in face to face encounters (defined as unscheduled sick visits, urgent care visit, emergency room visits, and hospital admissions) for respiratory related illness with the prophylactic use of azithromycin in the 12 months

Participant timeline | 13 | See Table 2.

Table 2: Study Schedule

	Study Period		
	Enrollment	Allocation	Post allocation
Timepoint	Oct-Dec	Oct-Dec	Oct-March
ENROLLMENT:			
Eligibility Screen	X		
Informed consent	X		
EKG	X		
Nasal Aspirate	X		X
Oscillometer	X		
Allocation		X	
INTEVENTION:			
Take Medicine			X
Take Placebo			X
ASSESSMENT:			
Demographics	X		
Unscheduled Clinic visits			X
Unscheduled Hospital visits			X
ER visits			X
Evaluation of Nasal Aspirates			
Analysis			
Publication			

Sample size | 14 | Based on data from our HRCC, we expect the control group to have 1.6 encounters per child-year (SD=1.66). Assuming a two-sided alpha = 0.05, a sample size of 92 (46/group) will have 80% power to detect a difference of 1 in the encounter rate between placebo and azithromycin groups (i.e., 1.6 vs 0.6 in encounter rate or 38% reduction).

Recruitment | 15 | We will ensure patient recruitment by screening both clinics weekly during the enrolment process. Eligible patients will be approached by the research coordinator and one of the providers. Patients who have further questions will be given time to think and re approach with a phone call or next time they are in clinic.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation | 16a | Biostatistician will create the sequence generation sequence and will upload it into redcap

Allocation concealment mechanism	16b	Patients will be randomized to one of two different branches by the REDCap database's randomization program.
Implementation	16c	Redcap will assign patients to either group.
Blinding (masking)	17a	This is a double-blinded placebo study. Both participants and people directly related with study procedures, including the providers, will be blinded.
	17b	If a significant adverse reaction occurs, the medication will be discontinued. If an allergic reaction (such as rash or shortness of breath) is noted, the blind will be broken by the statistiican, who is not involved with patient care or data collection. This un-blinding will be done to note if it is an allergy to the medication.

Methods: Data collection, management, and analysis

Data collection methods	18a	All data will be kept in REDCap database. REDCap is a HIPPA compliant and safe database that can be accessed from any computer with an internet access. It is backed up on a regular basis. Collected data at baselines includes: demographic information, baseline EKG, baseline nasal aspirate and baseline oscillometer reading. At the conclusion of the study the EKG, nasal aspirate will be repeated. If any patient presents to the clinic, ER or hospital with respiratory symptoms during business hours, a nasal aspirate and oscillometer reading will be collected. Data on when symptoms started and the symptoms experienced will also be collected.
	18b	We will call patients once a month to follow up on their medication status and to assess for any possible side effects.
Data management	19	Data can only be entered into RedCap by the research coordinator. At the end of the medication period, the research coordinator will ensure that all encounters were entered correctly by doublechecking the medical records.

Statistical methods	20a	Standard frequentist and Bayesian analyses will be performed using an intent-to-treat approach. Total hospital days (counting each hospitalization as an event, and length of hospital stay), total ER visits (counting one day for each ER visit), and unscheduled clinic visits (counting one day for each visit) will be analyzed and related to treatment group (Azithromycin vs Placebo), with logistic regression models and the treatment group as a covariate and random intercept to account for within patient correlation (due to multiple ED visits). To assess the probability of benefit, we will use Bayesian hierarchical models with interaction terms between treatment groups (Azithromycin vs Placebo) and predefined potential moderators. The groups will be stratified by use of palivizumab and by current tracheostomy. The conservative Bayesian approach of Dixon and Simon allows us to shrink the subgroup estimates to the overall mean treatment effect. For all Bayesian analyses, prior distributions for main effects will be neutral ~N(0,1000), and Uniform (0,1000) for SD parameters. Neutral and skeptical priors will be used for interaction terms. Point estimates of treatment effect and 95% credible intervals will be reported along with probability of treatment benefit.
	20b	NA
	20c	Intent-to-treat analysis. We will continue to collect data on hospitalizations and ER visits even if we cannot get a hold of them.
Methods: Monitoring		
Data monitoring	21a	Dr. Cody Arnolds and Claudia Pedroza will act as the Data Monitoring Committee. Neither of them is involved in the daily aspects of the project but in case of any adverse events reported they will be in charge of the unblinding.
	21b	Since this is a pilot study no interim analysis or stopping rules will be applied.
Harms	22	Parents will be called once a month in order to see if any experience any adverse events. If there is a complain, the providers will be made aware and if their professional opinion they believe the event is an adverse reaction to the medication, it will be recorded in the RedCap database and parents will be advise to stop the medication immediately. In case of any serious adverse events, the IRB will also be notified.
Auditing	23	NA
Ethics and dissemination		
Research ethics approval	24	UTHealth IRB approval was sought.

Protocol amendments	25	Research coordinator will communicate any changes to the protocol or staff to the UTH IRB using iris.
Consent or assent	26a	Parental informed consent will be collected. The research coordinator and a provider will talk to parent and explain the project, answer any questions and give a thorough explanation of study procedures, risk and benefits. Once the parents agree to participate, they will sign an IRB approved parental consent, detailing study procedures, risks and benefits. This parental consent will be scanned and uploaded into Redcap and paper copies will be saved in a binder that will be kept in a locked office.
	26b	NA
Confidentiality	27	Patients will be identified using an ID number. All data will be entered into a HIPPA compliant database and all papercopies will be kept in a locked office to ensure patient confidentiality.
Declaration of interests	28	No financial interest to declare.
Access to data	29	Claudia Pedroza will have access to the final trial database. If any other investigators need to access the data, they will need to contact her.
Ancillary and post-trial care	30	No arrangements have been made for compensation to those who suffer harm from trial participation. This has been stated in the informed consent.
Dissemination policy	31a	The results of the trial will be published in a peer reviewed journal.
	31b	NA
	31c	We plan on submitting the protocol to the trials journal once the enrolment begins.

Appendices

Informed consent materials	32	Parental consent form is obtained.

Biological specimens	33	Microbiologic studies for exploratory outcomes will be conducted. The study involves collecting a minimum of 2 nasal aspirates from each patient. Nasal aspirate samples will be tested to measure the levels of myeloperoxidase and Lactate Dehydrogenase; we will also use ELISA tests to measure the levels of specific pro-inflammatory cytokines, including IL-8 and IL-6, and PCR, in order to screen for several major respiratory viral pathogens. Nasal aspirate specimens will be collected at the first visit, the final visit, and during any episodic respiratory sick visits at clinic or at Children's Memorial Hermann Hospital. Once collected the specimen will be immediately diluted (1:1 solution) with viral transport medium (15% glycerol in Iscove's media). The final solution (aspirated specimen mixed with viral transport medium) will contain a maximum of 9 mls (with a range of 3-9 mls). After dilution, the specimens will be immediately refrigerated at 37 degrees, until transportation to the freezers, where they will be stored at -80^{o}C. The samples will be frozen and store until the completion of the trial. The level of myeloperoxidase will be measured using reagent tests, and several cytokines that are markers of inflammation will be tested using ELISA tests, details of the primers and probes to be used are found in a 2005 article by Beckham et al.[10] In addition, RT-PCR assays will be performed using TaqMan-based primers and probes to detect the presence of 5-6 major respiratory viruses, and the remaining volume of aspirate will be stored for future studies of additional biomarkers. Nasal aspirate samples will be collected for assay of up to 4 cytokines and chemokines. Samples will be tested according to the manufacturer's instructions. Samples and serial dilutions of the cytokine standards will be incubated with anti-human cytokine-coated beads in a 96-well filtration plate.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The anti-inflammatory effect of prophylactic macrolides on children with chronic lung disease: a protocol for a double blinded randomized controlled trial

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The anti-inflammatory effect of prophylactic macrolides on children with chronic lung disease: a protocol for a double blinded randomized controlled trial.

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ABSTRACT

Introduction: Recent studies suggest that the high mortality rate of respiratory viral infections is a result of an overactive neutrophilic inflammatory response**. Macrolides have anti-inflammatory properties**, including the ability to down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbations. In this study, we will test **the hypothesis that prophylactic macrolides** will reduce the severity of respiratory viral illness in children with chronic lung disease by preventing the full activation of the inflammatory cascade.

Methods and analysis: A randomized double-blind placebo controlled trial that will enroll 92 children to receive either azithromycin or placebo for a period of 3-6 months during two **respiratory syncytial virus (RSV)** seasons (2015-2016 and 2016-2017). We expect a reduction of at least 20% **in the total number of days of** unscheduled face to face encounters in the treatment group as compared to placebo group. Standard frequentist and Bayesian analyses will be performed using an intent-to-treat approach.

Discussion: We predict that the prophylactic use of azithromycin will reduce the morbidity associated with respiratory viral infections during the winter season in patients with chronic lung disease as evidenced by a reduction in the total number of days with unscheduled face to face provider encounters.

Ethics and dissemination: This research study was approved by the Institutional Review Board of the University of Texas Health Science Center in Houston on October 9th, 2014. Upon completion, the results will be published.

Trial registration: clinicaltrials.gov identifier: NCT02544984

INTRODUCTION

For the past 3 years, the High Risk Children's Clinic (HRCC) at the University of Texas Health Science Center at Houston (UTHealth) has been providing a medical home for medically complex children. We have demonstrated major benefits of fewer hospital admissions and emergency room visits while providing health care savings for high-risk chronically ill children including patients with chronic lung disease (CLD).[1] These benefits have not been previously shown for medical homes for patients of any kind or age.[2, 3] These benefits result primarily from 24/7 access by phone to health care providers. The clinic offers same day appointments and provides coordination of care for this population. We now aim to further cut morbidity rates by developing specific outpatient interventions to augment the care for each major disorder that we treat.

Almost half (44%) of our patients in the **HRCC** are chronically ill children who have some form of CLD including patients with bronchopulmonary dysplasia (BPD).[1] **CLD**, as defined by the **American Thoracic Society (ATS)** statement from 2002, is "a heterogeneous group of respiratory diseases of infancy that usually evolves from an acute respiratory disorder experienced by a newborn infant" [4] specifically, infants with bronchopulmonary dysplasia, defined as the need for supplemental oxygen therapy in children over 28 days old that were born before 32 weeks gestation.[4] These infants often incur long-term pulmonary function abnormalities including oxygen dependency after discharge, recurrent respiratory infections, and other reactive airway diseases. From our data, we have learned that many of the hospital admissions in our group of patients were related to respiratory infections (37%) during the winter season. Despite vaccination rates of nearly 100%, administration of Palivizumab to all of those eligible patients, and access to our comprehensive care clinic, viral respiratory illnesses continue to cause considerable morbidity and high healthcare costs in this patient population.[1] Innovative new prophylactic treatments are needed.

Macrolides have received considerable attention for their anti-inflammatory and immunomodulatory actions. Such properties may ensure some efficacy against a wide spectrum of respiratory viral infections.[5] Recent studies, including a study performed in our lab with elderly BALB/c mice infected with **respiratory syncytial virus (RSV),** have shown that the high mortality rate of respiratory virus infections is a result **of an overactive neutrophilic** inflammatory response.[6, 7] A recently published study examined the inflammatory response in hospitalized infants with RSV and evaluated the predictive value of cytokines in nasopharyngeal aspirate in comparison to disease severity and found an increase in Th1 and Th2 cytokines.[8] Respiratory viral infections are characterized by the appearance of cytokine storms which **involve an** extreme production and secretion of numerous pro-inflammatory cytokines. Severity of infection is closely related to virus-induced cytokine dysregulation, which is responsible for the development of fatal clinical symptoms, such as massive pulmonary edema, acute bronchopneumonia, alveolar hemorrhage, and acute respiratory distress syndrome.[5] Macrolides down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbation.[5]

Clinical trials have demonstrated controversial results in the effects of macrolides in respiratory viral infections.[9,10,11] To date, studies have only evaluated macrolide use as a treatment, not as a prophylactic therapy. Long-term therapy with the macrolide antibiotic erythromycin was shown to alter the clinical course of diffuse pan bronchiolitis in the late 1980s.[12] Since **then**, macrolides have been found to have a large number of anti-inflammatory properties in addition to their antimicrobial effect. These observations provided the rationale for many studies performed over the last decade to assess the usefulness of macrolides in other inflammatory airways diseases including cystic fibrosis, asthma, COPD, and bronchiolitis obliterans syndrome.[13] One randomized controlled Trial (RCT) looked at the daily use of macrolides for up to six weeks to prevent bronchopulmonary dysplasia in premature infants in a **neonatal intensive care unit (NICU)** setting and found the neonates had better outcomes without an increase in adverse effects.[14] However, the chronic use of macrolides **to prevent respiratory infection complications in patients with CLD of infancy has not been studied**. We will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness by preventing the full activation of an inflammatory cascade.

METHODS AND ANALYSIS

Study Design:

A single-site double-blinded RCT that will enroll 92 children **age 6 months** to 6 years **who** have CLD secondary to bronchopulmonary dysplasia (BPD) during two **respiratory viral seasons** defined as October 1st to March 31st of each year (2015-2016 Season and 2016-2017 Season). At the conclusion of the first season, an interim analysis will be performed to justify the need for the second season. Clinic electronic health care records will be screened to determine eligibility.

Study intervention:

This will be a pragmatic study design with a rolling enrollment time period from October 1st until December 31st. At enrollment, patients who have parental consent will **undergo** a baseline **electrocardiogram (ECG),** a nasal aspirate, **and** an oscillometer reading (over 2 years of age only). **At the initial study visit (which will be done during a regularly scheduled follow up clinic visit), enrollees will receive a six month supply** of either the medication or the placebo. **All patients will take the medication until the end of the treatment phase (March 31st). Patients will therefore end up receiving the medication for a time period ranging from 3-6 months and extra medication will be discarded. The medication or placebo** will be taken once a day three days a week (Monday, Wednesday and Friday). The azithromycin medication will be dosed at 5 mg/kg/day. Any child **who** is eligible to receive Palivizumab will **receive it** every 28-30 days in clinic as per usual care. Patients will be monitored closely for adverse reactions over phone, in clinic during their regularly scheduled appointments, and/or during any necessary illness visits. Any children with adverse reactions will discontinue the medication, but will continue to be followed clinically. At any clinic visit in which a child presents with respiratory infections including pneumonia, upper respiratory illness, bronchiolitis, etc., he/she will have an additional nasal aspirate and/or tracheal aspirate (if applicable), and an oscillometer reading

(only for children >2 years) performed. **At the completion of the treatment phase**, each child will have a final nasal aspirate and/or tracheal aspirate, and an oscillometer reading performed. Data will continue to be collected for the following 2 months (April 1st to May 31st), to monitor for respiratory illnesses and possible side effects.

Study Population:

High risk children, born before 37 weeks gestation with a current diagnosis of CLD secondary to BPD between the chronological age of 6 months and <6 years who attend either the HRCC or the High Risk Infant Clinic (HRIC) at **UTHealth at the McGovern Medical School in Houston, Texas USA** will be screened by the clinic providers. The high risk infant clinic follows premature infants born before 32 weeks gestation for their first 2 years of life; the high risk pediatric clinic follows medically complex children who have had at least 3 **emergency department (ED)** visits, 2 hospitalizations, and/or 1 **pediatric intensive care unit (PICU)** visit within the last year for a chronic health condition. We have chosen to exclusively recruit from these two clinics because ~90% of premature children with CLD from the UTHealth System are followed up at either one of these two clinics**. We will ensure patient recruitment by screening both clinics weekly during the enrollment process. Eligible patients will be approached by the research coordinator and/or one of the providers. Patients who have further questions will be given time to think and will be approached later with a phone call or next time they are in clinic.**

Inclusion/Exclusion Criteria:

All children who currently attend either the HRCC or the HRIC **who** are between 6 months and 6 years at the time of enrollment **and who** meet the **American Thoracic Society (ATS)** definition of CLD secondary to BPD will be screened. Chronic lung disease, as defined by the ATS statement from 2002, is "a heterogeneous group of respiratory diseases of infancy that usually evolves from an acute respiratory disorder experienced by a newborn infant."[4] BPD is defined as either **as** (1) infants born less than 32 weeks **who** need supplemental oxygen for at least 28 days **or (2) infants** born between 32 weeks and 36 weeks **who** need for supplemental oxygen for at least 56 days[4]. All screening will be done by clinic providers (Table 1).

Exclusion criteria include children with cystic fibrosis or bronchiectasis, [15] because the prophylactic use of macrolides has already demonstrated value and become usual care for these patients. Children with cardiac arrhythmias will be excluded, due to the potential increase in cardiovascular death that has been shown in the adult population.[16] Patients with known cyanotic heart disease will be excluded. Children with colitis or short bowel syndrome will also be excluded due to the potential effects to the gastrointestinal flora or malabsorption. In addition, any child with a known macrolide allergy or who is taking any medication that has a known interaction with macrolides, and any child with kidney or liver failure will also be excluded.

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• 6 months-6 years of age **during respiratory viral season (Oct 1 – Dec 31)** • Diagnosis of chronic lung disease (CLD) secondary to bronchopulmonary dysplasia (BPD) as defined by **the American Thoracic Society (ATS).** • Receive primary care at High Risk Infant Clinic **(HRIC)** or High Risk Children's Clinic **(HRCC)**	• Cystic **fibrosis** or bronchiectasis • Cardiac arrhythmias • Cyanotic heart disease • Colitis • Known **macrolide** allergy • Taking medications known to interact with macrolides • **Short bowel syndrome** • **Kidney or liver failure**

Study Procedures:

After patients are screened as eligible, they will be approached during a routine office visit in the clinic. If the **patient is interested in participating in the study**, a baseline **ECG** will be conducted to ensure that patients enrolled do not have a prolonged QT **interval** or any other undiagnosed arrhythmias. If **the ECG** is normal, written informed consent will be obtained in the clinic from the parent or legal guardian of each eligible child by any of the co-investigators or the research nurse at the time of enrollment.

Once a patient is deemed eligible, he or she will be randomized to either azithromycin or placebo using the REDCap randomization module. Allocation ratio will be 1:1 and will be stratified by use of Palivizumab and presence of tracheostomy. The statistician will create the randomization sequence using labeling of A or B for the two groups and will upload it into REDCap. This will be double-blinded as neither the providers nor the patients will know whether they are receiving placebo or medication. Allocation concealment will be ensured, as the allocation sequence is only known to the study statistician (who is also blinded to the labeling of the groups) and is not made available in REDCap until after the patient has been recruited into the trial.

After randomization, the research nurse or another clinical member of the HRCC team will then collect a nasal aspirate sample at the first study visit (description in Laboratory section). The nasal aspirate will be stored and studied after the conclusion of the treatment phase for its levels of **myeloperoxydase,** cytokines, respiratory virology, and **microbiome**. In addition, all patients 2 and older who are able, will have a spirometry reading performed using a TremoFlo airway oscillometry system (AOS) manufactured by Thorasys. Patients will be recruited **and enrolled** for this study on a rolling basis from October 1st to **December 31st. Once enrolled, all participants** will complete the intervention phase of the protocol on 31st March. Half of the patients will receive azithromycin at a dose of 5 mg/kg to be given once a day on Monday,

Wednesday, and Friday. The other half, the control group, will be provided with a placebo medication of similar taste, color, texture, and consistency, also to be taken once a day on Monday, Wednesday, and Friday. Both the study medication and the placebo will have a fish-oil base to ensure a shelf life of more than six months, and flavored with citrus to improve palatability. Parents will be contacted monthly, either in clinic or by phone, to monitor for their progress and potential adverse reactions. If a significant adverse reaction occurs, the medication will be discontinued. If an allergic reaction (such as rash or shortness of breath) is noted, the blind will be broken by the statistician, who is not involved with patient care or data collection. This **unblinding** will be done to note if it is an allergy to the **azithromycin or the preparation. Confidentiality will be maintained as patients will be identified using only a study identification number. All data will be entered into a HIPAA compliant database and all paper copies will be kept in a locked office to ensure patient confidentiality.**

After the initial appointment, at any face to face encounter (unscheduled sick visit or hospital or ED admission Monday through Friday) in which the patient presents with respiratory symptoms, the patient will be evaluated by the research nurse or one of the co-investigators. Specifically, if a patient presents with the following symptoms: cough, wheeze, tachypnea, rhinorrhea, increased respiratory secretions, hypoxemia, and/or an increased oxygen requirement, an additional nasal aspirate sample and, if applicable, a tracheal aspirate will be done. Oscillometer reading will also be performed for those above 2 years of age when the patients are in clinic during each sick clinic visit for respiratory illness or after the study. At the conclusion of the 3-6 months treatment phase, a final nasal aspirate and/or tracheal aspirate and an **ECG** will be collected. Additionally an **oscillometer** reading will also be collected in those patient 2 years of age and older. There will be no expected/additional study visits and no compensation will be provided for parents or patients.

Risks:

Potential risks include an allergic reaction or adverse reaction to the medication or placebo. Examples of potential side effects include nausea, vomiting, diarrhea, and skin reactions. In addition, there may be an increased risk of diaper rash and/or oral thrush with the increased use of antibiotics. Other adverse outcomes associated with azithromycin include an increase in pyloric stenosis with both prenatal and infant exposure for the first 4 months of life;[17] however, our population for this project will be >6 months of age as approved **by the Institutional Review Board (IRB). Any changes to the protocol or staff will be communicated by the research coordinator will any to the UTHealth IRB using the Integrated Research Information System (iRIS).** Each of these risks and any other unexpected outcomes will be monitored at any visits to the clinic, or with monthly phone **calls to see if any patients experience any adverse events. If there is a complaint, the providers will be made aware and if in their professional opinion they believe the event is an adverse reaction to the medication, it will be recorded in the REDCap database and parents will be advised to stop the medication immediately. In case of any serious adverse events, the IRB will also be notified and if needed the statistician will break the blind.** With the anti-inflammatory properties of the macrolide, we predict an overall reduction in the severity of respiratory illnesses during the study period. Additionally, we will

continue to monitor patients for a 2 month period following the last azithromycin administration as the medication may lead to a more lasting reduction in the number of unscheduled office visits, emergency room visits, and hospital admissions. With less face to face provider encounters, there will be less opportunity for potential exposure to other viruses, as well as less time away from home or work for the patients' parents and, potentially, decreased health care related costs.

Study Outcomes

Our primary outcome is the total number of days of unscheduled face to face encounters for all diagnoses (defined as unscheduled sick visits, urgent care visits, emergency room visits, and hospital admissions) during the 3-6 month treatment phase of the study.

Secondary outcomes:

1. **Individual component of the primary outcome: unscheduled sick visits, urgent care visits, emergency room visits, and hospital admissions.**
2. **Adverse side effects including gastrointestinal upset (vomiting/diarrhea) and diaper rash.**
3. **Total hospital and clinic costs from a health care system perspective. We expect the intervention to be cost-effective from a healthcare system perspective defined as:**
   a. **Decreased days of care without increasing cost**
   b. **Decreased cost without increasing days of care**
   c. **Decreased days of care while decreasing cost**
4. **Level of myeloperoxidase in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter.**
5. **Level of pro-inflammatory cytokines in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter.**
6. **Level of airway obstruction as measured by a TremoFlo Airwave Oscillometry System during the 3-6 month treatment phase during respiratory illness and at the conclusion of the intervention phase.**

Laboratory Evaluation

Microbiologic studies for exploratory outcomes will be conducted. The study involves collecting a minimum of 2 nasal aspirates from each patient. Nasal aspirate samples will be tested to measure the levels of myeloperoxidase **(MPO)** and **lactate dehydrogenase (LDH)**; we will also use **enzyme-linked immunosorbent assay (ELISA)** tests to measure the levels of specific pro-inflammatory cytokines, including **interleukin 8 (IL-8)** and **interleukin 6 (IL-6)**, and **polymerase chain reaction (PCR),** in order to screen for several major respiratory viral pathogens.
Nasal aspirate specimens will be collected at the first visit, the final visit, and during any episodic respiratory sick visits at clinic or at Children's Memorial Hermann Hospital during regular office hours. Once collected, the specimen will be immediately diluted (1:1 solution) with viral transport medium (15% glycerol in Iscove's media). The final solution (aspirated

specimen mixed with viral transport medium) will contain a maximum of 9 **ml** (with a range of 3-9 **ml**). After dilution, the specimens will be immediately refrigerated at 37 degrees Fahrenheit until transportation to the freezers, where they will be stored at -80^{o}C. The samples will be frozen and store until the completion of the trial (table 2).

Laboratory Procedures:

The level of myeloperoxidase will be measured using reagent tests, and several cytokines that are markers of inflammation will be tested using ELISA tests, details of the primers and probes to be used are found in a 2005 article by Beckham et al.[18] In addition, **reverse transcription polymerase chain reaction (RT-PCR)** assays will be performed using TaqMan-based primers and probes to detect the presence of 5-6 major respiratory viruses, and the remaining volume of aspirate will be stored for future studies of additional biomarkers. Nasal aspirate samples will be collected for assays of up to 4 cytokines and chemokines. Samples will be tested according to the manufacturer's instructions. Samples and serial dilutions of the cytokine standards will be incubated with anti-human cytokine-coated beads in a 96-well filtration plate.

Table 2: Study Schedule

	Study Period			
	Enrollment	Allocation	Post allocation	Close out
Timepoint	Oct-Dec	Oct-Dec	Oct-March	March-Oct
ENROLLMENT:				
Eligibility screen	X			
Informed consent	X			
Electrocardiogram (ECG)	X			
Nasal aspirate	X		X	
Oscillometer	X			
Allocation		X		
INTERVENTION:				
Take medicine			X	
Take placebo			X	
ASSESSMENT:				
Demographics	X			
Unscheduled clinic visits			X	X
Unscheduled hospital visits			X	X
Emergency room visits			X	X
Evaluation of nasal aspirates				X
Analysis				X
Publication				X

Data Collection, Management and Analysis Plan

All data will be kept in REDCap database. REDCap is a HIPAA compliant and safe database that can be accessed from any computer with an internet access. It is backed up on a regular basis. Collected data at baselines includes: demographic information, baseline ECG, baseline nasal aspirate and baseline oscillometer reading. At the conclusion of the study the ECG, nasal aspirate will be repeated. If any patient presents to the clinic, ED or hospital with respiratory symptoms during business hours, a nasal aspirate and oscillometer reading will be collected. Data on when symptoms started and the symptoms experienced will also be collected.

Data can only be entered into REDCap by the research coordinator. At the end of the medication period, the research coordinator will ensure that all encounters were entered correctly by double checking the medical records. Each patient will be called once a month to follow up on their medication status and to assess for any possible side effects.

Standard frequentist and Bayesian analyses will be performed using an intent-to-treat approach. **Total number of days with unscheduled face to face encounters**, total hospital **admissions** (counting each hospitalization as an event), total ER**/urgent** care visits (counting one day for each ER**/urgent care** visit), and **total** unscheduled clinic visits (counting one day for each visit) will be analyzed and related to treatment group (Azithromycin vs Placebo) **using Poisson** regression models **with robust standard errors to estimate relative risks (RRs) and 95% confidence intervals. All models will include treatment group and stratification variables (use of palivizumab and current tracheostomy) as covariates and length of follow-up as an offset.** To assess the probability of benefit, we will use Bayesian models with interaction terms between treatment group (Azithromycin vs Placebo) and **two** predefined potential moderators: use of palivizumab and current tracheostomy. The conservative Bayesian approach of Dixon and Simon allows us to shrink the subgroup estimates to the overall mean treatment effect. For all Bayesian analyses, prior distributions for **all regression coefficients will be centered at RR of 1.0 (Normal with mean 0 and standard deviation of 1 in the log scale), and half-Normal(0,1) for standard deviation parameters.** Point estimates of treatment effect and 95% credible intervals will be reported along with probability of treatment benefit.

Sample size and power

Based on data from our HRCC, we expect the **placebo** group to have 1.6 encounters per child-year (SD=1.66). Assuming a two-sided alpha **of** 0.05, a sample size of 92 (46/group) will have 80% power to detect a difference of 1 in the encounter rate between placebo and azithromycin groups (i.e., 1.6 vs 0.6 in encounter rate or 38% reduction). Power will be more limited for secondary outcomes but Bayesian analyses will provide an estimate of the probability of benefit in these outcomes. A reduction of 1 encounter per child-year in the HRCC and the HRIC is clinically significant and realistically achievable. Since our clinics have been proven to decrease the number of days of care given outside the home by providing comprehensive care, we believe that the reduction of face-to-face provider encounters could be more pronounced in usual care.

Ethics and Dissemination

This research study was approved by the Institutional Review Board (IRB) of the University of Texas Health Science Center in Houston on October 9th, 2014 (HSC-MS-14-0476) (Appendix A). Parental informed consent was obtained at time of enrollment by either the provider who approached the family or the research coordinator. Details concerning the enrollment process can be found in the SPIRIT checklist (Appendix B).

Results from this trial will be published upon completion in a peer reviewed scientific journal.

DISCUSSION

A substantial portion of our high risk chronically ill children have some form of chronic lung disease, including patients with tracheostomies, bronchopulmonary dysplasia, and chronic respiratory failure requiring mechanical ventilation. Most of the hospital admissions in this

group of patients were related to respiratory infections in children less than 6 years of age (37% during the winter season for the previous 3 years). Despite vaccination rates of nearly 100% and 24/7 access to our clinic, viral respiratory illnesses continue to cause considerable morbidity and high healthcare costs. [1] Innovative new prophylactic treatments are needed.

With this proposal, we will determine if the prophylactic use of azithromycin will 1) reduce the total number of days when unscheduled medical treatment health care related encounters was given outside the home, 2) reduce the number of emergency room/urgent care visits, hospitalizations and clinic visits due to respiratory illness, 3) reduce the level of myeloperoxydase and pro-inflammatory cytokines during viral illnesses requiring face to face physician interaction, 4) demonstrate a reduction in airway obstruction as measured by an oscillometer 5) have a similar safe profile compared to the placebo, and 6) demonstrate cost-effectiveness of macrolides use. Understanding the anti-inflammatory effects of azithromycin when used as a prophylactic drug will provide important insight into the prevention of more serious sequelae of respiratory infections. In particular, this study will contribute to understanding disease in children ages 6 months to 6 years with chronic lung disease, a population that has a higher rate of hospitalizations for respiratory symptoms.

We predict that the prophylactic use of azithromycin will reduce the morbidity associated with respiratory viral infections during the winter season in patients with chronic lung disease as evidenced by a reduction in the days with unscheduled face to face provider encounters based on the preliminary results from our laboratory study that indicated that prophylactic azithromycin can effectively reduce airway inflammation and disease severity in a RSV-infected mouse model.[6,7] Recent studies have shown that the high morbidity rate of respiratory virus infections is a result of a neutrophilic overactive inflammatory. [5] Macrolides down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbation.[15]

TRIAL STATUS:
Actively screening and enrolling patients.

LIST OF ABBREVIATIONS:

HRCC: High Risk Children's Clinic
CLD: Chronic Lung Disease
BPD: bronchopulmonary dysplasia
RCT: Randomized controlled trial
HRIC: High Risk Infant Clinic

COMPETING INTERESTS:
All authors have no conflict of interest to report.

AUTHORS' CONTRIBUTIONS:

R. Mosquera, G. Colasurdo, C. Jon, K. Smith, J. Stark, A.Yadav, K. McBeth, T. Gonzales, E. Avritscher, C. Pedroza, C. Samuels, T. Harris, A. Gomez-Rubio, S. Wootton, P. Piedra, and J. Tyson, were involved in the Concept and design of the study.

R. Mosquera, C. Jon, A.Yadav, K. McBeth, T. Gonzales, C. Samuels, T. Harris, A. Gomez-Rubio were involved in Enrollment.

C. Samuels, T. Harris, A. Gomez-Rubio were involved in Daily research activities.

R. Mosquera, J. Stark, E. Avritscher, C. Pedroza, C. Samuels, A. Gomez-Rubio, S. Wootton, P. Piedra, and J. Tyson, were involved in the manuscript preparation and approved the final manuscript. R. Mosquera wrote the initial protocol. A. Gomez-Rubio helped with the editing of tables and figures and the refining of the protocol. J. Stark helped in editing the protocol and gave suggestions to improve the methodology of the protocol. C. Pedroza was involved in writing the statistical analysis section of methods. S. Wootton and P.Piedra were involved in the planning and writing of the microbiology/laboratory portion of the protocol. J. Tyson mentored and adviser the primary investigator and was involved in the revision of the protocol. C. Samuels helped create, write, finalize, edit and refine the protocol from the rough draft to the final protocol.

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Dr. Ricardo Mosquera
UT-H - MS - Pediatrics-Pulmonary

NOTICE OF APPROVAL TO BEGIN RESEARCH October 09, 2014

HSC-MS-14-0476 - The prophylactic use of macrolides during the winter to improve outcomes and decrease health care utilization in high-risk children with chronic lung disease: A randomized control trial

Number of Subjects Approved: Target: 92 **/Screen:** 425

PROVISIONS: This approval relates to the research to be conducted under the above referenced title and/or to any associated materials considered at this meeting, e.g. study documents, informed consent, etc.

NOTE: If this study meets the federal registration requirements and this is an investigator-initiated study, or if the PI is the study sponsor or holds the IND/IDE applicable to this study, and no one else has registered this trial on the national registry, **you are required to register at** https://register.clinicaltrials.gov/ **before enrollment or no later than 21 days after the first patient is enrolled.** For website access and further information visit https://www.uth.edu/ctrc/regulatory/clinicaltrials.gov-registration.htm or contact clinicaltrials@uth.tmc.edu or call 713-500-3622.

APPROVED: At a Convened Meeting on 09/05/2014

EXPIRATION DATE: 08/31/2015

CHAIRPERSON: Rebecca Lunstroth, JD

Subject to any provisions noted above, you may now begin this research.

CHANGES: The principal investigator (PI) must receive approval from the CPHS before initiating any changes, including those required by the sponsor, which would affect human subjects, e.g. changes in methods or procedures, numbers or kinds of human subjects, or revisions to the informed consent document or procedures. The addition of co-investigators must also receive approval from the CPHS. ALL PROTOCOL REVISIONS MUST BE SUBMITTED TO THE SPONSOR OF THE RESEARCH.

INFORMED CONSENT DETERMINATION:
Signed Parental Consent/One Parent Signature

INFORMED CONSENT: Informed consent must be obtained by the PI or designee(s), using the format and procedures approved by the CPHS. The PI is responsible to instruct the designee in the methods approved by the CPHS for the consent process. The individual obtaining informed consent must also sign the consent document. Please note that only copies of the stamped approved informed consent form can be used when obtaining consent.

HEALTH INSURANCE PORTABILTY AND ACCOUNTABILITY ACT (HIPAA):

HIPAA Authorization required:
HIPAA Authorization within consent form

Waiver for Screening and Recruitment granted:
Information to be accessed:
Information to be retained:

Waiver for Retrospective Chart Review granted:
Information to be accessed: Date of Birth, Subject name, and Treatment/Service dates Information to be retained: Date of Birth, Subject name, and Treatment/Service dates

DEVICE STUDIES: Non-Significant Risk

UNANTICIPATED RISK OR HARM, OR ADVERSE DRUG REACTIONS: The PI will immediately inform the CPHS of any unanticipated problems involving risks to subjects or others, of any serious harm to subjects, and of any adverse drug reactions.

RECORDS: The PI will maintain adequate records, including signed consent documents if required, in a manner that ensures subject confidentiality.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	**The anti-inflammatory effect of prophylactic macrolides on children with chronic lung disease: a protocol for a double blinded randomized controlled trial.**
Trial registration		NCT02544984
Protocol version	3	September 23, 2015
Funding	4	No funding available

Roles and responsibilities	5a	Ricardo A. Mosquera, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study, enrollment and the manuscript preparation and final approval. Tomika Harris, PNP. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrollment, daily research activities and the manuscript preparation and final approval. Ana M. Gomez-Rubio, MPH. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrollment, daily research activities and the manuscript preparation and final approval. Aravind Yadav, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study, enrollment, and the manuscript preparation and final approval. James Stark, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study and the manuscript preparation and final approval. Cindy Jon, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrollment, and the manuscript preparation and final approval. Katrina McBeth, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrollment, and the manuscript preparation and final approval. Traci Gonzales, PNP. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrollment, and the manuscript preparation and final approval. Elenir Avritscher, MD PhD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Claudia Pedroza, PhD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Keely Smith, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Giuseppe Colasurdo, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study. Susan Wootton, MD. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study and the manuscript preparation and final approval. Pedro Piedra, MD. Department of Virology and Microbiology, Baylor College of Medicine, Houston, Texas. He was involved in the concept and design of the study, laboratory procedures and the manuscript preparation and final approval. Jon E. Tyson, MD, MPH. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. He was involved in the concept and design of the study and the manuscript preparation and final approval. Cheryl Samuels, PNP. Department of Pediatrics, McGovern Medical School at University of Texas Health Science Center at Houston, Houston, Texas. She was involved in the concept and design of the study, enrollment, daily research activities and the manuscript preparation and final approval.

	5b	NA
	5c	NA
	5d	NA

Introduction

Background and rationale	6a	Macrolides have received considerable attention for their anti-inflammatory and immunomodulatory actions. Such properties ma ensure some efficacy against a wide spectrum of respiratory viral infections. Recent studies, including a study performed in our lab with elderly BALB/c mice infected with **respiratory syncytial viru (RSV),** have shown that the high mortality rate of respiratory virus infections is a result **of an overactive neutrophilic** inflammatory response. A recently published study examined the inflammatory response in hospitalized infants with RSV and evaluated the predictive value of cytokines in nasopharyngeal aspirate in comparison to disease severity and found an increase in Th1 and Th2 cytokines. Respiratory viral infections are characterized by th appearance of cytokine storms which **involve an** extreme production and secretion of numerous pro-inflammatory cytokines Severity of infection is closely related to virus-induced cytokine dysregulation, which is responsible for the development of fatal clinical symptoms, such as massive pulmonary edema, acute bronchopneumonia, alveolar hemorrhage, and acute respiratory distress syndrome. Macrolides down-regulate the inflammatory cascade, attenuate excessive cytokine production in viral infectio and may reduce virus-related exacerbation. Clinical trials have demonstrated controversial results in the effects of macrolides in respiratory viral infections. To date, studies have only evaluated macrolide use as a treatment, not as a prophylactic therapy. Lon term therapy with the macrolide antibiotic erythromycin was show to alter the clinical course of diffuse pan bronchiolitis in the late 1980s. Since **then**, macrolides have been found to have a large number of anti-inflammatory properties in addition to their antimicrobial effect. These observations provided the rationale fo many studies performed over the last decade to assess the usefulness of macrolides in other inflammatory airways diseases including cystic fibrosis, asthma, COPD, and bronchiolitis oblitera syndrome. One randomized controlled Trial (RCT) looked at the daily use of macrolides for up to six weeks to prevent bronchopulmonary dysplasia in premature infants in a **neonatal intensive care unit (NICU)** setting and found the neonates had better outcomes without an increase in adverse effects.[14] Howeve the chronic use of macrolides **to prevent respiratory infection complications in patients with CLD of infancy has not been studied**. We will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness by preventing the full activation of an inflammatory cascade.

	6b	No other standard of care
Objectives	7	With this RCT, we will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness and that this is associated with the prevention of the full activation of an inflammatory cascade.
Trial design	8	With this RCT, we will test the hypothesis that prophylactic macrolides are effective in reducing the severity of respiratory viral illness and that this is associated with the prevention of the full activation of an inflammatory cascade.

Methods: Participants, interventions, and outcomes

Study setting	9	High risk children, born before 37 weeks gestation with a current diagnosis of CLD secondary to BPD between the chronological age of 6 months and <6 years who attend either the **High Risk Children's Clinic (HRCC)** or the High Risk Infant Clinic (HRIC) at **UTHealth at the McGovern Medical School in Houston, Texas USA** will be screened by the clinic providers.
Eligibility criteria	10	All children who currently attend either the HRCC or the HRIC **who** are between 6 months and 6 years at the time of enrollment **and who** meet the **American Thoracic Society (ATS)** definition of CLD secondary to BPD will be screened. Chronic lung disease, as defined by the ATS statement from 2002, is "a heterogeneous group of respiratory diseases of infancy that usually evolves from an acute respiratory disorder experienced by a newborn infant." BPD is defined as either **as** (1) infants born less than 32 weeks **who** need supplemental oxygen for at least 28 days **or (2) infants** born between 32 weeks and 36 weeks **who** need for supplemental oxygen for at least 56 days. All screening will be done by clinic providers (Table 1). Exclusion criteria include children with cystic fibrosis or bronchiectasis, because the prophylactic use of macrolides has already demonstrated value and become usual care for these patients. Children with cardiac arrhythmias will be excluded, due to the potential increase in cardiovascular death that has been shown in the adult population. Patients with known cyanotic heart disease will be excluded. Children with colitis or short bowel syndrome will also be excluded due to the potential effects to the gastrointestinal flora or malabsorption. In addition, any child with a known macrolide allergy or who is taking any medication that has a known interaction with macrolides, and any child with kidney or liver failure will also be excluded.

Interventions	11a	Both medications will be taken once a day three days a week: Monday, Wednesday and Friday. The azithromycin medication will be dosed at 5 mg/kg/day.
	11b	Any children with adverse reactions will discontinue the medication, but will continue to be followed clinically.
	11c	**For this study, patients will receive a monthly phone call from the research coordinator to assess for side effects and to remind them to take their medications. This should help improve adherence.**
	11d	NA

Outcomes	12	Our primary outcome is the total number of days of unscheduled face to face encounters for all diagnoses defined as unscheduled sick visits, urgent care visits, emergency room visits, and hospital admissions during the 3-6 month treatment phase of the study. Secondary outcomes: 1. Individual component of the primary outcome: unscheduled sick visits, urgent care visits, emergency room visits, and hospital admissions. 2. Adverse side effects including gastrointestinal upset (vomiting/ diarrhea) and diaper rash. 3. Total hospital and clinic costs from a health care system perspective. We expect the intervention to be cost-effective from a healthcare system perspective defined as: a. Decreased days of care without increasing cost b. Decreased cost without increasing days of care c. Decreased days of care while decreasing cost 4. Level of myeloperoxidase in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter. 5. Level of pro-inflammatory cytokines in the nasopharyngeal secretions collected during respiratory illnesses that require a face to face provider encounter. Level of airway obstruction as measured by a TremoFlo Airwave Oscillometry System during the 3-6 month treatment phase during respiratory illness and at the conclusion of the intervention phase.

Participant timeline 13 See Table 2.

Table 2: Study Schedule

	Study Period			
	Enrollment	Allocation	Post allocation	Close out
Timepoint	Oct-Dec	Oct-Dec	Oct-March	March-Oct
ENROLLMENT:				
Eligibility screen	X			
Informed consent	X			
Electrocardiogram	X			
Nasal aspirate	X		X	
Oscillometer	X			
Allocation		X		
INTERVENTION:				
Take medicine			X	
Take placebo			X	
ASSESSMENT:				
Demographics	X			
Unscheduled clinic visits			X	X
Unscheduled hospital visits			X	X
Emergency room visits			X	X
Evaluation of nasal aspirates				X
Analysis				X
Publication				X

Sample size 14 Based on data from our HRCC, we expect the control group to have 1.6 encounters per child-year (SD=1.66). Assuming a two-sided alpha = 0.05, a sample size of 92 (46/group) will have 80% power to detect a difference of 1 in the encounter rate between placebo and azithromycin groups (i.e., 1.6 vs 0.6 in encounter rate or 38% reduction).

Recruitment 15 We will ensure patient recruitment by screening both clinics weekly during the enrollment process. Eligible patients will be approached by the research coordinator and/or one of the providers. Patients who have further questions will be given time to think and will be approached later with a phone call or next time they are in clinic.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Biostatistician will create the **randomization** sequence **using a 1:1 allocation ratio and permuted block sizes. The sequence will be uploaded into REDCap.**
Allocation concealment mechanism	16b	**Allocation concealment will be ensured, as the allocation sequence is only known to the study statistician (who is also blinded to the labeling of the groups) and is not made available in REDCap until after the patient has been recruited into the trial.**
Implementation	16c	**Research coordinator will use the REDCap randomization module to obtain a patient's group assignment.**
Blinding (masking)	17a	This is a double-blinded placebo controlled study. Both participants and people directly related with study procedures, including the providers, will be blinded.
	17b	If a significant adverse reaction occurs, the medication will be discontinued. If an allergic reaction (such as rash or shortness of breath) is noted, the blind will be broken by the statistician, who is not involved with patient care or data collection. This un-blinding will be done to note if it is an allergy to the medication.

Methods: Data collection, management, and analysis

Data collection methods	18a	All data will be kept in REDCap database. REDCap is a **HIPAA** compliant and safe database that can be accessed from any computer with an internet access. It is backed up on a regular basis. Collected data at baselines includes: demographic information, baseline **ECG**, baseline nasal aspirate and baseline oscillometer reading. At the conclusion of the study the **ECG**, nasal aspirate will be repeated. If any patient presents to the clinic, ER or hospital with respiratory symptoms during business hours, a nasal aspirate and oscillometer reading will be collected. Data on when symptoms started and the symptoms experienced will also be collected.
	18b	We will call patients once a month to follow up on their medication status and to assess for any possible side effects.
Data management	19	Data can only be entered into **REDCap** by the research coordinator. At the end of the medication period, the research coordinator will ensure that all encounters were entered correctly by double checking the medical records.

Statistical methods	20a	Standard frequentist and Bayesian analyses will be performed using an intent-to-treat approach. **Total number of days of unscheduled face to face encounters, total hospital admissions (counting each hospitalization as an event), total ER/urgent care visits (counting one day for each ER/urgent care visit), and total unscheduled clinic visits (counting one day for each visit) will be analyzed and related to treatment group (Azithromycin vs Placebo) using Poisson regression models with robust standard errors to estimate relative risks (RRs) and 95% confidence intervals. All models will include treatment group and stratification variables (use of palivizumab and current tracheostomy) as covariates and length of follow-up as an offset.** To assess the probability of benefit, we will use Bayesian models with interaction terms between treatment group (Azithromycin vs Placebo) and **two** predefined potential moderators: use of palivizumab and current tracheostomy. The conservative Bayesian approach of Dixon and Simon allows us to shrink the subgroup estimates to the overall mean treatment effect. For all Bayesian analyses, prior distributions **for all regression coefficients will be centered at RR of 1.0 (Normal with mean 0 and standard deviation of 1 in the log scale), and half-Normal (0, 1) for standard deviation parameters.** Point estimates of treatment effect and 95% credible intervals will be reported along with probability of treatment benefit.
	20b	NA
	20c	Intent-to-treat analysis. We will continue to collect data on hospitalizations and ER visits even if we cannot get a hold of them.
Methods: Monitoring		
Data monitoring	21a	Drs. Cody Arnolds and Claudia Pedroza will act as the Data Monitoring Committee. Neither of them is involved in the daily aspects of the project but in case of any adverse events reported they will be in charge of the unblinding.
	21b	Since this is a pilot study no interim analysis or stopping rules will be applied.
Harms	22	Parents will be called once a month in order to see if any experience any adverse events. If there is a complaint, the providers will be made aware and if in their professional opinion they believe the event is an adverse reaction to the medication, it will be recorded in the **REDCap** database and parents will be advised to stop the medication immediately. In case of any serious adverse events, the IRB will also be notified.

Auditing	23	NA

Ethics and dissemination

Research ethics approval	24	UTHealth IRB approval was sought.
Protocol amendments	25	Research coordinator will communicate any changes to the protocol or staff to the **UTHealth** IRB using IRIS.
Consent or assent	26a	Parental informed consent will be collected. The research coordinator and a provider will talk to parent and explain the project, answer any questions and give a thorough explanation of study procedures, risk and potential benefits. Once the parents agree to participate, they will sign an IRB approved parental consent, detailing study procedures, risks and benefits. This parental consent will be scanned and uploaded into Redcap and paper copies will be saved in a binder that will be kept in a locked office.
	26b	NA
Confidentiality	27	Patients will be identified using **a study identification** number. All data will be entered into a **HIPAA** compliant database and all paper copies will be kept in a locked office to ensure patient confidentiality.
Declaration of interests	28	No financial interest to declare.
Access to data	29	Dr. Claudia Pedroza will have access to the final trial database. If any other investigators need to access the data, they will need to contact her.
Ancillary and post-trial care	30	No arrangements have been made for compensation to those who suffer harm from trial participation. This has been stated in the informed consent.
Dissemination policy	31a	The results of the trial will be published in a peer reviewed journal.
	31b	NA
	31c	We plan on submitting the protocol to the trials journal once the enrolment begins.

Appendices

Informed consent materials	32	Parental consent form is obtained.

Biological specimens	33	Microbiologic studies for exploratory outcomes will be conducted. The study involves collecting a minimum of 2 nasal aspirates from each patient. Nasal aspirate samples will be tested to measure the levels of myeloperoxidase and Lactate Dehydrogenase; we will also use ELISA tests to measure the levels of specific pro-inflammatory cytokines, including IL-8 and IL-6, and PCR, in order to screen for several major respiratory viral pathogens. Nasal aspirate specimens will be collected at the first visit, the final visit, and during any episodic respiratory sick visits at clinic or at Children's Memorial Hermann Hospital. Once collected the specimen will be immediately diluted (1:1 solution) with viral transport medium (15% glycerol in Iscove's media). The final solution (aspirated specimen mixed with viral transport medium) will contain a maximum of 9 mls (with a range of 3-9 mls). After dilution, the specimens will be immediately refrigerated at 37 degrees, until transportation to the freezers, where they will be stored at -80^{o}C. The samples will be frozen and store until the completion of the trial. The level of myeloperoxidase will be measured using reagent tests, and several cytokines that are markers of inflammation will be tested using ELISA tests, details of the primers and probes to be used are found in a 2005 article by Beckham et al.[10] In addition, RT-PCR assays will be performed using TaqMan-based primers and probes to detect the presence of 5-6 major respiratory viruses, and the remaining volume of aspirate will be stored for future studies of additional biomarkers. Nasal aspirate samples will be collected for assay of up to 4 cytokines and chemokines. Samples will be tested according to the manufacturer's instructions. Samples and serial dilutions of the cytokine standards will be incubated with anti-human cytokine-coated beads in a 96-well filtration plate.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.